Abstracts

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Abstracts for the oral and poster presentations are provided in this special on-line supplement.
Pharmacological Inhibition of PCSK9 in Hyperlipidemic Mice Significantly Reduces Serum LDL-C While Increasing Hepatic Low-Density Lipoprotein Receptor Protein Abundance

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a member of the proprotein convertase family of proteases that has been previously demonstrated to promote the degradation of the low density lipoprotein receptor (LDLr) through an undefined mechanism. Mutational analysis in humans has demonstrated that genetic polymorphisms which inactivate PCSK9 produce dramatic reductions in serum LDL-C and further, appear to reduce the risk of coronary artery disease (CAD). These encouraging epidemiological findings have been corroborated using PCSK9 knockout mice, which exhibit only the same phenotypic profile (i.e. decreased LDL-C and increased hepatic LDLr). For this reason, we have developed second generation antisense oligonucleotide inhibitors (ASOs) which target both human and mouse forms of PCSK9 mRNA to define their hydropolymorphic effects both in vitro and in vivo. Due to their optimal pharmacokinetic/pharmacodynamic properties, 2'MOE-modified ASOs have been extensively explored to inhibit a broad range of therapeutically attractive liver gene targets such as apoE, ApoA1, and ACAT2. Administration of the PCSK9 ASO (ISIS 394814) in high fat fed mice for 6 weeks (i., 100mg/kg/wk) resulted in reductions in both total cholesterol and LDL-C, 53% and 38%, respectively. In addition, hepatic mRNA and protein analysis revealed that ISIS 394814 reduced PCSK9 mRNA expression by 92% while increasing LDLr protein levels greater than 2-fold relative to controls. The magnitude of these effects is consistent with results previously reported in PCSK9 knockout mice. Based on these data, additional studies are in progress in LDLr deficient and additional mouse models to further demonstrate the specificity and pharmacological efficacy of this drug. These promising in vivo results suggest that inhibiting PCSK9 may indeed represent a novel therapeutic approach for reducing LDL-C in man.

Intestinal Cholesterol Absorption is Required for the LXR Agonist to Increase Plasma HDL Cholesterol in Mice

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Genetic manipulation has established the liver as the major source of HDL in normal mice. However, there is about 30% of plasma HDL contributed to the small intestine. It has been reported that liver X receptor (LXR) agonist GW3965 raises plasma HDL cholesterol levels in wildtype and liver-specific ABCA1 knockout mice but not in mice lacking intestinal ABCA1, indicating that the intestine plays an important role under circumstance in the biogenesis of plasma HDL cholesterol. The source of cholesterol for intestinal HDL formation is unclear. We hypothesize that cholesterol absorption from the gut lumen plays a role in the LXR agonist-stimulated HDL formation. To test our hypothesis, mice lacking Niemann-Pick C1-Like 1 (NPC1L1) (L1-KO) mice, a gene that is essential for intestinal cholesterol absorption, were treated with the LXR agonist T0901317 at 25 mg/kg BW/day for 7 days. As expected, T0901317-treated wildtype mice showed a dramatic increase in the plasma HDL cholesterol level but this effect was almost abolished in the L1-KO mice in which a much greater fecal cholesterol excretion was observed instead. The intestinal ABCA1 mRNA level was about 4-fold lower in the untreated L1-KO versus wildtype mice, and increased 4.4-fold and 7.6-fold in the T0901317-treated wildtype and L1-KO mice, respectively. Hepatic ABCA1 failed to respond to T0901317 in both wildtype and L1-KO mice although hepatic ABCA1/G8 mRNA levels were higher in the T0901317-treated versus untreated animals. In conclusion, intestinal cholesterol absorption is required for LXR agonist to increase plasma HDL cholesterol in mice.
Tetrahydrobiopterin Reverses Preexisting Hypertension-Induced Ventricular Remodeling by Recoupling eNOS

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Background: Pressure overload triggers eNOSs as a prominent source of myocardial ROS that contribute to dilatatory remodeling and cardiac dysfunction. Administration of tetrahydrobiopterin (BH4) can partially prevent pressure-mediated remodeling. The aim of the study was to investigate that BH4 can reverse established non-decompensated heart failure by interacting with uncoupled eNOS, and on this way prevent the evolution to end-stage heart failure. Methods: Compensated cardiac remodeling was induced in 60 mice by transverse aortic constriction (TAC). After 4wks, mice were randomized in pBH4 or placebo groups (n=30) for the following 5wks. Echocardiography, MRI and PV-loop analysis were performed. Conclusion: BH4 was evaluated to perform eNOS dimer/monomer. ROS generation was evaluated with dihydro-ethidium (DHE) staining and chemiluminescence. ROS activity and downstream NOS pathway were determined. Isolated myocyte studies were performed. Myocyte dimensions and fibrosis (score 0: absent - 3 pronounced) were histologically evaluated. Overexpression of endothelial GTPase, the rate limiting enzyme of BH4 synthesis, was evaluated in this TAC model (n=15 mice). Results: BH4 significantly reversed cardiac hypertrophy (heart weight 240±17 mg at 4wks, 324±16 mg at 9wks and 212±11 mg at 9wks with BH4 p<0.001; idem for myocyte dimensions, wall thickness and calculated LV mass) and diminished fibrosis (score 2.1±0.4 vs. score 0.6±0.4 with BH4, p=0.05). BH4 prevented the evolution towards cardiac decompensation (fraction f=45.7±1.6% at 4wks, 34.7±2% at 9wks and 53.4±4.5% at 9wks with BH4, p<0.001 and confirmed by MRI and PV loop analysis). BH4 reciprocated the already uncoupled eNOS and increased its activity back to the normal level. Superoxide generation (total and NOS-dependent) was markedly reduced by BH4, BH4 improved fractional shortening and calcium kinetics in isolated myocytes. Endothelial upregulation of BH4 by GTPON-Tg had no beneficial effect on remodeling. Conclusion: BH4 can reverse established cardiac remodeling by re-coupling uncoupled eNOS and as a consequence less NOS dependent ROS is generated, leading to less hypertrophy and fibrosis and an amelioration of cardiac function.

New Pathway to Gene Expression in Human Endothelial Cells: Filamin B Translation Is Preserved by Internal Ribosome Entry in Virally Infected EC

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Introduction: Eukaryotic translation initiation occurs either at the 5' cap of the untranslated region (UTR) or at an internal ribosome entry site (IRES). Messenger RNA containing IRESs may still be translated despite cellular stress that usually downregulates translation. Whether IRs-mediated translation occurs in stressed EC is unknown. Hypothesis: IRES-containing mRNAs are selectively enriched in polyribosomes from human EC when cap-dependent translation is impaired by viral infection. Methods: Cap-dependent translation in primary human umbilical vein EC was disabled using poliovirus- or mock-treated EC was analyzed by whole genome microarray with later verification by real-time RT-PCR. The presence of an IRES in the 5'UTR of candidate mRNA was confirmed using a dicistronic luciferase reporter assay. Results: Infection of human EC was verified by immunofluorescence (poliovirus I antigen). Four hours post-infection, loss of polyme in ribosomes indicates reduced translation of effector mRNAs on western blot. Translation repression of cap-dependent translation. On microarray analysis, 277 mRNAs were enriched >2-fold in poliovirus fractions from infected v control EC including several known to contain an IRES (Cyr 61, Pim1). Sequences enriched in poliovirus from infected EC with >100 fold change likely to contain an IRES. 20 were selected for further analysis, including filamin B (not previously known to have an IRES). Real-time PCR confirmed the microarray findings for 18/20 mRNAs. The filamin B 5'UTR (165 bp) was cloned into a dicistronic luciferase reporter plasmid. Translation of the downstream luciferase product after transcription of the reporter construct into EAHY surrogate EC confirmed the presence of an IRES in the filamin B 5'UTR. Results: (1) Translation of filamin B, an actin-binding protein implicated in mechanotransduction and cell signaling, is preserved in virally infected EC via an IRES-mediated mechanism, identifying another mechanism by which EC regulate the pattern of expressed protein in response to microbial challenge and stress; (2) Microarray analysis of poliovirus-infected EC is an effective large-scale method to screen for IRES-containing mRNAs in EC.

Reoxygenation Leads to Dissociation of Histone Deacetylase 7 from Hypoxia-inducible Factor-1a

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Interruption of cerebral blood flow causes hypoxia leading to the death of neurons and glia. Hypoxia-inducible factor-1a (HIF-1a) plays an important role in cell survival under oxygen deprivation by regulating the transcription of genes involved in glucose metabolism, cell proliferation and angiogenic pathways. Administration of tetrahydrobiopterin (BH4) can reduce vascular endothelial growth factor (VEGF), erythropoietin, and glucose transporter-1, which play a role in improved cell survival under hypoxia. When oxygenation is normal, HIF-1a is rapidly degraded through the ubiquitin-proteasome pathway, which is triggered by the oxygen-dependent hydroxylation of proline residues (Pro402 and Pro564) in BH4. These hydroxylated proline residues are recognized by the von-Hippel-Lindau tumor suppressor protein (pVHL), a component of an E3 ubiquitin ligase complex (pVHL, Elongin B, and Elongin C). The E3 ubiquitin ligase complex promotes ubiquitination of HIF-1 leading to degradation of HIF-1. BH4 increased the expression of some hypoxia-inducible factor-1a under re-oxygenation. Our findings suggest that degradation of HIF-1a was correlated with translocation of HIF-1a from the nucleus to the cytoplasm upon re-oxygenation. Using a mutant of HIF-1a (Pro402A/P564A), we also found that the stabilized HIF-1a mutant localized in the nucleus upon re-oxygenation whereas HIF-1a was exported to the cytoplasm. The amino acids substitution mutations of nuclear export sequences (NES) in HIF-1a (Nes mut.) blocked translocation of HIF-1a to the cytoplasm and stabilized HIF-1a in the nucleus upon re-oxygenation. Moreover NES mut HIF-1a bound HIF-1 upon re-oxygenation. Taken together, these results suggest that the dissociation of HIF-1a from HIF-1 and translocation of HIF-1a to the cytoplasm may lead to degradation of HIF-1a upon re-oxygenation.

Role of Autotaxin, a Plasma Lysophospholipase D2, in Hemostasis and Thrombosis

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Lysophosphatic acid (LPA) is a bioactive lipid mediator produced by platelets and found in plasma and atherosclerotic plaques. LPA stimulates platelets, leukocytes, endothelial cells, and smooth muscle cells to regulate cell growth, differentiation, survival, motility, and contractility. LPA is thus poised to serve as a key regulator of vascular cell function. LPA is generated in large part by the secreted lysosphospholipase D autotaxin (ATX, ep252). Mice with only one ATX gene (ep252/-) have plasma LPA levels that are 50% of wild type mice. ATX deficiency in mice (ep252/-) results in embryonic lethality due to vascular defects, implicating ATX and potentially LPA in vascular development. We observed excessive bleeding in three founder lines of transgenic FVB mice globally overexpressing ATX (ATX-Tg). To characterize the bleeding defect further, tail vein bleeding times were performed. The mean bleeding time in control FVB mice was 3 ± 2.7 min (n = 6), whereas none of the ATX-Tg mice (n = 10) stopped bleeding within 10 min (p <0.001). Platelet counts were similar in control (292 ± 184 × 10^10/ml) and ATX-TG (808 ± 156 × 10^10/ml), and platelets from control and ATX-TG mice displayed similar levels of platelet membrane glycoprotein IIb/IIIa (GPIIb/IIIa) as measured by flow cytometry. Upon stimulation by different agonists (ADP, collagen, thrombin), platelets from ATX-Tg mice also expressed P-selectin and bound fibrinogen as did control platelets. No differences in shear-induced platelet aggregation in whole blood from control and ATX-TG mice were observed (surface coverage 10.25 ± 1.73% and 9.21 ± 1.6%, respectively, p = 0.59). Thus, ex vivo studies were not able to recapitulate a platelet function defect. Additionally, clotting times were normal in the ATX-TG mice. To determine if thrombosis was altered in the ATX-TG mice, mice were observed in the ferric chloride-induced carotid artery thrombosis model. In control mice, thrombus formation occluded the vessel in 10 ± 1.4 min (n = 3), whereas in the ATX-TG mice (n = 3) stopping occurred within 20 min (p<0.001). In summary, our results suggest that ATX, and potentially LPA, regulate hemostasis and thrombosis through effects on vascular function.

Variable Growth Rates and Diameter-Dependent Expression of Vascular Endothelial Growth Factor Receptors in Experimental Abdominal Aortic Aneurysms

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Purpose: Transmural inflammation and adventitial neovascularization are important pathophysiological correlates of both human and experimental abdominal aortic aneurysm disease progression. We hypothesized that adventitial VEGF receptor expression would be increased in dilated aortic segments of E-deficient mice versus E-mutant II a murine model.

Methods: Appropriate E deficient mice with a C57BL/6 background were infuened with Angiotensin II (1000 ng/kg/min) via a subcutaneous osmotic pump. The mice were maintained on a high fat diet. Luminal diameter was determined in vivo via serial transabdominal ultrasonad imaging examinations (n= 22). Greatest lumen diameter was recorded. Near-infrared fluorescent imaging of VEGF receptors with single chain VEGF-Cys 5 conjugate injected
intravenously (10μg/mouse) was performed on select mice (n = 3) once AAD diameter reached at least 175% of baseline aortic diameter. Results: All AADs were identified as suprarenal and exhibited a variable growth rate. By postoperative day 12, 45% of the mice demonstrated a large AAD (defined as 175% or greater lumen diameter dilation compared to normal) with transabdominal ultrasound. We visualized large AADs in 82% of the mice by postoperative day 24, and 95% of the mice by postoperative day 30 with ultrasound. In vivo and ex vivo fluorescence imaging of VEGF receptors with scVEGF-Cys5 in select mice demonstrated increased signal in the larger AAD (245% dilated) compared to the smaller AAD (183% dilated).

Conclusion: We have demonstrated a variable growth pattern and size dependent enhancement in VEGF-C2 expression in a mouse model of AAD disease. VEGF receptors may prove useful as a clinical marker of AAD progression.

Critical Role of Endothelial Notch1 Signaling in Postnatal Angiogenesis

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Notch receptors are important mediators of cell fate during embryogenesis, but their role in adult physiology, particularly in postnatal angiogenesis, remains unknown. Of the Notch receptors, only Notch1 and Notch4 are expressed in vascular endothelial cells. Here we show that blood flow recovery and postnatal neovascularization in response to hindlimb ischemia in haploinsufficient global or endothelial-specific Notch1−/− mice, but not Notch4−/− mice, were impaired compared with wild-type mice. The expression of vascular endothelial growth factor (VEGF) in response to ischemia was comparable between wild-type and Notch mutant mice, suggesting that Notch1 is downstream of VEGF signaling. Treatment of endothelial cells with inhibitors of phosphatidylinositol 3-kinase/protein kinase Akt or infecting endothelial cells with Atox1 deficient mouse fibroblasts and by knockdown of Atox1 using siRNA. Taken together, we provide the first evidence that Atox1 functions as a copper dependent transcription factor for cyclin D1 and thus stimulates cell proliferation, thereby promoting neovascularization induced by tissue ischemia.

Candidate Susceptibility Loci for Vascular Remodeling Identified Through a Genome-wide Association Study of In-stent Restenosis

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Introduction: Risk-conferring genes responsible for complex diseases are characterized by variable expressivity, low penetrance, epistasis and locus heterogeneity, making the analysis of complex genetic traits challenging. For complex cardiovascular diseases, candidate gene approaches have achieved limited success, making genome-wide association study (GWAS) designs appealing. Molecular and genetic studies suggest that in-stent restenosis (ISR) is primarily an inflammatory and proliferative disease, with distinct roles for cell cycle proteins, growth factors, and inflammatory cytokines. Methods: To investigate the genetic basis of ISR, we designed a case control GWAS on a dataset of ~116,000 single nucleotide polymorphisms (SNPs) assayed in 407 patients (150 cases, 257 controls). We undertook a haplotype analysis of regions highlighted by the presence of two or more SNPs within 250 kb of one another and with p < 0.001 (unadjusted) in univariate tests of allelic association. Haplotypes were defined in the regions and tested for association with ISR, using a Bonferroni correction for all haplotype and tumor angiogenesis data. VEGF expression was assessed by quantitative PCR in FACS isolated human monocytes. We then tested whether these 12 identified SNPs were associated with VEGF expression. Results: We identified two regions with significant associations with VEGF expression, and five genes: NOV, ARNTL, TAF4B, IPK4 and a hypothetical protein FLJ21986. We observed expression of these genes by RNA and protein analysis in normal, atherosclerotic and restenotic human coronary arteries, supporting the hypothesis that these genes may mediate vascular homeostasis and adverse vascular remodeling. We estimated the subject’s haplotypes and examined the time to development of ISR as a quantitative trait in a Cox regression model and demonstrated a significant effect of allele dose for each of the eight regions identified (p < 0.00038 to 0.035, after Bonferroni correction), suggesting that allele copy number contributes to ISR. We additionally added the data from our unbiased BRLLM algorithm to call genotypes and identified some of the same genes and e38
regions demonstrate significant allele dose effect as well (p<0.003). Conclusions: Haplotype analysis of GWAS using SNP markers is a useful approach to identify candidate genes for complex vascular diseases. These areas of association with ISR warrant further investigation.

17 Ambient Particulate Pollutants in the Ultraltra Range Promote Atherosclerosis and Systemic Oxidative Stress

Atherosclerosis is a disease of the vascular endothelium characterized by the formation of fatty plaques on the inner lining of arteries. It is caused by the accumulation of lipids, macrophages, and smooth muscle cells, which lead to the formation of atherosclerotic plaques. These plaques can rupture, leading to the formation of blood clots and potentially blocking blood flow to vital organs. Environmental factors, such as air pollution, have been implicated in the development of atherosclerosis. In this study, the authors aimed to investigate the role of ambient particulate pollutants in the ultraltra range (PM<0.1 μm) in promoting atherosclerosis and systemic oxidative stress.

The study was conducted in a genetically-modified mouse model, where the authors induced apoptosis in established atherosclerotic lesions. Apoptosis is a process that involves the programmed cell death of cells that are no longer needed. The authors used a new genetically-modified mouse model, where they inactivated macrophage fatty acid synthase (FAS) using Cre-lox technology. This model allowed them to study the role of FAS in atherosclerosis development. They found that FAS deficiency in macrophages significantly reduced atherosclerotic lesion formation.

The study also revealed that exposure to PM<0.1 μm particles increased systemic oxidative stress, which is a key factor in the development of atherosclerosis. The authors suggested that future studies should focus on the role of other pro-oxidative chemicals in PM particles and their impact on atherosclerosis development.

18 Induction of Apoptosis in Established Atherosclerotic Lesions Promotes Inflammation and Monocyte Recruitment in ApoE-/- Mice

Apoptosis is a process of programmed cell death that plays a crucial role in the development and progression of atherosclerosis. In this study, the authors aimed to investigate the role of apoptosis in atherosclerosis using a new genetically-modified mouse model, where they inactivated macrophage fatty acid synthase (FAS) using Cre-lox technology. This model allowed them to study the role of FAS in atherosclerosis development.

The authors found that apoptosis in atherosclerotic lesions promoted inflammation and monocyte recruitment. They also observed an increase in the expression of pro-inflammatory chemokines and the recruitment of macrophages to the atherosclerotic lesions. These results suggest that apoptosis associated with impaired apoptotic cell clearance may promote inflammation and monocyte recruitment in established atherosclerotic lesions.

19 Distinctive Expression of Chemokines and Transforming Growth Factor-β Signaling in Human Atrial Endothelium During Atherosclerosis

Atrial endothelial cells play a crucial role in the regulation of cardiac function. In this study, the authors aimed to investigate the role of chemokines and transforming growth factor-β (TGF-β) signaling in human atrial endothelium during atherosclerosis.

The authors found that the expression of chemokines and TGF-β signaling in human atrial endothelial cells was significantly increased in atherosclerotic lesions. They also observed an increase in the expression of pro-inflammatory chemokines and the recruitment of macrophages to the atherosclerotic lesions. These results suggest that apoptosis associated with impaired apoptotic cell clearance may promote inflammation and monocyte recruitment in established atherosclerotic lesions.

20 Inactivation of Macrophage Fatty Acid Synthase Decreases Atherosclerosis

Fatty acid metabolism is disturbed in atherosclerotic lesions but whether it affects lesion formation is unknown. To test this hypothesis, the authors inactivated macrophage fatty acid synthase (FAS) in apoE-deficient Cre-lox mice. They found that FAS deficiency in macrophages significantly reduced atherosclerotic lesion formation.

The authors suggested that future studies should focus on the role of other pro-oxidative chemicals in PM particles and their impact on atherosclerosis development.

21 A Cluster of Basic Residues Within the Factor V B-domain Contributes to Preserving the Procofactor State

Factor V is a coagulation factor that plays a crucial role in blood clotting. In this study, the authors aimed to investigate the role of a B-domain in preserving the procofactor state of Factor V.

The authors found that a B-domain that lacks a cluster of basic residues significantly reduced the procofactor state of Factor V. They also observed a decrease in the generation of active Factor Va (FVa), which is necessary for blood clotting.

The authors suggested that future studies should focus on the role of other pro-oxidative chemicals in PM particles and their impact on atherosclerosis development.

22 The Utility of Quantitative Calf Muscle Near-Infrared Spectroscopy in the Follow-up of Acute Deep Vein Thrombosis

Near-infrared spectroscopy (NIRS) is a non-invasive technique that can be used to measure tissue oxygenation. In this study, the authors aimed to investigate the utility of NIRS in the follow-up of acute deep vein thrombosis (DVT).

The authors found that NIRS can be used to measure tissue oxygenation in the calf muscle and that this can be used to monitor the progress of DVT treatment. They also observed that NIRS can be used to identify patients who are at high risk of DVT recurrence.

The authors suggested that future studies should focus on the role of other pro-oxidative chemicals in PM particles and their impact on atherosclerosis development.
of 78 limbs with an acute deep vein thrombosis (DVT) involving 156 anatomic segments were evaluated with duplex scanning and near-infrared spectroscopy (NIRS) at 1 month, 3 months, 6 months, and 1 year. Venous segments were examined whether they were occluded, partially recanalized, and totally recanalized, and the development of venous reflux was noted. The NIRS was used to measure calf muscle HbI levels. Calf venous blood filling index (HbFill) was calculated from standing, of the lower extremity venous index (HbIn) and the venous retension index (HbR) were obtained after exercise. RESULTS: The segments investigated were the common femoral vein (CFV; 38 segments), femoral vein (FV; 37), popliteal vein (POPV; 44), and calf veins (CV; 37). At 1 year, thrombi had fully resolved in 67% of the segments, 27% remained partially recanalized, 6% were occluded. The venous occlusion was predominant in the FV (24%) at 1 year. On the contrary, rapid recanalization was observed in CV than proximal veins at each examination (p < 0.01). Venous reflux was predominant in POPV (55%), followed by FV (19%), and no reflux was found in CV. At 1 year, the HbFill and HbIn in POPV reflux patients was significantly higher than the other segments (HbFill resolution (0.19 ± 0.05 vs. 0.14, 0.11 ± 0.05 g/100 ml, p = 0.009, respectively). Similarly, there was a significant difference in the HbR between the two groups (3.08 ± 1.91, 1.42 ± 1.56, p = 0.002, respectively). In patients with FV occlusion, the value of HbR was significantly higher than these with complete resolution (2.58 ± 1.50, 1.42 ± 1.56, p < 0.001, respectively). CONCLUSIONS: The lower extremity venous segments show different proportions of occlusion, partial recanalization, and total recanalization. The CV shows more rapid recanalization than proximal veins. The NIRS-derived HbFill and HbR could be promising parameters as the overall venous function in the follow-up of acute DVT. These findings might be very helpful for physician in detecting patients who require much longer follow-up studies.

A Unique Function for Low-density Receptor–Related Protein-1: A Component of a 2-Receptor System Mediating Specific Endocytosis of Plasma-derived Factor V by Megakaryocytes

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Factor V is endocytosed by megakaryocytes from plasma via a specific, receptor-mediated, clathrin-dependent mechanism to form the functionally and physically unique platelet-derived factor V pool. Because of its ability to endocytose proteins involved in hemostasis, the role of low density lipoprotein receptor-related protein-1 (LRP-1) in factor V endocytosis by the megakaryocyte-like cell line, CMK, was examined. Equilibrium binding of [125I]Factor V to CMK cells is defined by a sigmoidal binding isotherm, suggesting that factor V binding is cooperative and mediated by a two-receptor system. Furthermore, 125I-Factor V binding is reversible and partially sensitive to receptor associated protein (RAP), a known LRP-1 ligand. Based on these observations a two-receptor model for factor V binding to megakaryocytes was hypothesized. In this model, factor V binds to a specific receptor facilitating binding of another factor V molecule to LRP-1 or a like molecule, which subsequently endocytoses factor V. The purpose of the current study was to identify which member of the LRP-1 receptor family is involved. Using BC20, expression of an LRP-1 transcript in CMK cells was demonstrated. In contrast, transcripts representing other LRP family members could not be identified. Cell surface expression of LRP-1 antigen by CMK cells was confirmed by flow cytometry using a monoclonal anti-LRP-1 antibody. Greater than 70% of the CMK cells expressed LRP-1 on their cell surface. Co-localization of endocytosed Alexa Fluor 488–factor V and LRP-1 demonstrated that all of the factor V positive cells expressed LRP-1. These same anti-LRP-1 antibodies were used to displace ~40% of the bound 125I-factor V from the surface of the cells. Furthermore, factor VIII, a known ligand of LRP-1, inhibited 125I-factor V endocytosis equally as well as factor V when present at a 25-fold molar excess. These combined observations confirm our model of the receptor events regulating factor V binding, and represent a novel paradigm whereby an essential coagulation protein is endocytosed from plasma and modified intracellularly to yield a functionally distinct molecule. Furthermore, a role for LRP-1 in endocytosis of a protein not destined for lysosomal degradation is unique.

Induction of Tissue Factor and Loss of Thrombomodulin Activities upon Inflammatory Stimulation in Vivo

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Introduction: Induction of organ specific changes in expression of tissue factor (TF) and thrombomodulin (TM) antigen, but it is unknown whether this translates to a net procoagulant phenotype in vivo. Hypothesis: induction of TF and suppression of TM activities contribute to the organ-specific changes in response to inflammatory stimuli, including endotoxin (LPS) and particulate mater (PM). Design and results: in C57BL/6 mice TF activity in the lungs was induced more by intraperitoneal LPS (25.8 ± 5.3 pm) than by saline (12.7 ± 1.7 pm, p < 0.05); TF activities in other organs (brain, heart, spleen, liver, kidney) were comparable for LPS and saline. Addition of lung homogenate from control mice to plasma markedly attenuated the plasma endogenous thrombin potential (ETP) (148 vs. 175 pmol[1021]/sec, p < 0.002, respectively). Increased expression of the NAD(P)H oxidase subunit p47phox, an index of activity, was observed in Ang II-infused controls. This was blunted in TG/Ang–1–7 mice. Phosphorylation of cardiac c-Src was increased by Ang II in controls (2.5-fold) but not in TG/Ang–1–7 mice. Activation of redox-sensitive growth signaling molecules, Akt and p38MAPK, was increased by Ang II but not in TG/Ang–1–7 mice. Angiotensin II (2–3-fold, p < 0.05) increased Ang II infusion (TG/Ang–1–7) mice developed less ventricular hypertrophy and fibrosis versus controls, in spite of developing similar levels of hypertension (p < 0.05). Increased expression of Ang II, the Neg modulatory effects of natriuretic peptides dilate blood vessels and inhibit cell cycle regulatory proteins. Our findings suggest that the negative modulatory effects of Ang–1–7 may represent a protective mechanism whereby potentially deleterious actions of Ang II are counterbalanced.

25 Modulation of LPS-induced Inflammation and Coagulation by the PI3K-Akt Pathway

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Lipopolysaccharide (LPS) stimulation of monocytes/macrophages activates various intracellular signaling pathways, including the mitogen-activated protein kinases (MAPKs) and the phosphatidylinositol-3-kinase (PI3K)-Akt pathway. LPS activation of the MAPKs pathways is required for the expression of inflammatory cytokines and tissue factor (TF), the principal activator of blood coagulation. We have shown that pharmacologic inhibition of PI3K enhances LPS signaling and the induction of inflammation and coagulation, both in monocyctic cells and in endotoxemic mice. To extend these findings, we determined the effect of genetically reducing or enhancing activation of the PI3K-Akt pathway on LPS induction of inflammatory mediators and TF in peritoneal macrophages (PMs) and in mice. We utilized PMs lacking p85α, which have decreased PI3K-Akt activity and PMs lacking the phosphatase PTEN, which have increased PI3K activity. LPS signaling and PI3K-Akt expression, TF, IL-6 and TFα2 were enhanced in p85α−/− PMs. Furthermore, inflammation and coagulation were enhanced in endotoxemic wild type mice lacking p85α−/− in hematopoietic cells. LPS activation of the MAPKs and the expression of TF, IL-6 and TFα2 were reduced in PTEN−/− PMs. Our results indicate that LPS activation of the PI3K-Akt pathway in macrophages inhibits the MAPK signaling pathways and reduces inflammation and coagulation. Activation of PI3K or inhibition of PTEN may represent novel strategies to reduce inflammation and coagulation in endotoxia and sepsis.

Angiotensin II-induced Hypertrophy and Fibrosis is Prevented by Angiotensin 1–7 Overexpression in the Heart

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Background: In vitro studies demonstrate that Ang–1–7 opposes many actions of Ang II. Whether similar effects occur in vivo remains unclear. Objectives: We tested the hypothesis that Ang–1–7 protects the heart from damage induced by Ang II and assessed some signaling pathways whereby this occurs. Methods and results: Transgenic mice over-producing Ang–1–7 (TG/Ang–1–7) exclusively in the heart (6-fold) were generated. Basal blood pressure and cardiac contractility were similar between groups, we evaluated whether Ang–1–7 exerts direct cardioprotective effects, TG/Ang–1–7 and control mice were infused with Ang II (350ng/kg/min, 19 days). Ang II-infused TG/Ang–1–7 mice developed less ventricular hypertrophy and fibrosis versus controls, in spite of developing similar levels of hypertension (p < 0.05). Increased expression of Ang II, the Neg modulatory effects of natriuretic peptides dilate blood vessels and inhibit cell cycle regulatory proteins. Our findings suggest that the negative modulatory effects of Ang–1–7 may represent a protective mechanism whereby potentially deleterious actions of Ang II are counterbalanced.

26 Requirement of RhoA Serine 188 Phosphorylation for cGMP Kinase-mediated Vascular Protection

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[Background] cGMP agonists such as natriuretic peptides dilate blood vessels and inhibit vascular remodeling. Small GTPase RhoA and its effector ROCK, crucial mediators of
vasoconstriction and vascular growth, antagonize cGMP effects. We previously demonstrated that cGMP-dependent protein kinase type I (cGKI I), the direct cGMP effecter, phosphorylates Rhoades at Ser188 and inhibits its downstream signaling. However, the in vivo relevance of this interaction remains elusive. This study assessed our hypothesis that cGKI I phosphorylation of Rhoades mediates vascular protection by cGMP agonists. [Results] Ser188 phosphorylation of Rhoades was increased in response to cGMP-specific antibodies, was diminished in the aortas of cGKI I haploinsufficient mice (cGKI I-/-), whereas RhoA/RhoK activity was enhanced. Brain natriuretic peptide transgenic mice (Bnp-Tg), with 3- to 4-fold increase in plasma cGMP levels, showed augmented phosphorylation of Rhoades with less ROCK activity as compared to controls. cGKI I/-/- mice treated with angiotensin II (Ang II) developed exaggerated BP elevation, cardiac hypertrophy, coronary artery medial thickening (MT) and peripheral fibrosis (PF), and expression of fibrogenic cytokines and extracellular matrix (ECM) proteins. ROCK inhibitor Y-27632 markedly normalized these changes except elevated BP. To assess the role of Ser188 phosphorylation of Rhoades in cGKI I-mediated vascular protection, we generated transgenic mice that express cGKI I-unphosphorylatable mutant Rhoades (A188RhoA) or wild-type Rhoades (wtRhodA) in arterial smooth muscle using SM2Zaapia promoter. A188RhoA-Tg exhibited 2.7-fold augmented RhoA activity and 2-fold increase in MT and PF compared to non-Tg, whereas these changes were milder in wtRhoA-Tg mice. With similar Bnp-Tg double Tg (DTg) displayed normalized MT and PF, those of A188RhoA/BNP-Tg DTg remained worse (1.5-fold) than non-Tg. Ang II treatment induced comparable increase (1.5- to 4-fold) in MT, PF and ECM gene expression in A188RhoA/BNP DTg and non-Tg mice. By contrast, these changes induced by Ang II were blunted in wtRhoA/BNP DTg and BNP-Tg mice. [Conclusion] Our data suggest that Ser188 phosphorylation and inhibition of Rhoades by cGKI I is required for cGMP/cGKI I-mediated protection against vascular remodeling.

Cyclooxygenase-2 Expression Increases Vascular Inflammation and Abdominal Aortic Aneurysm Formation in Mice

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Abdominal aortic aneurysms (AAAs) are associated with a profound inflammatory response within the vessel wall throughout propagation of the disease. Increased expression of cyclooxygenase-2 (COX-2) is suggested to contribute to the disease in humans. We examined the hypothesis that COX-2-dependent proliferation or inflammation in the abdominal aorta contributes to AAA formation. AAAs were induced by angiotensin II infusion and compared between COX-2-deficient mice and their wild-type littermate controls. COX-2-deficient mice showed significantly reduced AAA incidence at multiple time-points following angiotensin II infusion (day 3, 31% for COX-2-/- versus 6% for COX-2+/-; P<0.07; day 7, 43% for COX-2-/- versus 12% for COX-2+/-; P<0.01; day 21, 37% for COX-2-/- versus 4% for COX-2+/-; P<0.01; day 28, 54% for COX-2-/- versus 0% for COX-2+/-; P<0.01). COX-2 has been previously shown to be required for angiotensin II-induced smooth muscle cell proliferation. To examine the role of proliferation in COX-2-dependent AAA formation, we determined the effect of COX-2 expression on activation of Akt and Erk1/2. As determined by western blot, the levels of phosphorylated Akt or Erk1/2 were not significantly different between angiotensin II-infused COX-2-/- and COX-2+/- mice, suggesting that COX-2 does not contribute to altered proliferation during AAA formation. Angiostatin II-induced AAA formation, we determined the effect of COX-2 expression on expression of MMP-1 and Timp-1. In conclusion, increased COX-2 expression in the abdominal aorta may contribute to AAA formation by enhancing macrophage recruitment.

Integrin Signaling is Critical for Pathological Angiogenesis

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The process of postnatal angiogenesis plays a crucial role in propagation of numerous diseases, including but not limited to tumor growth/metastasis, diabetic retinopathy, and in tissue remodeling upon injury. However, the molecular events underlying this complex process are not well understood and numerous issues remain controversial, including the regulatory function of integrin receptors. To analyze the role of integrin phosphorylation and signaling in angiogenesis, we generated knock-in mice that express a mutant beta3 integrin unable to undergo tyrosine phosphorylation. Two distinct models of pathological angiogenesis revealed that beta3 knock-in is impaired in mutant beta3 knock-in mice. In an ex vivo angiogenesis assay, mutant beta3 knock-in endothelial cells did not form complete capillaries in response to vascular endothelial growth factor (VEGF) stimulation. At the cellular level, defective tyrosine phosphorylation in mutant beta3 knock-in cells resulted in impaired adhesion, spreading, and migration of endothelial cells. At the molecular level, VEGF stimulation resulted in reduced expression of a number of endothelial markers, including VEGF receptor-2 and integrin beta-2 in wild-type but not in mutant beta3 knock-in endothelial cells. Moreover, phosphorylation of VEGF receptor-2 was significantly reduced in cells expressing mutant beta3 compared to wild type, leading to impaired integrin activation in these cells. These findings provide novel mechanistic insights into the role of integrin-VEGF axis in pathological angiogenesis.

Matrix Metalloproteinase-1: Role in Aneurysm Formation in Vivo and Regulation by Nicotine in Vascular Smooth Muscle Cells

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The role of matrix metalloproteinase-1 (MMP-1) and the molecular mechanisms regulating its expression in aneurysm remain unknown. Transgenic mice expressing human MMP-1 in macrophages were crossed into the ApoE-null / Timp-1 (Tissue inhibitor of metalloproteinases-1) knockout background, which develops a high number of micro- aneurysms associated with atherosclerosis. Transgenic mice (n=11) and their littermates (n=10) were given a high-fat diet for ten weeks and sacrificed. Transgenic mice had larger aneurysms (45,216±28,111 μm3, n=33) compared to controls (26,185±22,026 μm3, n=28, P<0.005). These aneurysms were also characterized by the bulging of the diseased area leading into the adventitia. In addition, we demonstrate that nicotine induces MMP-1 expression in smooth muscle cells via the MAP kinases extracellular signal-regulated kinases (ERK), at concentrations found in the circulation of moderate smokers (10 ng to 100 μg). After 24 hours, nicotine increased the expression of MMP-1, both at the mRNA and protein levels (P<0.05). This up-regulation was abrogated by specific inhibitors of p38 and ERK, suggesting that nicotine induces MMP-1 through the MAP kinases extracellular signal-regulated kinases (ERK) pathway. Western blot of cell lysates showed that nicotine activates the Jak/STAT kinase pathway, leading to increased phosphorylation of Jak2, ERK, p38, Jnk, and Stat3, and subsequent induction of MMP-1 expression. Our data demonstrates that MMP-1 enhances the severity of mouse aneurysms, and that nicotine induces MMP-1 expression in smooth muscle cells through the Jak/STAT kinase pathway. This study suggests that increased susceptibility to aneurysm formation and rupture in smokers might result from augmented collagenase activity in the vessel wall, due to a chronic exposure to circulating nicotine.
conclusion, these studies demonstrated that co-expression of CCL21 with Fk-1 improved T cell-mediated immune responses against Fk-1 and reduced lesion progression in hyperlipidemic mice.

**33** Diet-induced Dyslipidemia Is Associated with Acceleration of Disease and Mortality in Lupus-susceptible LDLr<sup>−/−</sup> Mice

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Individuals suffering from systemic lupus erythematosus (SLE) are predisposed to accelerated atherosclerosis. Unfortunately, the underlying mechanisms for increased vascular disease in lupus are not well understood. Our laboratory has recently developed an animal model of SLE-deranged atherosclerosis. We have shown that radiation chimeras consisting of SLE-derived hematopoietic cells transferred to LDLr<sup>−/−</sup> mice have increased atherosclerosis. Surprisingly, feeding mice high-fat diet for 8 weeks resulted in significant mortality in SLE-susceptible mice compared to controls. Based on these data, we hypothesized that increased dyslipidemia characterized by an accumulation of non-HDL lipoproteins, is associated with increased atherosclerosis. To test this hypothesis, we created radiation chimeras of LDLr<sup>−/−</sup> mice that were either SLE-susceptible (LDLR.Sle) or resistant (LDLR.B6). Eight weeks following bone marrow reconstitution, mice were placed on a normal chow or high fat (21% fat, 0.15% cholesterol) diet for eight weeks. All animals fed a high-fat diet had significantly increased total cholesterol and triglycerides compared to chow fed mice, however there were no significant differences between groups within LDLR.B6 control and LDLR.Sle mice. Compared to all chow-fed animals and high-fat fed LDLR.B6 controls, high-fat fed LDLR.Sle mice exhibited increased mortality (37%) and were mildly, but not significantly hypotensive. In addition, ECHO analyses showed that 60% (3 of 5 mice) of the LDLR.Sle mice fed high fat diet had increased left ventricular mass compared their LDLR.B6 counterparts. Increased blood pressure did not overtly appear to be due to advanced renal disease as serum creatinine and urea levels between LDLR.Sle mice on chow or high-fat diet did not differ significantly. These data demonstrate that increased dyslipidemia resulting from feeding a high-fat diet can exacerbate atherosclerosis and associated vascular complications.

**34** Chemical Genetic Analysis Reveals the Central Role of Phosphatidylinositol-3 Kinase and MAP Kinase/ERK Signaling Pathways in Artery/Vein Specification

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How the endothelial progenitor cells are specified to either the arterial or venous fates is a fundamental biological question with significant clinical implications. We reasoned that the arterial specification during development is amenable to genetic analysis, in a manner analogous to the classical genetic analyses, which have been instrumental in elucidation of numerous biological pathways in proaryocytes and intervertebrates. Specifically, we hypothesized that small molecules found to suppress zebrafish model of arterial deficiency will disrupt talin-mediated integrin activation by generating platelet-specific talin1 knock-out mice (PKO). The phenotype of PKO mice was due to lack of talin binding to integrin llb.

**35** ER Stressors PromoteTLR4/SRA-dependent Macrophage Apoptosis Through Ca<sup>2+</sup>-mediated CalMIIK Activation


The macrophage (Mö) scavenger receptor-A (SR-A) and toll-like receptor 4 (TLR4) are pattern recognition receptors (PRRs) of the innate immune system. In vitro data suggest that the SR-A and TLR4 contribute to the development of atherosclerosis, although the mechanistic links between these PRRs and atherosclerosis have not been elucidated. Our laboratory has focused on a critical event in the formation of necrotic atherosclerotic plaques, namely, Mö death. We recently discovered that SRA ligands trigger Mö apoptosis in an SRA- and TLR4-dependent manner. SRA ligands activate the pro-apoptotic TRA-JNK pathway but, unlike others, silence the pro-survival TFIR-A/JNK pathway. We also found that ER stressors that induce Ca<sup>2+</sup>-mobilization enhance the TRA-JNK-dependent signaling and that chelating Ca<sup>2+</sup> inhibits the pro-apoptotic STRAT activation and apoptosis. In the current study we show that LPS, which normally requires to cell surface TLR4, induces apoptosis in various SRA ligands such as fucoidan, β-amyloid, and advanced glycation products (AGEs). These SRA ligands do not induce apoptosis when added in the absence of LPS. Moreover, Mö apoptosis induced by LPS and SRA ligands was markedly amplified in the setting of Ca<sup>2+</sup> releasing ER stressors such as thapsigargin. To probe mechanism, we investigated the role of Ca<sup>2+</sup>-regulated calmodulin dependent protein kinase II (CalMIIk). We found that apoptosis-enhancing Ca<sup>2+</sup>-releasing ER stressors contribute to two events necessary for Mö apoptosis: enhancement of TRA-JNK dependent apoptosis through activation of CalMIIk and induction of the pro-apoptotic CHOP branch of the ER stress pathway through depletion of ER Ca<sup>2+</sup> stores. These findings reveal a novel link between ER stress, Ca<sup>2+</sup> mobilization, and PRR signaling and have potential implications for Mö death and plaque necrosis in advanced atherosclerosis and perhaps other diseases where PRRs, ER stress, and cell death are known to play a role.

**36** Signal-Dependent Splicing of Tissue Factor Pre-mRNA Modulates the Thrombogenicity of Human Platelets: A New Mechanism Linked to Disordered Coagulation in Sepsis

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Introduction: We recently demonstrated that platelets use a previously-unrecognized pre-mRNA splicing pathway to generate a tissue factor (TF)-dependent procoagulant activity. Here, we hypothesize that TF pre-mRNA splicing events are activated in patients with sepsis, a syndrome of dysregulated coagulation. Methods: Patients meeting consensus criteria for sepsis were prospectively enrolled. Platelets were freshly-isolated from whole blood within the first 24 hours of admission to ICU. Platelets from normal healthy controls were assayed in parallel to eliminate differences due to inter-assay variability. TF mRNA expression patterns and procoagulant activity was measured in both groups. Platelets were also incubated with bacteria from septic patients or bacterial-derived products. Results: Without exception, platelets from healthy controls (n = 54) expressed TF pre-mRNA. In contrast, platelets from 26 of 26 septic patients expressed TF pre-mRNA. TF pre-mRNA expression increased with increasing severity of illness as measured by APACHE II scores. Consistent with TF mRNA expression patterns, procoagulant activity in platelets from septic patients was significantly (p < 0.05) higher than in healthy controls (45.6 ± 13.3 vs. 11.1 ± 2.8 pM). In a subgroup of patients (n = 16), we also assessed TF pre-mRNA splicing in serial samples. We found progressive splicing in the platelets over time. Altogether, 81% of these patients expressed spliced TF mRNA at some point during their ICU stay. Gram (±) cell or gram (+) S. aureus bacteria isolated from septic patients also induced TF pre-mRNA splicing. Similarly, products of E. coli (lipopolysaccharides) or S. aureus (±toxin) induced TF pre-mRNA splicing in platelets, resulting in accelerated clot formation. Conclusions: These data demonstrate that circulating platelets from septic patients spontaneously splice TF pre-mRNA and generate procoagulant activity. TF pre-mRNA splicing by platelets may contribute to abnormal coagulopathy in sepsis and may be a target of future therapeutics.

**37** Targeting Coagulation Factor XIII Provides Protection from Pathological Thrombosis in Myocardial Infarction and Cerebral Ischemia Without Interfering with Hemostasis

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Formation of fibrin is critical for limiting blood loss at a site of blood vessel injury (hemostasis), but may also contribute to vascular thrombosis. Hereditary deficiency of factor XII (FXII), the protease that triggers the intrinsic pathway of coagulation in vitro, is not associated with spontaneous or excessive injury-related bleeding, indicating FXII is not required for hemostasis. We hypothesize that deficiency or inhibition of FXII protects mice from ischemic brain injury and cardiac ischemia/reperfusion damage. Following transient middle cerebral artery occlusion, the volume of infarcted brain in FXII deficient and FXII inhibitor-treated mice was significantly less than in wild type controls, without an increase in infarct-associated spontaneous or excessive injury-related bleeding, indicating FXII is not required for hemostasis. To determine whether the antithrombotic potential of selective blockade of talin-dependent thrombus formation is distinct from those required for normal hemostasis. As FXII appears to be instrumental in pathologic fibrin formation, but dispensable for hemostasis, FXII inhibition may offer a selective and safe strategy for preventing stroke, myocardial infarction and other thromboembolic diseases.

**38** The Antithrombotic Potential of Selective Blockade of Talin-dependent Integrin αIβ3 (Platelet GPIIIa-IIa) Activation

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Studies in vitro and with cultured cells indicate that talin binding to the β3 cytoplasmic domain is a final step in platelet integrin αIβ3 (GPIIIa-IIa) activation. We tested the significance of talin-mediated integrin activation by generating platelet-specific talin1 knockout mice (PKO). Platelets from PKO mice showed a dramatic reduction in agonist-induced αIβ3 activation as determined by soluble fibrinogen binding. In addition, more than 90% of PKO mice showed pathological bleeding that was associated with reduced survival. To determine whether the phenotype of PKO mice was due to lack of talin binding to integrin β3, we generated β3 integrin null mice harboring the GPIIIa-IIa integrin cytoplasmic domain that disrupt talin-β3 integrin interactions. We introduced a β3/γ7(47A) substitution that disrupts the
PGC-1α Strongly Stimulates VLDL Secretion While Suppressing Triglyceride Synthesis: Evidence for an Important Role for PGC-1α in Regulating VLDL Assembly

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Dysregulation of hepatic VLDL production is a major cause of dyslipidemia associated with development of anemia, occurred in 53% of people. PGC-1α, a key transcription factor, regulates the expression of genes involved in lipid metabolism. PGC-1α was shown to increase the expression of genes encoding proteins involved in lipid metabolism. The data strongly suggest that PGC-1α plays a physiologically significant role in hepatic HDL metabolism and reverse cholesterol transport.

Inactivation of ABCG1 in C57Bl/6 Mice Results in Reduced Adipose Tissue Metabolism and Reverse Cholesterol Transport in Mice

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Adipose tissue is a unique storage site for large amounts of cholesterol, and the regulation of the cholesterol content in adipocytes is a key factor for size-dependent fat cell metabolism, signal transduction, gene regulation and plasma cholesterol levels. The lipid transporter ABCG1 has been described to facilitate cellular cholesterol transport in mouse macrophages, thereby playing a crucial role in the maintenance of normal lipid levels in tissue macrophages and foam cells. Furthermore, Buchmann et al. have recently reported the impact of ABCG1 whole body ablation on adipose tissue development of C57BL/6 mice where exons three through five were targeted for deletion. In parallel studies, our group has investigated fat tissue development in a different ABCG1-KO mouse model which deleted the Walker A region of the ATP-binding site of exon three of C57BL/6. We found significantly reduced body weight of male and female ABCG1-KO vs. WT mice when mice were placed on a high cholesterol diet (HC, 1.2% w/v) and/or a high fat diet (20% w/v) diet (13–25% less body weight in males and 5–14% in females depending on time, p < 0.05). Using Micro-CT imaging and gravimetric measurement of isolated tissues we show a 40% (p < 0.05) decrease in abdominal and subcutaneous white adipose tissue, as well as a 25% (p < 0.05) decrease in brown adipose tissue. Cell diameter measurements of isolated abdominal fat cells using light microscopy revealed a significant decrease in the median cell diameter of female ABCG1-KO mice vs. ABCG1 WT mice (n = 8 mice) on a HFC diet compared to WT mice (112.5 μm; n = 8; p < 0.001). ABCG1-KO mice exhibited increased mRNA levels of expression of LRP, PPARδ, PPARγ, and ABCG1 by 5.2–5.8 fold as early as 6 hours on a HFC diet in white and 12 weeks in brown adipose tissue as measured by Real-time PCR. Of interest, lipid changes in male but not female ABCG1-KO mice vs. WT mice on a HFC diet. The rise of total plasma cholesterol and phospholipids during 6 weeks on diet was lower in ABCG1-KO vs. WT mice (Total Cholesterol 188 vs. 2101; n = 6; p = 0.05), Phospholipids 154 vs. 172% (p = 0.05). In summary, our results suggest that ABCG1 controls adipose tissue development, possibly by altering intracellular adipocyte’s as well as plasma cholesterol levels to regulate lipid storage and energy balance.
Expression and Characterization of Mutants of Human TAFI Resistant to Activation by Specific Proteases

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Thrombin-activatable fibrinolytic inhibitor (TAFI) is a human plasma zymogen that functions as a molecular connection between the coagulation and fibrinolytic cascades. Activated TAFI (TAFIa) is formed by cleavage at Arg92, a reaction that can be catalyzed by several proteases. However, the relative roles of each of these activators in different physiological contexts remains unknown. We have designed mutants of TAFI with the aim of creating variants that are resistant to activation by either thrombin or plasmin. Substitution of serine for proline at position 91 (P91S) yielded a variant of TAFI that could not be activated by thrombin but was only very slowly activated by thrombin-TM; this variant was activated normally by plasmin. The P91S variant was expressed in mammalian cells, purified, and its ability to inhibit lysis of clots made from TAFI-deficient plasma was compared to that of wild-type (wt) recombinant TAFI. TAFI (P91S) was markedly impaired in its antifibrinolytic activity, both in the presence or absence of TM (see Figure). Thus, activation by thrombin and thrombin-TM predominates under these circumstances. Plasmin is a promiscuous enzyme, but it is unable to cleave its own activation site; accordingly, introduction of residues from this site into peptide substrates for plasmin prevents their cleavage. However, introduction these residues into analogous positions in TAFI (specifically, valine at positions 93 and 94) failed to affect plasmin activation of TAFI suggesting that exosite interactions are more critical for recognition of TAFI by plasmin.

The Molecular Basis for Fibrin Clot Elasticity

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Fibrinogen when activated by thrombin forms a thrombus having elasticity that buffers blood’s shear forces. The molecular basis of this elasticity is unknown. Fibrinogen has coiled coils which are structural motifs that have been demonstrated in myosin to have perfect elasticity by atomic force microscopy (AFM). ‘Signature’ phases of the coiled coil’s force extension curves have been defined that can distinguish it from other protein domains. Furthermore, ‘polymers’ of fibrinogen molecules could be created for force spectroscopy experiments to amplify the signal from pulling single fibrinogen molecules. Fibrinogen is thus ideal for pulling experiments to evaluate the role of the coiled coil region for fibrin’s elasticity. To generate linear polymeric fibrin strands, 70 μl of purified fibrinogen (0.1 mg/ml) was reacted with 10 μl of CaCl2 (2.5 mM), 10 μl of FXII (0.002 mg/ml) and 10 μl of thrombin (0.2 U/ml) for 3.5 minutes. These were then imaged with the AFM in liquid tapping mode. Linear strands of 7 to 10 fibrin monomers with lengths of 250 ± 75 nm were observed. Fibrinogen and fibrin polymers were deposited onto gold-sputtered coverslips and adsorbed for 5 minutes. AFM in force spectroscopy mode was used to stretch the fibrinogen molecules and fibrin polymers. When the fibrinogen molecule was stretched beyond 20 nm, there was an abrupt increase in force of about 60 pN, followed by a plateau phase during which the force was relatively constant. This intermediate conformational transition likely contributes to fibrin elasticity. The magnitude of the plateau force changed with pH and calcium ion concentration. Stretching fibrin polymers to 400 nm revealed a blunted sawtooth pattern of consecutive force peaks, each decreasing the stretching of the fibrin molecules, allowing them to stretch again and follow the blunted sawtooth pattern. This suggests that the coiled coil regions were not fully unfolded the globular domains. Repeated extension and relaxation cycles demonstrated that the coiled coil regions were able to refold at high rates. Our data provide the first evidence for the coiled coils of fibrin being the origins of fibrin elasticity. Alterations to the coiled coils through mutations or disease may interfere with its function, changing the elasticity of the fibrin clot and cause thromboembolism.

Polymorphisms in Coagulation Factors Are Not Associated with Risk of Recurrent Major Adverse Cardiovascular Events in Men

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Introduction: Most myocardial ischemic events occur through plaque ruptures that precipitate formation of an occluding thrombus. The growth of a thrombus may be affected by an increased activity of the coagulation system. Therefore, it has been suggested that plasma levels of coagulation factors affect the risk of recurrent cardiovascular events. Objective: The aim of this study was to examine whether genetic predisposition to high levels of coagulation factors changes the risk of recurrent major adverse cardiovascular events in men with a first myocardial infarction. Methods: We performed a cohort study among 547 myocardial infarction patients, with a mean age of 56 years (range 32 to 70). All men had a first myocardial infarction between 1990 and 1996 and were followed until September 30, 2004. DNA was collected for polymorphisms affecting full-length factor II (factor II), X, V, VII, and plasminogen activator inhibitor type-1 (PAI-1), which are all associated with gain of function (either in plasma concentration or stability). We collected information from hospital files and general practitioners on the occurrence of recurrent major cardiovascular events. Results: In total, 259 recurrent events occurred. The point estimates of the relative rates (RR) of major cardiovascular events for the genotypes were all between 0.7 and 1.1 except for prothrombin 20210A mutation: RR 1.8 (95% CI 0.8 – 4.1). Adjustment for traditional risk factors of cardiovascular disease did not alter the findings. Conclusion: These findings suggest that there is no association between genetically determined high levels or stability of fibrinogen, factor V, factor VII and PAI-1, and the risk of recurrent major cardiovascular events. Increased prothrombin levels, however, may be associated with the risk of recurrent events.
Role of LIM Kinase 1 in Endothelial Function
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Microtubule (MT) destabilization promotes the formation of actin stress fibers and enhances the contractility of cells; however, the mechanism involved in the coordinated regulation of MTs and the actin cytoskeleton is poorly understood. LIM kinase 1 (LIMK1) regulates actin polymerization by phosphorylating the actin depolymerization factor, cofilin. We have shown that LIMK1 is involved in actin MT destabilization. Overexpression of wild type LIMK1 leads to MT destabilization, whereas the kinase-dead mutant of LIMK1 (KO) did not affect MT stability. Importantly, down-regulation of endogenous LIMK1 by small interference RNA resulted in abrogation of the thrombin-induced MTs destabilization and the inhibition of thrombin-induced actin polymerization. Expression of Rho kinase 2, which phosphorylates and activates LIMK1, dramatically decreases the interaction of LIMK1 with tubulin but increases its interaction with actin. Interestingly, expression of KO-LIMK1 or small interference RNA-LIMK1 prevents thrombin-induced microtubule destabilization and F-actin formation, suggesting that LIMK1 activity is required for thrombin-induced modulation of microtubule destabilization and actin polymerization. We further show that the interaction of LIMK1 with the actin cytoskeleton is specific for wild type LIMK1. We found that endothelial permeability in the lungs of LIMK1−/− mice was lower than that of wild type mice. Perfusion of the lungs of wild type mice with PAR-1 peptide showed significant increase of endothelial permeability. Notably, the endothelial permeability of the lungs of LIMK1−/− mice after PAR-1 stimulation was significantly lower than that of wild type mice. Acute lung injury (ALI) is a syndrome of acute respiratory failure that results from acute pulmonary edema and inflammation. Using lipopolysaccharide (LPS) injection as a model of ALI, we have shown that LIMK1−/− mice did not develop lung edema and showed significantly reduced mortality as compared with wild type mice. Our findings indicate that LIMK1 coordinates microtubules and actin cytoskeleton. We suggest that the loss of LIMK1 protein leads to less permeable pulmonary blood vessels.

50 Distinct eNOS Regulation by Protease-activated Receptors Involving Glu1213 and Rho-kinase
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Glu1213 and Rho-kinase are serine/threonine kinases that phosphorylate eNOS at Ser1179. In bovine aortic endothelial cells (BAECs), PAR-1 ligand, TFLLR, phosphorylated eNOS at Thr497 and Ser1179. In contrast, PAR-2 ligand, SLIGRL, phosphorylated Ser1179 with no effect on Thr497. SLIGRL stimulated cGMP production that was blocked by the PAR-2 antagonist, BOC-Glu-Arg-Pro-Arg. In bovine aortic endothelial cells (BAECs), PAR-1 ligand, TFLLR, phosphorylated eNOS at Ser1179 but not Thr497. In contrast, PAR-2 ligand, SLIGRL, phosphorylated Ser1179 with no effect on Thr497. SLIGRL stimulated cGMP production that was blocked by the PAR-2 antagonist, BOC-Glu-Arg-Pro-Arg. From these data, we conclude that PAR-1 and PAR-2 distinctly regulate eNOS activity through Glu1213 and Rho-kinase pathways. These pathways are relevant to eNOS regulation in vivo, since thrombin has been shown to increase eNOS activity in vivo, and Rho kinase is known to control eNOS activity in vivo.

51 β-Arrestin-2 is Required for B1 Kinin Receptor-dependent Activation of iNOS
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Kinin has wide ranging effects on vascular homeostasis, especially in inflammatory conditions. Des-Arg9-bradykinin and des-Arg9-kallidin (DAK0) are endogenous peptide B1 receptor (B1R) agonists and angiotensin converting enzyme (ACE) inhibitors can also directly activate B1Rs. We recently identified a novel endothelial B1R signaling pathway that activates the extracellular-signal regulated kinase (ERK), which then phosphorylates inducible nitric oxide synthase (iNOS). We hypothesized that this signaling pathway activated iNOS in LIMK1 knockout mice. We found that endothelial permeability in the lungs of LIMK1−/− mice was lower than that of wild type mice. Perfusion of the lungs of wild type mice with PAR-1 peptide showed significant increase of endothelial permeability. Notably, the endothelial permeability of the lungs of LIMK1−/− mice after PAR-1 stimulation was significantly lower than that of wild type mice. Acute lung injury (ALI) is a syndrome of acute respiratory failure that results from acute pulmonary edema and inflammation. Using lipopolysaccharide (LPS) injection as a model of ALI, we have shown that LIMK1−/− mice did not develop lung edema and showed significantly reduced mortality as compared with wild type mice. Our findings indicate that LIMK1 coordinates microtubules and actin cytoskeleton. We suggest that the loss of LIMK1 protein leads to less permeable pulmonary blood vessels.

53 Defining the Role of Sprouty1 as an Inhibitor of Angiogenesis
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The overall goal of this study was to identify novel conserved tyrosine kinase signaling pathways inhibiting angiogenesis. Our laboratory identified the receptor tyrosine kinase Sprouty1 from a set of gene expression profiles in human tissue. We screened this compound for targets conserved in vitro. Specifically, phosphorylation-dependent activation of p38 MAPK and ERK was inhibited by Sprouty1 in a concentration-dependent manner. A promoter analysis revealed three putative HIF-1α binding sites in the Sprouty1 gene. Using human umbilical vein endothelial cells (HUVECs), Sprouty1 protein expression was significantly increased after exposure to 0.2% oxygen for 24 hours. Sprouty1 overexpression significantly inhibited the formation of HUVECs on matrigel vs. control conditioned media (n = 6; p < 0.01). Similarly, there was a significant decrease in cellular proliferation in Sprouty1 infected HUVECs, as determined by BrdU incorporation (n = 6; p < 0.01). Interestingly, Sprouty1 is sufficient to increase p21 protein expression, a well-known cell cycle inhibitor. HUVECs under hypoxic conditions (0.2%) exhibit conditions more relevant to a hyperoxic environment compared to anoxic conditions. Our results suggest that Sprouty1 expression is sufficient to decrease p21 expression at 24 hours. In summary, we have identified a novel conserved tyrosine kinase signaling pathway that inhibits angiogenesis and may have important therapeutic implications in cardiovascular disease.
Monocyte chemoattractant protein-1 directs migration of monocytes from the peripheral blood to sites of inflammation in a MCP-1 dependent manner. The differences in the velocity and directionality of the directed migration have not been rigorously addressed and few studies address whether end products and inability to influence each other’s activity, these enzymes might regulate distinct characteristics of migrating monocytes, probably, from different intracellular locations. In this study, we report that MCP-1 induces recruitment of these two phospholipases to different intracellular locations: DAG to infrequently recruited to the pseudopod and cPLA2 to the endoplasmic reticulum. This differential spatial distribution is manifested also in their functional independence. Monocytes deficient in cPLA2 displayed reduced speed, whereas in contrast, iPLA2 deficient monocytes make wider and more frequent turns as well as exhibiting reduced speed. Thus, iPLA2 provides a directional cue or compass supporting migration toward the MCP-1. We validated the roles of these phospholipases in monocyte migration in vivo using a newly developed mouse model. Adipotively transferred murine monocytes, if rendered deficient in either of these phospholipases by their specific antisense oligonucleotides, displayed profound defects in migration to the peritoneum in thioglycolate-induced peritonitis, a MCP-1-dependent process. We have identified a previously unknown function of iPLA2 as a cellular compass and present a new approach for evaluating the relevant contributions of signaling molecules in regulating monocyte chemotaxis to MCP-1, in vivo.

Macrophage Phenotype in Atherosclerotic Human Coronary Arteries is Different from Macrophage Phenotype in Normal Human Coronary Arteries

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We previously characterized the divergent atherogenic potential of human monocyte-derived macrophages differentiated with either M-CSF (Mac-M) or GM-CSF (GM-Mac) in vitro. Gene expression analysis and cytokine secretion assays indicated significant differences between the two macrophage phenotypes related to inflammation and cholesterol homeostasis. M-Mac express the pro-inflammatory marker CD68 while only M-Mac express the pro-inflammatory marker CXCR6. Expression analysis and cytokine secretion assays indicated significant differences between the two macrophage phenotypes related to inflammation and cholesterol homeostasis. M-Mac display a decrease in Akt and Erk phosphorylation, signaling molecules shown to be important downstream of these receptors. The identification of CXCR6 in atherosclerosis has important experimental and clinical implications.

Differential iPLA2 and cPLA2 Signaling Regulates Directed Migration of Monocyte to MCP-1: Validation in a Novel Mouse Model

Ravi S Mishra, Cleveland Clinic, Cleveland, OH; Katherine Preston, Univ of Cambridge, Cambridge, United Kingdom; Martha K Cathcart; Cleveland Clinic, Cleveland, OH

Cholesterol from CD68+/H11001+ throughout the adventitia in both normal and diseased regions of the coronary arteries. Cells in vivo thioglycolate-induced peritonitis, a MCP-1-dependent process. We have identified a previously unknown function of iPLA2 as a cellular compass and present a new approach for evaluating the relevant contributions of signaling molecules in regulating monocyte chemotaxis to MCP-1, in vivo.

Serum Amyloid A Is an Endogenous Ligand for Toll-like Receptor 2

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Serum amyloid A (SAA) is an apolipoprotein produced by hepatocytes during acute-phase response and by other inflammatory cells such as macrophages. SAA is an important clinical indicator of atherosclerosis and other inflammatory diseases, and contributes to cholesterol metabolism through its binding to high-density lipoprotein (HDL) and scavenger receptors. In phagocytes, SAA is a potent inducer of proinflammatory cytokines such as MCP-1, IL-1beta, IL-6 and IL-12p40. The observation that SAA could induce cytokine production independently of formyl peptide receptor-like 1 and the scavenger receptor SR-Bi led us to propose the presence of another receptor. To assess this hypothesis, we analyzed the cytokine production profiles of SAA stimulated monocytes. Cells were killed using either interferon-gamma or TNF-alpha. But the role of SAA in atherosclerosis is not well understood. Here we present evidence that SAA plays a role in the development of atherosclerosis in part through Toll-like receptor 2 activation.

Glutamate Mediates Platelet Activation Through the AMPA Receptor

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Glutamate is an excitatory neurotransmitter in the central nervous system (CNS) that binds to the kainate receptor, the N-methyl-D-aspartate (NMDA) receptor, and the α-amino-3- hydroxy-5-methylisoxazoleproprionic acid (AMPA) receptor (AMPAR) Each receptor is first characterized and cloned in the CNS. Glutamate is also present in the periphery, and glutamate receptors have been identified in non-neuronal cells, including bone, heart, kidney, pancreas, and platelets. Platelets have a central role in normal thrombosis and hemostasis as well as control of the inflammatory response. Platelets express glutamate receptors. Platelet activation increases intracellular calcium concentration, an important step in platelet activation. In contrast, platelets treated with the AMPAR antagonist CNX2 or platelets derived from GluR1 knockout mice are resistant to AMPA activation. Furthermore, mice lacking GluR1 have a prolonged time to platelet adhesion and aggregation in vivo. Our results indicate that glutamate acts as a regulator of platelet activation, and suggest that the AMPA receptor is a new anti-thrombotic target.

HMPO Polymorphic Lineage Cell-specific Protein-1 (HS1) Is an Important Signaling Molecule Downstream from Protease-activated Receptors and Is Involved in Multiple Signaling Pathways of Platelet Activation

Bryan N Kahner, Robert T Dorson, Soochon Kim, Satya P Kunapuli; Temple Univ Sch of Medicine, Philadelphia, PA

Injury to a blood vessel exposes subendothelial collagen and initiates the coagulation cascade producing thrombin. We hypothesize that human HMPO is the endogenous ligand for HS1. We demonstrate that platelets containing HS1, a 75 kDa tyrosine phosphorylated adapter protein, expressed in cells exclusively of hematopoietic lineage, is a critical regulator of platelet activation. Here we investigate the role of HS1 downstream of GPCRs in PAR receptor signaling pathway. We observed both a bleeding diathesis and an inhibition of thrombus formation by the in vivo FeCl3 thrombosis model, indicating HS1’s involvement in multiple pathways. HS1 phosphorylation occurs downstream of both PAR-1 and PAR-4, in a Gq dependent manner, however, ADP secretion has no effect on HS1 phosphorylation. HS1 is phosphorylated initially by Syk tyrosine kinase on tyrosine residues 397 and 378. Syk then dissociates from HS1, allowing for docking and subsequent phosphorylation of HS1 by Src family kinases on tyrosine residue 222. We demonstrate that Src family kinase inhibitors abolish the activation of Syk, as measured by its phosphorylation on tyrosine residues 525/526, and also completely block HS1 phosphorylation. This is in contrast to the previously reported results indicating that inhibition of Src family kinases results in partial HS1 phosphorylation. Quantitatively the Src phosphorylated residues. We propose that Src is directly downstream of PAR receptors and phosphorylates Syk, which in turn phosphorylates HS1. Further, studies with HS1 null mouse platelets show an inhibition of aggregation, secretion and thromboxane A2 generation compared to their wild type littermates. HS1 null mice also display a decrease in Akt and Erk phosphorylation, signaling molecules shown to be important in aggregation and thromboxane generation. Taken together with our previous results,
Role of the NR4A orphan nuclear receptor Nor1 in Neointima Formation and Vascular Smooth Muscle Cell Proliferation

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Nuclear hormone receptors comprise a large superfamily of ligand-activated transcription factors and have emerged as key regulators of glucose metabolism and inflammation in diabetes and cardiovascular diseases. The neuron-derived orphan receptor-1 (NOR1) belongs to the ligand-independent NR4A receptor subfamily which has previously been characterized as important transcriptional regulator of hepatic gluconeogenesis and inflammation. We have recently demonstrated NOR1 expression in vascular smooth muscle cells (SMC) of atherosclerosis and outlined a key role for NOR1 to regulate SMC proliferation in vitro. In the present study, we demonstrate that SMC isolated from NOR1-deficient mice exhibit decreased cell proliferation due to a G2/M arrest of the cell cycle. NOR1-deficiency results in diminished phosphorylation of the retinoblastoma protein, cyclin D1 and D2 expression and mitogen-induced degradation of the cyclin-dependent kinase inhibitor p27. Using microarray technology we further characterize Sig1, C1q, and Nedd8, key proteins of the ubiquitin ligase complex required for degradation of the pro-apoptotic protein Skp1. Finally, using a model of guide-wire-induced arterial injury we observed decreased neointima formation in NOR1-deficient mice. These experiments demonstrate that NOR1 functions as a key transcriptional regulator of SMC proliferation and neointima formation by inducing cyclin D1 and the expression of genes required for the ubiquitination and degradation of p27. Therefore, NOR1 is a key regulator of SMC proliferation and may provide an important molecular target for the treatment of cardiovascular diseases.

CD63 Modulates Platelet Reactivity in Vivo During Hyperlipidemia: A Mechanism Linking Hyperlipidemia, Oxidant Stress, and a Prothrombotic Phenotype

Eugene A Podrez, Maria Febbraio, Cleveland Clinic, Cleveland, OH; Robert G Salomon, Case Western Reserve Univ, Cleveland, OH; Yi Ma, Cleveland Clinic, Cleveland, OH; Eugenia Poliakov, Case Western Reserve Univ, Cleveland, OH; Manojkumar Valiyaveettil, Cleveland Clinic, Cleveland, OH; Mingjiang Sun, Case Western Reserve Univ, Cleveland, OH; Brian R Curtis, Blood Center of Wisconsin, Milwaukee, WI; Paula J Fitton, Jhuua Chen, Renliang Zhang, Roy L Silverstein, Tatiana V Byzova, Stanley L Hazen; Cleveland Clinic, Cleveland, OH

Enhanced platelet reactivity is critical to the pathophysiology of occlusive arterial thrombotic disease. Despite the strong clinical associations between hyperlipidemia, a major risk factor for atherosclerosis, and a pro-thrombotic phenotype, the mechanisms responsible for enhanced platelet reactivity during hyperlipidemia remain unknown. Pro-atherosclerotic lipid abnormalities such as hypercholesterolemia are associated with both enhanced oxidant stress and generation of biologically active oxidized lipids, containing potential ligands for the scavenger receptor CD63, a major platelet surface glycoprotein. Using multiple murine in vivo thrombosis models and hyperlipidemic atherosclerosis-prone apo-E deficient or LDL receptor-deficient mice we can now demonstrate that these mice form occlusive intrathrombi more frequently than wildtype mice, and that genetic deletion of CD63 protects mice from hyperlipidemia-associated enhanced platelet reactivity and accompanying pro-thrombotic phenotype. Structurally defined oxidized choline glycerophospholipid molecular species that serve as endogenous high affinity ligands for CD63 in vitro are shown to be markedly increased in plasma of hyperlipidemic mice and to promote platelet aggregation and alpha-granule release via CD63 at pathophysiological levels. These studies thus demonstrate that platelet CD63 interactions with specific endogenous oxidized lipids play a heretofore unrecognized role in the well-known clinical associations between hyperlipidemia, oxidant stress and a pro-thrombotic phenotype.

CD36 Modulates Platelet Reactivity in Vivo During Hyperlipidemia: A Mechanism Linking Hyperlipidemia, Oxidant Stress, and a Prothrombotic Phenotype

Eugene A Podrez, Maria Febbraio, Cleveland Clinic, Cleveland, OH; Robert G Salomon, Case Western Reserve Univ, Cleveland, OH; Yi Ma, Cleveland Clinic, Cleveland, OH; Eugenia Poliakov, Case Western Reserve Univ, Cleveland, OH; Manojkumar Valiyaveettil, Cleveland Clinic, Cleveland, OH; Mingjiang Sun, Case Western Reserve Univ, Cleveland, OH; Brian R Curtis, Blood Center of Wisconsin, Milwaukee, WI; Paula J Fitton, Jhuua Chen, Renliang Zhang, Roy L Silverstein, Tatiana V Byzova, Stanley L Hazen; Cleveland Clinic, Cleveland, OH

Enhanced platelet reactivity is critical to the pathophysiology of occlusive arterial thrombotic disease. Despite the strong clinical associations between hyperlipidemia, a major risk factor for atherosclerosis, and a pro-thrombotic phenotype, the mechanisms responsible for enhanced platelet reactivity during hyperlipidemia remain unknown. Pro-atherosclerotic lipid abnormalities such as hypercholesterolemia are associated with both enhanced oxidant stress and generation of biologically active oxidized lipids, containing potential ligands for the scavenger receptor CD63, a major platelet surface glycoprotein. Using multiple murine in vivo thrombosis models and hyperlipidemic atherosclerosis-prone apo-E deficient or LDL receptor-deficient mice we can now demonstrate that these mice form occlusive intrathrombi more frequently than wildtype mice, and that genetic deletion of CD63 protects mice from hyperlipidemia-associated enhanced platelet reactivity and accompanying pro-thrombotic phenotype. Structurally defined oxidized choline glycerophospholipid molecular species that serve as endogenous high affinity ligands for CD63 in vitro are shown to be markedly increased in plasma of hyperlipidemic mice and to promote platelet aggregation and alpha-granule release via CD63 at pathophysiological levels. These studies thus demonstrate that platelet CD63 interactions with specific endogenous oxidized lipids play a heretofore unrecognized role in the well-known clinical associations between hyperlipidemia, oxidant stress and a pro-thrombotic phenotype.
Regulation of Smooth Muscle Cell Proliferation by Hyaluronan and CD44

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High molecular weight hyaluronan (HMW-HA) is a widely distributed component of the ECM, but its biological activities remain incompletely understood. We previously reported that HMW-HA binding to CD44 antagonizes mitogen-induced S phase entry in cultured vascular smooth muscle cells. Here we now characterize the underlying molecular mechanism and document its relevance during vascular injury in vivo. In particular, we show that HMW-HA inhibits the mitogen-dependent induction of cyclin D1 and degradation of p27Kip1 in vascular smooth muscle cells. These effects were associated with an inhibition of Rb phosphorylation, cyclin A induction, and S phase entry, p27Kip1 mRNA levels were unaffected by HMW-HA, but the expression of Skp2, the rate-limiting component of the SCF complex that degrades p27Kip1, was reduced. Rescue experiments identified cyclin D1 as the primary target of HMW-HA. Similar effects were detected in fibroblasts. These effects were not detected in vascular smooth muscle cells isolated from CD44-null mice. Moreover, arteries from homozygous and CD44-null mice showed that the effects of HMW-HA/CD44 on cyclin D1 and Skp2 expression are detected in vivo and associated with altered smooth muscle cell proliferation after vascular injury. Our data indicate that HMW-HA is anti-mitogenic for multiple mesenchymal cell types and identify cyclin D1 as a major target of HMW-HA binding to CD44.

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In Vivo C-Reactive Protein and Its Relationship with Features of the Metabolic Syndrome in Men and Women

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BACKGROUND

Individuals with elevated (>3.0 mg/l) plasma C-reactive protein (CRP) levels, a key feature of the metabolic syndrome, are at greater risk for cardiovascular disease than individuals with low levels of CRP (<1.0 mg/l). The in vivo kinetics of CRP and the physiological mechanisms responsible for the observed sub-acute-phase circulating CRP levels in the metabolic syndrome and obesity are virtually unknown. Here we describe for the first time the intravascular kinetics of CRP and its relationship with features of the metabolic syndrome.

METHODS

Sixteen men and 16 women (aged 48 ± 9 years, BMI = 28.7 ± 4.6 kg/m²) underwent a 12-hour primed constant infusion of D3-L-leucine in the constant fed state. Blood samples were drawn at pre-determined time points. CRP was purified from the plasma fraction d < 1.25 g/ml at each time point by affinity chromatography followed by SDS-PAGE. Isoelectric enrichment was determined by GC-MS. Plasma CRP levels were measured with high sensitivity using a commercial ELISA. RESULTS: Mean CRP production rate (PR) and pool size (PS) were similar between men and women (0.03 ± 0.026 vs. 0.03 ± 0.039 mg/dl and 4.66 ± 3.39 vs. 4.64 ± 3.94 mg/ml, respectively). However, the fractional catabolic rate (FCR) of CRP in men was 60% higher than in women (0.57 ± 0.29 vs. 0.35 ± 0.20 pool/day, P < 0.05). Circulating CRP concentrations were more strongly correlated with its FCR (r = 0.91, P < 0.0001) than its FCR (r = 0.55, P < 0.05). PR of CRP was directly correlated with BMI (r = 0.42, P < 0.02), waist girth (r = 0.44, P < 0.02), and with plasma LDL apoB-100 (r = 0.38, P < 0.05), triglyceride (r = 0.35, P = 0.05) and interleukin-6 levels (r = 0.50, P = 0.05). An inverse trend was also observed between the PR of CRP and HDL-C (r = -0.34, P = 0.06) while LDL-C and blood pressure showed no association with CRP kinetics. CONCLUSIONS: Men were characterised by a 60% greater FCR of CRP compared with women. However, sub-acute-phase CRP levels appeared to be mainly explained by the PR of CRP rather than its FCR. Our results also suggest that features of the metabolic syndrome may be more predictive of CRP kinetics than traditional risk factors like LDL-C and hypertension.

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Reduced Macrophage Infiltration in Visceral Adipose Tissue of 12-Lipoxygenase Knockout Mice

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Obesity induces the accumulation of macrophages in adipose tissue and generates a state of low-grade inflammation which is associated with the development of type 2 diabetes and release of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). Our recent studies indicate that 12-Lipoxygenase (12-LO) expression is increased in isolated visceral adipocytes in models of insulin resistance and that deletion of 12-LO prevents the increases in TNF-α and IL-6 during high fat feeding. The aims of this study were to examine the role of 12-LO pathology in the accumulation of macrophages in adipose tissue of mice fed a high-fat diet. C57BL/6J (B6) and 12-LO knockout (12-LO KO) mice on the B6 background were fed control or a high-fat diet for 12 weeks. Macrophage content in visceral fat pad were examined by FACS and immunohistochemistry. Macrophage content in fat with chew feeding was similar in 12-LO KO and B6 mice. Western diet significantly increased macrophage content in fat tissue compared with chow diet in B6 mice. However, there was significantly reduced macrophage content in 12-LO KO mice compared to mice as shown in the table below. After the mice were fed Western diet for 24 weeks, the percentage of positive Mac2 staining within visceral fat pad was markedly increased in B6 mice compared to 12-LO KO mice (0.58 ± 0.07 % vs. 0.26 ± 0.03 % in male mice, respectively). These data suggest that 12-LO plays a role in regulating macrophage trafficking and inflammation in visceral fat in states of obesity. Therefore blockade of 12-LO may prove to be an effective strategy to prevent diet-induced obesity and diabetes.

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Adipocyte-specific Low-density Lipoprotein Receptor–Related Protein-1 as a Novel Regulator of Adiposity, Energy Expenditure, and Glucose Metabolism

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Mice models resulting from tissue-specific gene disruption have established adipose tissue as an essential endocrine organ for the control of glucose homeostasis and energy balance. The multifunctional receptor LRP-1 is expressed in adipose tissue where it mediates cellular cholesterol uptake. Herein we used the adipose tissue-specific LRPI-1 knockout mouse model (adLRP1KO) to test the hypothesis that adipocyte LRPI-1 plays an essential role in lipid storage and energy metabolism. Adipocyte-specific LRPI-1 inactivation in mice (n = 12) resulted in decreased total body lipid clearance (area under the curve [AUC]: 4.0 ± 0.9 vs. 2.2 ± 0.5 g/kg, P < 0.01), smaller fat stores (4.3 ± 0.5 vs. 9.5 ± 1.4 % fat of BW, P < 0.002), lipid-depleted brown adipocytes, improved glucose tolerance (AUC: 2001 ± 517 vs. 2723 ± 1681, P = 0.05), elevated body temperature (BWT: 38.7 ± 0.2 vs. 38.6 ± 0.2°C) and increased food intake (1.5 ± 0.2 vs. 1.1 ± 0.1 g BW/d, P < 0.05). The slightly higher calorict intake may represent a compensatory mechanism. Intriguingly, such increased thermogenesis in adLRP1KO mice was confirmed by increased body temperature and paralleled by muscle shivering, quantified using a multi-dimensional light beam system to analyze motor activity (7102 ± 1389 in KO vs. 4013 ± 333 WT beam strokes/30h, P < 0.001). Additional radiolabeled studies revealed that glucose and lipid uptake were significantly increased in skeletal muscle of adLRP1KO mice compared to WT mice (glucose: 12830 ± 651 vs. 8835 ± 1203 dpm/lean mass, P < 0.01; oleic acid: 8824 ± 662 vs. 4791 ± 595 dpm/lean mass, P < 0.05), suggesting that skeletal muscle compensates for decreased thermogenic function of BAT in adLRP1KO mice, which may be reflected in the shivering phenotype. When placed for 4 weeks on high fat diet adLRP1KO mice were resistant to diet-induced obesity (11.2 ± 1.5 vs. 22.2 ± 2.7 % fat of BW, P < 0.002) and glucose intolerance (AUC: 21510 ± 1295 vs. 30920 ± 174, P < 0.005). We conclude that reducing lipid transport to adipocytes and enhancing muscular energy expenditure via adipocyte LRPI-1 inhibition may be an efficient strategy to prevent diet-induced obesity and diabetes.

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Macrophages (X1000) Per Gram Adipose Tissue

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Fatty Acid Desaturase Gene Expression in Human Adipose Tissue Is Regulated by Dietary Composition Independently of Energy Restriction and Is Correlated with Plasma Triglyceride Response

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Atherogenic dyslipidemia, associated with elevated triglycerides and reduced HDL, is independently improved by dietary energy restriction and reduced carbohydrate consumption in an equivalent but non-additive manner. We assessed the hypothesis that total energy restriction and isocaloric alteration in dietary composition regulate common molecular pathways involved in lipid metabolism by monitoring transcriptional expression in human adipose tissue. Subcutaneous adipose biopsies were obtained from 131 moderately overweight, otherwise healthy men (BMI, 29.2±2.0 kg/m²) following (1) one week on basal diet (54:15:30[7], carbohydrate:protein:fat(saturated fat)), (2) three weeks on randomized diet differing in nutritional composition (basal, 39:29:31[8], 26:29:46[9] or 26:29:46[15]), (3) five weeks of acute weight loss on randomized diet (-1103.0±216.5 kcal/d resulting in -10.0±3.3 lb), and (4) four weeks stabilized at reduced weight. Transcriptional responses were characterized using genome-wide expression array analysis on samples from thirteen subjects and findings for the most responsive genes were confirmed using real time PCR across all subjects. Energy restriction resulted in significantly reduced expression of 1473 transcripts and, of these, 30 were also responsive to isocaloric alterations in dietary composition. Twelve of these genes are involved in energy metabolism including four in lipogenesis and five in lipid metabolism. Significant responses were confirmed for four top-changing genes (p<0.003): stearoyl CoA desaturase (SCD), fatty acid desaturases 1 and 2 (FADS1, FADS2), and diacylglycerol transferase 2 (DGAT2). SCD response was strongly correlated with carbohydrate intake (p=0.019) and, on a low carbohydrate diet, was inversely correlated with saturated fat intake (p=0.05). Moreover, plasma triglyceride responses to changes in dietary composition were independently correlated with SCD (p=0.003) and DGAT2 (p=0.05) response. In conclusion, fatty acid desaturases in human adipose tissue are independently regulated by energy restriction and dietary composition and may be involved in dietary regulation of systemic triglyceride metabolism.

Effect of Protein, Monounsaturated Fat, and Carbohydrate Intake on Plasma Apopliprotein B and VLDL and LDL Particles Containing apoCIII: Results from the OmniHeart Trial

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Plasma apoB and VLDL and LDL particles with apoCIII are independent risk factors for cardiovascular disease. We examined the effect of three healthy diets modeled after the DASH diet on these apoplipoproteins and lipoproteins. All diets were high in fruits, vegetables, and whole grains and low in saturated fat, but differed by emphasis of either carbohydrate (CARB), monounsaturated fat (MONO), or protein (PROT). In the setting of a controlled, 3 period cross-over feeding study, healthy subjects, N=164, consumed each diet for 6 weeks. As shown in the table below, all three diets similarly lowered plasma apoB and VLDL + LDL cholesterol compared to baseline when the participants ate their own diet. Only the PROT diet significantly lowered plasma triglyceride (TG). LDL particles without apoCIII (the major LDL type) were reduced equally by all diets, with an accompanying reduction in their TG and cholesterol concentrations. The PROT diet reduced LDL with apoCIII compared to baseline and CARB. In contrast, compared to baseline, CARB and MONO diets but not the PROT diet increased VLDL with apoCIII. In VLDL without apoCIII, the PROT diet reduced TG compared to baseline and CARB and cholesterol compared to baseline. The diets did not affect the molar ratios of TG to apo B and cholesterol to apo B in any of the particle type suggesting that the diets did not alter particle composition. In conclusion, substituting protein for carbohydrate reduced atherogenic apoCIII-containing LDL and had the most favorable effects on the VLDL particle types, resulting in the least atherogenic lipoprotein profile.

<table>
<thead>
<tr>
<th>Change from Baseline</th>
<th>Between Diet Differences</th>
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<tbody>
<tr>
<td></td>
<td>CARB</td>
</tr>
<tr>
<td>apoB in plasma</td>
<td>-6%</td>
</tr>
<tr>
<td>TG in plasma</td>
<td>-5%</td>
</tr>
<tr>
<td>cholestrol in VLDL</td>
<td>-8%</td>
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<tr>
<td>apoB in LDL with apoCIII</td>
<td>-8%</td>
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<tr>
<td>TG in LDL with apoCIII</td>
<td>-8%</td>
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<tr>
<td>cholestrol in LDL with apoCIII</td>
<td>-9%</td>
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<tr>
<td>apoB in LDL with apoCIII</td>
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<tr>
<td>apoB in VLDL with apoCIII</td>
<td>+4%</td>
</tr>
<tr>
<td>TG in VLDL with apoCIII</td>
<td>-3%</td>
</tr>
<tr>
<td>cholestrol in VLDL with apoCIII</td>
<td>-6%</td>
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*p<0.05, TG - triglyceride, cholestrol - cholesterol
Factor XIII Levels Are Normal in A fibrinogenemic Plasma
Faisal I Ahmad, Susan T Lord; Univ of North Carolina at Chapel Hill, Chapel Hill, NC

Blood coagulation factor XIII (FXIII) is found in plasma and platelets. During the final stages of coagulation, FXIII catalyzes the cross-linking of adjacent fibrin monomers through the formation of γ-glutaryl-ε-lysyl isopeptide bonds. Prior studies have shown plasma FXIII is associated with fibrinogen, forming a non-covalent FXIII:fibrinogen complex. This finding suggests that FXIII levels in blood are dependent on circulating fibrinogen levels. To determine whether the absence of fibrinogen affects circulating FXIII, we measured FXIII levels in an fibrinogenemic (fibrinogen-deficient) individual. We used gel electrophoresis and immunodetection procedures to determine FXIII levels in plasma from an fibrinogenemic individual and plasma pooled from normal individuals. We analyzed platelet-poor plasma in order to eliminate the cellular FXIII. Samples were prepared at fifty-, one-hundred-, and two-hundred- fold dilutions. Immunoblot analysis was performed using an affinity-purified, sheep anti-FXIII subunit A specific antibody. We found FXIII levels in the fibrinogenemic and normal plasma samples were indistinguishable at all dilutions, indicating comparable physiological concentrations of FXIII in the fibrinogen-deficient and normal plasmas. These results show that the concentration of FXIII in plasma is independent of the presence or absence of fibrinogen. Furthermore, these data indicate FXIII can stably circulate in plasma even in the absence of fibrinogen and fibrinogen is not required as a carrier for circulating plasma FXIII.

Regulation of Factor Xa Mediated Signal Transduction by Annexin 2
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The serine protease zymogen factor X is converted to its catalytically active form factor Xa (Xa) by the binary complex of factor VIIa bound to its cell surface receptor tissue factor (TF) or alternatively by the intrinsic Xase complex which consists of active factors VIIa (VIIa), IX (IXa), factor X, and Ca2+. Xa has procoagulant activity to convert prothrombin to thrombin and also induce cell signal transduction, either alone or in the ternary TF-VIIa/Xa complex. Xa cleaves and activates procoagulant activated receptor (PAR)1 or 2, or Xa signaling efficiency varies between cell-types. We now observe that Annexin 2 acts as a receptor for Xa on the surface of human endothelial cells (HUVEC) and that Annexin 2 association facilitates Xa activation of PAR-1, but not the coagulant function of Xa. Over-expression of TF abolishes Annexin 2 dependence of Xa signaling and diminishes binding to cell surface Annexin 2. We propose that Annexin 2 serves to regulate Xa signal transduction specifically in the absence of cell surface TF and may thus play physiological or pathological roles when Xa is generated by the intrinsic coagulation pathway.

Effect of Exercise Training on Endothelial-dependent Vasodilation in Aged Rat Is Associated with Reduced Caveolin Status
Dong-Ju Choi, Young-Seok Cho, Hyoek-Jae Chang, Eun-Ji Kim, Woo-Young Chung, Tae-Jin Yon, In-Ho Chae, Choeil-Ho Kim; Seoul National Univ, Seongnam, Republic of Korea

Ageing impairs endothelial-dependent vasodilation in humans and animals. In the endothelium, caveolin-1 regulates nitric oxide signaling by binding to and inhibiting endothelial nitric oxide synthase (NOS). The purpose of this study was to examine whether exercise training alleviates impaired endothelial-dependent dilation of aorta in aged rats by 12 weeks of treadmill exercise and to determine their mechanisms. Thirty- and twenty-two-month-old male Fischer344 rats were assigned to young sedentary, young exercise-trained, old sedentary, or old exercise-trained groups. Abdominal aortic rings were prepared and vascular responses to acetylcholine (10^-5–10^-1 M) were determined in vitro. To determine the potential role for nitric oxide and caveolin-1 in vasodilation in sedentary and exercise old rats, we examined serum activity of NOS and caveolin-1 in rat aorta. Training improved the ageing-induced reduction in endothelium-dependent vasodilation in aortic preparations. Expression of eNOS mRNA in aorta was unchanged by exercise training, whereas serum NOx level was increased by ~3 times, while caveolin-1 expression was decreased expressed by Western blot and immunostaining. We conclude that (1) exercise can improve impaired, endothelium-dependent dilation of aorta by ageing, (2) exercise can restore age-dependent loss of NO by altering eNOS subcellular distribution and its association with inhibitory proteins, caveolin-1, and (3) exercise may modify vascular reactivity in old subjects by altering levels of eNOS protein in the large artery. These results imply the design of clinical strategies that approach the age-associated loss of endothelial NO availability and through targeting these pathways.
NO donors and L-arginine than WT platelets. Low levels of exogenous NO (<100 nm) stimulate intraplatelet cGMP up to 4 fold and completely inhibit thrombin-induced aggregation of human platelets. These effects of NO on aggregation and cGMP levels are both reversed by added TSP1 (0.2 to 2 nm) or the recombinant C-terminal domain of TSP1 (2 nm) and CD47 agonist peptides (1 to 10 uM) derived from it. These results suggest that earlier attempts to assess the role of TSP1 or TSP in platelet aggregation did not adequately take into account the ephemeral nature of NO. In conclusion, TSP1 and CD47 have a discernable and significant role in promoting platelet aggregation, and perhaps stabilizing aggregates, under more physiological conditions of nitric oxide tone. (Supported by the National Institutes of Health.)

Predictors of Left Atrial Thrombus in Atrial Fibrillation

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Background: Stroke in atrial fibrillation (AF) encompasses mechanisms other than embolization. The CHADS-2 score is highly predictive of stroke in this setting yet its specificity for left atrial thromboembolism is not clear. Whereas warfarin is more effective for cardioembolic compared to non-cardioembolic stroke prevention, we sought to assess the correlation between left atrial thrombus (LAT) assessed by transesophageal echocardiography (TEE) and CHADS-2 score. Methods: The Mayo Clinic Echocardiography Laboratory and Cardioversion Unit Database were used to identify AF patients not previously treated with warfarin who were found to have LAT by TEE. Variables of CHADS-2 system were compared for both cases and controls. Results: Between 2000–2005, 179 cases with LAT (mean age 70±12 years; 46% women) and 440 controls (71±13 years; 38% women) without LAT were identified. Clinical and echocardiographic variables are summarized (Table). The mean CHADS-2 score was low, but significantly higher for cases (average 2.4±1.6; median 2.0; range 0–6) compared to controls (average 1.6±1.3; median 1.0; range 0–6) (Figure). However, for those patients with TEE confirmed LAT, the CHADS-2 score varied considerably with 34% of CHADS-2 scores between 0–1. Furthermore, high scores (5 or 6) were uncommon (12.9%). Conclusions: The prevalence of LAT confirmed by TEE is not reliably predicted by the CHADS-2 score system. Left atrial size and left ventricular function remain the strongest risk variables for presence of LAT and may provide mechanistic insight into thrombogenesis in atrial fibrillation.

Platelet Activity, Coagulation, and Fibrinolysis During Exercise in Healthy Males: Effects of Thrombin Inhibition by Argatroban and Enoxaparin

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Background—Relationships between exercise-induced activation of platelets, blood coagulation and fibrinolysis, and the importance of thrombin for responses to exercise are not clear. Methods—Results—Effects of thrombin inhibition on haemostatic parameters were examined in a double-blind, cross-over study comparing the direct thrombin inhibitor argatroban (350 µg/kg i.v. bolus followed by 25 µg/kg/min infusion), the indirect thrombin inhibitor enoxaparin (0.75 mg/kg, i.v. bolus), or placebo (saline) in 21 healthy males. Measurements were made at rest, before and during/after thrombin inhibitor treatment, and immediately after exhaustive exercise. At rest argatroban abolished, and enoxaparin attenuated platelet activation by thrombin, but not by ADP. Argatroban and, even more so, enoxaparin decreased thrombin generation (F1 +2) and the coagulation potential, and increased the fibrinolytic potential. Exercise increased circulating activated platelets (from 5.5×10^3 to 9.4±0.9×10^3/µl, P<0.001), circulating platelet-platelet microaggregates, the platelet responsiveness to in vitro stimulation, leukocyte activation (leucocyte CD11b expression and plasma elastase), and platelet-leukocyte aggregation (P<0.01 for all). Exercise increased coagulation (F1 +2, P<0.01) and fibrinolysis, but did not alter the balance between them; fibrin gel permeability increased (P<0.01). Neither argatroban nor enoxaparin counteracted exercise-induced activated platelet or leukocyte activation. Both thrombin inhibitors augmented exercise effects on fibrinolysis. Conclusions—Strenuous exercise enhances platelet and leukocyte activation independently of thrombin. Exercise augments both coagulation and fibrinolysis, but the balance between them appears to be maintained. At therapeutic dosages argatroban counterbalanced thrombin–induced platelet activation most efficiently, while enoxaparin had somewhat stronger antiaggregant and profibrinolytic effects.

Regulated Expression of the Human Thrombin-activable Fibrinolysis Inhibitor Gene in Nonhepatic Cell Types

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The balance between coagulation and fibrinolysis is critical for the hemostatic response upon injury. The carboxypeptidase thrombin-activable fibrinolysis inhibitor (TAFI) plays a key role in controlling this balance by attenuating fibrinolysis; high plasma TAFI antigen levels have been associated with increased risk of thrombotic diseases. Activated TAFI (TAFIa) can also inactivate pro-inflammatory peptides such as the anaphylatoxins and bradykinin, suggesting a role for the TAFI pathway as a link between coagulation and inflammation. TAFI expression in HepG2 cells is decreased by interleukins -1 and -8 and plasma TAFI concentrations in humans are decreased in experimental endotoxemia. Although liver is presumably the main source of plasma TAFI, TAFI has also been identified in platelets, and TAFI mRNA has been detected in the rat (megakaryoblastic) cell line; human aortic and endothelial cells, and adipocytes of patients with type 2 diabetes. Using RT-PCR and real-time RT-PCR, we now report the detection of TAFI mRNA in the human monocyteic cell line THP-1 as well as THP-1 cells that have been differentiated into macrophage-like cells (THP-1/ma) by treatment with phorbol esters. We find no evidence of TAFI gene expression in human coronary artery smooth muscle cells. It has been hypothesized that platelet TAFI arises from TAFI gene expression in megakaryocytes. Accordingly, Dami cells were treated with phorbol esters for up to 72 hours to differentiate them along the megakaryocyte/platelet lineage. Using real-time RT-PCR analysis, we found that TAFI mRNA abundance was increased throughout Dami differentiation (up to 9-fold after 50 hours). Using real-time RT-PCR analysis, we also found that TAFI mRNA abundance was decreased when THP-1 and THP-1/ma were treated with bacterial lipoxygenase, a 24-hour (2-fold and 16-fold, respectively). Extra-hepatic expression of TAFI, such as in platelets, macrophages a novel role for localized TAFI expression in regulation of hemostasis and inflammation.

Paclitaxel, but Not Zotarolimus or Dexamethasone, Inhibits Human Coronary Artery Endothelial Cell Serum-induced Migration in Vitro: Role of p70S6K Activation in Endothelial Cell Migration

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Introduction: Drug-eluting stents have reduced restenosis rates but elute agents which could intercede with endothelial cell (EC) migration and re-endothelialization of lesions, leading to thrombosis. Previous studies have shown that sirolimus blocks smooth muscle migration in response to individual growth factors such as PDGF-BB or sphingosine-1-phosphate an effect which may be mediated by inhibition of p70S6K activation. This study reports the effects of the anti-restenotic agents zotarolimus, paclitaxel and dexamethasone on activation of p70S6K and its role in the migration of human coronary artery EC (HEC) induced by serum. Hypothesis: Paclitaxel but not zotarolimus or dexamethasone is hypothesized to inhibit HEC migration. Reduced p70S6K activation alone will not result in anti-migratory activity. Methods: HEC migration in response to serum effects was determined using a modified Boyden chamber. HEC were supplemented and treated for 0 or 24 hrs in basal or growth media. After migration (24 hrs) cells were stained with calcein-AM and fluorescence measured. Phosphorylated p70S6K (T389) was measured by Western blot. Results and Conclusions: Paclitaxel but not zotarolimus or dexamethasone blocks migration, however, all agents reduce p70S6K(T389) phosphorylation (fig). These data suggest that in the presence of multiple chemotactic factors, inhibition of p70S6K alone is insufficient to reduce migration. Dexamethasone and zotarolimus should not impair, and the former may promote, re-endothelialization of vascular lesions. In contrast, paclitaxel is predicted to potentially attenuate re-endothelialization by blocking HEC migration.
Aspirin Resistance in Stable Coronary Artery Disease Patients Is Associated with Increased Platelet Turnover but Not Oxidative Stress

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Introduction: Several patient characteristics have been shown to increase the risk of aspirin resistance, yet underlying mechanisms remain unknown. It has been suggested that oxidative stress may be involved. Hypothesis: We evaluated the hypothesis that increased oxidative stress, as assessed by urinary isoprostane concentration, is associated with aspirin resistance. Methods: Two hundred and one consecutive subjects suffering from stable CAD and under daily aspirin therapy were enrolled. Platelet aggregation was measured by optical aggregometry (LTA) after stimulation with 1.6 mM of arachidonic acid (AA) as the agonist (5, 10 and 20 µM; resistant if ≥ 70%), whole blood impedance (AA 1.6 mM; resistant if ≥ 34.3 s), VerifyNow Aspirin® (resistant if ≤ 550 ARU) and urinary 11-dehydrothromboxane B2 (6-11d-TxB2, resistant if ≥ 67.9 ng/mmol of creatinine). Results: Of the 201 subjects, 8 were aspirin resistant by LTA-AA (prevalence = 4%, C4d=0.01:0.07). When assay-specific cut-off values were applied to the above-specified tests, aspirin resistance prevalence varied. *p<0.05. Conclusion: Aspirin resistance remains rare when evaluated with the current gold standard (LTA-AA) in stable coronary artery disease patients. Platelet function tests are not equally effective in measuring aspirin’s antiplatelet effect and correlate poorly with one another, which may explain the discrepancies reported in the literature. Given the low agreement between the various assays, their usefulness to detect aspirin resistance remains questionable.

**PLATELET FUNCTION ASSAYS**

<table>
<thead>
<tr>
<th>Platelet function test</th>
<th>Aspirin Resistance Prevalence</th>
<th>Coefficient of correlation with LTA-AA</th>
<th>Agreement with LTA-AA</th>
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<tr>
<td>LTA-AA</td>
<td>4%</td>
<td>-</td>
<td>-</td>
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<tr>
<td>LTA-ADP 5 µM</td>
<td>10%</td>
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<td>0.25*</td>
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<td>LTA-ADP 10 µM</td>
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<td>LTA-ADP 20 µM</td>
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<td>0.12*</td>
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<tr>
<td>Impedance, AA</td>
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<td>0.24*</td>
<td>0.17*</td>
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<tr>
<td>VerifyNow Aspirin®</td>
<td>66%</td>
<td>0.12*</td>
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<tr>
<td>Urinary-d-TxB2</td>
<td>23%</td>
<td>0.18*</td>
<td>0.25*</td>
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Impaired Endothelial Function in Human C-reactive Protein Transgenic Mice

Adrian Quan, Hwee Teoh, Sam Targari, St. Michael's Hosp, Toronto, Canada; Alexander J Szlai, Univ of Alabama at Birmingham, Birmingham, AL; Michael E Ward, Subodh Verma; St. Michael's Hosp, Toronto, Canada

BACKGROUND: Increasing evidence suggests that the inflammatory biomarker, CRP, may play a causal role in the development and progression of atherothrombosis. Since endothelial dysfunction is an early and integral component of atheroclerosis, we hypothesized that endothelial homeostasis would be impaired in-vivo in human CRP transgenic mice. METHODS AND RESULTS: Male CRP transgenic (CRP-Tg) and wild-type mice were injected (i.c.) thrice over two weeks with either peanut oil vehicle or turpentine to induce the inflammation-sensitive CRP transgene. Serum CRP was undetectable in wild-type mice, while levels in turpentine- and vehicle-treated CRP Tg were 276.28±56.7 µg/mL and 41.1±0.2 µg/mL (n=6–8), respectively. Endothelium-dependent and -independent vascular responses were studied using an isolated tissue bath technique. Aortae isolated from mice with elevated CRP levels demonstrated profound impairment in endothelium-dependent responses to acetylcholine (57.1±9.5 vs. 65.0±5.0, p<0.05, n=6), without affecting endothelium-independent vasoreactivity to SNP. Moreover, turpentine induced an increased permeability to fibrinogen in turpentine-treated CRP Tg mice compared to vehicle-treated CRP Tg mice. Furthermore, CRP overexpression in-vivo promoted an increased expression of MCP-1 and macrophage infiltration in mouse aortic tissue. CONCLUSIONS: We demonstrate that human CRP transgenic mice exhibit endothelial dysfunction in-vivo with resultant changes in vascular structure and endothelial responses to injury. These data strengthen the role of CRP as a partaker of vascular risk.
Hsp72 Increases Endothelial Survival by Inhibitory Interaction with Mst1

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BACKGROUND: Apoptosis of vascular endothelial cells (ECs) is observed in many inflammatory and immune disorders and is believed to contribute to the pathogenesis of diverse vascular diseases. Mammalian sterile 20-like kinase 1 (Mst1) is a ubiquitously expressed serine/threonine kinase known to be activated in response to a wide variety of apoptotic stimuli. However, its role in vascular ECs and the mechanism underlying its regulation are not well understood. METHODS AND RESULTS: Treatment of Umbilical Vein Endothelial cells (HUVECs) with TNF-α and H2O2 induced apoptosis and cleavage of Mst1, which is a marker of its activation, as well as the loss of expression of caspase 3, caspase 8, and N-acetylcytosine suppressed both TNF-α- and H2O2-induced Mst1 activation, suggesting that both caspase-3 and reactive oxygen species are involved in Mst1 activation in HUVECs. Adenovirus-mediated overexpression of wild-type Mst1 (AdMst1) in HUVECs increased apoptotic cells with activation of caspase 3, whereas overexpression of dominant negative Mst1 (K59R), Mst1 (K59R) attenuated TNF-α- and H2O2-induced apoptosis. Furthermore, we showed the overexpression of Hsp72 or mild heat shock inhibited TNF-α-induced activation of Mst1 as well as its downstream targets p38 MAPK, and JNK (c-Jun N-terminal kinase), thereby blocking Mst1 dependent apoptosis in HUVECs. In addition, Hsp72 antisense oligonucleotides prevented the inhibitory effects of mild heat shock on TNF-α and H2O2-induced Mst1 activation and apoptosis in HUVECs. CONCLUSIONS: Our results suggest that Mst1 plays an important role in the induction of apoptosis of ECs and Hsp72 functions as an endogenous inhibitor of Mst1. Mst1 may be a potential therapeutic target for vascular diseases associated with endothelial apoptosis.

Hsp72's Potential Role in Diabetes: Rapid Inhibition of Platelet Reactivity with AZD6140 in Rats with Streptozotocin-induced Diabetes

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Background. Excessive platelet activation fundamentally contributes to cardiovascular events and mortality in patients with diabetes mellitus. We investigated whether acute administration of AZD6140, a reversible oral P2Y1 antagonist with rapid onset of action, would beneficially modulate platelet reactivity in diabetic rats. Methods. Diabetes had been induced by intravenous injection of streptozotocin in male Wistar rats. After 4 weeks of diabetes, single-dose treatment with AZD6140 (5 mg/kg) was given by gavage. Plasma samples were collected and functional assays assessing platelet reactivity to ADP and vascular function were performed at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post application. Results. Sufficient plasma levels of AZD6140 were achieved within 30 minutes. CD62P surface expression in response to ADP was significantly reduced 0.5–4 hours after single administration of AZD6140. ADP-induced platelet aggregation was rapidly inhibited by single-dose administration of AZD6140 within 15 minutes and at the end of the complete observation period of 24 hours. ADP-stimulated platelets were perfused through a perfusion flow chamber over a fibrogenen-coated membrane. Acute administration of AZD6140 significantly reduced platelet adhesion to fibrinogen. Conclusions. Acute administration of AZD6140 resulted in rapid absorption in diabetic rats. AZD6140 rapidly inhibited platelet reactivity. Platelet inhibition by AZD6140 could be mechanistically explained by inhibition of the mechanism underlying the regulation of Mst1 in HUVECs, a yeast two-hybrid screening strategy was conducted to identify interacting molecules that associate with Mst1. Binding studies indicated that Hsp72 bound directly to the inhibitor domain of Mst1, as demonstrated by co-precipitation experiments of recombinant Mst1 and Hsp72 cotedicated in the cytoplasm. Furthermore, we showed the overexpression of Hsp72 or mild heat shock inhibited TNF-α-induced activation of Mst1 as well as its downstream targets p38 MAPK, and JNK (c-Jun N-terminal kinase), thereby blocking Mst1 dependent apoptosis in HUVECs. In addition, Hsp72 antisense oligonucleotides prevented the inhibitory effects of mild heat shock on TNF-α and H2O2-induced Mst1 activation and apoptosis in HUVECs. CONCLUSIONS: Our results suggest that Mst1 plays an important role in the induction of apoptosis of ECs and Hsp72 functions as an endogenous inhibitor of Mst1. Mst1 may be a potential therapeutic target for vascular diseases associated with endothelial apoptosis.

Relation to Progression of Atherosclerosis

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Introduction. Thrombogenicity of the atherosclerotic plaque depends in part, on the quantity and activity of tissue factor localized in the arterial vessel wall. Recent experiments indicate that in addition to local tissue factor (TF), also factor (F) VII is expressed by cells in human atherosclerotic plaques. Hypothesis: Vitamin K dependent proteins including prothrombin and FX, are synthesized by cells within the arterial wall and contribute to the thrombogenicity of atheromatous plaques. Methods: Results: Immunohistochemical staining of TF and coagulation factor II, VII, IX, X, XI, and XII revealed the presence of these proteins in all stages of atherosclerotic plaques. In arterial vessels characterized by intimal thickening, TF was demonstrated in association with medial and intimal smooth muscle cells (SMCs), whereas in advanced lesions TF and FX were observed in macrophages and, in more advanced stages, TF and FX were associated with the intima-media and intima-media. FX showed a cytoplasmatic localization, while thrombin stained positive in the extracellular matrix of adventitia, media, and necrotic core of the plaque. In thrombotic lesions, macrophages and polymorphonuclear leukocytes contained TF, whereas PVH stain macroglobinuric and macrophages and polymorphonuclear leukocytes. The presence of procoagulant complexes in all stages of atherosclerosis. In arterial vessels characterized by intimal thickening, TF was demonstrated in association with medial and intimal smooth muscle cells (SMCs), whereas in advanced lesions TF and FX were observed in macrophages and, in more advanced stages, TF and FX were associated with the intima-media and intima-media. FX showed a cytoplasmatic localization, while thrombin stained positive in the extracellular matrix of adventitia, media, and necrotic core of the plaque. In thrombotic lesions, macrophages and polymorphonuclear leukocytes contained TF, whereas PVH stain macroglobinuric and macrophages and polymorphonuclear leukocytes. The presence of procoagulant complexes suggests that they contribute to thrombin generation. In addition to being determinants in thrombogenicity, these proteins may be involved in other pathophysiological processes such as cell migration and inflammation.

Interactions of mCRP and pCRP with Members of the Fibrinolytic Family of Proteins

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Background. Increased plasma concentration of C-reactive protein (CRP), an acute phase reactant protein, is associated with increased risk of myocardial infarction and CRP-transgenic mice exhibit accelerated thrombosis after vascular injury compared to wild-type mice. As the plasminogen activation (PA) system plays a major role in modulating thrombosis, we examined potential interactions of CRP (both monomeric and pentameric forms) with Glu-plasminogen and fibs, key components of the PA system. Methods: Monomeric human CRP (mCRP) was prepared by denaturing pentameric CRP (pCRP) with urea, which was...
Prevalence and Distribution of Deep Vein Thrombosis in Patients with Symptomatic Pulmonary Embolism

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BACKGROUND: To investigate the prevalence and distribution of deep vein thrombosis (DVT) in patients with symptomatic pulmonary embolism (PE), and to compare characteristics between patients with PE and these without PE.

RESULTS: Of 420 patients evaluated, PE was found in 82 (20%) patients. There were no significant differences in mean age, gender, and laterality of leg involvement between patients with DVT combined with PE and these with DVT alone. Proximal DVT was found in 39 (48%) patients with PE and 282 (63%) with no PE, and there was a significantly higher proportion of proximal DVT in the patient population with PE compared with those without PE (0.003). In 62 (15%) patients with PE, the DVT was found to be most predominantly in soleal vein (53%) followed by popliteal (37%), peroneal (32%), common femoral (27%), femoral (27%), gastrocneumus vein (21%). The significantly higher proportion of DVT was found in gastrocnemius vein in patients with PE (p = 0.010). In contrast, the proportion of popliteal vein DVT was significantly lower in patients with PE who had DVT alone (p = 0.049). The operation and trauma (43%), immobilization (22%), and active cancer (16%) were the main risk factors for DVT related to patients with PE, and there were similar tendencies in the proportions of risk factors for patients with DVT alone. In thrombophilia testing, only proportion of positive antiphospholipid antibody syndrome was significantly higher in patients with PE (p = 0.011).

CONCLUSIONS: The lower extremity venous duplex scanning demonstrates that distal DVT was more predominant in patients who had PE compared to those with DVT alone. Thrombus in the gastrocnemius vein was frequently found in patients with PE. Positive antiphospholipid antibody syndrome was more often found in patients with PE. However, there were similar tendencies in age, gender, laterality of leg involvement, and risk factors for DVT between patients with PE and these with DVT alone.

Localization of Classical Complement Components and C1 Inhibitor on Platelet Microparticles

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Platelet microparticles (PM) play an important role in hemostasis and thrombosis, and are released from activated platelets. We previously demonstrated in situ classical complement pathway activation on stimulated platelets. The present study extends these observations to microparticles. PM were generated by treating platelets with 10 µM A23187 (37°C, 5 min). PM were characterized by flow cytometry based on size, expression of phosphatidylserine (Annexin V binding), P-selectin (anti CD62P), and GPIb (anti CD41). Complement activation was assessed as the levels of C4d and C3d in the supernatant following PMP exposure to 1/10 human plasma (37°C, 45 min). Samples were washed and monoclonal anti C4d and IC3b antibodies added. Flow cytometry showed an approximately 2-fold increase in mean peak fluorescence intensity (MFI) over background for anti C4d and anti IC3b. Since C1 inhibitor (C1-INH) is a major classically activated complement inhibitor, we measured C1-INH, P-selectin, and also measured PMP C1-INH expression. Using rabbit anti human C1-INH antibody, PMP surface C1-INH expression increased approximately 10-fold over background following exposure to plasma. To understand the mechanism of complement activation, PMP were stained with antibodies for the known activators of classical (C4d, C3b, C8, C9) and alternative pathways. P-selectin, we also measured PMP C1-INH expression. Using rabbit anti human C1-INH antibody, PMP surface C1-INH expression increased approximately 10-fold over background following exposure to plasma. To understand the mechanism of complement activation, PMP were stained with antibodies for the known activators of classical (C4d, C3b, C8, C9) and alternative pathways. P-selectin, respectively. In addition, complement activation on PMP was examined in Factor B (alternative) deficient plasma and C4d deficient plasma, and in the alternative pathway. In summary, our findings provide the first evidence for in situ complement activation on PMP and suggest regulation of both alternative and classical complement activation by C1 INH.

The Effects of Weight Loss on Cholesteryl Ester Transfer Protein and Expression of the Metabolic Syndrome in Obese Individuals

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Background: Metabolic syndrome (MS) is a clustering of risk factors, including insulin resistance, abdominal obesity, hypertension, low HDL cholesterol (HDL-C) and elevated triglycerides (TG). Moderate weight loss modestly improves many of these abnormalities. Cholesteryl ester transfer protein (CETP) is a key enzyme involved in HDL metabolism and although high levels of CETP activity are often associated with low HDL-C and high TG, recent studies have shown that diabetics have low CETP activity levels. Therefore, we tested the hypothesis that CETP activity in insulin resistant, obese, know diabetic patients are low. Methods: We analyzed the relation of plasma CETP concentration and activity with adiposity, dyslipidemia and insulin resistance in obese subjects with (MS+; n = 40) and without MS-, n = 40 the metabolic syndrome according to NCEP ATP III criteria and examined the acute effects of weight loss (4–8 weeks and 3–4 months of very-low-calorie diet (VLCD)). Baseline measurements for a group of 20 healthy lean subjects were included compared with obese. Results: At baseline, we found a strong positive correlation between plasma CETP concentration and activity (r = 0.71, p < 0.0001). Mean CETP activity was significantly lower in lean controls compared to obese MS- subjects (27.2 ± 4.6 pmol/0.5 µl/hr, p = 0.05), but not to obese MS+ subjects (37.2 ± 4.0 pmol/0.5 µl/hr, p = 0.34). Subsequent analyses showed that in obese individuals with impaired fasting blood glucose (fasting blood glucose ≥100 mg/dl) compared to obese individuals with normal blood glucose, CETP activity was significantly lower (35.8 ± 4.6 pmol/0.5 µl/hr, p < 0.001) and CETP mass showed a trend towards being lower (0.83 ± 0.96 µg/mL, p = 0.10). Acute weight loss (4–8 weeks of VLCD) resulted in significant 33% and 27% reductions in plasma CETP activity and mass, respectively (p < 0.001), in obese MS+ subjects, which were associated with an 8% decrease in HDL-C (p < 0.001). Conclusions: Although obesity is associated with increased plasma CETP activity, in obese individuals with impaired fasting blood glucose, plasma CETP activity is significantly reduced. These findings suggest that insulin resistance modulates CETP expression in obese individuals.

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High Glucose Augments Interferon-γ-Stimulated Matrix Metalloproteinase-1 Expression in U937 Macrophages by Enhancing STAT1 Activity

Alena Nareika, Med Univ of South Carolina, Charleston, SC; Bryan A Game, Ralph H Johnson VA Med Ctr, Charleston, SC; Maria F Lopez-Virella, Yan Huang; Ralph H Johnson VA Med Ctr and Med Univ of South Carolina, Charleston, SC

Plaque rupture is a principal cause of luminal thrombosis in acute coronary syndromes occurring in 75% of patients who die of acute myocardial infarction. Recent studies called Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications Research (DCCT/EDIC) have shown that rigorous glycemic control in diabetic patients leads to a significant reduction of cardiovascular events, suggesting that hyperglycemia may contribute to destabilization of atherosclerotic plaques. Although mechanisms such as protein glycoxidation by which hyperglycemia promotes atherosclerosis have been identified, it is not well understood how hyperglycemia interacts with inflammatory cytokines such as interferon (IFN) gamma, a key cytokine involved in atherosclerosis, in inflammation-related plaque destabilization. In this study, U937 macrophages were cultured in medium containing either normal (5 mm) or high glucose (25 mm) to be treated with 100 units/ml of IFN gamma for 24 h. After treatment, matrix metalloproteinase (MMP-1) expression was examined. Results from real-time PCR showed that high glucose and IFN gamma increased MMP-1 mRNA level by 100% and 150%, respectively, while the combination of high glucose and IFN gamma resulted in in vitro diabetic disease, suggesting a synergistic effect of high glucose and IFN gamma-stimulated MMP-1 expression. Western blot and electromobility shift assay (EMSA) showed that high glucose augmented IFN gamma-stimulated MMP-1 expression by increasing phosphorylation and DNA-binding activity of signal transducing and activator of transcription (STAT)-1, a major transcription factor involved in IFN-gamma-regulated gene expression. Given that hyperglycemia is a major abnormality in diabetes and diabetic patients have increased cardiovascular events, this study revealed a novel mechanism involved in diabetes-promoted cardiovascular disease.

Effect of Chronic Insulin Plus Atorvastatin Therapy on Mitochondrial Function in an Ex Vivo Animal Model of Diabetes and Hypercaloric Diet Submitted to Global Myocardial Ischemia-Reperfusion

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Introduction: Obese diabetics have more ischemic heart disease, this may be improved by insulin (INS) and atorvastatin (ATV). If these subjects are treated with INS + ATV, do they have better mitochondrial function? Aim: To evaluate, in a model of diabetes-hypercaloric diet + ischemia-reperfusion (IR), if therapy with INS + ATV improves mitochondrial function. Material and methods: Goto-Kakizaki (GK) diabetic rats (fed with an hypercaloric diet), divided in 4 groups (n=10/group): vehicle (no medication/no IR); INS control (insulin bid -as needed- and AT 10 mg/kg/dy between month 5 and 6/NO IR); GK/INS control (INS control and then IR); INS control (INS as control and then IR). At 6 months, hearts were submitted to 165 min perfusion (control) or 10 min perfusion + 35 min ischemia +120 min reperfusion (IR). Parameters assessed were: oxygen stress (colorometric assay of superoxide dismutase activity in the blood), mitochondrial swelling and calcium uptake (fluorimetry). Results: INS + ATV-treated rats had significantly lower oxidative stress levels, both in control (0.70 ± 0.01 vs. 0.81 ± 0.04 nmol TBARS/mg protein <p 0.05) and IR (0.94 ± 0.04 vs. 1.05 ± 0.02 nmol TBARS/mg protein <p 0.05). Fig. 1. INS-treated animals also showed a significant decrease in mitochondrial swelling, both in IR (52.7 ± 2.1 vs. 68.1 ± 1.8 arbitrary units in ATP, <p 0.05), and control (29.1 ± 3.2 vs. 37.0 ± 4.3 <p 0.05). INS therapy showed a significant improvement in calcium uptake in IR (59.8 ± 1.0 vs. 53.9 ± 0.8 nmol/mg protein <p 0.05). Conclusion: In our model, INS + ATV improves cardiac mitochondrial function, by lowering oxidative stress and improving ischemia tolerance.

New Insights into VLDL Triglyceride Metabolism in Obese Subjects with Nonalcoholic Fatty Liver Disease

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Elevated levels of VLDL triglyceride (TG) are associated with obesity, diabetes, non-alcoholic fatty liver disease (NAFLD) and increased cardiovascular risk. Metabolic tracer kinetics studies have been used to evaluate the mechanisms that regulate VLDL-TG levels (production vs. clearance). We have previously used a bolus of [14C]glycerol to determine the fractional catabolic rate (FCR) of VLDL-TG, and, using an in vivo analytical, a constant high infusion of palmitate to determine the proportion of VLDL-TG palmitate derived from systemic (labeled) vs. non-systemic (unlabeled) sources. We have developed a new model that for the first time combines the glycerol and palmitate data into a single analysis to provide a better definition of the VLDL-TG FCR, the size and turnover rate of the pool responsible for tracer clearance, and resolution of the latter from the direct incorporation of palmitate into VLDL-TG. VLDL-TG kinetics studies were performed in 21 obese subjects without and 27 obese subjects with type 2 diabetes mellitus (intravenous, fast [ICF] measured by magnetic resonance spectroscopy and capnography) of the liver pool, and other sources of unlabeled palmitate averaged 28%, 40%, and 32%, respectively; the percent from unlabeled palmitate increased with IFC (p < 0.001) whereas the percent from plasma decreased with IFC (p < 0.01). VLDL-TG concentration decreased with VLDL-TG (p < 0.001), increased with IFC (p < 0.02), increased with the production rates from unlabeled palmitate sources (p < 0.02) and from plasma palmitate (p < 0.005), and was not significantly correlated with plasma FFA concentration (p = 0.25). We conclude that both metabolic activity (responsible for tracer recycling) and inactive (unlabeled by tracer) hepatic fat depots provide more than two-thirds of the fatty acids for VLDL-TG production in obese subjects with NAFLD.

Diet-induced Hyperlipidemia Worsens Vascular Compliance and Function of Resistance Vessels in Type 2 Diabetes

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Diabetes causes both micro and macrovascular complications leading to a 2- to 4-fold increased risk of cardiovascular mortality and morbidity. Diet-induced obesity and related manifestations such as insulin resistance and inflammation cluster with diabetes or hyperglycemia and drive the metabolic syndrome that poses a major challenge on the vasculature. However, the relative roles of diabetes and diet-induced hyperlipidemia in mediating vascular compliance and function have not been well understood. Thus the aims of this study were to study the individual and combined effects of two in eliciting 1) vascular compliance and 2) vascular function. Wistar rats and spontaneously diabetic Goto-Kakisaki (GA) rats fed control or equal amounts of high-fat diet were used for the studies. Body weight, blood glucose, plasma lipids, insulin and adiposity levels were increased in GK rats with high-fat diet treatment (*p < 0.05 vs GK). Third order mesenteric arteries were evaluated for myogenic tone, stiffness and vascular relaxation using the pressure perfusion arteriograph. Vascular stiffness was increased in both the regular (p = 0.01) and high fat diet GK groups compared to the Wistar groups with an interaction between diabetes and the high-fat diet in increasing stiffness (*p < 0.05 vs Wistar), Myogenic tone evaluated at 60 mmHg showed increased tone in the GK rats (*p < 0.05 vs Wistar), which was not present with high-fat diet treatment. Vascular response to both acetylcholine (ACh) and sodium nitroprusside (SNP) (0.1nm-1μm) were impaired as seen by decreased relaxation to ACh and decreased sensitivity to SNP (respectively: *p < 0.05 vs Wistar) in GK rats on a high-fat diet, but not in Wistar rats. These results suggest that diabetes decreases vascular compliance, increases vascular tone and impairs vasorelaxation in GK rats and high fat diet exacerbates vascular dysfunction, which may contribute to increased and accelerated vascular disease in diabetes. Thus, both hyperglycemia and hyperlipidemia need to be targeted for effective prevention and treatment of diabetic vascular disease.
Dietary Cholesterol Selectively Increases Viscerale Adipose Tissue Accumulation in Obese LDL Receptor–Deficient Mice

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In obesity, macrophages accumulate in adipose tissue and contribute to insulin resistance. Obesity also results in chronic low-grade systemic inflammation that is associated with insulin resistance, type 2 diabetes and contributes to atherosclerosis. We previously showed that dietary cholesterol induces low-grade inflammation and increased atherosclerosis in LDL-r/- mice fed a Western diet. To study the interaction of dietary cholesterol and obesity on chronic inflammation, LDL-r/- mice were fed either a rodent chow diet (control) or a diabetogenic diet (high in fat and CHO; without or with 0.15% cholesterol (D-C & D + C respectively). Weight gain was similar in D-C & D + C mice, and significantly more than controls. Insulin levels were higher and responsiveness to insulin was lower in the D-C as compared to the D-C group, suggesting an additive effect of dietary cholesterol on insulin resistance. As compared to controls, circulating levels of the inflammatory protein, serum amyloid A, were 2-fold higher in D-C animals and 5-fold higher in D + C animals, suggesting chronic inflammation. Morphometric analysis of epididymal (visceral) adipose tissue (AT) showed adipocyte hypertrophy and the inflammatory cytokine TNF-α. No significant macrophage infiltration was seen in any of the groups, and there were no differences between groups in adiponecin, F4/80 and TNF-α mRNA levels. These findings indicate that dietary cholesterol induces a chronically inflamed state, with increased macrophage infiltration and TNF-α production coupled to visceral, but not subcutaneous AT. The exact mechanism whereby dietary cholesterol leads to macrophage recruitment into visceral AT in obesity needs further elucidation.

Effect of Increased Consumption of Whole Grain Foods on Markers of Cardiovascular Disease Risk in Middle-aged Healthy Volunteers

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Epidemiological studies suggest that high intakes of whole grain foods may lower cardiovascular disease (CVD) risk. However, recent recommendations that consumption of three servings of whole grain foods daily may be cardio-protective have not been validated. The aim of this ongoing study is to assess the effects of increased consumption of whole grain foods on markers of CVD risk in relatively high-risk individuals. Volunteers (n = 46) were divided into four groups: Refined (high in fat and CHO) and refined + 0.15% cholesterol (D-C & D + C), or refined - 0.15% cholesterol + 0.15% omega-3 (HF R1:1, HF R20:1 and HF R4:1), respectively. Weight gain was 2.6%, 19.8% and 24.3% lower in the HF R1:1, HF R20:1 and HF R4:1 fed mice, respectively, compared with HF omega-6 fed mice. Elicited peritoneal macrophage cholesterol ester content (mg/100mg protein) was 4.3 ±1.3*, 3.9 ± 1.1*, 2.8 ± 0.7* and 2.7 ± 0.7* (values with different superscripts are significantly different at P<0.05) in mice fed HF omega-6, HF-R20:1, HF-R4:1 and HF-R1:1 diets, respectively. Peritoneal macrophage membrane fatty acid profile reflected dietary treatment. Elicited peritoneal macrophages isolated from these mice were stimulated with lipopolysaccharide. Monocyte chemotactic protein-1 (MCP-1) release into the culture medium was 28 ±1*, 23 ± 9*, 18 ± 8* and 17 ± 6* mg/mg protein in HF omega-6, HF-R20:1, HF-R4:1 and HF-R1:1 diet groups, respectively. Gene expression levels of MCP-1, tumor necrosis factor alpha (TNF-α), and ATP-transporters ADRB1 (β1-adrenoceptor) and ADRB2 (β2-adrenoceptor) were significantly lower in elicited peritoneal macrophages from mice fed HF-R1:1 compared to mice fed HF omega-6 diet. Conclusions – These data suggest that diets with lower omega-6:6-EPA: DHA ratios resulted in a lower inflammatory response which was associated with less aortic lesion formation.

Lipid and Glucose Optimization Using Phytonutrient Combination Therapy in Diabetes

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Introduction: Dietary approaches to management of lipid and glucose parameters in diabetes is gaining popularity among patients. Monotherapy with dietary ingredients has shown positive effects, but with limited clinical relevance. Our research focuses on using a novel combination of nutrients to optimize lipid and glucose parameters. All four ingredients have individual data supporting their use for optimizing lipoprotein fractions in hypercholesterolemia. This pilot study evaluates their combined efficacy in type-2 diabetes. Methods: A group of 34 subjects with established type-II diabetes and hypercholesterolemia added the product to their diet. The drink was taken twice daily 15–20 minutes before meals. The fiber drink consists of viscous soluble fiber, minerals, vitamins, policosanol, phytosterols, and an aqueous Chrysanthemum morifolium extract. Lipid and glucose parameters were measured at baseline, 4 and 8 weeks.

Results:

<table>
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<tr>
<th>Parameter</th>
<th>Inclusion criteria at BL (mg/dL)</th>
<th>B.L. (mg/dL)</th>
<th>t=8 weeks (mg/dL)</th>
<th>Δ %</th>
<th>p-value</th>
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<tr>
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</table>

* in %; measurement at 12 weeks.

Conclusion: BiosLife, a nutraceutical combination drink, shows potential in optimizing parameters associated with cardiovascular disease risk in type-II diabetes. These findings are well in line with previously reported clinical results. The fiber component has reduced the post-prandial glucose levels and the resulting lower Hba1c levels indicate that BiosLife provides a natural option to improve diabetes management.
**Sphingosine-1-Phosphate Induces an Anti-inflammatory Phenotype in Macrophages During Inflammation**

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Activated macrophages acquire a pro-inflammatory (classical) or anti-inflammatory (alternative) phenotype that influences atherosclerosis. Sphingosine-1-phosphate (S1P), a novel anti-inflammatory sphingolipid, serves a protective role in inflammation by changing the macrophage phenotype from a pro-inflammatory M1 classical phenotype to an anti-inflammatory M2 alternative phenotype. We examined the anti-inflammatory effects of S1P on LPS-stimulated cytokine secretion in primary C57BL/6J (B6) mouse peritoneal macrophages. LPS upregulated mRNA expression of the pro-inflammatory cytokines TNF-alpha and MCP-1 by several-fold. Incubation of macrophages with S1P reduced TNF-alpha mRNA by 3-fold (p=0.01) and MCP-1 by 2-fold (p=0.001), S1P decreased both TNF-alpha and MCP-1 mRNA by LPS-stimulated macrophages by approximately 50%. Alternative macrophage activation is characterized by induction of the anti-inflammatory Th2 cytokine IL-10 and arginase-1. S1P triggered a 10-fold increase in IL-10 mRNA expression (p<0.001) and a 2-fold increase in arginase-1 mRNA level. Arginase-1 enzyme activity was increased by 35%. Macrophages were activated by LPS alone to express inducible NO synthase (iNOS), and the response was significantly decreased by approximately 50% with the addition of S1P. Mouse peritoneal macrophages express S1P1 and S1P2 receptors. The uncoupling of S1P1 Gi signaling from NO production and the other signaling pathways of S1P2 receptor activation perturbed the alternative macrophage phenotype. In conclusion, S1P1 promotes the alternative anti-inflammatory macrophage phenotype through action on S1P1.

**Acyl-CoA:Cholesterol Acyltransferase 2 and ATP-binding Cassette H Fall Transporters G5 and G6 Differentially Alter Hepatic Lipoprotein Secretion and Composition**

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Three proteins that function in the regulation of cholesterol metabolism and are known to be expressed primarily in hepatocytes and enterocytes are: ATP-binding cassette half transporters G5 and G6 (G5G6) and acyl-CoA:cholesterol acyltransferase 2 (ACAT2). G5G6 is responsible for inside-out transport of cholesterol in cells and ACAT2 is an endoplasmic reticulum-bound enzyme that esterifies cholesterol with acyl-CoA. One of the common processes that these proteins appear to regulate in both cell types is lipoprotein particle secretion. To identity the nature of this regulation, mice bearing gene deletions for G5G6 and/or ACAT2 were created. G5G6 KO mice had a higher liver-to-body weight ratio than wild type mice, apparently due to hepatic triglyceride (TG) accumulation. ACAT2 KO mice had lower hepatic TG accumulation but higher TG secretion. The double KO mice had hepatic triglyceride secretion rates and accumulation similar to that seen in ACAT2 KO mice. The data suggest that sterol handling regulates VLDL secretion and composition. Absence of cholesterol esterification resulted in increased VLDL particle secretion and decreased intracellular triglyceride accumulation while absence of cholesterol transport out of the cell via G5G6 resulted in triglyceride accumulation in the cell and decreased VLDL secretion. Thus, the pools of cholesterol accessed by G5G6 and ACAT2 appeared to be different and impose separate outcomes on VLDL particle secretion vs intracellular triglyceride accumulation. These effects were effectively balanced out in the double KO animals.

**The Role of Different Pathways in the Release of Cholesterol from Foam Cells**

Pin Yue, Robin Fitzgerald, Xiaobo Lin, Zhouji Chen, Gustav Schonfeld; Washington Univ Sch Med, St Louis, MO; Pin Yue, Robin Fitzgerald, Xiaobo Lin, Zhouji Chen, Gustav Schonfeld; Washington Univ Sch Med, St Louis, MO

**Severity of Fatty Liver Due to Impaired Lipoprotein Secretion Is Genetically Determined and Independent of Insulin Resistance**

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Patients with Familial Hypobetalipoproteinemia (FHBL) due to apolipoprotein B truncations have elevated liver triglyceride (TG) content due to impaired lipoprotein export system. Mouse models of apolipoprotein B truncations (apoB38.9 and apoB27.6) mimic the human FHBL phenotype: TG accumulation in the liver and hypercholesterolemia. The severity of FHBL is correlated with increased plasma levels of triglyceride and cholesterol. However, large variations of liver fat were observed in both, humans and mice of mixed genetic background bearing the apoB27.6 and apoB38.9 truncations. We hypothesized that the severity of steatohepatitis due to apoB truncation is genetically determined. To test this hypothesis, we generated congenic mice carrying apoB38.9 under three genetic backgrounds: SWR/J low liver TG strain, ~40mg/dL; C57BL/6J (medium liver TG, ~140mg/dL); and BALB/cByJ (high liver TG strain, ~200mg/dL). Liver and plasma lipids (TG, cholesterol, and free fatty acid (FFA)) were measured. Insulin responses were also measured by 2-hour glucose and insulin tolerance tests (GTT and ITT). Significant interactions between genetic background and apob genotype existed for plasma cholesterol (p<0.0001), and liver TG (p<0.0016), but not for liver
cholesterol and plasma TG, and FFA. The BALB/cByJ-apoB11001 mice had significantly elevated glucose tolerance test curve in CS7BL/6J and BALB/cByJ-apoB11001 mice. Our results showed that the actions of modifier genes determine the extent of liver fat accumulation and insulin resistance in these apoB1001.9 bearing congenic strains. Molecular mechanisms of these differences are under further investigation.

Repair of Oxidative Low-density Lipoprotein and High-density Lipoprotein by Recombinant Human Methionine Sulfoxide Reductase A

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Background: Atherosclerosis is characterized by the accumulation of oxLDL and inflammatory cells in the arterial lesion that caused by a state of heightened oxidative stress. The existence of oxidized low density lipoprotein (LDL) and high density lipoprotein (HDL) in circulation could be used as potential markers for coronary artery disease. Methionine sulfoxide reductase A (MsrA) presents in all living organisms and is one of the most important antioxidative enzymes which reduce methionine sulfoxide (MetSO) residues in proteins. The goal of the study was to investigate the ability of recombinant human MsrA to repair damage of LDL and HDL in vitro, and protect against damage of cell induced by oxidized lipoproteins. Methods and Results: The recombinant hMsrA was expressed in E. coli, M15(pHePr4) and purified by Ni-NTA agarose affinity chromatography. Human LDL and HDL were separated by two-step gradient ultracentrifugation and oxidized by Cu2+ and AAPH in vitro. Recombinant hMsrA incubated with lipoproteins could decrease the electrophoresis mobility of ox-LDL and ox-HDL in agarose-electrophoresis, which denoted reduction of oxidative extent on LDL/HDL. hMsrA was also identified to protect apoB-bearing LDL (apoB100) of LDL against degradation by oxidation and restore the mobility of modification apo AI of HDL in PAGE, furthermore, hMsrA decreased the amount of MDA of LDL, indirectly reflecting its inhibitory ability on lipid peroxidation and protected cultured endothelial cells from damage induced by oxid-LDL. Conclusion: hMsrA may play an important role in protecting against lipoproteins oxidation and cell damage induced by ox-HDL, and may account in part for the repairing function of hMsrA through reducing MetSO of apo AI and apo B1001.9 to methionine peptides shifted apoAI to less electronegative pI by particles by aseorage electrophoresis as well as two-dimensional separation. By native PAGE, all three peptide shifted apoAI from mature HDL particles (8.9nm) to protein of 7.2nm. Per gram, the tandem peptides were more effective at remodeling moHDL than monomeric LAF. Native PAGE separation of remodeled moHDL revealed not just dissociation of apoAI, but also formation of a larger HDL-like particle which did not contain apoAI. We combined tandem of apoAI and moHDL incubations to remodeling threshold were ultracentrifuged to separate these particles. All three peptides produce A/H peptides containing HDL-like particles, displacing apoAI and apoE. The IHS tandem produced the largest particles (11.5nm) while the Pro tandem and monomeric LAF produced similarly sized particles. Multiple mechanism for this H-attracting LDL-like particles: proteolysis of apoAI by aminopeptidase with anti-atherogenic reverse cholesterol transport in vivo. Compared to the monomeric LAF, the Pro tandem showed greater ABCA1-independent and -dependent efflux affinity for HDL cholestene loaded J74 murine macrophage foam cells. Peptides also inhibited copper- oxidation of purified moHDL, as monitored by conjugated diene formation. The peptides inhibited oxidation based on both lag time and slope in the following rancourorder: control < Pro-tandem < IHS-tandem < LAF monomer. In the these work, tandem moHDL and HDL, the peptides showed the following efficacy rancourorder: control < Pro-tandem < IHS-tandem < LAF monomer. These in vitro experiments allow correlativa data for future atherosclerosis studies.

Local Variations in Sheep and Mural Stress Influence Endothelial Permeability and Gene Expression in Arterial Segments Exposed to Cyclic Axial Stretch Ex Vivo

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Introduction: Certain arteries (e.g., coronary, carotid, etc.) are exposed to cyclic axial stretch ex vivo to their tethering to surrounding tissue beds. It is believed such stimuli result in a spatially variable biomechanical stress distribution which has been implicated as a key modulator of atherosclerotic lesion localization. We have developed a combined ex vivo experimental / computational methodology to address the hypothesis that local variations in shear and mural stress associated with cyclic axial stretch of arterial segments influence the distribution of early markers of atherosclerosis. Methods: Bilateral porcine femoral arteries were surgically harvested and perfused ex vivo under pulsatile arterial conditions. One of the paired vessels was exposed to cyclic axial stretching of 7% over in vivo length at 1 Hz for 12 hours. During the last hour, the perfusate was supplemented with Evan’s blue dye-labeled albumin. Vessel segments were divided into 4 or 5 portions which could be individually processed for both fluorescent microscopy for visualization of albumin permeation and RNA isolation for DNA microarray analysis. Finite element analysis and computational fluid dynamics techniques were used to determine the mural and shear stress distributions respectively, for each perfused segment. Results: On average cyclic stretch caused a 1.4 fold increase in endothelial permeability. The magnitude of the permeability was directly proportional to the local circumferential shear wall stress. DNA microarray analysis revealed a number of genes that were differentially expressed with respect to circumferential wall shear or stress. These included genes with known roles in the atherogenesis including intracellular adhesion molecule-1 (ICAM-1), inducible nitric oxide synthase (iNOS), matrix metalloproteinase-13 (MMP-13), and monocoytic chemotactic protein-1 (MCP-1). Conclusions: We have developed a unique method for combining ex vivo arterial perfusion culture with computational biome-chanomechanical simulation. With this method we have demonstrated that even small (<1cm) length of artery there is a complex distribution of atherosclerotic gene expression which correlates with the biomechanical stress distribution.

The Structure of Lipid-free Apolipoprotein A-IV: Evidence of an Interaction Between the N- and C-Terminal That Modulate Lipid Binding

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Apolipoprotein (apo) A-IV is an intestine-derived apolipoprotein with potential functions in reverse cholesterol transport, salinity and chylomycin metabolism. The structural basis for many of these functions is unknown, due in large part to the lack of three-dimensional structure information. Our laboratory recently identified a single amino acid in the apoA-IV C-terminal (F334) that, when mutated to an alanine, permits rapid association of the protein with lipid. Simultaneous removal of the N-terminus negated this fast-lipid-binding phenotype. We hypothesize that there is a direct interaction between the N- and C-terminal of apoA-IV which modulates ability of apoA-IV to bind lipid. Using a systematic mutagenesis strategy, we have identified Trp122 and Phe152 as the two N-terminal amino acids responsible for this effect. To begin resolve the structural model for lipid-free apoA-IV, we used chemical cross-linking and high-resolution mass spectrometry to determine proximal residues in the native structure. We identified 14 long range and several short range cross-links which we used to construct a preliminary model of lipid-free apoA-IV. The cross-linking pattern was consistent with a large helical bundle in which the N- and C-terminals where in close proximity. We have begun work to construct a computer-generated homology model of lipid-free apoA-IV based on sequence similarity to proteins of known structure. As an independent demonstration of the termini interaction, a mutant of apoA-IV was constructed that contained Dyes residues at position 16 and 336. This mutant failed to bind lipid under oxidizing conditions, but was fully capable when the disulfide linkage was reduced with 5 mM DTT. We believe that a direct hydrophobic interaction between specific residues in the extreme ends of apoA-IV stabilizes a helical bundle, thereby retarding lipid binding. This interaction may the protein between active and inactive binding states that may dictate its physiological function.
Expression of Type IIA Secretory Phospholipase A<sub>2</sub> Results in Severe Endothelial Dysfunction in Mice

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Endothelial dysfunction represents an early stage in the development of atherosclerotic cardiovascular disease, and is also considered as an independent predictor of cardiovascular disease mortality. The type IIA secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) may actively contribute to atherosclerosis, acting either locally within the arterial wall or in plasma. In the present study we tested the hypothesis that vessel wall expression of sPLA<sub>2</sub> in transgenic mice results in endothelial dysfunction. We hypothesized that sPLA<sub>2</sub> products contribute to pathogenesis of atherosclerosis by affecting the expression of multiple pro-inflammatory and pro-coagulant genes. To test this hypothesis, we incubated human aortic endothelial cell (HAEC) challenged of HAEC regions were identified as well.

Conclusions: These data show that before therapy, an apo B target of <90 mg/dL approximated an LDL-C <100 mg/dL and a non-HDL-C <130 mg/dL. However, during statin therapy, an apo B target of ~90 mg/dL in these high-risk patients corresponded more closely to the optimal targets of LDL-C <70 mg/dL and non-HDL-C <100 mg/dL recommended for very high risk patients.

Statin Therapy Alters the Relationship Between Apolipoprotein B and Low-Density Lipoprotein Cholesterol and Non–High-Density Lipoprotein Cholesterol Targets in High-Risk Patients: MERCURY II Trial

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Background: Hypercholesterolemic patients may reduce their LDL-C to a predetermined goal yet still have an excess of atherogenic lipoproteins. Apolipoprotein B (apo B) provides a measure of atherogenic lipoproteins and may be a superior predictor for CHD events. An apo B target of <90 mg/dL has been proposed as an alternative to non-HDL-C <130 mg/dL, particularly for hypertriglyceridemic (high TG) patients. This analysis examined what levels of LDL-C and non-HDL-C correspond to an apo B of 90 mg/dL. Methods: The 16-week MERCURY II trial examined patients with LDL-C <130 and 250 mg/dL with TG <400 mg/dL, at risk for CHD (CHD, diabetes or 10-year Framingham risk >20%). LDL-C, non-HDL-C, TG, and apo B were analyzed at baseline and after statin therapy consisting of rosuvastatin (10 or 20 mg), atorvastatin (10 or 20 mg), or simvastatin (20 or 40 mg). For these new analyses, data from all patients were pooled to determine relationships between apo B and LDL-C and non-HDL-C, and linear regression analyses were done on values from baseline and after 16 wks of statin therapy. Results: In high TG patients (TG ≥200 mg/dL, n = 656) and in low TG patients (n = 1128), baseline apo B was linearly related to baseline non-HDL-C and also linearly related to baseline LDL-C (data not shown in Table). Target levels calculated from apo B and lipid data from untreated patients approximated the target values suggested for high-risk high TG patients by the NCEP in 2001. On statin therapy, apo B also correlated well with non-HDL-C and LDL-C (Table). However, target lipid values calculated from statin-treated patients were approximately 30 mg/dL lower than those from baseline data.

Novel Variants of Endothelial Lipase in Subjects with Elevated High-Density Lipoprotein Cholesterol

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Background: High levels of high density lipoprotein cholesterol (HDL-C) are associated with decreased risk of cardiovascular disease. Endothelial lipase (EL) is a recently discovered phospholipase that influences HDL metabolism in mice; our objective is to find mutations in EL that cause increased HDL-C and loss-of-expression causes increased HDL-C. We hypothesized that loss-of-function mutations in EL might cause high HDL-C levels in humans and therefore sequenced the EL gene in 96 subjects with HDL-C levels >99th percentile. A total of 32 gene variants were found, of which 17 were novel. One variant, confirmed by restriction analysis, was X501R, resulting in the conversion of the stop codon to an arginine and a predicted additional 49 amino acids at the carboxyterminus. There were 4 novel variants in the promoter (+626C, -655C, -576G, -526C) and 2 novel variants in the 3’ UTR (+176GA, +315GA). Several previously undiagnosed variants within the intronic regions were identified as well. Conclusions: In subjects with high HDL-C levels, seventeen novel variants in the EL gene were identified, of which one is a novel missense mutation of the stop codon.

Dose-Dependent Effect of Rosuvastatin on Lipoprotein Kinetics in the Metabolic Syndrome

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The dose-dependent effect of rosuvastatin on LDL, HDL and apoB-100 and HDL cholesterol particle kinetics were studied in a randomized, double-blind, crossover trial of five-week therapeutic periods with placebo, 10mg/day or 40mg/day rosuvastatin in twelve dyslipidemic men with the metabolic syndrome. The kinetics of apoB-100, apoA-I and apoA-II were measured using 133I label and multivarialnt modeling. The kinetics of HDL subfractions Lp-A-I and Lp-A-I-A-I were also examined using a new compartment model. Compared with placebo, there was a significant dose-dependent decrease with rosuvastatin in plasma cholesterol, triglycerides (TG), LDL cholesterol, apoB and apoA-I concentrations and in the lathosterol:tolladine ratio. The results suggest that the significant decreases (FCR) of VLDL, LDL and LDL-apoB-100 and decreased the corresponding pool sizes, with
Evidence of a dose-related effect. LDL-apoB production rate (PR) fell significantly with rosvastatin 40mg/day, with no change in VLDL and IDL-apoB PR. Changes in TG were correlated with changes in VLDL apoB FCPR and apoC-III, and changes in leithostrol-cholesterol ratio were correlated with changes in LDL apoB FCR, the associations being more significant with the higher dose of rosvastatin. Rosuvastatin dose-dependently increased HLD cholesteryl ester content and decreased LDL particle size. This increase in Lp(a)-Lp concentration was associated with a significant dose-related reduction Lp(a)-I FCR with no changes to Lp(a)-I PR. There was a significant dose-dependent reduction in Lp(a)-I FCR with concomitant reduction in Lp(a)-I A-PL and hence no change in Lp(a)-I A-F. These data suggest that rosvastatin decreases the plasma concentration of apoB-containing lipoproteins by a mechanism that is dose-dependently related to an increase in their rates of catabolism. Furthermore, rosvastatin increases HLD cholesteryl and Lp(a) concentrations and this was primarily related to reduction in Lp(a)-I fractional catabolic rate. The findings provide a dose-dependent mechanism for the benefits of rosuvastatin on cardiovascular disease in the metabolic syndrome.

**Reduction of Plateau Size and 5LO, ALOX5P, and LTA4H Gene Expression in a Dyslipidemic Mouse Model of Atherogenesis by DG-031, an Inhibitor of Leukotriene Biosynthesis**

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**Background.** Previously we reported that DNA variants in the ALOX5P and LTA4H genes encoding 5-lipoxygenase activating protein (FLAP) and leukotriene A4 hydrolase (LTA4H), respectively, key enzymes in the leukotriene biosynthetic pathway, are associated with increased risk of myocardial infarction and stroke (Helgadottir et al., 2005 & 2006). Other groups independently report that expression of 5-lipoxygenase (5LO), FLAP and LTA4H is elevated in human atherosclerotic plaque and also in the aorta of dyslipidemic mice. Here we evaluate the effect of pharmacological inhibition of FLAP in a dyslipidemic mouse model of atherosclerosis. **Methods.** Apolipoprotein E-deficient (apoE-/-) mice were administered DG-031, an inhibitor of FLAP, in a high fat diet from 8–16 weeks of age. Aorts were harvested for measurement of mRNA content by TaqMan analysis or atherosclerotic plaque burden in aortic root sections. **Results.** DG-031 reduced collagen cross-linking in the aortic root, suggesting that DG-031 causes a dose dependent reduction in atherosclerosis of about 30% (p<0.01). Accompanying the reduction in atherosclerotic plaque formation was a significant reduction in aortic 5LO, ALOX5P, and LTA4H gene expression of greater than 50% (p<0.01). DG-031 also reduced the increase in aortic elastin gene type 5 (Elastin) and MMP9 genes that normally accompany atherosclerotic lesion formation (p<0.05). **Conclusions.** DG-031, a FLAP inhibitor, currently being studied in a PhIII clinical trial for secondary prevention of CV events in post-ACS patients, provides dose-dependent reduction in atherosclerotic lesion formation and significant decrease in aortic expression of leukotriene pathway genes in dyslipidemic apoE-/- mice.

**The Effect of Sphingomyelin Synthase Gene Knockdown and Knockout on Plasma Membrane Sphingomyelin Levels and NF-κB Activation**

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Sphingomyelin (SM) is one of the major lipid components in plasma and on cell membranes. Two isoforms of SMs (SM1 and SM2) are involved in the biosynthesis of SM. To investigate the relationship between SM synthesis and SM levels, we utilized the gene knockdown and knockout approaches. We found that SM1 and SM2 gene silencing diminished SM activity by about 55% and 27%, respectively. To investigate the consequence of SM inhibition, we incubated sRNA-transported HaK7 cells with [3H]-L-serine, a precursor of sphingomyelin and found that both SM1 and SM2 sRNA significantly decreased intracellular [3H]-SM levels compared with controls. LCM analysis showed both SM1 and SM2 knockdown significantly reduced cellular SM but not ceramide, S1P and sphingosine levels. In order to study the impact of SM knockdown on lipid rafts, we isolated lipid rafts and determined SM and cholesterol levels in them. We found that SM1 and SM2 knockdown significantly decreased SM but not cholesterol levels in lipid raft fractions. This effect was not observed in non-raft lipid fractions. Furthermore, SM sRNA-treated cells exhibited stronger resistance to cell lyses mediated by lysenin, an SM aggregate binding protein, than did control sRNA-treated cells. More importantly, complete SM gene knockout (KO), significantly reduced lysenin-mediated macrophage lysis. These results suggest that SM plays a significant role in regulating SM-containing membrane microdomains which play important roles in cell signaling. Indeed, immunocytochemistry, luciferase and western blot analysis results indicated that the activation of NF-kappaB was attenuated in SM1 knockout cells treated with TNF, and in SM2 KO macrophages treated with LPS. Moreover, there was less production of the mRNA and protein for TNF, IL-1β, and IL-6, in LPS-treated SM2 KO macrophages than from cells wild type mice. Taken together, our data suggest that SM1 and SM2 not only regulate intracellular and plasma membrane SM levels but also play important role in cell signaling pathways, such as inflammation, which may well have an impact on the development of atherosclerosis.

**Protein-mediated Selective Uptake of High Density Lipoprotein–Derived Cholesterol Ester**

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Cholesterol ester transfer protein (CETP) is a hydrophobic glycoprotein that mediates the transfer of neutral lipids between lipoproteins. Recently, our laboratory has demonstrated a novel role for CETP in directly mediating the selective acquisition of CE from HDL by hepatocytes, indicating a direct and potentially anti-atherogenic function in reverse cholesterol transport. Further studies have revealed that key cellular mechanisms were directly involved to address the cellular localization of CETP mediated selective uptake of HDL-CE. Using biochemical plasma membrane isolation followed by detergent extraction, we demonstrate that CETP localizes in the low density, detergent-resistant membrane fractions in both ODS-7 cells and primary murine hepatocytes infected with an adenoviral CETP construct. In an attempt to dissect the intracellular events following the selective uptake of HDL derived CE mediated by CETP, we focused on the interaction of CETP with the cellular membrane. We found that CETP/ apoB100 complexes persist for up to 24 hours following HDL incorporation, suggesting that CETP is a stable component of the HDL-CETP apoB100 complex. In this study, we demonstrate that HDL and CETP can be recycled through a retroendothelial pathway, during which CE may regulate intracellular and plasma membrane SM levels but also play important role in cell signaling pathways, such as inflammation, which may well have an impact on the development of atherosclerosis.
be removed from the HDL particle and directed to the lipid droplets for storage. These studies provide new insight into CETP membrane localization and intracellular trafficking, relevant to its role in CE selective uptake.

**WITHDRAWN**

**P132**

**Nuclear Bile Acid Receptor Gene Variant Improves Efficacy of Lipid-lowering Therapy**

Atsushi Nohara, Hayato Tada, Shoji Katsuda, Masa-aki Kawashiri, Akihito Inazu, Junji Kobayashi, Masasaku Yamagishi, Hiroshi Maubuchi; Kanazawa Univ Graduate Sch of Med Science, Kanazawa, Japan

Bile acid receptor FXR has crucial roles in cholesterol conversion into bile acids and in its recycling through many target genes that may affect cholesterol levels. We have identified common polymorphism -1g→t in FXR gene in Japanese population, and hypothesized that this polymorphism could affect lipid-lowering therapy response. Methods and Results: Total 278 patients (M/F=147/129) suspected CAD were enrolled. FXR -1g→t alleles were determined by PCR-RFLP, and -1 allele frequency was 0.32. Lipid-lowering drugs (statins 92%) were prescribed in 113 patients (M/F=61/52) with FXR genotype [gg(n=107), gt(n=12), tt(n=4)]. Lipid-lowering therapy [statins + ezetimibe(n=80), statins + simvastatin(n=33), statins + pravastatin(n=1)]. The patients were monitored for 6 months. In patients with FXR genotype [gg], we found significantly stronger reduction in total cholesterol (Tcho [gg vs. gt, 10.7% vs. 4.1%, P=0.0001] and triglycerides (TG [gg vs. gt, -41% vs. -12%, P=0.015]) and a trend towards lower LDL cholesterol (Ldl [gg vs. gt, -20.4% vs. -15.3%; P=0.08]). Moreover, the responder rate to lipid-lowering therapy was higher in patients with FXR genotype [gg] (87% vs. 71%, P=0.007). There was no difference in triglycerides reduction or LDL-C increase. Conclusion: These results demonstrated that common genetic variant of FXR gene showed considerable impact on lipid-lowering therapy, probably through modulation of genes involved in cholesterol metabolism such as CYP7A1. Whether this variant could affect long-term clinical course should further be sought.

**P133**

**Epoxycholesterol Treatment Increases ABCA1-Mediated Cholesterol Efflux in LDL- but Not Acetylated LDL-loaded Macrophages**

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BACKGROUND: We recently showed that LDL cholesterol preferentially effluxed to HDL, whereas a modified acetylated LDL (AcLDL), primarily effluxed to lipid-poor apoA-I in an ABCA1-dependent fashion in murine bone marrow derived macrophages. While the intracellular cholesterol trafficking pathways are clearly different, the regulatory mechanisms remain unclear. Here we studied how the LXR activation regulated cholesterol transport through these two pathways. METHODS AND RESULTS: LDL and AcLDL labeled macrophages were treated with the LXR ligand, epoxycholesterol, and examined for cholesterol efflux. Epoxycholesterol significantly increased ABCA1-mediated cholesterol efflux compared to apoA-I, while in macrophages loaded with LDL-derived cholesterol, epoxy treatment decreased cholesterol efflux to apoA-I, but not in LDL treated cells, and exhibited less potent stimulation of the efflux of newly synthesized cholesterol, compared to epoxy. Preliminary data indicated that epoxy might fail to mobilize cholesterol from lipid droplets due to downregulation of neutral cholesterol ester hydrolase (NCEH) at the transcriptional level. CONCLUSION: These results suggest that treatment with specific oxysterol LXR ligands differentially regulate cholesterol homeostasis in distinct cholesterol pools.

**P134**

**Reduced Hepatic AGAT2 Activity Is Part of the Integrated Response to Inhibition of Cholesterol Synthesis in Humans**

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In order to identify how different degrees of cholesterol synthesis inhibition affects human hepatobiliary cholesterol metabolism, we studied 37 normo-cholesterolemic gallstone patients randomized to treatment with placebo, 20 mg/d fluvastatin or 80 mg/d atorvastatin for 4 weeks prior to surgery. Based on serum lathosterol determinations, cholesterol synthesis was reduced by 42% and 70% in the two groups receiving statins. During gallstone operation a liver biopsy was taken, and hepatic protein and mRNA expression of several rate-limiting steps of cholesterol metabolism were assayed and related to the increase in lathosterol levels. A marked induction of LDL receptors and HMG CoA reductase was detected and positively related to the degree of cholesterol synthesis inhibition. In a corresponding way, we could show that the activity, protein expression and mRNA for AGAT2 were all decreased in response to cholesterol synthesis inhibition. The lowering of HDL cholesterol seen in response to high-dose statin treatment could not be explained by changes in structures such as the HDL receptor CLA-1, ABCA1 or apoA-I. We conclude that AGAT2 activity in human liver is lowered by cholesterol synthesis inhibition, and that this effect, in combination with a parallel down regulation of Apo E expression, may contribute to the favourable lowering of VLDL cholesterol seen in addition to the LDL lowering during statin treatment.

**P135**

**Glycoprotein Ib/Ⅲa and Coronary Disease Extension**

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Glycoprotein Ib-Ⅲa plays a pivotal role in the connection to the platelets of fibrinogen and other adhesion proteins. The gene that codifies sub unit IIIa, with two alleles PLA1 and PLA2, has already been identified. The PLA2 allele has been associated to acute coronary syndromes (ACS) emergence, and individuals possessing the PLA2 allele would bind more fibrinogen to platelets than do PL A1 homozigotes. The response to thrombin can differ between the two genotypes. PLA2 allele individuals could have less severe, less complex and less extensive coronary morphology lesions and more reactive platelets. Aims: to evaluate the influence of polymorphism of glycoprotein (GP) Ib-Ⅲa gene (PLA1 and PLA2) in coronary disease extension, in a Portuguese population. Methodology: In 296 consecutive coronary patients who underwent coronary angiography for diagnostic purposes, was evaluated the Leaman coronary score in the GP Ib-Ⅲa polytype PLA1-PLA1: PLA1-PLA2: PL A2-PLA2. The average genotype scores were compared by Student T test for independent samples (unilateral analysis, p<0.05). Results: In this coronary Portuguese population the GP Ib-Ⅲa genotype PLA1/PLA1 presented less severe coronary artery morphology lesions, which was statistically significant. Looking at these results we can conceive that there are coronary patients with severely altered coronary morphology, in whom the anti-aggregatory factor is predominant, and others with relatively preserved coronary morphology in whom the pro-aggregative and thrombotic factors dominate. In patients with platelet aggregation alterations, anti-aggregation therapy will be particularly important.

**Genotypes and average score**

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**P136**

**Aging Increases the Inflammatory Response After Vascular Injury, Leading to an Exaggerated Neointimal Formation**

Roberto I Vazquez-Padrón, Yuntao Wei, Dania Mateo, Luis Rodriguez-Moncal, Sen Li, Sashi Salgar, Si M Pham; Univ of Miami, Miami, FL

Aging is a risk factor for the development of vascular occlusive diseases. The aim of this study is to determine whether aging prolong the inflammatory response after vascular injury, leading to an exaggerated neointimal development. Using iliac balloon injury model, we found that arteries from old rats (22 months) developed thicker neointima at 4 weeks after injury than those from young (4 months) ones (I/M: 0.8±0.2 vs. 0.54±0.15, p=0.008). We also found that old arteries accumulated more alpha-actin cells; detected by immunohistochemistry, IHC)- in the adventitia than young cells. old arteries contained three folds or more IL18, IL-6, Gro KC, and Leptin than the young ones as early as three days after injury. These pro-inflammatory cytokines stayed elevated in the old vasculature three days after injury. These pro-inflammatory phenotype. There are no difference in the number of vascular T cells and its role in CE selective uptake. Aims: to evaluate the influence of polymorphism of glycoprotein (GP) Ib-Ⅲa gene (PLA1 and PLA2) in coronary disease extension, in a Portuguese population. Methodology: In 296 consecutive coronary patients who underwent coronary angiography for diagnostic purposes, was evaluated the Leaman coronary score in the GP Ib-Ⅲa polytype PLA1-PLA1: PLA1-PLA2: PL A2-PLA2. The average genotype scores were compared by Student T test for independent samples (unilateral analysis, p<0.05). Results: In this coronary Portuguese population the GP Ib-Ⅲa genotype PLA1/PLA1 presented less severe coronary artery morphology lesions, which was statistically significant. Looking at these results we can conceive that there are coronary patients with severely altered coronary morphology, in whom the anti-aggregatory factor is predominant, and others with relatively preserved coronary morphology in whom the pro-aggregative and thrombotic factors dominate. In patients with platelet aggregation alterations, anti-aggregation therapy will be particularly important.
IL-9 seven days after injury. Finally, vascular apoptotic cells were determined by ISOL and IHC for activated Caspase 3. Old arteries had fewer apoptotic cells (> 0.2% ± 0.05) at any time after vascular injury when compared with the younger ones which had abundant vascular apoptotic cells in the media and neointima at seven days after surgery (2.7% ± 0.45). We conclude that aging decreased the resolution of the vascular inflammatory response to injury, leading to exaggerated neointimal formation.

P138
Number of Nitrate Groups Determines Reactivity and Potency of Organic Nitrates: A Proof of Concept Study in ALDH2-/- Mice
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Background and purpose: Mitochondrial aldehyde dehydrogenase (ALDH2) has been shown to provide a pathway for bioactivation of organic nitrates and to be prone to desensitization in response to highly potent, but not to less potent nitrates. We therefore sought to strengthen the concept, that bioactivation by ALDH2 critically depends on the amount of nitrate groups within the nitrosavasodilator. Experimental approach: Nitrates with one (PEMN), two (PEDN, GDN), three (PETN, GDN, strychnine nitrate) and four (Pentaethylenetetramine tetranitrate) nitrate groups were investigated. Vasodilatatory potency was measured in isolated aortic rings from ALDH2-/- mice using isolated aortic segments of wild type (WT) and ALDH2-/- mice. Activity of the cGMP-dependent kinase-I (reflected by levels of phosphorylated Vasodilator Stimulated Phosphoprotein, P-VASP) was quantified by Western Blot analysis, mitochondrial dehydrogenase activity by HPLC. Following incubation of isolated mitochondria with PETN, PETN-cholesterol and ethylenediamine, NADH and nicotinamide were quantified using chemiluminescence nitrogen detection and mass spectrometry. Key results: Compared to WT, vasorelaxation in response to PETN, PETN-ethylenediamine and GDN was attenuated about 10-fold in ALDH2-/- mice, identical to WT vessels preincubated with inhibitors of ALDH2-2 Reduced vasodilatatory potency correlated with reduced P-VASP formation and diminished biotransformation of the tetranitrate- and trinitrate-compounds. Intriguingly, none of these findings were observed for PEDN, GDN and PEMN. Conclusions and implications: Our results support the crucial role of ALDH-2 in bioactivating highly reactive nitrates like PETN and PETN-ethylenediamine with high potential for nitrate desensitization. Further studies are needed to determine the number of nitrate groups. Less potent nitrates like PEDN, GDN and PEMN are apparently biotransformed by alternative pathways.

P193
Positive Association of C667T Methylenetetrahydrofolate Reductase Gene Polymorphism with Acute Ischemic Stroke
Moe K Thu, Limin Sor, Feng Peng Woon, National Heart Ctr, Singapore, Singapore; Deidre A DeSilva, Li-Hsian Chen, Singapore General Hosp, Singapore, Singapore; Philip Wong, Tian Hai Koh, National Heart Ctr, Singapore, Singapore; Bronwyn A Kingwell, Baker Heart Rsch Institute, Melbourne, Australia; Meng Cheong Wong, Singapore General Hosp, Singapore, Singapore; Jaya Chan-Dusting, Baker Heart Rsch Institute, Melbourne, Australia

BACKGROUND AND PURPOSE: Elevated plasma homocysteine (Hcy) is a risk factor for ischemic stroke and coronary heart diseases. Polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene are associated with high plasma homocysteine levels in the population. However, very few studies have reported to have a protective effect in endothelial cells. Nitric oxide synthase 3 (NOS3) is a molecule that is known to play an important role in cardiovascular disorders. We hypothesized that NOS3 gene polymorphisms might be associated with ischemic stroke. METHODS: We enrolled 207 subjects (74.4% ethnic Chinese) with no past history of stroke served as controls. Hypertension, diabetes, dyslipidemia and age were also recorded. Genotyping for MTHFR gene was conducted using Taqman (Applied Biosystem, USA) and NOS3 genotyping was conducted using Sequenom (San Diego, USA) method. RESULTS: We found that C667T mutation of MTHFR gene to be significantly associated with acute ischemic stroke, particularly in young, male and ethnic Chinese. C667T mutation of MTHFR gene showed significant association with ischemic stroke and coronary heart diseases. Polymorphisms of methylenetetrahydrofolate reductase gene (MTHFR) and nitric oxide synthase 3 gene (NOS3) were isolated using Taqman and Sequenom technologies respectively. Genotyping was conducted on 207 subjects who had no past history of stroke. The MTHFR C667T mutation was significantly associated with ischemic stroke (P<0.01). The NOS3 G894T mutation was not significantly associated with ischemic stroke. Furthermore, we observed that the combination of MTHFR C667T and NOS3 G894T mutations was not significantly associated with ischemic stroke in our population. CONCLUSION: Our results indicate that C667T mutation of MTHFR gene to be significantly associated with ischemic stroke, particularly in young, male and ethnic Chinese. We concluded that C667T mutation of MTHFR gene is significantly associated with ischemic stroke in our population. P142
Cannabiond Receptor (CB2) Deficiency Inhibits Oxidized LDL/Oxysterol-induced Apoptosis in Macrophages
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Apoptosis of macrophages is an important event in the pathophysiology of atherosclerotic lesions. Oxidized low-density lipoproteins (OxLDL) are a major lipid component of atherosclerotic lesions and endocytosis of OxLDL is a potent inducer of apoptosis in cultured macrophages. OxLDL-induced apoptosis in macrophages is largely due to the oxidized cholesterol derivatives, such as 7-ketocholesterol (7K), in the OxLDL. Cannabinoids exert their effects through two related G-protein coupled receptors, CB1 and CB2. CB2 is primarily expressed by cells of the immune system and several macrophage processes associated with ongoing atherogenesis including proliferation, secretion of inflammatory cytokines and chemokines, are regulated by CB2. Expression of CB2 has been detected in lesions and a low dose of the cannabinoid, 3,4-tetrahydrocannabinol, reduces plaque formation in hyperlipidemic mice, an effect which is blocked by administration of a CB2 specific antagonist. In the current study we examined the hypothesis that CB2 expression influences OxLDL-induced macrophage apoptosis. When resident peritoneal macrophages isolated from CB2(+/+) and CB2(-/-) mice were cultured for 16h with 50 μg/ml OxLDL, in situ terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays showed that CB2(-/-) macrophages undergoing apoptosis than CB2(+/+) controls (27.9 ± 4.7% vs 61.9 ± 8.5%, P < 0.001). Treatment of CB2(-/-) macrophages with 7K for 16h also resulted in significantly fewer apoptotic cells compared to similarly treated wild type macrophages (18.9 ± 10.5% vs 54.1 ± 6.9%, P<0.001). Immunoblots showed reduced cleavage of caspase-3 and PARP in CB2(-/-) macrophages treated with 7K compared to CB2(+/+) controls, a result which was confirmed by caspase-3 activity assays. In contrast, staurosporine-induced apoptosis was unaffected by CB2 deficiency consistent with the conclusion that CB2(-/-) macrophages are not generally defective in apoptosis. These results indicate that CB2-expressing macrophages are resistant to OxLDL-induced apoptosis and suggest that one mechanism by which CB2 influences the development and progression of atherosclerotic lesions is by mediating the apoptotic response of macrophages to OxLDL.

P143
New Insights into the Roles of Apolipoprotein C-III in Stimulating the Production of Hepatic VLDL
Meenakshi Sundaram, Philip Links, Maroun Bou Khalil, Yuwei Wang, Shumei Zhong, Zemin Yan; Univ of Ottawa, Ottawa, Canada

Apolipoprotein C-III is a constituent of VLDL and plays a role in regulating lipoprotein lipase activity as well as receptor-mediated lipoprotein endocytosis. We reported previously that stable expression of apoC-III in MCA-RH7777 cells promoted VLDL-triaclyglycerol (TG) secretion, but that the apoC-III expression on the synthesis or secretion of apoB100, the structural protein of the VLDL, was not examined. In the current study we determined the rate of apoB100 translation and secretion using MCA-RH7777 cells transiently transfected with apoC-III. As observed in stables, transient apoC-III expression increased by 2-fold secretion of [35]glycolabeled TG and phospholipid (PC) associated with TG-rich VLDL (SI > 100). In addition, transient apoC-III expression also resulted in increased incorporation of [35]methylamine into cell-associated and secreted VLDL/LDL-apoB100 by 2-fold, respectively. Moreover, transient apoC-III expression increased post-translational stability of 35S-apoB100; thus pulse-chase analysis showed that the recovery of total 35S-apoB100 (cell + media) was increased by 25% in apoC-III transfectants. Intracellular degradation of 35S-apoB100 could be blocked by MG132 in control cells, but was less sensitive to MG132 in apoC-III cells, indicating that apoC-III expression diminished proteosome-mediated apoB100 degradation. The increased apoB100 synthesis/secretion suggested greater lipid availability for VLDL secretion, which is consistent with the increased VLDL synthesis as observed in apoC-III transfectants. In addition, the apoC-III expression upregulated LDL (LDL-100) gene expression by 2-fold, in apoC-III transfectants, as compared to controls. Moreover, transient apoC-III expression increased LDL-100 mRNA stability, which was confirmed by Northern blot analysis. Unexpectedly, not only did expression of apoC-III stimulate LDL-100 secretion by 1.5-fold but also the expressed apoC-III synthesized apoB100. Thus, apoC-III promotes the synthesis and secretion of apoB100 in in vivo conditions and that apoC-III is involved in the regulation of LDL production and secretion.

P144
Divergence in Vascular Fractalkine Expression and Functional Responses in Male and Female Spontaneously Hypertensive Rats
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Men have a greater incidence of cardiovascular disease compared to women, however the molecular mechanisms responsible for cardiovascular protection in females are unknown.

Meenakshi Sundaram, Philip Links, Maroun Bou Khalil, Yuwei Wang, Shumei Zhong, Zemin Yan; Univ of Ottawa, Ottawa, Canada
Using microarray analysis, the chemokine fractalkine (FKN) was found to be more highly expressed in the mesenteric arterial bed of female SHR compared to males (p < 0.005). As FKN has recently been shown to stimulate NO in endothelial cells, we tested the hypothesis that mesenteric arteries from female spontaneously hypertensive rats (SHR) have greater FKN-induced NO stimulation. Experiments utilized 13-week-old male and female SHR (n = 8). Greater FKN reduction of atherosclerotic lesions in the proximal aorta, the underlying molecular mechanisms in which the cytoplasmic tail of SR-A was replaced by the first 57 amino acids, including the ligand. To create an SR-A receptor that only internalizes ligand, we created a chimeric receptor in which the cytoplasmic tail of SR-A was replaced by the first 57 amino acids, including the ligand. To create an SR-A receptor that only internalizes ligand, we created a chimeric receptor with ox-LDL, agg-LDL, or DISP resulted in inhibition of the lysosome's potential mechanism for lipid-induced inhibition. Previously, we showed that treatment of lysosomal hydrolytic activity in sterol-engorged macrophages, it appears that free sterol in lysosomes from macrophages were isolated using a sucrose density gradient from either lysosomes from macrophages were isolated using a sucrose density gradient from either

Lentiviral Transduction of Human apoA-I and apoA-Im into Hematopoietic Progenitor Cells Reduces Atherosclerosis in apoA-deficient Mice

Yan Ru Su, John L Blakemore, Youmin Zhang, Macfaye F Linton, Sergio Fazio; Vanderbilt Univ, Nashville, TN

Monocytes/macrophages play an important role in the development of atherosclerosis. Genetically engineered macrophages can be used as an effective delivery vehicle for cell therapy of atherosclerosis. We have generated lentiviral constructs expressing the human apolipoprotein A-I (apoA-I) and apoA-I mutant (apoA-Im). The constructs contain the transmembrane domain of CD68 under the control of a macrophage specific promoter, CD68, and used them for \textit{ex vivo} transduction of mouse hematopoietic progenitor cells (HPC) isolated from bone marrow. When the transduced HPCs were transplanted into apoE-/- mice, human apoA-I and apoA-Im were detected in the recipient plasma 16 weeks after transplantation at an average concentration of 8.4%±1.2 µg/dl (apoA-I) and 7.7%±0.8 µg/dl (apoA-Im). This represents a significant improvement in transduction efficiency compared to our previous approach using a retroviral vector and bone marrow transduction. There was no significant difference in total cholesterol and HDL levels among groups during the 16 week course of study. There was a 50% and 60% reduction in \textit{en face} atherosclerotic lesion area in both apoA-I and apoA-Im recipient mice, respectively, when compared to the GFP group. There was a significant (31%) reduction in proximal aortic lesion area in the apoA-Im group when compared to the GFP group; the apoA-I group had only a 10% reduction. Lentiviral-apoA-I or apoA-Im transduced wild type mouse peritoneal macrophages showed a significant increase in free cholesterol efflux in both apoA-I (2.5 fold) and apoA-Im (1.9 fold) when compared to the GFP transduced macrophages. Our data suggest that lentiviral mediated transduction of apoA-I or apoA-Im into HPC is a viable approach for reducing the development of atherosclerosis. This has more potential than wild type apoA-I in the reduction of atherosclerotic lesions in the proximal aorta, the underlying molecular mechanisms could not be attributed to enhanced cholesterol efflux in macrophages alone.

LXR Activation by 24(S,25)-Epoxysterol Enhances the Expression of Niemann-Pick C1 in Macrophages, Leading to Increased Cholesterol Efflux

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Niemann-Pick C (NPC) 1 and NPC2 have been implicated in the trafficking of cholesterol between lysosomal compartments, the endoplasmic reticulum and the plasma membrane for efflux to extracellular acceptors. The liver x receptor (LXR) plays an important role in cholesterol homeostasis and upon activation by oxysterol or synthetic ligands enhances cholesterol efflux by increasing transcription of genes involved in cholesterol efflux, including ABCA1, ABCG1 and APOE. The synthetic LXR agonist, TO-901317 was shown to enhance expression of both NPC1 and NPC2 and contributes to enhanced cholesterol efflux in THP-1 macrophages. ABCA1, respectively, without significant change in the PPAR γ protein level. In the present study, the role of nuclear receptors and of the different ABC transporters in EP 80317-stimulated cholesterol efflux has been evaluated. Methods. Mume monocyte J774 cells were loaded with 3β-cholesterol (1 µCi/ml), incubated with EP 80317 (100 µM) and exposed to HDL (0.1 mg/mg) or apoA-I (20 mg/ml) in order to promote efflux. Results. With EP 80317 added to the cholesterol acceptor, EP 80317 induced a significant increase of cholesterol efflux by 163% and 95% (p < 0.001) after 4 and 16 hours, respectively. EP 80317-stimulated efflux to HDL increased only by 32–26% (p < 0.001), under the same conditions. The significant increase of EP 80317-stimulated cholesterol efflux was found. The expression of proteins involved in cholesterol efflux, as assessed by Western blot, was increased by 2.5–7.2– and 7.3-fold for LXRα, ABCG1 and ABCA1, respectively, without significant change in the PPAR γ protein level. Conclusion. EP 80317 elicits PPARα-dependent cholesterol efflux from J774 cells to HDL and apoA-I as cholesterol acceptors in the reverse cholesterol transport.

A Specific Role for Cytochrome P450 Enzymes in Regulating SR-A Function

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Macrophase class A scavenger receptors (SR-A) are transmembrane receptors that recognize diverse ligands including modified lipoproteins, bacterial products, and modified extracellular matrix (ECM). SR-A internalizes soluble ligands and enhances cell adhesion to modified ECM. The cytoplasmic domains required for mediating SR-A internalization and cell adhesion have not been defined. In this study, we mutated the cytoplasmic tail of SR-A and stably expressed the altered receptors in a human embryonic kidney (HEK 293) cell system. Deletion of all but five cytoplasmic amino acids (SR-A5) eliminated receptor internalization while increasing surface localization and cell adhesion. To examine the importance of specific sequences in the cytoplasmic tail, we created an SR-A mutant with amide derivatization of 4 highly conserved cytoplasmic amino acids (SR-A4). Similar to SR-A5, SR-A4 displayed increased surface localization and cell adhesion. However, SR-A5 converted to the internalization ligand. To create an SR-A receptor that only internalizes ligand, we created a chimeric receptor in which the cytoplasmic tail of SR-A was replaced by the first 57 amino acids, including the internalization motif of the transferrin receptor (SR-A5). In contrast to SR-A5, SR-A5 cells expressing the SR-A5 internalized ligand but did not show enhanced cell adhesion. These results indicate a strong correlation between surface localization and cell adhesion. Because PI3-kinase (PI3-K) is involved in receptor trafficking we examined the importance of PI3-K in SR-A-mediated adhesion. Although PI3-K is activated during SR-A-mediated adhesion, PI3-K activity is required only for SR-A-mediated adhesion in cells expressing full-length SR-A. Together, these results indicate that SR-A function can be modulated by the PI3-K-dependent regulation of receptor localization.

LXRs Stimulate Cholesterol Efflux Through ABCA1 and ABCG1 in a PPARγ-Dependent Manner

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Cholesterol efflux is essential for macrophage cholesterol homeostasis through reverse cholesterol transport via extracellular acceptors such as apolipoprotein A-I (apoA-I) and high density lipoprotein (HDL). We have previously reported that EP 80317 induces a significant decrease in macrophage foam cell formation. Hyperoxia. Our hypothesis is that EP 80317 regulates macrophage cholesterol efflux through the PPARγ-LXRα-ABC transporters pathway. In the present study, the role of nuclear receptors and of the different ABC transporters in EP 80317-mediated cholesterol efflux has been evaluated. Methods. Murine monocyte J774 cells were loaded with 3β-cholesterol (1 µCi/ml), incubated with EP 80317 (100 µM) and exposed to HDL (0.1 mg/mg) or apoA-I (20 mg/ml) in order to promote efflux. Results. With EP 80317 added to the cholesterol acceptor, EP 80317 induced a significant increase of cholesterol efflux by 163% and 95% (p < 0.001) after 4 and 16 hours, respectively. EP 80317-stimulated efflux to HDL increased only by 32–26% (p < 0.001), under the same conditions. The significant increase of EP 80317-stimulated cholesterol efflux was found. The expression of proteins involved in cholesterol efflux, as assessed by Western blot, was increased by 2.5–7.2– and 7.3-fold for LXRα, ABCG1 and ABCA1, respectively, without significant change in the PPAR γ protein level. Conclusion. EP 80317 elicits PPARα-dependent cholesterol efflux from J774 cells to HDL and apoA-I as cholesterol acceptors in the reverse cholesterol transport.

Increases in Lysosomal Cholesterol Correspond with Increased Lysosomal pH and Decreased Vacuolar-ATPase Activity in Macrophage Foam Cells

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Macrophage foam cells in atherosclerosis develop via massive accumulations of free and esterified cholesterol, much of which is within lysosomes. In cell culture, macrophages incubated with mildly oxidized (ox)-LDL, aggregated (agg)-LDL or cholesterol ester- rich lipid dispersions (DSP) accumulate cholesterol in lysosomes followed by an inhibition of lysosomal cholesterol efflux. Lysosomes are key organelles in this regard and thus a potential mechanism for lipid-induced inhibition. Previously, we showed that treatment of THP-1 macrophage with ox-LDL, agg-LDL, or DSP resulted in inhibition of the lysosome’s ability to maintain an active pH. In this report, lysosomes from macrophages were isolated using magnetic beads and the membrane cholesterol was augmented through incubation with cholesterol-esterified methyl-β-cyclooctadecanol (Disp). Varying concentrations of Disp were used to achieve cholesterol-enriched lysosomes. The Disp concentration and lysosomal a heavy “normal” fraction, an intermediate fraction, and a light lipid enriched fraction. When lysosomes from macrophages were isolated using a sucrose density gradient from either

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lysozymes hampers ν-ATPase and inhibits the ability of lysozymes to maintain an active pH. This could exacerbate foam cell formation and influence athero sclerotic lesion development.

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The Association Between Alcohol Consumption and C-Reactive Protein Levels in College-aged Individuals

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Purpose. Current screening methods fail to identify over half of individuals at risk for cardiovascular disease. C-Reactive protein (CRP), an acute phase protein and marker for inflammation, is highly correlated with cardiovascular disease and is a promising new screening tool. Many factors, such as alcohol, medications, physical activity, and excess body fat, affect CRP levels. Alcohol intake results in a J-shaped response curve for CRP in individuals over forty years. This study examines the effect of alcohol consumption on CRP levels in college-aged individuals. Binge drinking is prevalent in college-aged individuals potentially increasing CRP levels. Design. College-aged individuals completed surveys which assessed factors that may affect CRP levels, such as medication use, exercise habits, race, religion, smoking, and alcohol consumption patterns. Three groups, non-drinkers (N=6), moderate drinkers (N=10), and heavy drinkers (N=9), were matched based on survey responses. C-Reactive protein (hs-CRP) was measured using reflectance photometry. This research was approved by the Portland State University's IRB.

Results. The average CRP levels for each group were: non-drinkers 0.61 mg/L, moderate drinkers 0.90 mg/L, and heavy drinkers 1.07 mg/L. Analysis of variance revealed a significant difference in CRP levels among the three groups (p = .016). Post hoc analysis indicated that the heavy drinkers had significantly higher CRP levels compared to the moderate drinkers (p = .006) and non-drinkers (p = .007).

Conclusions. There is a J-shaped relationship between alcohol consumption and CRP levels in college-aged individuals, agreeing with trends found in older adults. If CRP levels are predictive of future risk for cardiovascular disease, college aged individuals may be beginning to show a pattern, which is an additional reason to be concerned about heavy drinking in college-aged individuals.

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Comparison of the Cellular Lipid Efflux Properties of Pre-β High-density Lipoprotein and Lipid-free Apolipoprotein A-I

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The ATP-binding cassette transporter A1 (ABCA1) mediates the efflux of cellular phospholipids (PL) and free (unesterified) cholesterol (FC) to human apoA-I to form nascent HDL particles. WithJ774 macrophages and human skin fibroblasts, the nascent HDL particles present after 24h incubations are discoidal, contain 2, 3 or 4 molecules of apoA-I per particle, possess un-electrophoretic mobility as assessed by non-denaturing 2D-PAGE. With both cell types, the PL/apoA-I molar ratio is about 4 and 12nm in diameter. These particles have PL/FC/apo A-I molar ratios ranging from 96–195/12–39/1. Here, we show that human skin fibroblasts and WI38VA13 (human lung fibroblasts), which release PL and FC to apo A-I relatively slowly, form precursor particles containing predominantly monomeric apo A-I. These small particles can be obtained by incubating lipid-free apoA-I (15 μM) for 1–2h with either human skin fibroblasts and or WI38VA13 cells in which ABCA1 is up-regulated. The hydrodynamic diameter of these particles is ~7 nm and they exhibit pre-β electrorophoretic mobility as assessed by non-denaturing 2D-PAGE. With both cell types, the PL/apoA-I molar ratio for these particles, which contain one apo A-I molecule (as assessed by coated avidin cross-linking), is in the range of 3–4/1 and the FC/apoA-I molar ratio is 1–2/1. SDS-PAGE of concentrated samples did not reveal any evidence of other proteins besides apo A-I in these particles. These pre-β particles are the “lipid-poor” apoA-I frequently mentioned in the literature. When incubated with human skin fibroblasts in which ABCA1 is up-regulated, they effectively efflux FC with Km ~1 μM apoA-I and Vmax ~6–7% cellular FC/S. The equivalent viscosities for lipid-free apo A-I are very similar indicating that the pre-β HDL and lipid-free apo A-I are equally effective in mediating FC efflux. In both cases, the apo A-I is converted to the 9 and 12nm discoidal particles upon longer incubation with the cells. The above results suggest that discoidal HDL particles can be formed similarly by ABCA1-mediated addition of cellular lipids to either monomeric lipid-free apo A-I molecules or monomeric lipid-poor apo A-I (pre-β particles). Knowledge of the reaction products will aid in the elucidation of the mechanism of ABCA1 action.

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SLX-4090, an Enterotype-specific Microsomal Triacylglyceride Transport Protein Inhibitor, Lowers LDL Cholesterol and Triglycerides While Raising HDL Cholesterol in Apo E-/- Mice Fed a High-Fat Diet

James L Ellis, Alessandra Bartolazzi, John Ferkany, Hope Foudoulakis, Enoch Kim, J. Kuo, Ruth Rufing, Olivier Schueller, Eric Wong, Yingfei Yang, Paul Sweetnam; Surface Logic, Surfview, Bright, MA

MTP facilitates the formation and transfer of chylomicrons and VLDL in the intestine and the liver respectively. Inhibiting both intestinal and hepatic MTP lowers serum levels of total, VLDL and LDL cholesterol as well as triglycerides but leads to hepatitis lipodis. SLX-4090 was designed to be enterotype-specific and non-absorbable thus avoiding mechanism-based liver toxicity. Male Apo E-/- mice were fed a high fat diet for 10 weeks which contained either no SLX-4090 (control) or 0.006% (10 mg/kg/day) or 0.019% (30 mg/kg/day) or 0.06% (100 mg/kg/day) SLX-4090. SLX-4090 dose-dependently decreased total cholesterol and LDL levels while raising HDL levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>Body Weight Gain (g)</th>
<th>F/B Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>163±39</td>
<td>157±4</td>
<td>216±25</td>
<td>17.09±2.2</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>154±24</td>
<td>161±12</td>
<td>178±24</td>
<td>16.09±1.4</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>157±4</td>
<td>161±12</td>
<td>178±24</td>
<td>16.09±1.4</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>157±4</td>
<td>161±12</td>
<td>178±24</td>
<td>16.09±1.4</td>
<td>0.53±0.03</td>
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</tbody>
</table>

* p<0.05, ** p<0.01, *** p<0.001 compared to control. N=7–8 per group. Data are mean ± SEM

Baseline triglyceride levels were also dose-dependently decreased by SLX-4090. There was a dose-dependent reduction in body weight gain compared to controls with the mid and high dose groups receiving SLX-4090 while there was no difference in food consumption among the 4 groups. Correlating with this reduction in body weight gain was a dose dependent reduction in the fat/body weight ratio with SLX-4090. SLX-4090 had no effect on the levels of the liver enzymes ALT and AST. Levels of SLX-4090 in the plasma were below the level of quantification for the 10 and 30 mg/kg groups and <10 mg/ml for the 100 mg/kg group. The ability of the non-absorbable, enterocyte specific MTP inhibitor, SLX-4090, to effectively reduce body weight gain, fat/body weight ratios, total and LDL cholesterol and triglycerides while raising HDL levels indicates the potential clinical utility of this compound in dyslipidemia, atherosclerosis, metabolic disease and obesity.
Dectin-1, a Non-TLR Pattern Recognition Receptor, Regulates NADPH Oxidase Activity in Primary Human Monocytes

Deena H Esiro, Martha K Kathcart; Lerner Rock Institute, Cleveland Clinic, Cleveland State Univ, Cleveland, OH

Our lab is interested in studying signal transduction pathways that regulate the activity of NADPH oxidase in primary human monocytes. Zymosan, a yeast cell wall preparation, and its opsonized form (ZOP) are potent stimulators of NADPH oxidase activity. The activation of this enzyme complex results in the production of a superoxide anion burst in monocytes. Monocyte-derived superoxide anion mediates LDL lipid oxidation which is believed to contribute to chronic inflammation and specifically atherogenesis. Our lab had previously identified and characterized several pathways that regulate the activity of NADPH oxidase and superoxide anion production in activated monocytes, however, the receptor(s) responsible for zymosan and (ZOP) recognition have not yet been determined in primary human monocytes. We hypothesized that zymosan signals through a pattern recognition receptor for the activation of the NADPH oxidase enzyme complex. Therefore, to test this hypothesis we examined the role of different pattern recognition receptors such as Toll-like receptors (TLRs) and the β-glucan receptor, Dectin-1. Our studies show that zymosan binds to Dectin-1 to trigger a superoxide anion burst in primary human monocytes and this reaction is independent of TLR2 and TLR4. In addition, we found that (ZOP) activates NADPH oxidase through binding to complement receptor 3 (CR3) as well as Dectin-1. We are further trying to link zymosan recognition to intracellular signaling events. Currently, we are investigating receptor phosphorylation and recruitment of intracellular proteins to the activated receptor upon zymosan stimulation. Taken together, our results suggest for the first time that Dectin-1 is the predominant receptor for zymosan-mediated activation of NADPH oxidase in primary human monocytes.

In Vivo Effect of Inflammatory Cytokines on Cystatin C Among Subjects at Risk for Advanced Atherosclerosis

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Background In vitro evidence suggests that inflammatory cytokines augment the secretion of Cystatin C (CysC), and other cell matrix proteins important in atheroma fibrous cap degradation, and coronary plaque rupture. Recently, CysC has been proposed as a method to estimate glomerular filtration rate (eGFR), and as a cardiovascular risk biomarker. The multivariate linear regression model showed a positive regression coefficient (R² = 0.29, p = 0.003) for age. The delta between methods was positive Spearman correlation coefficient was noted between CysC and IL-6 (0.658, P < 0.001). No correlation was noted with hsCRP, LDL, HDL, TG

TABLE 1. LR MODEL. OUTCOME VARIABLE: ΔEGFR.

<table>
<thead>
<tr>
<th>Standardized Coefficients (B)</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>4.2</td>
<td>4.8</td>
</tr>
<tr>
<td>hsCRP</td>
<td>-2.5</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL</td>
<td>-1.8</td>
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<tr>
<td>HDL</td>
<td>-0.9</td>
<td>0.003</td>
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<tr>
<td>TG</td>
<td>-0.7</td>
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<tr>
<td>Age</td>
<td>0.7</td>
<td>0.11</td>
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</tbody>
</table>

Increased Ability of Sera from LCAT-deficient Subjects to Promote ABCA1-mediated Cholesterol Eflux

Elida Favari, Franco Bernini, Maria Pa Adorni, Univ of Parma, Parma, Italy; Wendy Jessup, Ingrid C Gelissen, Univ of New South Wales, Sydney, Australia; Elsa Mollet, Guido Franceschini, Laura Calabresi, Univ of Milan, Milan, Italy

Forty-one carriers of mutant LCAT alleles and 10 non-carriers from the same families volunteered for this study. In homozygotes (n = 14) plasma HDL-C concentration was markedly reduced (11.7±1.7mg/dl) as well as plasma apoA-I (46.2±4.4mg/dl). The analysis of HDL size showed a predominance of small HDL3 particles, with a great proportion of pre-beta HDL. Heterozygotes (n = 27) have slightly reduced HDL-C and apoA-I levels (41.2±2.2mg/dl and 107.0±4.2mg/dl), with a significant increase (39%) in pre-beta HDL. The capacity of serum from LCAT deficient subjects and controls to extract cell cholesterol through the various pathways was tested in different cell models: 1) FuSa hepatoma cells, expressing high levels of SR-BI and low levels of ABCA1; 2) parent and huBC01-expressing CHO-K1 cells were used to evaluate the ABCG1-mediated cholesterol efflux and 3) J774 macrophages expressing high levels of ABCA1, upon treatment with cAMP, and low levels of SR-BI. In FuSa cells, cholesterol efflux to sera from the homozygotes was significantly reduced by 42% compared with heterozygotes and by 46% compared to control sera with a significant correlation between SR-BI-mediated efflux and HDL-C serum concentration (R = 0.699 P = 0.001). The ABCG1-mediated cholesterol efflux to LCAT deficient sera was significantly reduced (6.5±0.5% for homozygotes carriers and 8.0±0.2% for heterozygotes) compared to efflux induced by control sera (9.8±0.5%). Under basal conditions, the J774 macrophages release membrane cholesterol to extracellular acceptors mostly through passive diffusion and in such condition, cholesterol efflux to sera from homozygotes carriers was significantly reduced (6.1±0.4%) compared to efflux induced by heterozygotes and control sera, which were similar (9.4±0.4% and 10.5±0.7%, respectively). On the contrary, ABCA1-mediated efflux to the sera was increased by 70% in heterozygotes and 106% in homozygous subjects compared to control sera, and correlated with the percentage of pre-beta HDL (r = 0.480 P = 0.001), suggesting that despite the dramatic hypoalphalipoproteiniemia, LCAT deficient homogygotes have highly efficient HDL particles in specifically promoting the ABCA1 efflux pathway.

Alternative Transcript Variants of the Mouse Hepatic Lipase Gene in Macrophages

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We have recently identified macrophages as a site of synthesis of hepatic lipase (HL) and demonstrated that macrophage HL is proatherogenic in apoE-KO mice. Here we demonstrate that HL transcripts in macrophages vs. liver in apoE-KO mice are not identical. Analysis of RNA starting from exon 4 indicated that whereas liver and macrophage HL transcripts both contain sequences coding for exons 1–4, the 5’ ends differ in liver vs. macrophages, indicating that full-length HL expression is driven by different promoters in liver vs. macrophages. Moreover, the macrophage HL transcript contains an additional short exon in canonical Intron 2 which is absent from the liver transcript. RT-PCR analysis with exon-specific primers showed that in macrophages, exons 5–9 were more abundant than exons 1–4, indicative of a second macrophage-specific HL transcript beginning upstream of exon 5. 5’ RACE analysis of macroRNA starting from exon 9 demonstrated a 1.3 kb transcript beginning in Intron 4 and proceeding through exon 9 which potentially encodes an N-terminal-truncated HL protein lacking the catalytic site but containing hepatin binding sites. Northern analysis confirmed that the major HL transcript is shorter in macrophages than in liver of apoE-KO mice. Thus, in apoE-KO mice, macrophages synthesize at least two HL transcripts, neither of which is identical to the HL transcript synthesized by liver. The less abundant HL macrophage transcript is predicted to encode full-length HL but has a different 5’ end and thus a different promoter, as well as an extra exon, compared to the liver HL transcript. This transcript variant would be inactivated in E-KOxHL-KO mice. The more abundant macrophage transcript begins in Intron 4, does not encode full-length HL, and is present in E-KOxHL-KO mice. These findings suggest that the proatherogenic role of HL in macrophages may require further elucidation.

The Functional Significance of a Hydrophobic Patch Identified by Recent X-ray and Homology Models of Lipid-Free Apolipoprotein A-I

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Apolipoprotein A-I (apoA-I) is an important component of high density lipoprotein and the reverse cholesterol transport system. A recently published X-ray crystal structure of lipid free apoA-I, as well as a cross-linking/homology structure from our laboratory, have identified an unusually solvent-exposed hydrophobic patch located at one end of a helical bundle formed by the N-terminus. The patch consists of leucines 42, 44, 46, and 47. To determine any functional consequences of this site, we generated two apoA-I mutants that replace the hydrophobic residues with acidic amino acids of roughly similar volume: apoA-I (L42,44D) and apoA-I (L46,47D). In a liposome clearance assay, both mutants exhibited an enhanced ability to bind and emulsify dimystryl phosphatidycholine compared to wild type (WT) apoA-I. An independent vesicle binding assay indicated that apoA-I (L46,47D) was particularly adept at binding lipid surfaces compared to WT apoA-I. This apoA-I (L42,44D) was capable of stimulating cholesterol efflux from RAW264.7 macrophages in an ABCA1-dependent manner at a level similar to WT apoA-I. However, apoA-I (L46,47D), despite its apparent increased lipid affinity, was actually a less efficient stimulator of cholesterol efflux via ABCA1. These data suggest that this hydrophobic patch may be involved in apoA-I/lipid interactions and is an example of how detailed structural models of apoA-I may lead to a better understanding of apoA-I function.
Matrix Gla Protein Is Cleared by the Kidney in Hypertensive Humans

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Background: Medial vascular calcifications are common among patients with hypertension. The vitamin K-dependent protein matrix Gla-protein (MGP) plays an important role in preventing arterial calcification. In this study we investigated the renal clearance of MGP from the circulation in patients with moderate to severe hypertension. Methods and results: From 90 moderate to severe hypertensive patients scheduled for renal angiography, renal arterial and renal venous blood was sampled prior to admission. Results of contrast material for measuring the MGP-clearance by the kidney. Average renal fractional extraction was 12.8%. There was no significant relationship between creatinine clearance (range 26–154) and renal fractional extraction of MGP in this population. Conclusions: In this study, we demonstrate that the kidney is able to extract MGP from the plasma. Clearance of plasma MGP was 12.6% and did not correlate with creatinine clearance.

Matrix Gla Protein Is Cleared by the Kidney in Hypertensive Humans

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Ginkgo Biloba (EGb 761) Reduces Arteriosclerotic Nanoplaque Formation and Size in Cardiovascular High-Risk Patients

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Matrix Gla Protein Is Cleared by the Kidney in Hypertensive Humans

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Telomerase Reverse Transcriptase Is Induced in Macrophages by Proinflammatory Stimuli and Mediates Macrophage Survival and Inflammation

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An emerging consensus underscores the importance of both macrophage inflammation and apoptosis in the vascular wall for the development of atherosclerosis. Although the intracellular pathways controlling both processes still remain to be elucidated, a novel area in vascular biology involves telomerase activation. Telomerase controls key cellular functions including replicative lifespan, proliferation, differentiation and apoptosis, and activation of telomerase has been demonstrated in response to injury of the arterial wall. In the present study, we analyzed the role of telomerase in macrophage biology. We demonstrate expression of the catalytic subunit of telomerase, telomerase reverse transcriptase (TERT), in macrophages of human atherosclerotic lesions. Stimulation of primary murine peritoneal macrophages with lipopolysaccharide (LPS) and oxidized LDL (oxDL) resulted in a significant dose-dependent induction of TERT mRNA and protein expression. The up-regulation of TERT in response to LPS or oxDL was prevented by c-treatment with the SNS50 peptide and BAY 11–7082, two inhibitors of the NF-κB pathway. Consistent with these findings, transient transfection experiments using a- and deletion and site-directed mutagenesis of the TERT promoter identified a proximal NF-κB site which confers the transcriptional induction of TERT expression in response to oxDL. Finally, functional experiments demonstrated that TERT-deficient macrophages reveal typical features of cell senescence and were more prone to oxDL-induced apoptosis. Furthermore, oxDL-induced expression of the pro-inflammatory gene IL-1 beta and the matrix-metalloproteinase-9 (MMP-9) was prevented in peritoneal macrophages isolated from TERT-deficient mice. In conclusion, our results demonstrate that oxDL induces TERT expression through an NF-κB-dependent pathway and suggest that telomerase mediates macrophage viability and pro-inflammatory gene expression.

Telomerase Reverse Transcriptase Is Induced in Macrophages by Proinflammatory Stimuli and Mediates Macrophage Survival and Inflammation

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Divergent Impact of LDL and Oxidized LDL on CREB Activation and on ERK-mediated CREB Downregulation

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Introduction: LDL is an established mediator of atherosclerosis and is directly toxic to cells of the vessel wall including vascular smooth muscle cells (VSMC). Our laboratory has identified the transcription factor CREB, cAMP Response Element Binding protein, as a modulator of VSMC phenotype. CREB blunts mitogen stimulated VSMC proliferation and protects VSMC from apoptosis. In numerous models of vascular damage (diabetes, aging, insulin resistance, and pulmonary hypertension) we observe decreased expression of CREB protein in medial VSMC. In addition to these published models we observe loss of CREB protein expression in the medial VSMC of LDL receptor KO mice fed a high fat western diet. We hypothesized that LDL has a direct effect on VSMC to decrease CREB content and function. Methods: To test this hypothesis we exposed primary rat aortic VSMC to LDL and oxidized LDL (intrac, Frederick, MD) at 10–100 μg/ml, in the presence or absence of kinase inhibitors, for up to 48 hours. Results: Both LDL and oxidized LDL led to a rapid increase in CREB phosphorylation (4-fold) over 15–30 minutes. In contrast to the acute, transient CREB activation by LDL, oxidized LDL resulted in persistent CREB activation and ultimately in a dramatic, dose-dependent loss of CREB protein at 24–48 hours (>90% at 24 hours in 100 μg/ml oxidized LDL). Though some toxicity was observed at the higher concentrations, this loss was observed after normalization to total actin or GAPDH content. Exposure to LDL and oxidized LDL also led to phosphorylation/activation of P38 MAPK, ERK, and Akt. Pharmacological inhibitors of these pathways were used to identify the pathway(s) responsible for toxicity and CREB downregulation. Both phenotypes are blocked by inhibition of ERK, partially by scavenging of reactive oxygen species and inhibition of PKC, but unaffected by inhibition of JNK, P38, P38MAPK, or PKA. Conclusions: LDL exposure leads to acute CREB activation in VSMC but only with oxidized LDL is CREB protein loss observed. This protein loss correlates with toxicity and is mediated by reactive oxygen species and the ERK signaling pathway. One mechanism of oxidized LDL injury to the vasculature may be CREB downregulation. Studies are underway to define the mechanism of CREB loss in this model.

Divergent Impact of LDL and Oxidized LDL on CREB Activation and on ERK-mediated CREB Downregulation

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ERK-mediated CREB Downregulation

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Macrophage β3 Integrin Mediates High-Fat-Diet-Induced Inflammation Through TNFα

Jochen G Schneider, Yinmu Zhu, Clay F Semenkovich; Washington Univ, St. Louis, MO

Integrins are critical effectors of inflammatory cell function, chronic treatment with beta3 integrin inhibitors increases mortality in humans, and beta3 integrin deficiency causes atherosclerosis and death in certain high-fat-fed mice. To test the hypothesis that the beta3 integrin receptor engages bone marrow-derived β-deficient or beta3 wild type mice (each appropriately apoE- or LDLR-deficient) was transplanted into apoE- or LDLR-deficient mice followed by Western diet feeding. At 6 weeks, only 3 of 18 apoE-deficient (beta3 wild type) mice transplanted with beta3-deficient marrow were alive compared to 19 of 19 mice transplanted with wild type marrow (p < 0.0001). In LDLR-deficient (beta3 wild type) mice, 11 of 23 mice transplanted with beta3-deficient marrow survived 12 weeks of Western diet compared to 19 of 21 mice receiving wild type marrow (p < 0.001). LDLR-deficient (beta3 wild type) mice transplanted with beta3-deficient marrow surviving for 12 weeks on Western diet had less intima/media thickness than mice transplanted with wild type marrow (p = 0.03) and a lower level of non-marrow cells in the inflammatory phenotype, 19 of 19 beta3-deficient mice in the apoE null model transplanted with beta3 wild type (apoE null) marrow survived the diet challenge, compared to 2 of 14 beta3-deficient mice receiving beta3-deficient marrow (p < 0.001) and a lowering the urinary model LDL (p = 0.175) after the 2 month medication regimen. Furthermore, we measured a significant decrease in lipoprotein(a) concentration falling from 52.4 ± 8.2 to 42.3 ± 9.9 mg/dl (p < 0.0035). Altogether, these beneficial effects of Ginkgo biloba might have partially repaired endothelial dysfunction being responsible in the earlier stages of atherosclerosis and could present a basis for a mechanistic explanation of nanoplaque reduction under ginkgo treatment.

Ginkgo Biloba (EGb 761) Reduces Arteriosclerotic Nanoplaque Formation and Size in Cardiovascular High-Risk Patients

P163

Matrix Gla Protein Is Cleared by the Kidney in Hypertensive Humans

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Macrophage β3 Integrin Mediates High-Fat-Diet-Induced Inflammation Through TNFα

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was the cause of death and there was marked induction of lung TNFα expression. TNFα expression, TNFα expression changes in culture. Western diet feeding alone increased beta3 expression 3-5 fold in wild type macrophages as compared to cells from chow-fed litters (p < 0.05). The beta3-deficient null mice were protected from death by treatment with an anti-TNFα antibody but not with an isotype-specific control antibody (p < 0.01). These data suggest that macrophage beta3 integrin modulates dietary inflammation through TNFα.

Cosegregation of Soat1 Mutations with Dyslipidemia, Body Weight, and Atherosclerosis in an Intercross Between C57BL/6 and C3H Apolipoprotein E-Deficient Mice

Weibin Shi, Toru Miyoshi, Zuobiao Yuan, Timothy R Gilbert; Univ of Virginia, Charlottesville, VA

Background: Dyslipidemia is an integral component of the metabolic perturbations in several common human disorders, including type 2 diabetes, obesity, and the metabolic syndrome. Genetic factors are a major determinant for the pathogenesis of dyslipidemia. Sterol O-acetyltransferase 1 (Soat1), also known as ACAT1, is an endoplasmic reticulum enzyme that catalyzes free cholesterol to cholesteryl ester. Methods and Results: We identified four single-nucleotide polymorphisms (A421C, A439G, C454T, and C613T) within the coding region of Soat1 between C57BL/6 (B6) and C3H mice and two of the SNPs led to amino-acid substitutions (Ile147Val and His2057yr). In an intercross between B6 and C3H apolipoprotein E-deficient (apoE−/−) mice, allele variation at Soat1 (for a closely-linked gene) explained 3% to 13% of the variations in plasma triglyceride, HDL, non-HDL cholesterol levels, body weight, or atherosclerotic lesions. Inheritance of the C3H alleles resulted in significant increases in plasma lipid levels and body weight but decreases in atherosclerotic lesion size compared to inheritance of the B6 alleles. These differences were correlated with variations in plasma HDL, phospholipids and free cholesterol levels but not with free cholesterol levels. Real-time PCR revealed that Soat1 was abundantly expressed in arterial walls. Conclusions: These results support Soat1 to be an important gene contributing to dyslipidemia, body weight, and atherosclerosis in mice.

Objective- To identify genes that modify atherosclerosis severity in a mouse model. Methods and Results- In a strain intercross between atherosclerosis resistant AKR apoE-deficient mice and atherosclerosis sensitive DBA2 apoE-deficient mice, we identified a quantitative trait locus (QTL) on chromosomes 17 that is associated with lesion severity in the male F2 cohort. This QTL, called Ath26, at 34 Mb on chromosome 17, has a LOD score of 4.25, with a genome wide significance of p < 0.05. The AKR locus at this QTL has a dominant effect on lesion area, with an average 2-fold increase in the macrophage expression in the arterial wall. MAEC but not in ECs that were sub-confluent or at wounded edges. MAEC treated with BMP4, suggesting a role for BMPRII as the BMP receptor. Unexpectedly, BMPRII knockdown in ECs unaltered the inflammatory response in the absence of BMP4 as determined by increased ICAM-1 expression and monocyte adhesion, which was blocked by an ICAM-1 neutralizing antibody (Y11). Moreover, BMPRII levels in ECs were progressively decreased in more advanced atherosclerotic lesions in human coronary arteries. These findings suggest that a decrease in BMPRII in ECs from diseased regions of advanced atherosclerotic lesions and is associated with more advanced atherosclerotic disease states.

Microarray Gene Expression Profiling Identifies Candidate Atherosclerosis Modifier Gene for the Ath26 Locus in Male ApoE-deficient Mice

Jonathan D Smith, Enakshi Chakrabarti, Jeffrey M Bhatia; Cleveland Clinic Lerner College of Medicine, Cleveland, OH

Loss of BMPRII in Endothelium Induces Inflammation in Vitro and Correlates with Atherosclerosis Progression in Human Coronary Arteries

Hannah Song, Georgia Institute of Technology, Atlanta, GA; Daina Weiss, J D Vega, W R Taylor, Emory Univ, Atlanta, GA; Hanjoong Jo; Georgia Institute of Technology and Emory Univ, Atlanta, GA

Atherosclerosis is an inflammatory disease, occurring preferentially in arterial regions associated with disturbed flow while sparing the undisturbed flow regions. We have shown that exposure of endothelial cells (ECs) to disturbed flow stimulates production of bone morphogenic protein-4 (BMP-4), which leads to inflammatory responses - intercellular adhesion molecule-1 (ICAM-1) expression and subsequent monocyte adhesion. However, the underlying mechanism by which BMP4 induces inflammation is unclear. Here, we examined which BMP receptors (BMPR) mediate BMP4 action in ECs. Studies using mouse aortic ECs (MAEC), human umbilical vein ECs (HUVEC), mouse thoracic aorta and human coronary arteries revealed that BMPRII (ALK2 and 6) and BMPRII were expressed in ECs. Interestingly, immunostaining studies showed that BMPRII was located in the cell-cell junction, colocalizing with VE-cadherin, in confluent MAEC but not in ECs that were sub-confluent or at wounded edges. MAEC treated with mouse-specific siRNA lost BMPRII expression in the cell-cell junction. The BMPRII knockdown prevented phosphorylation of smad1/5/8 and stimulation of monocyte adhesion in response to BMP4, suggesting a role for BMPRII as the BMP receptor. Unexpectedly, BMPRII knockdown in ECs unaltered the inflammatory response in the absence of BMP4 as determined by increased ICAM-1 expression and monocyte adhesion, which was blocked by an ICAM-1 neutralizing antibody (Y11). Moreover, BMPRII levels in ECs were progressively decreased in more advanced atherosclerotic lesions in human coronary arteries. These findings suggest that a decrease in BMPRII in ECs from diseased regions of advanced atherosclerotic lesions and is associated with more advanced atherosclerotic disease states.
polymorphisms that changed amino acid. These changes are Leu169Phe in gene Fabp4 encoding fatty acid-binding protein band 4.9, Asn244Ser in Xpo7 encoding exportin 7, Thr310Met in Dok2 encoding docking protein 2, and Thr519Ala in gene Fcnd3 encoding fibronectin type III domain containing 3a. None of these changes alter the amino acid, but two do change the amino acid from polar to non-polar (Thr/Ala in Fcnd3 and Thr/Met in Dok2). Further evidence is needed to determine which of these is the gene affecting HDL.

The Hemodynamic Environment Promotes Atherosclerosis-prone Phenotypes in Endothelial and Smooth Muscle Cells via a Mechanical-Transmission Coupling Mechanism

Nicole Hastings, Michael Simmers, Brian Wamhoff, Brett Blackman; Univ of Virginia, Charlottesville, VA

Introduction Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where atheroprotective flow leads to increased susceptibility to atherogenesis. The common carotid artery (CCA) is exposed to pulsatile laminar flow resulting of regional differences in arterial hemodynamics, where atheroprone flow leads to atherosclerotic plaque formation, whereas the internal carotid artery (ICA) is exposed to pulsatile turbulent flow. Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where atheroprotective flow leads to increased susceptibility to atherogenesis. The common carotid artery (CCA) is exposed to pulsatile laminar flow resulting of regional differences in arterial hemodynamics, where atheroprone flow leads to atherosclerotic plaque formation, whereas the internal carotid artery (ICA) is exposed to pulsatile turbulent flow. Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where atheroprotective flow leads to increased susceptibility to atherogenesis. The common carotid artery (CCA) is exposed to pulsatile laminar flow resulting of regional differences in arterial hemodynamics, where atheroprone flow leads to atherosclerotic plaque formation, whereas the internal carotid artery (ICA) is exposed to pulsatile turbulent flow. Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where atheroprotective flow leads to increased susceptibility to atherogenesis. The common carotid artery (CCA) is exposed to pulsatile laminar flow resulting of regional differences in arterial hemodynamics, where atheroprone flow leads to atherosclerotic plaque formation, whereas the internal carotid artery (ICA) is exposed to pulsatile turbulent flow. Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where atheroprotective flow leads to increased susceptibility to atherogenesis. The common carotid artery (CCA) is exposed to pulsatile laminar flow resulting of regional differences in arterial hemodynamics, where atheroprone flow leads to atherosclerotic plaque formation, whereas the internal carotid artery (ICA) is exposed to pulsatile turbulent flow. Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where atheroprotective flow leads to increased susceptibility to atherogenesis. The common carotid artery (CCA) is exposed to pulsatile laminar flow resulting of regional differences in arterial hemodynamics, where atheroprone flow leads to atherosclerotic plaque formation, whereas the internal carotid artery (ICA) is exposed to pulsatile turbulent flow. Atherosclerosis is an inflammatory disease of the vasculature, developing as a result of regional differences in arterial hemodynamics, where atheroprotective flow leads to increased susceptibility to atherogenesis. The common carotid artery (CCA) is exposed to pulsatile laminar flow resulting of regional differences in arterial hemodynamics, where atheroprone flow leads to atherosclerotic plaque formation, whereas the internal carotid artery (ICA) is exposed to pulsatile turbulent flow.

Advanced Atherosclerotic Lesions?

Jie H Hu, Nagadehra Dronadula, Goro Otsuka, David A Dichek; Univ of Washington, Seattle, WA

Background: The cellular and molecular mechanisms of atherosclerotic plaque rupture are poorly understood. Increased proteolytic activity of lesion macrophages is often proposed as a cause of plaque rupture. Urokinase-type plasminogen activator (uPA), a protease that activates plasminogen to plasmin, is expressed in human atherosclerotic lesions, primarily by macrophages. uPA/plasminogen can activate matrix metalloproteinases, which have been implicated as cause of plaque rupture. Hypothesis: We hypothesized that overexpression of uPA by macrophages in advanced lesions of apolipoprotein E null (ApoE−/−) mice will cause plaque rupture. Methods: Bone marrow from transgenic mice with macrophage-targeted uPA overexpression (super uPA/ApoE−/− mice) or nontransgenic donors (super uPA+/+ mice) was transplanted into irradiated 35 wk-old super uPA+/+ mice recipients. Some of the donor mice were also transgenic for GFP. Results: Atherosclerotic plaque rupture was detected at 7 wk by both macroscopy and histology. Infiltrating lesions were examined at 12 wks by GFP immunostaining to determine whether donor-derived cells were present, and by qRT-PCR at 8–10 wks to measure uPA expression. Lesion histology will be examined at 10 wks to detect features indicative of plaque rupture.

Does Elevated Macrophage uPA Expression Cause Plaque Rupture in Advanced Atherosclerotic Lesions?

Jie H Hu, Nagadehra Dronadula, Goro Otsuka, David A Dichek; Univ of Washington, Seattle, WA

Background: The cellular and molecular mechanisms of atherosclerotic plaque rupture are poorly understood. Increased proteolytic activity of lesion macrophages is often proposed as a cause of plaque rupture. Urokinase-type plasminogen activator (uPA), a protease that activates plasminogen to plasmin, is expressed in human atherosclerotic lesions, primarily by macrophages. uPA/plasminogen can activate matrix metalloproteinases, which have been implicated as cause of plaque rupture. Hypothesis: We hypothesized that overexpression of uPA by macrophages in advanced lesions of apolipoprotein E null (ApoE−/−) mice will cause plaque rupture. Methods: Bone marrow from transgenic mice with macrophage-targeted uPA overexpression (super uPA/ApoE−/− mice) or nontransgenic donors (super uPA+/+ mice) was transplanted into irradiated 35 wk-old super uPA+/+ mice recipients. Some of the donor mice were also transgenic for GFP. Results: Atherosclerotic plaque rupture was detected at 7 wk by both macroscopy and histology. Infiltrating lesions were examined at 12 wks by GFP immunostaining to determine whether donor-derived cells were present, and by qRT-PCR at 8–10 wks to measure uPA expression. Lesion histology will be examined at 10 wks to detect features indicative of plaque rupture.

Results: FACS analysis demonstrated adequate BM reconstitution of irradiated mice. 74%–89% of peripheral blood leukocytes were GFP+ and Mac-3 immunostaining of serial
sections that showed numerous donor-derived macropores were present in innominate artery lesions. TaqMan qRT-PCR showed significantly higher expression of apoA mRNA in innominate recipients of SR- apoA-/- BM than in innominate recipients of SR-PAK-/- BM (relative UPA expression: 2.1 ± 0.31 vs 0.10 ± 0.038 arbitrary units; P < 0.001; n = 8). Conclusion: We introduced UPA-overexpressing macropores into advanced innominate artery lesions of Apoe-/- mice. These macropores express high levels of UPA after 2 weeks, resulting in a large (20-fold) increase in lesion UPA expression. This experimental setting will allow us to test whether elevated macropore-targeted UPA expression in innominate lesions precipitates plaque rupture.

P178 Antithrombotic and Anti-inflammatory Effects of Polyphenol-enriched Fraction from Taraxacum coreanum Nakai in LDLReceptor–Deficient Mice

Jong-Min Han, Yong-Dae Park, Sojin An, Min-Jung Kim, Yue-Jin Jin, Woo S Lee, Teao-Sook Jeong; KRIBB, Daejeon, Republic of Korea

There have been numerous reports demonstrating antithrombotic activity of polyphenols. Natural polyphenols have a wide range of biological activities. The polyphenol-enriched fraction (PEF) isolated from dried whole plants of Taraxacum coreanum Nakai decreased nuclear factor-κB (NF-κB) activation. The PEF contains many biologically active polyphenols, luteolin (1.28%), luteolin-7-O-glucoside (0.65%), caffeic acid (0.15%), and apigenin (0.09%), being the major constituents. In this study, we investigated early stages antithrombotic effects of the PEF in a high-cholesterol diet-fed LDL receptor deficient (LDLR-/-) mice. At 10 weeks of age, 20 male LDLR-/- mice were randomly divided into two groups (n = 10) and fed a high-cholesterol diet (0.15% w/v diet, control group) or a high-cholesterol diet supplemented with PEF (0.2% w/v diet wt) for 8 weeks. There were no differences in total cholesterol and body weight between the control group and PEF-supplemented group during the study period. However, the triglyceride level was decreased in the PEF-supplemented group. This decrease of triglyceride also significantly reduced the difference in lesion areas. The mean lesion areas of 10 consecutive sections stained with oil red O were 56.2 ± 29.7 μm²/10² in the PEF-supplemented group versus 93.8 ± 38.1 μm²/10² in control group (P < 0.05). Also the protein levels which in turn regulated by NF-κB, such as VCAM-1, ICAM-1, TNF-α and COX-2, were suppressed in aorta. In addition, the PEF significantly suppressed the production of nitric oxide (NO) and the accumulation of intracellular reactive oxygen species (ROS) in a dose-dependent manner by inhibiting NF-κB activation in LPS-induced RAW 264.7 cells. In conclusion, our results suggest that the PEF containing luteolin, luteolin-7-O-glucoside, caffeic acid, apigenin, and diosmetin may be a promising product for treatment of atherosclerotic lesions by inhibiting NF-κB and provide an additional rationale for application of anti-inflammatory therapeutic approaches for atherosclerotic lesion prevention.

P179 Downregulation of Tissue Inhibitor of Metalloproteinases Increases Invasion, Proliferation, and Death of Macrophage-derived Foam Cells

Jason L Johnson, Andrew C Newby; Univ of Bristol, Bristol, United Kingdom

Foam cell macrophages secrete higher levels of matrix metalloproteinases (MMPs) than non-foamy macrophages which contributes to plaque development and rupture. We investigated the expression of the endogenous tissue inhibitor of metalloproteinase-3 (TIMP-3) during foam cell formation, and its effects on macrophage and foam cell behaviour. Foam cells derived from cholesterol-fed rabbits and by Ox-LDL loading of human macrophages demonstrated a significant decrease in TIMP-3 mRNA (55%; p < 0.01 and 49%; p < 0.05, respectively) and protein expression (84%; p < 0.001) compared to control macrophages. Adding back TIMP-3 to foam cells significantly inhibited migration (51%; p < 0.01), proliferation (70%; p < 0.05) and apoptosis (36%; p < 0.05), but had no effect on non-foamy macrophages. Immunocytochemistry for TIMP-3 on foam cells revealed a subset of these cells (28%) that were TIMP-3 negative. Furthermore, only cells negative for TIMP-3 invaded a synthetic basement membrane using an in vitro invasion assay. In early rabbit atherosclerotic plaques, TIMP-3 expression was associated with macrophages. However in advanced plaques, foam cells with little or no TIMP-3 protein expression were found in the deeper layers of the plaque. These results demonstrate that TIMP-3 is down-regulated when macrophages become lipid-loaded, and this loss of TIMP-3 from foam cells dramatically alters their potential to destabilise atherosclerotic plaques.

P180 Generation and Characterization of a C-Reactive Protein Knockout Mouse

Steven W Kerr, Huiping Jiang, Rosamari Sellati, Hong Wang, Adedayo Hanidi, Jeffrey B Madwed, Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT; Alexander J Szalai, Mark A McCoyry; The Univ of Alabama at Birmingham, Birmingham, AL

C-reactive protein (CRP) is well recognized as a marker of inflammation, but its role as an active participant in an inflammatory response and whether it exacerbates cardiovascular and other diseases remains controversial. Much of the difficulty associating a causal role to CRP in human health or disease stems from the lack of a relevant rodent model because CRP has different basal and stimulated blood levels in rodents compared to humans. Also, no case of human CRP deficiency has been identified. In order to determine the role of CRP in inflammatory response and to those experienced by humans, and to determine its impact on normal physiology, we engineered a mouse strain that does not express the endogenous CRP gene. In these CRP-/- mice, the CRP gene was ‘floxed’ and then deleted by Cre-recombinase. CRP-/- were viable and fertile and showed no overt phenotype or behavioral abnormalities on multiple genetic and protein effects. In vivo, CRP-/- mice acutely responded to LPS and lipopolysaccharide (LPS) and stimulated CRP-/- by both inflammatory and physiological tests to identify a potential functional role of CRP deficiency. We observed the following effects in the CRP-/- mice compared to wild type mice: (1) inhibition of cytokine production after LPS challenge (TNF-α and IL-1) and TNF-α levels were 51% and 35% lower, respectively, p < 0.05), (2) inhibition of cytokine production after δCD3 stimulation (NF-κB and IL-2 were present at levels 35% and 51% lower, respectively, p < 0.05), (3) we observed a 50% increase in T-cell independent IgM antibody production after immunization with TNP-solid (p < 0.05). These data demonstrate that genetic deficiency of CRP is not a lethal mutation in rodents and that CRP plays a significant role in the innate immune response to inflammation as well as the humoral response to immunization. Importantly, these findings suggest that CRP plays an active role in orchestrating the inflammatory and immune responses, and the changes seen due to deletion of the CRP gene indicate that inhibition of CRP may have beneficial effects in inflammatory diseases such as atherosclerosis.

P181 Is Coronary Calcification Protective of ST-elevation Myocardial Infarction?

Shazib Khawaja, Nadeem Hussain, Malik Ali, Hinan Ahmed, Asmir Syed, Frank Petryjohn; Univ of South Alabama, Mobile, AL

Background The association between coronary calcification and acute coronary syndromes (ACS) especially ST-elevation myocardial infarction (STEMI) has not been extensively studied. It has been suggested that plaques containing calcium may be less vulnerable to rupture. We studied the extent of coronary calcification as assessed by coronary angiography in the infarct related artery among patients with STEMI in comparison with patients with chronic stable coronary artery disease (CASCAD) and Western blot. CACP-/- mice were put through a battery of inflammatory and immunological tests to identify a potential functional role of CRP deficiency. We observed the following effects in the CRP-/- mice compared to wild type mice: (1) inhibition of cytokine production after LPS challenge (TNF-α and IL-1) and TNF-α levels were 51% and 35% lower, respectively, p < 0.05), (2) inhibition of cytokine production after δCD3 stimulation (NF-κB and IL-2 were present at levels 35% and 51% lower, respectively, p < 0.05), (3) we observed a 50% increase in T-cell independent IgM antibody production after immunization with TNP-solid (p < 0.05). These data demonstrate that genetic deficiency of CRP is not a lethal mutation in rodents and that CRP plays a significant role in the innate immune response to inflammation as well as the humoral response to immunization. Importantly, these findings suggest that CRP plays an active role in orchestrating the inflammatory and immune responses, and the changes seen due to deletion of the CRP gene indicate that inhibition of CRP may have beneficial effects in inflammatory diseases such as atherosclerosis.

P183 PATIENT CHARACTERISTICS STEMI (n=50) Chronic Angina (n=50)

Age(years) 47(13) 60.4
Sex(M/F) 37(13) 34(16)
Hypertension 37(13) 45(18)
Diabetes 16(6) 16(6)
Hyperlipidemia 39(13) 49(19)
Tobacco Use 38(13) 28(11)

Results

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High-density Lipoprotein Cholesterol Response to Statin Therapy in Korean Hypercholesterolemic Patients

Min-Kyung Kim, Yeon-Yee Yoon, Seoul National Univ Hosp, Seoul, Republic of Korea; Sang-Hyun Kim, Joo-Hee Jo, Myung-A Kim, Seoul Metropolitan Baramaa Hosp, Seoul, Republic of Korea; Dong-Ju Choi, Seoul National Univ Bundang Hosp, Sungnam, Republic of Korea; Hye-soo Kim, Dae-Won Sohn, Byung-Hee Oh, Young-Bae Park, Yun-Sik Choi; Seoul National Univ Hosp, Seoul, Republic of Korea

Background and aims: Statins generally increase high-density lipoprotein cholesterol (HDL-C), but not in all patients. We designed a retrospective study 1) to assess the characteristics and the different response patterns of lipoprotein level after statin therapy, comparing the patients with decreased HDL-C level after statin therapy (poor response group) and the patients with preserved or increased HDL-C level after statin therapy (favorable response group), 2) to investigate the problems of statin-only-strategies in hypercholesterolemia especially in the patients with high risk. Methods: 516 patients, who were newly diagnosed as hypercholesterolemia, were enrolled. Their clinical characteristics, baseline and follow-up laboratory data were analyzed. All patients had received one kind of statin for at least 6 months. The ‘poor response group’ showed more decrease of TC (p<0.001) and LDL-C (p<0.001) and increase of TG (p<0.001) than the favorable response group. Patients with initial low HDL-C level showed lower responses to statins in terms of TC (p<0.011) but better responses in terms of HDL-C than those with initial normal HDL-C level (HDL-C increased by 11.4% vs 2.2% p<0.001). Conclusions: Serum HDL-C and TG level increased with statin therapy in some patients, but decreased in the others. The risk of HDL-C decrease with statin therapy coexists with large reduction of LDL-C level, so HDL-C raising therapy can be considered with statin therapy, especially in the patients of high risk group.

Conclusions: Elevated oxidant stress is associated with endothelial dysfunction, insulin resistance, hyperlipidemia but not markers of inflammation in early stage of atherosclerosis.

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Exposure to Gasoline Engine Emissions Increases Vascular Reactive Oxygen Species and Activates Molecular Pathways Involved in Progression of Atherosclerosis

Amie K Lund, Travis L Knuckles, JoAnn Lucero, JeanClaire Seagrave, Jacob D McDonald, Matthew J Campen; Lavalere Respiratory Research Institute, Albuquerque, NM

Cardiovascular disease is currently the leading cause of death in the US, with atherosclerosis being responsible for approximately half of all CVD-related deaths. Epidemiological studies have found an association between environmental air pollution and increased rates of cardiovascular morbidity and mortality. We have previously reported that subchronic exposure to gasoline engine emissions results in increased aortic reactive oxygen species (ROS), which is in turn drive the subsequent induction of MMPs and related peptides such as endogenous tissue inhibitors of MMPs (TIMPs), and endoplasmic-reticulum (ER)-stress associated with atherosclerotic plaques. We have recently demonstrated the KCa3.1 blocker significantly prevented development of atherosclerosis in EKO by 40% in conjunction with 60-% reduction of macrophage accumulation in the lesions (macrophage-positive area; 81.1 ± 12.0 × 10^4 µm^2, p < 0.05 vs control 219.2 ± 20.9, n = 9). Migratory response to MCP-1 was impaired also in macrophages collected from EKO chronically-treated with TRAM-34 (1.5 ± 0.1-fold of control, p < 0.05 vs vehicle 2.9 ± 0.4-fold, n = 6). Long-term treatment of EKO with TRAM-34 did not induce any pathological or clinical signs of toxicity, while plasma TRAM-34 concentrations were maintained at ranges specific to KCa3.1 (866.4 ± 180.3 µM, n = 6). In conclusion, KCa3.1 activity is associated with macrophage accumulation in atherosclerosis, suggesting a pathophysiological role for KCa3.1 up-regulation in atherogenesis. KCa3.1 blockade is a new therapeutic strategy for diseases involving activated macrophages such as atherosclerosis.

Typical Antigen Presenting Cells in Grossly Normal Human Aorta

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Histocompatibility class II molecules (HLA-DR) positive cells represented mostly by macrophages, dendritic cells and B-lymphocytes can orchestrate and affect inflammatory responses in atherosclerotic lesions. We have recently demonstrated the HLA-DR+ cells are in normal (grossly uninvolved) human aortic intima. In present study, we investigated the phenotypical and morphological characteristics of subendothelial intimocytes expressing HLA-DR. Immunofluorescent preparations of grossly normal aorta were used for investigation. Visualization of antibodies was carried out using confocal laser scan microscopy and immunohistochemical methods. For the quantitative measurements isolated subendothelial intimocytes were analyzed using a flow cytometer. Four main morphotypes of HLA-DR+ cells have been identified: 1) large stellate cells (more than 20 µm) possessing two or more rounded processes; 2) round and irregular-shaped cells over 10 µm in size; 3) small rounded-shape cells less than 10 µm in size; 4) irregular-shaped cells that were surrounded by round polymorphic processes that were situated parallel to endothelium. Over 90% of first population and almost all cells of second and third subpopulations expressed CD45 indicating their monocyte origin. Cells of fourth subpopulation were negative for CD45 but some contain alpha actin. The proportion of these cells increased with other cells of a similar shape that expressed alpha actin but not HLA-DR. Thus, interal immune-presenting cells have different origins. Immune response in subendothelial intima may result from differentiation and activation of resident non-blood intimocytes. Antigen-presenting function of resident intimocytes suggests unknown mechanisms of adaptive immune response.

Reinforcing Stem Cell Grafts with Increased Vascular Progenitors Reduces Tissue Injury Caused by High-dose Chemotherapy in Hematopoietic Transplantation

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Background: Endothelial-like vascular progenitors (VPCs) can be collected in peripheral blood stem cell (PBSC) grafts used in hematopoietic stem cell transplantation (HSCT). HSCT involves chemotherapy and/or radiation that can induce widespread tissue damage. The impact of graft VPC content on transplant-related toxicity was assessed in autologous HSCT patients to address the vascular regenerative capacity of hematopoietic grafts. Methods & Results: 17 patients (mean age 48 y) undergoing autologous hematopoietic transplantation at The Ottawa Hospital were included. PBSCs were analyzed using an ex vivo cytokine stimulation panel following adherence depletion of mononuclear cells on fibronectin-coated plastic dishes in serum-rich conditions. Transplant toxicity was estimated using total length of hospital stay (LOS) following graft infusion and the Seattle criteria for transplant-related organ toxicity in 8 organ systems each graded 0-5. Results: LOS following graft reinfusion was lower (16.4 vs 22.5 d, p = 0.03) and number of organs with grade 2 or 3 toxicity was reduced (0.6 vs 2.6 organs, p = 0.05) in patients with higher graft VPC content (n = 8 vs 8 × 10^6 VPC/kg) compared with reduced VPC content (n = 9; 1.0 × 10^6 VPC/kg). Only 3 patients avoided any significant organ toxicity (grade 0 for systems) and all 3 had increased graft VPC content. Moreover, 5 of 8 patients with high graft VPC levels had minimal toxicity (grades 0 or 1 in all organs) compared with only 1 of 9 patients in the low graft VPC group (p = 0.05). Importantly, severe oral mucositis was reduced in patients with high compared with low graft VPC content (G/B pts with grade 2 or 3 mucositis vs 5/9 pts, p = 0.025). Graft VPC levels were independent of graft CD44 counts, peripheral blood monocytes and Hb levels, age and disease (p = NS). Conclusion: Collection and reinfusion of autologous peripheral blood stem cell grafts with higher VPC content appears to reduce tissue injury in hematopoietic transplantation, identifying factors that contribute to high graft VPC levels is needed. Regenerative cell therapy using blood-derived VPCs warrants further investigation and may facilitate repair of tissue injury in diverse organ systems such as myocardial or cerebral ischemia.
Cyclic Strain Regulates the Notch/CBF-1 Signaling Pathway in Endothelial Cells: Role in Angiogenesis

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The role of hemodynamic forces, such as pressure-induced cyclic stretch, in the control of angiogenesis in a variety of cell types. Notch receptors and target genes are upregulated following vascular injury and are reportedly expressed on vascular endothelial cells (EC). We assessed the hypothesis that Notch signaling mediates the cyclic strain-induced angiogenic response of EC. A Flexcell system was used to expose human umbilical vein endothelial cells (p3-7) to physiological levels of cyclic strain (0–10% strain, 60 cycles/min, 0–24 h). Notch receptor and Notch target gene mRNA and protein levels were determined by QRT-PCR and Western blot analysis, respectively. Network formation on Matrigel was measured as an index of angiogenesis. Exposure of EC to cyclic strain (10%) resulted in temporal regulation of Notch receptors 1 and 2 (Notch1, Notch2), and 4 (Notch4) mRNA and protein (n=4 for each strain condition, p<0.05). Notch1 (1.51-fold) and Notch2 (1.24-fold) and Notch4 (2.3-fold) were upregulated by cyclic strain in a time-dependent manner. Notch1 and Notch2 signaling was associated with increased mRNA abundance in the osteocalcin gene, a known angiogenic marker. Notch1 signaling was also associated with increased protein abundance in the osteocalcin gene, a known angiogenic marker. Notch1 and Notch2 signaling was associated with increased protein abundance in the osteocalcin gene, a known angiogenic marker. Notch1 and Notch2 signaling was associated with increased protein abundance in the osteocalcin gene, a known angiogenic marker. The results suggest that Notch signaling mediates the cyclic strain-induced angiogenic response of EC and that Notch1 and Notch2 may play a more significant role in the regulation of angiogenesis than previously thought.
Hyperbaric Oxygen Induces bFGF and HGF Expression and Enhances Blood Perfusion as well as Muscle Remodeling in Mouse Ischemic Hind Limbs

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Background: It is not clear how hyperbaric oxygen (HBO) affects ischemia-induced pathophysiologic responses such as angiogenesis and skeletal muscle remodelling. We studied the effects of HBO on the functional and morphological recovery of ischemic hindlimbs, blood perfusion and the local production of angiogenic growth factors in a mouse model. Methods and results: Mice were placed in pure oxygen under 3 atm one hour a day for 14 days after the removal of a segment of left femoral artery. HBO-treated mice showed better functional recovery and greater blood flow in the ischemic hindlimb than untreated mice. Histological examination revealed the unattractive muscle fibers with islands of small regenerating muscle cells and angiogenesis only in HBO-treated mice. Regeneration of muscle was confirmed by the increase in myf5 mRNA. The amount of mRNA for VEGF, HGF and bFGF was slightly increased in the ischemic hindlimbs. HBO eliminated the increase in VEGF mRNA. In contrast, the amount of mRNA for bFGF and HGF was increased by HBO treatment. HBO increased Egr-1 in ischemic hindlimbs by increasing the production of bFGF and HGF and by promoting muscle regeneration in mice.

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Pulmonary Hypertension–Related Shear Stress Induces Pulmonary Endothelial Dysfunction

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Introduction: Recent clinical measurements and subsequent computational modeling studies have shown that patients with pulmonary hypertension have higher peak shear flow stress (FSS) compared to normotensive subjects. Thus, we assessed the hypothesis that pulmonary hypertension-related FSS induces pulmonary arterial endothelial cell (PAEC) dysfunction, which may result in smooth muscle vasoconstriction and hypertrophy. Methods: Bovine PAECs were subject to a laminar FSS profile covering a range of conditions from low-pathological (FSS: 0 dyne/cm²), normal physiological (FSS: 5, 20, 60 dyne/cm²), and high-pathological (FSS: 90, 120 dyne/cm²). Endothelial function was evaluated by examining vasodilator protein expression (eNOS, PGIS, COX-1 and COX-2), vasoconstrictor expression (ET-1), and growth factor release (VEGF). Cell signal studies were performed using flow-conditioned endothelial media to culture smooth muscle cells (SMC). Subcellular mechanical structures (F-actin and VE-cadherin) were examined to provide mechanotransduction evidence. Results: Compared to normal physiological FSS exposure (20 dyne/cm²), low-pathological (0 dyne/cm²) and high-pathological FSS (90 or 120 dyne/cm²) significantly downregulated eNOS expression by 1.49 ± 0.20, 1.31 ± 0.16 and 1.41 ± 0.16, respectively. In the physiological FSS range (5, 20, and 60 dyne/cm²), eNOS expression significantly increased with increasing FSS. Expressions of PGIS, COX-1 and COX-2 did not increase significantly for FSS > 60 dyne/cm². These results suggest that increased FSS favors the expression of eNOS, PGIS, COX-1 and COX-2 only in the physiological range. Compared to 0 dyne/cm², FSS of 90 and 120 dyne/cm² significantly increased VEGF expression. SMC expression of SM-a and PCNA suggests hypertension-related FSS increases SMC hypertrophy and proliferation. Conclusions: We conclude that pulmonary hypertension related high FSS may play an important role in endothelial-dysregulated vasoconstriction and vascular remodeling.

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Palmitate, but not Oleate, Reduces β3 Integrin Expression in Vascular Smooth Muscle Cells

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A relationship between fatty acid metabolism and several human diseases may be mediated by changes in integrin expression patterns. Beta3 integrin, part of the promiscuous alphaVbeta3 receptor expressed on smooth muscle cells, appears to mediate inflammation induced by high fat diets containing palmitate in mouse models. Specifically, beta3 integrin-null mice have increased inflammatory markers and are more susceptible to atherosclerosis when challenged with a high fat diet. To test the hypothesis that fatty acids affect beta3 integrin expression, primary porcine aortic smooth muscle cells were treated with oleate- or palmitate-supplemented cell culture media. Palmitate treatment did not induce apoptosis as detectable by propidium iodide staining or fluorescent DNA end-labeling. Palmitate treatment (500 micM) reduced beta3 integrin mRNA by half compared to BSA treatment alone. This response was dose-dependent and maximal at 24 hours of palmitate exposure. A lower concentration of palmitate (250 micM) also caused a significant decrease in the beta3 integrin message level. Beta3 integrin protein mass was also reduced after palmitate treatment as shown by Western blot analysis. Unlike palmitate, 500 micM oleate treatment did not reduce beta3 integrin expression. Palmitate did not affect beta3 integrin transcription in beta3 integrin promoter/reporter gene construct experiments. Transcription inhibition followed by analysis of decay of the beta3 integrin mRNA revealed that downregulation of the beta3 integrin message occurs, at least in part, through mRNA destabilization. These results show that palmitate, the saturated fatty acid most commonly consumed in the diets of industrialized countries, down-regulates the expression of the beta3 integrin in smooth muscle cells through effects on mRNA stability, and suggest a potentially novel mechanism underlying the induction of inflammation in vascular cells by high fat diets.

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Statin Treatment of Vascular Endothelial Cells Disrupts Caveolae via Cholesterol Depletion and Redistribution of Caveolin-1 Expression

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Statin inhibits a rate-limiting enzyme in the biosynthesis of cholesterol, HMG-CoA reductase, and are widely used to treat atherosclerosis. In addition to its role in atherosclerotic plaque development, cholesterol is a requirement for the formation of caveolae, important signaling microdomains of many cell types including vascular endothelial cells. Therefore the current study tests the hypothesis that statin will have a direct effect on vascular endothelial cells, in particular affecting the relationship between cholesterol and caveolae abundance and localization. Experiments used human umbilical vein endothelial cells and bovine aortic endothelial cells with lovastatin or simvastatin treatments. Confocal imaging of caveolin-1-eGFP and fluorescein-tagged cholesterol analogues in live cells identified a concentration- and time-dependent reduction of caveolin-1 and cholesterol at the plasma membrane in response to statin, respectively. Immunoassay experiments determined that statin reduced the caveolin-1 expression in buoyant sucrose-gradient fractions of Triton-X100-solubilized lysates without a significant change in total cell caveolin-1 expression. However, high concentrations (>1mM, >4hr) of statin did reduce the global caveolin-1 expression but this correlated with an increase in expression of the p17 fragment of caspase 3 indicating this may be a cytotoxic effect of statin at high doses. Since caveolae are a site of endothelial nitric oxide synthase (nNOS) and interaction with caveolin-1 has been shown to negatively regulate eNOS activity, we measured nitric oxide production (using an electrochemical sensor) in real-time from statin-treated cells; responses elicited by bradykinin (10nM) treatment of statin-treated cells were >2-fold those of control-treated cells. In conclusion, the results indicate that statins, by acting directly on endothelial cells, can produce a cascade of events initially reducing cholesterol content of plasma membrane; a subsequent reduction in caveolin-1 expression at the membrane; followed by an increase in eNOS activity. In addition to the cholesterol-lowering effects of statins these direct actions of statin on endothelial cells may underlie some of their therapeutic benefit in vivo.

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Low Endothelial Shear Stress (ESS) Leads to Excessive Expansive Remodeling of Coronary Atherosclerotic Subsegments

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Background: The role of excessive expansive remodeling in the natural history of atherosclerosis has not been studied. We investigated the hypothesis that low ESS leads to the excessive expansive remodeling of coronary atherosclerotic subsegments. Methods: In 11 diabetic hyperlipidemic swine, IVUS based 3D reconstruction of the coronary arteries was performed at baseline (wk 23) and follow up (wk 36). Local ESS was assessed using computational fluid dynamics with plaque-free segments and 12% or 19% of stenosis. The coronary arteries (n=31) were harvested at follow up, cryosectioned at the subsegments of interest, stained histologically and intima/media ratio, lipid deposition (oil red O), inflammation (CD45) and collagen content (picrossirius red) were quantified. Local remodeling behavior was assessed by a novel ab initio based on the information content of wall thickness and the remodeling characteristics of the artery as a whole and classified into excessive expansive (EER), compensatory expansive (CER) and inadequate remodeling (IR). Results: Subsegments with EER had larger plaques with more lipid deposition, inflammation and collagen content (p<0.01) than CER or IR (Fig a). Baseline ESS was 1.31±0.39 dynes/cm² lower in subsegments with EER as compared to those with IR (Fig b). Follow up ESS remained low in subsegments with EER or CER, and slightly increased in subsegments with IR (Fig b). Conclusion: Low ESS leads to EER in atherosclerotic subsegments, and in this setting, the adverse low ESS environment persists, thereby fostering continued lipid accumulation, inflammation, matrix degradation, and development of a high risk plaque.
Therapeutic Approach of Human Embryonic Stem Cell–Derived Endothelial Cells in Mouse Ischemic Heart and Hind Limb

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Human embryonic stem cells (HESCs) have been established as a potential resource for cell replacement therapy. Here, we determined the engraftment and reconstitution of functional endothelial-like cells differentiated from HESCs (HES-ECs) in NODSCID mice. HES-ECs were obtained from blast colonies and displayed endothelial characteristics by expression of KDR, VE-cadherin, vWF, Tie-2, and uptake of LDL. To monitor engraftment of HES-ECs, HES-ECs were transfected with lentivirus-GFP/luciferase. HES-ECs (1x10^6) were injected into either myocardial infarcted (MI) mouse heart or ischemia hind limb (HL). Medium-only injections served as a control (n=6). Cell engraftment was determined at 2w and 4w (weeks) of cell transplantation in both MI hearts and HL by luciferase imaging. Doppler perfusion imaging was also performed to evaluate limit blood flow by comparison of the ratio of blood flow in HL to that in non-HL. Mice were sacrificed at 4w of cell transplantation to determine myocytes regeneration and new vessel formation derived from the HES-ECs in both MI hearts and HL. A positive Luciferase image was obtained in both MI hearts and HL at 2w and 4w of cell transplantation. Doppler imaging indicated that HES-EC transplantation significantly improved blood flow as early as 1w with a flow ratio (HL/non-HL) of 0.6:0.2 in the HES-EC group versus the control group 0.3:0.1 (blood flow ratio at day 3 of ischemia ligation was 0.2:0.1). The blood flow in HES-EC mice was greatly improved thereafter in comparison to control mice. At 4w, cells positively stained with cardiac specific protein cTnI or NKx2.5 were also positive for GFP in MI hearts, which indicated regeneration of myocytes from transplanted HES-ECs. New vessel formation was demonstrated by human specific vWF staining in both MI hearts and HL with vascular-like structure. Our data suggest that HESC-derived endothelial precursors could be used therapeutically for the treatment of ischemia heart and limb.

The Frequency of Cyclic Stretch Determines the Proliferative and Apoptotic Capacity of Vascular Smooth Muscle Cells in Vitro

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Cyclic stretch is a key factor in determining the proliferative and apoptotic capacity of vascular smooth muscle cells (SMC). Changes in SMC growth are critical to vascular remodeling and restenosis following injury. The aim of this work is to investigate the effect of cyclic stretch applied at different frequencies on the proliferation and apoptosis of SMC. Bovine aortic SMC were subjected to 5% cyclic stretch at different frequencies (0, 0.5 and 1 Hz) for up to 96 h using a Flexercell Tension Plus FX-4000™ system with an applied equibiaxial heartbeat waveform before cell proliferation and apoptosis were evaluated. The Vybrant™ CFDA-SE dye and Vybrant™ Alexa Fluor 488™ Annexin V and propidium iodide were utilized to determine cell proliferation and apoptosis, respectively, using FACS analysis. Cell counts were also performed using a hemocytometer to confirm changes in cell growth after a 24, 48 and 96 h period of the applied stretch. Using both FACS analysis and cell counting it was found that cyclic stretch decreased SMC proliferation in a time and frequency dependent manner. Moreover, when cells were exposed to the lower frequency, SMC proliferation was inhibited to a greater extent. (Fig 1). In parallel cultures using FACS analysis, the level of SMC apoptosis following exposure to cyclic stretch was also frequency dependent. There was a reduced level of apoptosis after 96 h at the higher frequency. We conclude that cyclic stretch has a temporal antiproliferative effect on SMC in vitro. When cells are subjected close to the physiological frequency condition, the growth and apoptotic activity of these cells is reduced. Fig 1: Temporal effect of 5% cyclic stretch on SMCs under varying frequencies after 96 hours.

PTEN Depletion Enhances Vascular Smooth Muscle Cell Proliferation and Accelerates Neointima Formation

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A change in proliferative phenotype of vascular smooth muscle cells (SMC) from a highly quiescent to a rapidly proliferating phenotype is a key component of neointima formation. The PI3K/Akt pathway is a principal pathway involved in SMC proliferation and phenotypic modulation. PTEN is a tumor suppressor gene that negatively regulates the PI3K pathway; our previous research has shown that early inactivation of PTEN in response to vascular injury is a critical event involved in neointima formation. The goal of this study was to determine the role of PTEN in SMC function and neointima formation using both in vitro and in vivo techniques. Rat aortic SMC were transfected with control or PTEN-specific siRNA oligonucleotides. Western blotting was used to determine levels of total PTEN and phospho-Akt and SMC proliferation was determined by BrdU immunocytochemistry. To study the effect of PTEN depletion in vitro, we performed carotid artery ligation experiments on wild type mice (WT), global PTEN heterozygous mice (PTENfl/fl), and SMC-targeted PTEN heterozygous mice (SM22α-Cre;PTENfl/fl−/−), and SMC-targeted PTEN heterozygous mice (SM22α-Cre;PTENfl/fl−/−) generated by crossing PTENfl/fl mice to transgenic mice expressing Cre recombinase (Cre) under the control of the SM22α promoter. At day 13 following injury, mice were injected with BrdU and tissues harvested on day 14. Uninjured and injured carotid arterial sections were examined for neointima size and were stained for SMε−/−-actin and BrdU using immunofluorescence/histochemistry. Compared to controls, SMCs transfected with PTEN siRNA exhibited significantly decreased PTEN levels with corresponding increases in phosphorylated-Akt and enhanced proliferation under both basal and serum-stimulated conditions. Injured carotid
Ca²⁺ is a ubiquitous second messenger and controls many cellular processes in vascular smooth muscle, including proliferation. Activating Ca²⁺-permeable channels of the transient receptor potential (TRP) family contributes to smooth muscle proliferation. Specific TRP channel isoforms can be regulated by the sarcoplasmic reticulum (SR) Ca²⁺ stores, such that depolarizing the SR of Ca²⁺-coupled to influx of Ca²⁺. We tested the hypothesis that Osobaw pigs that manifest the metabolic syndrome when fed excess fat/cholesterol diet have greater TRP channel-mediated Ca²⁺ influx with Gd³⁺ treatment of normoxic human coronary artery endothelial (HCAE) cells increased NO production compared to normoxic cells. In addition, hypoxia preferentially with COX-2. Preservation of PGE₂ biosynthesis in front of profound suppression of PGI₂ in endothelial cells, at physiological level of steady LSS, by selective inhibitors of COX-2 (the hydrolysis product of PGI₂) and PGE₂ by Endothelial Cells in Response to LSS, 6700 and 6376 Paola Patrignani, Luigia Di Francesco, GdAnnunzio Univ, Chieti, Italy; Antonio Piccoli, Mario Negri Sud, Santa Maria Imbaro Chieti, Italy Prostacyclin (PGI₂) and prostaglandin (PG)E₂, the major prostanoids released from endothelial cells, are produced by cyclooxygenase (COX) isozymes. Prostacyclin and Prostaglandin E₂ by Endothelial Cells in Response to LSS while only COX-2 contributed to the generation of the prostanoid in response to LSS, 6700 and 6376 Paola Patrignani, Luigia Di Francesco, GdAnnunzio Univ, Chieti, Italy; Antonio Piccoli, Mario Negri Sud, Santa Maria Imbaro Chieti, Italy PGI₂ Biosynthesis in Endothelial Cells in Response to LSS, 6700 and 6376 Paola Patrignani, Luigia Di Francesco, GdAnnunzio Univ, Chieti, Italy; Antonio Piccoli, Mario Negri Sud, Santa Maria Imbaro Chieti, Italy PGI₂ biosynthesis is regulated by the expression of COX-2 and Prostaglandin E₂ synthase (PGES). The expression of COX-2 is regulated by the transcription factor nuclear factor kappa B (NFkB), which is upregulated by inflammatory stimuli. In this study, we investigated the role of COX-2 in the regulation of PGI₂ biosynthesis in endothelial cells. Our results showed that COX-2 expression was increased in endothelial cells treated with proinflammatory stimuli, such as lipopolysaccharide (LPS) and tumor necrosis factor-alpha (TNF-α), compared to untreated cells. This upregulation was associated with increased PGI₂ production. To further investigate the role of COX-2 in PGI₂ biosynthesis, we used siRNA to inhibit COX-2 expression and found that this treatment significantly reduced PGI₂ production. In conclusion, our results suggest that COX-2 plays a critical role in the regulation of PGI₂ biosynthesis in endothelial cells. This study provides new insights into the mechanisms underlying the regulation of PGI₂ production and may have implications for the development of therapeutics targeting COX-2 in the treatment of cardiovascular diseases.

**References:**


**Activin A and Transforming Growth Factor-β1 Have Different Functions in Smooth Muscle Cell Migration**

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Activin A and Transforming Growth Factor-β (TGF-β) are members of the TGF-β superfamily. Both modulate vascular lesion formation beneficially, however, they involve distinct mechanisms and processes. Activin A inhibits the formation of smooth muscle cell (SMC)-rich lesions, whereas TGF-β1 does not. In addition, TGF-β1 induces production of extracellular matrix components, resulting in a larger but more stable vascular lesion. Activin A and TGF-β1 bind unique cell-surface receptors, but both involve Smad-2, 3 and 4 in downstream signaling pathways. To understand how Activin A and TGF-β1 affect SMC-rich lesion formation differently, we first investigated the presence of Activin receptor-like kinases (ALK) in SMCs. We demonstrate that ALK-1, 2, 4, and 5 are all expressed in human SMCs. Western-blot analyses showed that both Activin A and TGF-β1 induce Smad-2 phosphorylation, however, also Smad-1 phosphorylation was observed in response to both treatments. Furthermore, our knowledge of ALK-1/2 has not been reported before to phosphorylate Smad-1, this was unexpected. Secondly, we performed micro-array experiments followed by gene-set enrichment analyses on SMCs stimulated with either Activin A or TGF-β1. Several pathways were discovered to be differentially regulated by these factors, including cellular migration. Further, we confirmed that Activin A does not affect SMC migration (323.6±22.7 µm vs control: 330.1±17.2 µm, after 24 hours; n=8, p>0.05), whereas TGF-β1 inhibits migration (249.9±17.1 µm vs control: 330.1±17.2 µm, after 24 hours; n=6, p<0.01), most likely through increased expression of extracellular matrix proteins promoting firm attachment of the cells. In conclusion, gene expression profiling experiments provide evidence that Activin A and TGF-β1, in spite of the activation of intracellular signaling pathways, provoke different significances in cellular responses of SMCs, which may clarify their distinct effects on the vessel wall.

**Calcium/CaMulin-Dependent Protein Kinase II-δ Regulates Vascular Smooth Muscle Cell Proliferation**

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There is accumulating evidence that Ca²⁺-dependent signaling pathways regulate proliferation and migration of SMC cells contributing to the intimal accumulation of VSM observed in many vascular diseases. In this study we investigated the role of the multifunctional serine/threonine kinase, CaMKII, as a mediator of Ca²⁺ signals regulating VSM cell proliferation. Differentiated VSM cells isolated from rat aortic media express primarily CaMKIIγ gene products while passaged primary cultures of de-differentiated VSM cells express primarily CaMKIIα. Experiments examining the time course of CaMKII isoform modulation revealed the process was rapid following initial dispersion of aortic VSM with a significant increase in CaMKIIα protein and significant decrease in CaMKIIγ protein within 30 hrs, coinciding with onset of DNA synthesis and cell proliferation. Attenuating the initial upregulation of CaMKIIα, in primary cultured cells using siRNA resulted in decreased serum-stimulated DNA synthesis and cell proliferation. In passaged VSM cells, suppression of CaMKIIα activity by overexpression of a kinase-negative mutant, or suppression of endogenous CaMKIIα gene using multiple siRNAs, significantly attenuated serum-stimulated DNA synthesis and cell proliferation. Cell cycle analysis following either inhibitory approach indicated decreased proportion of cells in G1, an increase in proportion of cells in G2/M, and an increase in polyploidy corresponding with accumulation of multi-nucleated cells. In vivo we observed CaMKIIα isoform modulation in response to vascular injury in the rat carotid artery balloon angioplasty model. Three days after injury to the left carotid artery, there is a significant increase in CaMKIIα and a significant decrease in CaMKIIγ. At 14 days after injury the neointima contained primarily the δ isoform. Incubation of the injured vessel with siRNA to the δ isoform prevented the upregulation of the δ isoform and inhibited neointimal formation by 70% . PNA detection in the medial wall was significantly attenuated at 3 and 7 days in siRNA-treated vessels. These results indicate that CaMKIIδ is specifically induced during modulation of VSM cells to the synthetic phenotype and is a positive regulator of proliferation.

**Patterns of Protein Kinase C and Rho Activation During Cerebral Vasospasm After Subarachnoid Hemorrhage**

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Delayed Cerebral Vasospasm (CV), the phenomenon of sustained vascular contraction is seen in about 40% of Subarachnoid Hemorrhage (SAH) cases that survive the initial hemorrhagic event. This complex pathological vasospasm that occurs between 3–10 days after SAH results in worsening of neurological deficits and increased morbidity. Despite intensive research efforts, the cause of and molecular mechanisms of CV still remain to be elucidated. Our laboratory has shown that Bilirubin Oxidation Products (BOXes) are present in greater concentrations in the cerebrospinal fluid (CSF) of patients with SAH who suffer from vasospasm than those who do not. Previous in vivo studies from our lab have shown that BOXeXes are vasoactive and potentiate smooth muscle contraction similar to protein kinase C (PKC) activators and phosphatase inhibitors. Our in-vitro model of CV after SAH uses CSF from SAH patients with (CSF+D) and without (CSF-) CV. We compared the effects these with laboratory-prepared BOXes on porcine carotid arteries (PCA) and examined the activation of regulatory proteins PKCα, PKC δ and Rho. PCA rings are pre-stretched to a tension length and placed in control solution (MOPS), CSF, CSF+BOXes (25 µM) for 20 minutes. Significant differences in cellular responses of SMCs, which may clarify their distinct effects on the vessel wall.
Cyclic Strain of Vascular Endothelial Cells Reduces Vascular Smooth Muscle Cell Proliferation, Possibly via an MMP-2-dependent Mechanism

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Introduction: Hemodynamic forces, namely shear stress and cyclic strain play an important role in physiological control of vascular tone, remodelling and associated pathologies. Furthermore, these forces indirectly impact vascular smooth muscle cell fate decisions by modulating vascular endothelial cell functions. MMPs (Matrix metalloproteinases) metabolic processes in vascular cell fates via degradation of extracellular matrix substrates. Recent studies demonstrate that these forces can modulate MMP expression and activity in different vascular cell types. For these investigations, we examined how MMP-2 and MMP-9, induced in BAECs in response to cyclic strain, putatively impact on BASMC proliferation. Methods: BAECs were exposed to a defined level of equibiaxial cyclic strain using the Flexercell® Tension Plus™ FX4000™ apparatus (10% strain, 60 cycles/min, 24 h, cardiac waveform). BAECs were harvested for Real Time PCR to monitor MMP-2 mRNA levels while BASMCs were incubated with different strain conditions (0-10% strain), and cell counts and analysed for proliferation by FACs analysis and cell counting. Results: Cyclic strain of BAECs increased MMP-2 and MMP-9 mRNA levels by (1.2±0.1 fold) relative to control. Subsequent incubation of BASMCs with BSM decreased proliferation to (0.86±0.1 and 0.82±0.1 fold) respectively as compared to control. Conclusion: These findings indicate that cyclic strain of BAECs reduces BASMC proliferation. Therefore, blocked MMP-2 induction but not MMP-9 appeared to reverse this effect, suggesting a regulatory role for strain-induced endothelial-derived MMP-2.

Three-day High-flow Loaded Rabbit Carotid Artery Remodels Automatically Ex Vivo

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[Discussion]We proved that 3-day high-flow loaded rabbit carotid artery gaps observed in the controls. MIB-5 positive smooth muscle cells were observed in the media layer of high-flow loaded samples. Therefore, it is concluded that 3-day high-flow loaded rabbit carotid artery would not remodel automatically ex vivo. The cause of remodeling is still under investigation. [Conclusion]We conclude that 3-day high-flow loaded rabbit carotid artery would remodel automatically ex vivo. We hope that this model is useful for understanding the mechanism of remodeling.

Vascular smooth muscle cell (VSMC) proliferation is a key component of vascular lesion formation and progression. We previously showed that high flow (HF) loading of rabbit carotid arteries, which is modeled in vivo by the Flexercell® Tension Plus™ FX4000™ apparatus (10% strain, 60 cycles/min, 24 h, cardiac waveform), increases vascular smooth muscle cell proliferation (SM22α) compared to control. We hypothesized that increased HF loading could negatively impact carotid artery remodeling in vivo. In order to test this hypothesis, we exposed carotid arteries from 10-week-old male New Zealand White rabbits to HF loading for 3 days. After 3 days, we harvested the carotid arteries and performed a morphological analysis using light microscopy. We observed that high-flow loading increased SM22α positive cells in the carotid artery media. Therefore, we conclude that high-flow loading decreases carotid artery remodeling. In order to further investigate this phenomenon, we performed in vitro experiments using rabbit carotid arteries. We isolated rabbit carotid arteries and treated them with different concentrations of HF loading (0-10%). We observed that increased HF loading decreased SM22α positive cells in the carotid artery media. Therefore, we conclude that high-flow loading decreases carotid artery remodeling in vitro. In order to understand the mechanism behind this phenomenon, we performed in vivo experiments using rabbit carotid arteries. We isolated rabbit carotid arteries and treated them with different concentrations of HF loading (0-10%). We observed that increased HF loading decreased SM22α positive cells in the carotid artery media. Therefore, we conclude that high-flow loading decreases carotid artery remodeling in vivo. In order to understand the mechanism behind this phenomenon, we performed in vitro experiments using rabbit carotid arteries. We isolated rabbit carotid arteries and treated them with different concentrations of HF loading (0-10%). We observed that increased HF loading decreased SM22α positive cells in the carotid artery media. Therefore, we conclude that high-flow loading decreases carotid artery remodeling in vitro.
Synergetic Interaction Between Aldosterone and Angiotensin II: Differential Effects on RhoA, Akt, and MAP Kinase Signaling in Vascular Smooth Muscle Cells

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Aldosterone (Aldo) exerts a synergistic mitogenic effect with angiotensin II (Ang II) through ERK5, p38 and JNK activation in vascular smooth muscle cells. We investigated whether similar interactions influence contractile and migratory signaling pathways remain unclear. Here we investigated cross-talk of c-Src, MAP kinases (ERK 5, JNK and p38) and calcium signaling between Aldo and Ang II. Cultured rat mesenteric vascular smooth muscle cells were studied. Activation of MAP kinases and Akt was determined by immunoblotting using pro-apoptotic- or specific antibodies. Rho activity was assessed using the G-LISA Rho activation assay kit. c-Src activity was measured by the Light Scatter assay. Ca2+ signaling was assessed by Fura-2 imaging. These modules were also enriched in pathways relevant to vascular inflammation. We have used human microvascular endothelial cells (HMEC) to study the combined effects of a model air pollutant, diesel exhaust particles (DEP), and oxidized phosphatidic acid (ox-PAPC) on genome-wide gene expression. We treated HMEC in triplicate wells with an organic DEP extract (5 * 10^-8 mol/L) of Aldo and Ang II significantly increased activation of c-Src (5 * 10^-8 mol/L) of Aldo and Ang II significantly increased activation of c-Src (5 * 10^-8 mol/L). ERK 5, p38 and JNK were unaffected by low levels of Ang II stimulation. The selective receptor (Af)-R antagonist, ebselen (10^-6 mol/L), or a mineralocorticoid receptor (MR) antagonist, eplerenone (10^-6 mol/L). Our results suggest that Aldo/Ang II, through MR/AT/R, synergistically exerts a positive effect on c-Src, RhoA and [Ca2+]i, and a negative effect on Akt. However, signaling events involving ERK5, JNK and p38 are independent of Aldo/Ang II interactions. These findings support the hypothesis that PM is associated with increased cardiovascular morbidity and mortality, and that these pro-oxidative chemicals could synergize with oxidized lipid components generated in low-density lipoprotein (LDL) particles to enhance vascular inflammation and atherosclerosis.

Air Pollutant Chemicals and Oxidized Lipids Exhibit Genome-wide Synergistic Effects on Endothelial Cells

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Background - Ambient air pollution is associated with increased cardiovascular morbidity and mortality. We have recently found that exposure to ambient ultrafine particle matter, highly enriched in redox cycling organic chemicals, promotes atherosclerosis in mice. We hypothesize that these pro-oxidative chemicals could synergize with oxidized lipid components generated in low-density lipoprotein (LDL) particles to enhance vascular inflammation and atherosclerosis. We have used human microvascular endothelial cells (HMEC) to study the combined effects of a model air pollutant, diesel exhaust particles (DEP), and oxidized phosphatidic acid (ox-PAPC) on genome-wide gene expression. We treated HMEC in triplicate wells with an organic DEP extract (5 * 10^-8 mol/L) of Aldo and Ang II significantly increased activation of c-Src (5 * 10^-8 mol/L). ERK 5, p38 and JNK were unaffected by low levels of Ang II stimulation. The selective receptor (Af)-R antagonist, ebselen (10^-6 mol/L), or a mineralocorticoid receptor (MR) antagonist, eplerenone (10^-6 mol/L). Our results suggest that Aldo/Ang II, through MR/AT/R, synergistically exerts a positive effect on c-Src, RhoA and [Ca2+]i, and a negative effect on Akt. However, signaling events involving ERK5, JNK and p38 are independent of Aldo/Ang II interactions. These findings support the hypothesis that PM is associated with increased cardiovascular morbidity and mortality, and that these pro-oxidative chemicals could synergize with oxidized lipid components generated in low-density lipoprotein (LDL) particles to enhance vascular inflammation and atherosclerosis.

Methods and Results - We have used human microvascular endothelial cells (HMEC) to study the combined effects of a model air pollutant, diesel exhaust particles (DEP), and oxidized 1-palmitoyl-2-acyl-sn-glycero-3-phosphorylcholine (ox-PAPC) on genome-wide gene expression. We treated HMEC in triplicate wells with an organic DEP extract (5 * 10^-8 mol/L) of Aldo and Ang II significantly increased activation of c-Src (5 * 10^-8 mol/L). ERK 5, p38 and JNK were unaffected by low levels of Ang II stimulation. The selective receptor (Af)-R antagonist, ebselen (10^-6 mol/L), or a mineralocorticoid receptor (MR) antagonist, eplerenone (10^-6 mol/L). Our results suggest that Aldo/Ang II, through MR/AT/R, synergistically exerts a positive effect on c-Src, RhoA and [Ca2+]i, and a negative effect on Akt. However, signaling events involving ERK5, JNK and p38 are independent of Aldo/Ang II interactions. These findings support the hypothesis that PM is associated with increased cardiovascular morbidity and mortality, and that these pro-oxidative chemicals could synergize with oxidized lipid components generated in low-density lipoprotein (LDL) particles to enhance vascular inflammation and atherosclerosis.
Pulmonary Diesel Pollution Exposure Dysregulates Key Cardiac and Pulmonary Transcripts

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Air pollution accelerates atherosclerosis and cardiovascular disease. We examined the effect of diesel exhaust particles on cardiac and pulmonary gene expression in vivo, using transgenic mice. Tie2-GFP mice express green fluorescent protein regulated by the endothelial-specific promoter Tie2. Tie2-GFP mice were subjected to intratracheal instillation of a single dose of 100 μg of diesel exhaust particles (DE). Within 1 and 5 days after exposure to diesel exhaust particles, a subset of 11 transcripts detected by microarray were consistently dysregulated by more than 2 fold within heart. Within 1 and 5 days after exposure of diesel exhaust particles, an overlapping subset of 18 transcripts was consistently dysregulated by greater than 2 fold within 5 mmHg). Pven was did not change (P=0.13).

The Environmental Pollutant, Polychlorinated Biphenyl 77, Augments Angiostatin II-induced Abdominal Aortic Aneurysm Formation in Apolipoprotein E-deficient Mice

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Objectives: Polychlorinated biphenyls (PCBs) are omnipresent industrial pollutants that have been linked to increased cardiovascular disease. Previous studies demonstrated that co-planar PCBs (PCB77) increase proinflammatory gene expression in endothelial cells, macrophages and adipocytes. Infusion of angiostatin II (AngII) to hyperlipidemic mice results in the formation of abdominal aortic aneurysms (AAAs). Angiostin-induced AAAs are associated with pronounced inflammation. The purpose of this study was to determine if PCB77 would augment AngII-induced AAAs. Methods and results: Male ApoE−/− mice (3 months of age) were injected twice (i.p.) with either (vehicle) or PCB77 (170 μM/kg) at 1 week prior to and 1 week after implantation of miropump minipumps for infusion of saline or AngII (1,000 ng/kg/min) for 28 days. Administration of PCB77 increased body weight gain in saline- and AngII-infused mice (vehicle, 1.4 + 0.3; saline/PCB77, 3.6 + 0.3; AngII/PCB77, 2.2 + 0.4 g P < 0.0036). Elevations in body weight were associated with adipocyte hypertrophy in mesenteric adipose tissue and liver steatosis from PCB77-infected mice. PCB77 increased systolic blood pressure in saline-infused mice (vehicle, 102 ± 3; PCB77, 117 ± 4 mmHg). Infusion of AngII increased blood pressure to a similar extent in vehicle (146 ± 6 mmHg) and PCB77-infected mice (140 ± 4 mmHg). Both AAA incidence (AngII/vehicle, 45%; AngII/PCB77, 90%) and aortic diameter measured by ultrasound (day 28; vehicle, 0.92 + 0.01; AngII/vehicle, 1.66 ± 0.21; AngII/PCB77, 2.32 ± 0.15 mm) were increased by PCB77. PCB77 also increased the severity of AAAs, including mortality from ruptured AAAs. Unexpectedly, PCB77 increased mortality in aortic infusion. Following the results of our initial study, we submitted a manuscript to a major cardiovascular journal.}

Diesel Exhaust Enhances Vascular Oxidative Stress, Vasocostriction, and Venous Congestion in a Cardiomyopathic Hamster Model

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Environmental air pollution has been associated with increased hospital admissions and death due to heart failure. However, the exact mechanism(s) by which environmental air pollution affects the heart and vasculature is currently unknown. Recent studies have found that exposure to environmental air pollution ensues vasocostriction in humans. We hypothesized that diesel exhaust (DE), a major component of ambient urban air, could enhance vasoconstriction and produce venous congestion in the presence of a failing heart. To test this hypothesis, we examined transcriptomic responses to DE-induced cardiac dysfunction in cardiomyopathic hamsters (CH). Fractional shortening 0.15–0.20, normal ~0.60) to freshly derived DE. HCM were exposed to 300 μg/m3 of DE for 4 hours/day for 2 days and venous pressures (Pven) were obtained via radiotelemetry. Pven was significantly increased to ~13 mmHg compared to control animals (Pven ~9 mmHg). Pven was not increased in early- and mid-phase of hypoxic control. This finding suggests that pre-existing cardiac disease is prerequisite for the development of venous congestion. DE also enhanced vascular oxidant generation in exposed animals as measured by lucigenin-enhanced chemiluminescence. Interestingly, HCM animals exposed to gasoline exhaust at 60 μg/m3, a 1:12 dilution with filtered-air, for 3hr for 1 day did not have enhanced oxidant generation unlike DE exposed animals. In a related series of studies, the vasocostrictive effects of the volatile organic components of DE were determined ex vivo using mouse mesenteric arteries and veins. DE-induced vasoconstriction is enhanced by the depressor effects of ET-1 in mesenteric arteries and veins. Furthermore, mesenteric vessels exposed to a combined acetalddehyde, formaldehyde, acetone, hexadecane and octadecane saline solution at concentrations found in DE-exposed saline also had enhanced ET-1 vasoconstriction. However, the individual compounds did not appear to mediate these effects. These findings suggest that DE may induce venous congestion in susceptible subjects through enhanced vasocostric- tion due to vascular oxidative stress.

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Increased Blood Borne Tissue Factor Activity Following Total Knee Arthroplasty: A Contributor to Venous Thrombosis?

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Introduction Blood-borne tissue factor (TF) is increased in several disorders associated with thromboembolism, and cell-associated TF appears to be important in thrombogenesis. Elevated blood TF has been demonstrated primarily in patients with increased risk of arterial thrombosis, but evidence for an association of elevated blood TF with risk of venous thromboembolism (VTE) is lacking. Total knee arthroplasty (TKA) is associated with a risk of venous thrombosis which is 40–80% without thromboprophylaxis. Hypothesis We hypothesized that elevated
blood TF is a contributing factor to venous thrombosis following TKA. Methods: Fifteen male subjects who had unilateral TKA gave written, informed consent. Venous blood samples were obtained before surgery, 5 min following tourniquet release and daily until hospital discharge at day 4–7. All patients received prophylactic dalteparin and none developed VTE. Platelets and mononuclear cells were isolated from EDTA anticoagulated blood by differential centrifugation. A modified prothrombin time (PT) assay was used to quantify TF procoagulant activity. Intron was employed as the PT calibration standard. The encrypted TF procoagulant activity was fully expressed by pretreatment with 20 μM monomycin in the presence of 12.5 mM CaCl2 and 5 mM activated factor VII. Results: Blood mononuclear TF activity increased following TKA, peaked at day 4 and returned to near baseline by day 7 (Table). Platelet TF activity did not change significantly. The peak rise in TF activity preceded the median time to diagnosis of venous thrombosis following TKA (7 days) observed in previously reported studies. Conclusion: Increased mononuclear cell-derived blood TF activity is a delayed response to TKA which is predominantly independent of acute tissue injury. The close temporal relationship of the rise in TF to the occurrence of VTE strongly suggests that blood-borne TF contributes to VTE following TKA.

**TF ACTIVITY (PG/TF/MIL BLOOD; MEAN ± SEM) **<p>0.05 COMPARED TO PRE SURGERY</p>
...and other endogenous ligands) may increase thrombus formation and exacerbate the development of atherosclerosis. We confirmed that TLR4 agonists activate platelets and increase ROS formation. We also found that LPS, and the TLR2 agonist bacterial lipopolysaccharide, induce platelet P-selectin expression, and that ROS production was partially blocked by pretreatment with antioxidant Trolox (water-soluble vitamin E analogue). LPS also potentiated platelet aggregation and maximal inhibition was reduced to 50% in the presence of ROS scavengers. To test the postulate that TLR4- and TLR2 agonists may have induced expression of aspirin-inhibitable cyclooxygenase-2 (COX-2). We detected COX-2 in agonist-treated platelets using real-time RT-PCR, confocal microscopy, FACS, and Western blot. Trolox treatment significantly down-regulated COX-2 protein expression in LPS-activated platelets. TxA2 synthesis in LPS-treated cells was less sensitive to aspirin, but sensitive to COX-2 inhibitor NS398. These observations suggest that the induction of COX-2 in platelets by the agonists of TLR4 and TLR2 mediate aspirin-resistant production of TxA2, and so upregulation of COX-2 in platelets may be one mechanism leading to aspirin resistance. Application of antioxidants together with COX-2 inhibitors may be useful in the treatment of acquired aspirin resistance.

The Role of Akt in Glycoprotein Ib-IX-Mediated Platelet Activation

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Under high shear flow conditions, initial platelet adhesion at the site of vascular injury is mediated by the interaction between von Willebrand factor (vWF) and its receptor, the glycoprotein Ib-IX-GPIX complex. This interaction also initiates a signaling cascade, leading to activation of the platelet integrin, αIIbβ3. The signaling mechanism of GPIb-IX is not totally clear. The cytoplasmic domain of GPIb-IX has been suggested to interact with signaling molecules such as phosphoinositide 3-kinase (PI3-K), which has been shown to be involved in the GPIb-IX signaling. An important signaling molecule that is activated by PI3-K is the protein kinase, Akt. Thus, to understand the downstream signaling pathway of GPIb-IX signaling, we investigated the role of protein kinase, Akt, in vWF-induced, GPIb-IX-mediated platelet activation. We showed that GPIb-IX-vWF interaction-dependent platelet aggregation induced by botrocetin or ristocetin was impaired in Akt-deficient mouse platelets or human platelets treated with an Akt inhibitor, SH-6. In contrast, botrocetin-induced vWF binding to platelets or platelet agglutination was not significantly affected. Similarly, GPIb-IX and integrin-dependent platelet spreading on vWF was partially inhibited in Akt-1 deficient or Akt-1 null platelets.

Under high shear flow conditions induced by the cone-and-plate rheometer, GPIb-IX- and integrin-dependent stable platelet adhesion on immobilized vWF was attenuated in Akt-1 deficient or SH-6 treated platelets. Thus, Akt is important in GPIb-IX and integrin-dependent platelet adhesion, spreading and aggregation. The role of Akt-1 is independent of integrin outside-in signaling, because Akt inhibitor-treated or Akt-1 null platelets are not different from control platelets in spreading on immobilized fibronectin, which requires integrin outside-in signaling but not integrin activation. Thus, Akt-1 plays an important role in GPIb-IX-mediated platelet activation signaling. In signaling inhibition of integrin outside-in signaling, as noted above, Akt was required.

Blood-induced vWF-induced platelet aggregation was partially inhibited in Akt-1 deficient or Akt-1 null platelets treated with the phosphatidylinositol 3-kinase (PI3-K) Akt phosphorylation. Taken together, our data indicate a role for Akt in GPIb-IX-mediated platelet activation signaling.

Blood and Hair Hg Levels Increase Blood Pressure but Do Not Affect Vascular Reactivity

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Introduction: Some studies suggest that high levels of urine, hair or toenail Hg increase the risk of atherothrombotic diseases, an effect that may be explained by oxidative damage to the vascular endothelium. Hypothesis: We tested the hypothesis that high Hg levels impair the vascular reactivity, but did not affect oxidative stress. Methods: We measured the association between high blood and hair Hg and brachial artery flow mediated vasodilation (FMD), middle cerebral artery reactive dilation to CO2 (MCAR), heart rate variability (standard deviation of normal RR intervals, SDNN), and hypertensive status in a consecutive sample of 101 subjects participating in the Wisconsin Sleep Cohort study (mean age = 54.8 years; range: 44–75, 52.5% males). Whole blood total Hg and hair total Hg were tested using inductively coupled plasma mass spectrometry and cold vapor atomic fluorescence spectrometry, respectively. Results: Geometric mean blood and hair Hg were 1.16 μg/L (0.98, 1.38) and 270.1 ng/g (226.1, 322.6). Blood and hair Hg were positively associated with FMD (%), MCAR (%), SDNN, and SDNN/HR (Table). However, after adjusting for gender, age, body mass index, and fish intake, people in the upper quartile of hair Hg were more than 4 times more likely to be hypertensive (odds ratio: 4.19; 95% confidence interval: 1.28, 13.76). Similarly, hypertension was 1.4 times more likely in those in the upper quartile of blood Hg, although this difference was not statistically significant (odds ratio: 1.40; 95% confidence interval: 0.92, 2.12). Conclusion: High hair and blood Hg levels do not seem to influence vascular reactivity, but may increase the risk of hypertension. This could be explained by deleterious effects on kidney function.

DIFFERENCE IN VASCULAR FUNCTION PARAMETERS IN SUBJECTS IN THE UPPER AND LOWER QUARTILES OF HG LEVELS

<table>
<thead>
<tr>
<th>Blood Hg Difference</th>
<th>Hair Hg Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD%</td>
<td>0.47 (0.50, 1.42)</td>
</tr>
<tr>
<td>MCAR%</td>
<td>0.14 (0.04, 0.67)</td>
</tr>
<tr>
<td>SDNN</td>
<td>0.95 (2.98, 4.88)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.40 (0.92, 2.12)</td>
</tr>
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p

-0.50 (-1.81, 2.81)  0.22
-0.63 (-1.24, 0.47)  0.02
-1.45 (1.28, 3.76)  0.02
Nitric Oxide Release from Pulmonary and Coronary Vasculature with the Use of Intravenous Ioprost and Nitroglycerin in Diabetic Patients Undergoing Valvular Heart Surgery

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Introduction: The aim of this study is to compare the effects of intravenous ioprost and nitroglycerin in patients with type II diabetes mellitus undergoing valvular heart surgery. Methods: Twenty-five patients undergoing valvular replacement with pulmonary hypertension > 25 mmHg were randomized to be given ioprost or nitroglycerin via a central pulmonary catheter, and the levels of nitro/nitrate were evaluated before incision (T1) and 20 minutes after incision (T2). Coronary, aortic, coronary and coronary-pulmonary mixed blood samples were taken at the T1 and T4 time periods and the release of nitric oxide from the coronary vasculature was determined by the difference between the aortic and coronary sinus concentrations of nitrate and nitrite. Results: Apoptosis scores according to TUNEL staining were higher in groups A1 and A2 than in group B (P < 0.05). Moreover, the release of nitric oxide from the coronary vasculature was determined by the difference between the aortic and coronary sinus concentrations of nitrate and nitrite. Results: Apoptosis scores according to TUNEL staining were higher in groups A1 and A2 than in group B (P < 0.05). However, after the removal of the cross-clamp, a significant increase in nitric oxide is observed in the group 2 at T4 comparing to group 1 (26.1 ± 13.8 μM/mL versus 34.2 ± 2.8 μM/mL, P < 0.05). Conclusion: This study has shown that in patients with type II diabetes mellitus undergoing valvular heart surgery the ioprost group did not show an increase in the release of nitric oxide from the coronary vascular bed after aortic cross clamp and during reperfusion period. This finding suggests that ioprost and nitroglycerin act via different mechanisms on vascular smooth muscle cells during their use for pulmonary hypertension.

Induction of Thrombotic Plaque Erosion in Balloon-injured Arteries of Atherosclerotic Rabbids

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Objective: Plaque erosion triggers thrombosis, serving as a vital pathologic basis of coronary thrombotic events. This research sought to establish a model of thrombotic plaque erosion in atherosclerotic rabbit aorta by stent-soresringer-triggered endothelial apoptosis. Methods: Atherosclerotic plaques were established in 33 New Zealand rabbits by post-balloon-injury high-cholesterol feeding for 3 months. The animals were randomized into two groups and their susceptibility to carotid artery thrombosis was measured using a photochemical injury model. Thrombotic occlusion occurred more rapidly in the hypercholesterolemic group compared to the normocholesterolemic group (P < 0.05) and greater at 9 months compared with 5 months (P < 0.05). In the reversed group, aortic sinus lesion area at 9 months was similar to the hypercholesterolemic group at 5 months, but greater than the hypercholesterolemic group at 9 months (P < 0.05), indicating that atherosclerosis did not progress between 5 and 9 months. Susceptibility to carotid arterial thrombosis was measured using a photochemical injury model. Thrombotic occlusion occurred more rapidly in the hypercholesterolemic group than in the normocholesterolemic group at both 5 months (P < 0.05) and 9 months (P < 0.05). Accelerated thrombosis was found toward normal or hypercholesterolemic atherosclerotic plaques (11:2 months; P < 0.05 vs. hypercholesterolemic group). Conclusions: Reversal of hypercholesterolemia normalizes thrombotic susceptibility in atherosclerotic mice.

Pentoxifylline Lowers Plasmaactivity Activator Inhibitor-1 Levels in Obese Human Subjects

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Obesity, an increasingly important risk factor for cardiovascular disease in men and women, is associated with the premature development of atherosclerosis. A classical perspective of cardiovascular risk does not adequately explain all of the cardiovascular events associated with obesity. Elevations in plasma levels of PAI-1 (plasminogen activator inhibitor) and CRP (c-reactive protein) are biochemical hallmarks of obesity and inflammation that likely contribute to the increased risk of atherosclerotic events in patients with obesity. While PAI-1 and CRP are known to be synthesized in vascular tissue, the liver and adipose tissue, the source and mechanisms of increased PAI-1 and CRP in obesity are incompletely understood. There is strong experimental evidence that TNF-α (tumor necrosis factor-α) is an important cytokine involved in the pathogenesis of obesity-related inflammation. Plasma PAI-1 and CRP levels are measured at days 0, 28 and 56. There was a strong correlation between baseline PAI-1 and hs-CRP levels in obese patients (r = 0.766, P = 0.002). PAI-1 levels decreased by 5.5 mg/L in the pentoxifylline arm while the placebo arm saw an increase in plasma levels of 5.4 mg/L over the 56 days. Pentoxifylline successfully reduced to hs-CRP levels (1.8: ± 0.3 mg/L compared to placebo (0.2: ± 0.3 mg/L), but this did not achieve statistical significance (P = 0.08). TNF-α levels were essentially unchanged throughout the study. These data suggest that pentoxifylline may be an effective agent in lowering PAI-1 and CRP in obese subjects, which could portend a better cardiovascular risk profile in this patient population.

Small-molecule ALK5 inhibitor in a vascular fibrosis model and suggest the potential therapeutic application of these inhibitors in vascular fibrosis.

Reversal of Hypercholesterolemia Prevents Progression of Atherosclerosis and Protects Against Arterial Thrombosis in Mice

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Introduction: Hypercholesterolemia promotes atherosclerosis and increases thrombotic risk, but little is known about the effect of cholesterol lowering on susceptibility to experimental thrombosis. Hypothesis: We tested the hypothesis that reversal of hypercholesterolemia induces regression of atherosclerosis and restores normal susceptibility to carotid artery thrombosis in Reverse mice. Methods and Results: Male Reverse (Ldr::ApoB^{tm1a_Tgt}Hprt::Mx1^- Cre+) mice were fed a high fat diet and studied at 5 or 9 months of age (total cholesterol (TC) 784 ±66 and 726 ±65 mg/dL, respectively). To prevent hypercholesterolemia, Reverse mice were injected with p/o-polysinic-polycylic acid (pl-pC) at one month of age to induce Mx1-Cre and switch off hepatic Hprt gene expression, fed a control diet, and studied at 5 or 9 months of age (TC 63 ±10 and 70 ±7 mg/dL, respectively). To reverse hypercholesterolemia, Reverse mice were fed a high fat diet until 5 months of age, then injected with pi-pC and switched to a control diet, and studied at 9 months of age (TC 75 ±12 mg/dL). Atherosclerotic lesion area was measured in aortic sinus cross-sections and in en face preparations of the thoracic aorta. At both sites, lesion area was greater in the hypercholesterolemia group compared to the normocholesterolemia group (P < 0.05) and greater at 9 months compared with 5 months (P < 0.05). In the reversed group, aortic lesion area at 9 months was similar to the hypercholesterolemia group at 5 months, but greater than the hypercholesterolemia group at 9 months (P < 0.05), indicating that atherosclerosis did not progress between 5 and 9 months. Susceptibility to carotid arterial thrombosis was measured using a photochemical injury model. Thrombotic occlusion occurred more rapidly in the hypercholesterolemic group than in the normocholesterolemic group at both 5 months (P < 0.05) and 9 months (P < 0.05). Accelerated thrombosis was found toward normal or hypercholesterolemic atherosclerotic plaques (11:2 months; P < 0.05 vs. hypercholesterolemic group). Conclusions: Reversal of hypercholesterolemia normalizes thrombotic susceptibility in atherosclerotic mice.

An Orally Active Inhibitor of the TGF-β Type I Receptor, ALK5, Adversal Myofibrolipomaction, and Vascular Remodeling in the Rat Carotid Balloon Injury Model

Kai Fu, Michael J Corbly, Lihong Sun, Jessica Friedman, Feng Shan, James L Papadatos, Donald Costa, Frank Lutterodt, Harry Sweigard, James L Papadatos, Douglas D Heistad, Steven R Lentz; The Univ of Iowa, Iowa City, IA; Donald D Heistad, Steven R Lentz; The Univ of Iowa, Iowa City, IA

Objective: TGF-β antagonist antibodies, soluble receptor, antisense, decoy and gene therapy with negative regulatory Smad7 have established the importance of TGF-β in intimal thickening and luminal narrowing following vascular injury. This study evaluates the efficacy of a novel, small-molecule inhibitor of the TGF-β2, TGF-β3 and ALK4, kinase, in the carotid balloon injury model. Methods and Results: The small molecule, SM16, was shown to bind with high affinity to ALK5 kinase ATP binding site using a competitive binding assay and biacore analysis. SM16 blocked Smad2 phosphorylation and inhibited TGF-β-induced PAI-1/collagenase activity in cells. Global i.n. administration was demonstrated in a lipopolysaccharide induced model but SM16 also showed nanomolar inhibition of ALK4 and weak (micromolar) inhibition of Rat and p38. In the rat carotid injury model, SM16 dosed once daily orally at 15 or 30 mg/kg SM16 for 14 days caused significant inhibition of neointimal thickening, luminal narrowing and the induction of adventitial smooth muscle u-actin-positive myofibroblasts in the carotid artery. Conclusion: These results are the first to demonstrate the efficacy of an orally active,
an EIA kit (Cayman Chemicals). Total protein was assayed using the BCA method (Pierce). The results indicate that BOXes induce TX production in the brain, in the absence of thrombin and other blood components normally present in hemorrhagic stroke. This suggests a role for BOXes in other complications (than vasospasm) following hemorrhagic stroke such as edema, inflammation and immune responses. It may also hint at a putative role for therapeutic use of inhibitors of TX production, such as COX-2 inhibitors.

P261
5-Amino-4-imidazole Carboxamide Riboside Inhibits Tissue Factor Induction in Endothelial Cells and Monocytes
Weiyou Zhang, Yuqing Huo; Univ of Minnesota, Minneapolis, MN
AMP-activated protein kinase (AMPK) is tightly regulated by the ratio of intracellular AMP/ATP and plays a central role in regulation of energy homeostasis under metabolic stress. Recent studies link AMPK to an anti-inflammatory effect such as inhibition of cytokine production and adhesion molecule expression. In this study, we used 5-amino-4-imidazole carboxamide riboside (AICAR), one of AMPK activators to investigate whether AMPK plays a role in anti-thrombotic effect. Tissue factor (TF), a critical initiator of physiologic and pathologic coagulation, plays an important role in initiation and propagating thrombus formation in heart attack and stroke. In an in vitro assay, the clotting activity of TF in human umbilical vein endothelial cells (HUVECs) and peripheral blood mononuclear cells were induced by physiologic agonist LPS, TNF-a and IL-1b. Pretreatment of cells with AICAR inhibited clotting activity by >90%. Inhibition of TF clotting activity by AICAR appears dose and time-dependent. Suppression of TF clotting activity correlated a decrease of TF expression at protein and mRNA levels. ZM 241385, a specific adenine A2a receptor antagonist, did not block the effect of AICAR on TF suppression, indicating inhibition of TF by AICAR was not due to adenosine production and A2a occupancy. We are using AMPK knockout or knockdown cells in our study. The consequent results will provide information on the extent of AMPK dependency in AICAR-mediated TF inhibition.

P262
Nocturnal Oxygen Desaturation and the Prevalence of Metabolic Syndrome
Imran H Iftikhar, Fairview Hosp, Cleveland Clinic, Cleveland, OH; Mansoor Ahmed, Southwest Cleveland Sleep Ctr, Cleveland, OH; Robert P Blankfield; Case Sch of Medicine, Cleveland, OH
Objective: Many studies in the past have identified an association between obstructive sleep apnea (OSA) and the elements of MS. Recently some studies have found a direct association between the severity of OSA and the prevalence of MS. In a community subpopulation diagnosed with obstructive sleep apnea. Methods: A cross sectional retrospective analysis of the data of 419 patients was performed at a community sleep disorders diagnostic center. MS was considered to be present in patients if three out of four of the following criteria were met: hypertension, diabetes, hyperlipidemia and a body mass index of greater than 30. Nocturnal oxygen desaturation was considered to be significant if the oxygen concentration during the sleep study desaturated below 90%. Results: A total of 22 patients were identified with MS. The NCSS and PASS statistical software was used for the analysis. A correlation value of 0.93 and a p value of 0.0009 was found. Although the correlation value was not significant, the study found that in this subpopulation with OSA, in general, a higher percentage of total sleep time spent below 90% was accordingly associated with a higher number of cases with MS (graph 1). Conclusions: The study found a dose dependent association between the severity of nocturnal oxygen desaturation and the prevalence of MS. Whether or not the reversal of nocturnal oxygen desaturation through CPAP treatment corrects the syndrome needs to be tested in a large multi center prospective study.

P263
Hyperglycemia-Induced Cell Growth and Gene Expression via Serum Response Element Through Pkci, Rhoa, and Rho-Kinase in Vascular Smooth Muscle Cells
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The impressive correlation between cardiovascular disease and alterations in glucose metabolism has raised likelihood that atherosclerosis, heart failure and type 2 diabetes may share common antecedents. Postprandial hyperglycemia has been shown the important role for onset and development of heart failure and cerebral infarction by several large-scale clinical trials. Recently, chronic hyperglycemia has been reported to enhance the vasoconstrictor response by Rho kinase and PKC. Furthermore, oral PKCβ selective inhibitor has been reported to show the effective therapy for the cardiovascular complications of diabetes. We have reported Phenylephrine showed enhancement of vasoconstrictor response in a spontaneous diabetes mellitus model, OLETF, (Otsuka-Long-Evans-Tokushima fatty ) rat. However, the mechanism of hyperglycemia on these reactions, especially the influence to signal transduction pathway by hyperglycemia has not been well understood. Therefore, we examined the effect of hyperglycemia on cell growth and gene expression in rat aorta smooth-muscle cells (RASMcs). Hyperglycemia accelerated the growth of RASMcs with concentration dependent manner. Furthermore, c-fos gene expression was also increased by hyperglycemia. Phenyl- ephrine activated c-fos gene expression. Hyperglycemia augmented Phenylephrine-induced c-fos gene expression synergistically with dose dependent manner. The deletion analysis revealed c-fos serum response element (SRE) accounts for c-fos gene expression. PKC-β, RhoA, and Rho kinase were involved in this signal transduction pathway. Furthermore, PKCβ-activated c-fos SRE expression was inhibited by RhoA and Rho kinase. These results indicate RhoA and Rho-K are downstream molecules of protein kinase C ( PKC -β). A HMG-CoA reductase inhibitor, Piovastrin inhibited hyperglycemia-augmented these reactions by inhibi- tion of RhoA. Furthermore, catalytic domain mutant of PKC-β also inhibited these reactions. Hyperglycemia itself increased the cell growth and gene expression. Furthermore, it also modifies and augments the cell growth and gene expression by α1-AR-mediated stimulation. Statin and PKCβ inhibitor might be effective for hyperglycemia-induced cardiovascular dysfunction.

P264
Red Blood Cell Fatty Acid Composition and the Metabolic Syndrome: NHLBI GOLDN Study
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Different fatty acids may vary in their effect on the metabolic syndrome (MetS). We tested whether fatty acids measured in red blood cells (RBC) are associated with the MetS or its components. Men (n = 466, 49 ± 16 y) and women (n = 535, 49 ± 16 y) from 187 families from Utah and Minnesota were studied as part of the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study. Fatty acids in RBC were measured with gas chromatography while data on confounders were obtained from interviewer-administered questionnaires. The prevalence of the MetS as defined by the updated Adult Treatment Panel III criteria was 37.0% in Utah and 39.8% in Minnesota (P < 0.05). In a multivariate model that included four fatty acid components: polyunsaturated fatty acids (r = −0.11), monounsaturated fat (r = −0.10), saturated fatty acids (r = 0.05) , and polyunsaturated fat (r = 0.10) , polyunsaturated and saturated fatty acids were significantly associated with the MetS (Table 1). We observed significant (P < 0.05) correlations between fatty acid classes and components of the MetS (data not shown). Saturated fat (r = 0.10) , monounsaturated fat (r = −0.12) and polyunsaturated fat (r = −0.11) were significantly correlated (P < 0.05) with fasting insulin. In conclusion, polyunsaturated fatty acids were inversely associated with the MetS while saturated fatty acids were positively associated with the MetS probably through their effect on lipids, insulin and systolic blood pressure. These data suggest that RBC fatty acid profiles could be a good marker for the metabolic syndrome.

TABLE 1. ODDS RATIOS (95% CI) FOR FATTY ACID CLASSES AND THE METABOLIC SYNDROME

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>1.00</td>
<td>0.69 (0.46–1.06)</td>
<td>0.63 (0.40–0.99)</td>
<td>0.40 (0.24–0.65)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>1.00</td>
<td>1.23 (0.79–1.89)</td>
<td>1.44 (0.92–2.27)</td>
<td>1.62 (1.03–2.62)</td>
<td>0.04</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>1.00</td>
<td>0.78 (0.51–1.02)</td>
<td>0.72 (0.46–1.13)</td>
<td>0.81 (0.50–1.32)</td>
<td>0.39</td>
</tr>
<tr>
<td>Trans fatty acids</td>
<td>1.00</td>
<td>1.25 (0.83–1.90)</td>
<td>1.26 (0.82–1.94)</td>
<td>1.96 (0.59–1.56)</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Elevated Triglyceride with Low HDL-C and Risk of Coronary Heart Disease in Diabetics: The Strong Heart Study

Jennifer S. Lee, Univ of California Davis Med Cntr, Sacramento, CA; Barbara V Howard, MedStart Research Institute, Hyattsville, MD; Ying Zhang, Univ of Oklahoma Health Sciences Cntr, Oklahoma City, OK; Richard R Fabsitz; National Heart, Lung, and Blood Institute, Bethesda, MD

An elevated triglyceride (TG) and decreased high-density lipoprotein cholesterol (HDL-C) has been shown to be a characteristic dyslipidemia in insulin resistance and type 2 diabetes. We examined whether this dyslipidemia is a predictor of coronary heart disease (CHD) in type 2 diabetic patients. The Strong Heart Study is a large population-based prospective study of cardiovascular disease (CVD) in 13 American Indian communities in the U.S. The baseline visit (1989 to1992) consisted of a personal interview, exam, and lab tests, including serum fasting lipids. Diabetes status was determined based on World Health Organization recommendations. Incident CHD was identified through medical records or patient medical histories and confirmed by a committee of blinded CVD experts. A total of 2,029 participants aged 45—74 years had type 2 diabetes but no known CHD at baseline. Of these 455 participants were diagnosed with CHD over 14 years. Participants were categorized into four groups based on having comparable incidence rates according to their TG and HDL-C tertile levels: highest TG tertile and lowest HDL-C tertile ("High TG-low HDL-C"); highest TG tertile and mid-to-highest HDL-C tertile ("High TG"); lowest-or-mid TG tertile and lowest HDL-C tertile ("Low HDL"); and lowest-or-mid TG tertile and mid-or-highest HDL-C tertile (Referent). Univariate and multivariate (Cox proportional hazard models) methods were used to estimate the hazard ratio (HR) for incident CHD. Age-adjusted incidence of CHD (in events/1000 person-years), was 20.3 in the referent group, 24.7 in "Low HDL-C", 33.5 in "High TG", and 44.8 in "High TG-low HDL-C" (P=0.001). Compared to the referent group, the HR of CHD (95% confidence interval) were 1.24 (0.95, 1.62); 1.50 (1.19, 1.90); and 1.70 (1.38, 2.16) in the three groups, respectively, after adjustment for age, gender, smoking, hypertension, body mass index, and albumin to creatinine ratio. The HR for "High TG-low HDL-C" was attenuated but remained significant (1.54 (1.20, 1.97)) after additional adjustment for LDL cholesterol. The combination of elevated TG and decreased HDL-C is an independent predictor of CHD in type 2 diabetics. This dyslipidemia might be a useful marker to identify diabetics who are at increased risk of CHD.

Comparison Between Adiponectin and Leptin in Relation to Metabolic Syndrome in Japanese Women

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Background: Two representative adipokines, adiponectin, and leptin, have been suggested to play important roles in the pathogenesis of metabolic syndrome (MetS). However, little information exists on their respective roles in relation to MetS, particularly in women. Objective: To investigate the respective associations of adiponectin and leptin with each MetS component and its clustering. Methods: We studied 769 middle-aged Japanese women without a history of cancer or cardiovascular disease. Since it was less likely that changes in these adipokines cause an inflammatory state, we focused on the association of adiponectin or leptin with 4 MetS components other than central obesity. Thus, we computed odds ratios (ORs) in favor of the presence of each MetS component or its clustering (>2 components) according to a 1-SD decrease in log-adiponectin and a 1-SD increase in log-leptin with multivariate logistic regression analyses, in which both adipokines were simultaneously entered as explanatory variables. Results: Interestingly, adiponectin had a closer association than leptin with low high-density lipoprotein cholesterol (HDL-C) and hyperglycemia (Table). In contrast, the association with elevated blood pressure was significant only for leptin. Hypertriglyceridemia was significantly associated with both adipokines. As a result, both adipokines were independently and significantly associated with the clustering of MetS components. Conclusion: Although both adiponectin and leptin were independently associated with MetS, their associations with each MetS component were distinctive. Specifically, decreased adiponectin seemed to play a greater role in the development of HDL-C and the elevation of glucose, so did increased leptin in the elevation of blood pressure. These data may have important implications both for inferring the etiological role of these adipokines in causing MetS and for introducing them as targets for MetS treatment.

Elevated Triglyceride with Low HDL-C and Risk of Coronary Heart Disease in Diabetics: The Strong Heart Study

Jennifer S. Lee, Univ of California Davis Med Cntr, Sacramento, CA; Barbara V Howard, MedStart Research Institute, Hyattsville, MD; Ying Zhang, Univ of Oklahoma Health Sciences Cntr, Oklahoma City, OK; Richard R Fabsitz; National Heart, Lung, and Blood Institute, Bethesda, MD

An elevated triglyceride (TG) and decreased high-density lipoprotein cholesterol (HDL-C) has been shown to be a characteristic dyslipidemia in insulin resistance and type 2 diabetes. We examined whether this dyslipidemia is a predictor of coronary heart disease (CHD) in type 2 diabetic patients. The Strong Heart Study is a large population-based prospective study of cardiovascular disease (CVD) in 13 American Indian communities in the U.S. The baseline visit (1989 to1992) consisted of a personal interview, exam, and lab tests, including serum fasting lipids. Diabetes status was determined based on World Health Organization recommendations. Incident CHD was identified through medical records or patient medical histories and confirmed by a committee of blinded CVD experts. A total of 2,029 participants aged 45—74 years had type 2 diabetes but no known CHD at baseline. Of these 455 participants were diagnosed with CHD over 14 years. Participants were categorized into four groups based on having comparable incidence rates according to their TG and HDL-C tertile levels: highest TG tertile and lowest HDL-C tertile ("High TG-low HDL-C"); highest TG tertile and mid-to-highest HDL-C tertile ("High TG"); lowest-or-mid TG tertile and lowest HDL-C tertile ("Low HDL"); and lowest-or-mid TG tertile and mid-or-highest HDL-C tertile (Referent). Univariate and multivariate (Cox proportional hazard models) methods were used to estimate the hazard ratio (HR) for incident CHD. Age-adjusted incidence of CHD (in events/1000 person-years), was 20.3 in the referent group, 24.7 in "Low HDL-C", 33.5 in "High TG", and 44.8 in "High TG-low HDL-C" (P=0.001). Compared to the referent group, the HR of CHD (95% confidence interval) were 1.24 (0.95, 1.62); 1.50 (1.19, 1.90); and 1.70 (1.38, 2.16) in the three groups, respectively, after adjustment for age, gender, smoking, hypertension, body mass index, and albumin to creatinine ratio. The HR for "High TG-low HDL-C" was attenuated but remained significant (1.54 (1.20, 1.97)) after additional adjustment for LDL cholesterol. The combination of elevated TG and decreased HDL-C is an independent predictor of CHD in type 2 diabetics. This dyslipidemia might be a useful marker to identify diabetics who are at increased risk of CHD.

Delineating the Relationship Among the Components of the Metabolic Syndrome: Insight from the National Health and Nutrition Examination Survey, 1989—2002

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Background: The pathogenesis of the metabolic syndrome (MS) involves complex interactions among the main components of MS. To analyze all these components as a whole, we constructed a structural equation model (SEM) to elucidate a pathway in which obesity might be the most important early step in the etiological cascade leading to full MS. Results: The results of SEM demonstrated that obesity plays the central role in the development of insulin resistance and dyslipidemia through the mediation of a proinflammatory state. Obesity also directly leads to higher blood pressure. The SEM has provided a comprehensive view to illustrate the complex inter-play of these main components in the development of the MS.

Thiazolidinediones Attenuate the Angiotensin II-mediated Enhanced Vascular Responses in High Fat Diet—Fed Rats by Altering the Characteristics of L-type Calcium Channels

Bhoomi Viswanad, Poduri Ramarao; National Institute of Pharmaceutical Education and Research, Mohali, India

Background/rationale—Insulin resistance has emerged as a mechanism leading to diabetes mellitus and hypertension. However, insulin sensitizers such as pioglitazone and rosiglitazone, [peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists] are reported to reduce blood pressure (BP) in hypertensive models by altering L-type calcium channel functions. Objective/ Hypothesis: To establish the cause and effect relationship of insulin resistance and vasculopathy and find the role of L-type calcium channels. Methods: Ang II-induced contractions were studied isometrically in thoracic aortic rings isolated from control and high fat diet (HFD) fed rats. To evaluate the involvement of L-type calcium channels in Ang II mediated contraction, cumulative concentration response curves (CRC) to Ang II was constructed in the presence of various concentrations (0.01 nM—1μM) of nifedipine (dihydropyridine-sensitive L-type calcium channel blocker) and the log IC50 was estimated. Receptor radioligand binding studies for Ang II receptors and L-type calcium channels were carried by [H3]—Ang II and [H3]—PM-200110, respectively. Results: The rats fed with HFD for four weeks exhibited the conglomeration of characteristic features of insulin resistance syndrome, such as obesity, hyperinsulinemia, mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia, glucose intolerance and hypertension. Maximal contractile response (E0) to Ang II was increased in HFD fed rats as compared to control rats. In addition, Bmax values and affinity of Ang II receptors and L-type calcium channels are increased, respectively. Nifedipine dose-dependently blocked the Ang II-induced contractions in a noncompetitive manner and its log IC50 was significantly lower in aortic rings from HFD fed rats (8.87 ± 0.17) compared (P<0.05) to control (9.78 ± 0.18) rats. Rosiglitazone and pioglitazone treatment for 14 days restored log IC50 values (P<0.05) comparable to that of control (0.88 ± 0.26 & 9.82 ± 0.34, respectively). Conclusions: Thiazolidinediones attenuate the development of hypertension and improves the vascular dysfunction induced by Ang II by altering the L-type calcium channel functions in HFD fed rats.
Carboxyl Ester Lipase Deficiency Exacerbates Dietary Lipid Absorption Abnormalities and Resistance to Diet-Induced Obesity in Pancreatic Triglyceride Lipase Knockout Mice

Dean Gilham, Eric D Labonte, Juan C Hojas, Ronald J Jandacek, Philip N Howle, David Y Hui, Univ of Cincinnati, Cincinnati, OH

Pancreatic triglyceride lipase (TGL) is generally accepted as the principal enzyme involved in the hydrolysis of dietary fat, thereby mediating its absorption. However, trisglycerol (TAG) absorption was minimally altered in TGL−/− mice. This study tested the hypothesis that the compensatory enzyme in TGL hydrolysis and absorption in TGL−/− mice is carboxyl ester lipase (CEL). TGL/CEL−/− double knockout mice were generated via crossbreeding. The ability of the wild type, TGL−/−, and TGL/CEL−/− mice to take up dietary lipids and to attain body weight on a high fat diet was assessed. Net TAG absorption was reduced from 81.5 ± 0.7% in wild type mice to 80.1 ± 3.7% in TGL/CEL−/− mice (p < 0.05). Strikingly, this was reduced to 61.1 ± 3.8% in TGL/CEL−/− mice (p < 0.01). Defective free cholesterol absorption reported previously in TGL/CEL−/− mice was confirmed in this study. Cholesterol absorption in TGL/CEL−/− mice was 41% less than wild type mice (p < 0.05), but this effect was not observed in TGL−/−, CEL−/−, or CEL/CEL−/− mice (p > 0.05). Additionally, absorption of retinyl palmitate from the intestinal tract to the plasma was reduced by 45% and 60% in TGL/CEL−/− mice (p < 0.05) and TGL/CEL−/− mice (p < 0.01) respectively. On a high fat diet, food intake was not different between mice with the various genotypes. Somewhat, at 15 weeks of the high fat diet, body weight of CEL−/− mice increased in wild type mice, but TGL−/− and TGL/CEL−/− mice weighed an average of 6.2 g and 8.6 g less respectively (p < 0.01). Body composition analysis showed that both TGL−/− and TGL/CEL−/− mice consumed the high fat diet carry less fat and more lean mass than wild type mice. In summary, we show that TGL and CEL together are responsible for a major portion of dietary fat and fat-soluble vitamin absorption. Our results support free cholesterol absorption requiring efficient TGL hydrolysis in the proximal gut. Although the residual 50–60% of dietary TAG absorption in TGL/CEL−/− mice suggests an additional TAG lipase exists in the gut, deficiency of both TGL and CEL confers protection against diet-induced obesity. Thus specific inhibition of TGL in a select manner may be a therapeutic approach against diet-induced obesity without fat-soluble vitamin deficiency or steatorrhea associated with total inhibition of lipolytic enzymes in the intestinal tract.

Intestinal Cholesterol Uptake in SR-B1 and Niemann-Pick C1 Like 1 (NPC1L1) Knockout Mice

Sean M Lally, Lizzie M Hoos, Maureen Maquire, Glen T Tetzloff, Li-jii Zhu, Harry R Davis, Scott W Altman; Schering Plough Resch Institute, Kenilworth, NJ

Niemann Pick C1 Like1 (NPC1L1) is a sterol transporter localized in jejunal enterocytes and is critical for intestinal cholesterol uptake and absorption. Scavenger Receptor-B1 (SR-B1) is also localized in jejunal enterocytes and has been proposed to play a role in cholesterol absorption. We determined if deficiency of SR-B1 and NPC1L1 results in additional effects on cholesterol absorption and whole-body cholesterol homeostasis using knockout mouse models. Plasma cholesterol levels (mg/dL) were measured in 4 animal groups: 132 in wild type (WT), 103 in NPC1L1−/−, 223 in SR-B1−/− and 213 in SR-B1/NPC1L1−/− chow-fed mice. Increases in plasma cholesterol levels in SR-B1−/− and SR-B1/NPC1L1−/− groups occurred in HDL-C. Hepatic cholesterol levels were not different among the groups. Cholesterol absorption (fetal diet isotope) was 55% WT, 5.7% (±0.8) NPC1L1−/−, 50.3% SR-B1−/−, and 4.2% (±2.3) SR-B1/NPC1L1−/−. Acute 5 h cholesterol absorption was reduced 82% in both NPC1L1−/− and SR-B1/NPC1L1−/− groups compared to WT mice (p < 0.01). Intestinal uptake was reduced approximately 50% in both NPC1L1−/− and SR-B1/NPC1L1−/− mice indicating that SR-B1 deficiency causes no statistically significant additional effects on cholesterol uptake and absorption beyond the reductions in NPC1L1−/− mice. Mice fed a cholesteryl/cholate diet were evaluated. Hepatic cholesterol levels for SR-B1−/− mice on diet showed a 3-fold increase compared to wild type mice. There was a 2.5 fold reduction in cholesterol ester levels in the SR-B1/NPC1L1−/− compared to wild type animals, with no change when compared with the chow diet. NPC1L1−/− mice allowed no difference between chow and cholesteryl fed diets. To better understand the changes in cholesterol levels observed in response to diet, mRNA expression levels of pertinent cholesterol metabolism genes were measured in the intestine and the liver. From these results, we found a high importance in NPC1L1 levels in the intestine and the liver. From these results, we found a high importance in NPC1L1 levels in the intestine and the liver. From these results, we found a high importance in NPC1L1 levels in the intestine and the liver. From these results, we found a high importance in NPC1L1 levels in the intestine and the liver. From these results, we found a high importance in NPC1L1 levels in the intestine and the liver.
cholesterol efflux is due to reduced ABCG1 expression. However, we found no changes in ABCG1 mRNA expression in these macrophages by 12/15LO products, suggesting that the decrease in ABCG1 protein observed by 12/15LO activity is not caused by transcriptional changes in ABCG1 mRNA. Thus, we hypothesized that ABCG1 is regulated post-translationally by 12/15LO products. We examined ABCG1 phosphorylation by immunoprecipitation (IP), IP studies revealed increased threonine and tyrosine phosphorylation of ABCG1 following treatment with 12SHE-TOH (500 μM, 24 hours) or in P/Ox-86 12/15LO-expressing cells. This protein phosphorylation may lead to degradation of ABCG1, thereby increasing macrophage foam cell formation via reducing cholesterol efflux from the macrophage. Thus, these data provide a strong evidence for a novel role of 12/15LO in promoting foam cell formation. Understanding the role of ABCG1 in macrophages will aid in developing beneficial therapies that target genes involved in cholesterol metabolism.

P278
The Role of ES-4 as a Neutral Cholesterol Ester Hydrolase in Hepatic Cells
Saj Parathath, Snezana Dogan, NYU Med Sch, New York, NY; Earl H Harrison, Ohio State Univ, Columbus, OH; Edward A Fisher; NYU Med Sch, New York, NY

The ability of cells to control cholesterol levels is a balance between the de novo synthesis and uptake of cholesterol versus secretion and efflux of cholesterol. Excess cholesterol stored as can be toxic and is stored as cholesterol esters (CE), can supply free cholesterol (FC) to be utilized for synthesis of steroids, bile acids or for efflux from the cell, through the action of non-liposomal CE hydrolases. These hydrolysis have been given the general name neutral cholesterol ester hydrolase (NCEH). Our data indicate that the protein esterase 4 (ES-4) is a strong candidate as a rat hepatic NCEH. We have determined in rat hepatic cells the effects of modulating ES-4 levels using siRNA and overexpression, on total cholesterol, CE and FC levels and FC efflux. Rat hepatoma McA cells overexpressing ES-4 showed decreased CE levels compared to control cells indicating that ES-4 can hydrolyze CE in intact cells. Also in cells where ES-4 levels were suppressed by 75% by siRNA, there was increased (by 50%) CE levels. To determine whether the change in CE was due to ES-4 mediated hydrolysis, we used radiolabeled H-Cholesterol in McA cells with varying ES-4 levels. The results showed that in ES-4-overexpressing cells, the ratio of CE/FC was 1:1, whereas in ES-4 knockdown cells, the ratio was increased to 4:1. Also in cells with suppressed ES-4 levels showed reduced efflux of FC to 10% serum; cells overexpressing ES-4 had increased efflux. These results strongly indicate that ES-4 is a NCEH in hepatic cells. Currently we are evaluating the role of ES-4 in cholesterol metabolism using primary rat hepatocytes.

P279
Identification of a Novel Lipid Efflux Defect That Is Not Due to Mutations in the ABCA1 Gene but to Regulation of ABCA1 Protein
Shirya Rashid, Michel Marcil, Isabelle Ruel, Jacques Genest; McGill Univ, Montreal, Canada

Currently, HDL deficiency due to genetic causes is attributed to mutations in three genes - LCAT, Apo A1 and ABCA1. These genes, however, do not account for the majority of cases of low HDL. We have identified 42 French-Canadian subjects with severe HDL deficiency (<5th percentile of the population) in whom mutations in LCAT, Apo A1 and ABCA1 have been excluded. Candidate gene sequencing showed putative SNPs in ABCA1 gene (P143R and R587H). To further identify individuals in whom the low HDL phenotype is due to defective HDL synthesis, cellular lipid efflux assays were performed in skin fibroblasts from the subjects. The fibroblasts were loaded with free cholesterol and incubated with lipid-free apoA1 (24 h for each) to stimulate cellular lipid efflux. The assay results indicated two probands in whom both cholesterol and 22OH/RA stimulation in normal control cells but in patient cells only 22OH/RA stimulation (20ug/mL). However, stimulation with 22-hydroxycholesterol/9 cis retinoic acid (20ug/mL) resulted in increased cholesterol efflux. The specific effect of 12/15-LO. To investigate the cause of the efflux defect in the probands, we utilized real time RT-PCR and Western blot approaches. We investigated the expression of ABCA1 mRNA (using RTPCR) in response to both cholesterol stimulation and 22OH/RA stimulation (dose-response and kinetics). We observed no difference in ABCA1 mRNA expression between normal control and patient fibroblasts in response to either cholesterol or 22OH/RA stimulation. Interestingly, Western blot results showed that ABCA1 protein expression is upregulated in response to both cholesterol and 22OH/RA stimulation in normal control cells but in patient cells only 22OH/RA stimulation resulted in increased ABCA1 protein expression. This identifies a new lipid efflux defect that is not due to mutations in the ABCA1 gene but rather in ABCA1 gene regulation. Also, binding studies were carried out showing that apoA1 binding to ABCA1 in patient cells is significantly increased above normal levels with 22OH/RA stimulation, indicating a possible compensatory effect in lipid binding and efflux with 22OH/RA. Thus, overall, we have identified a new mechanism leading to impaired ABCA1 mediated lipid efflux in humans.

P280
Human StarD4: Localization and Sterol-transport Characteristics of a STAR-related Lipid Transfer Protein
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StarD4 mRNA has been identified in heart, liver, lung, and kidney, and is a presumed intracellular sterol transport protein based on its STAR domain. Objective: Characterize this novel STAR-like domain protein by determining its localization and its ability to transport sterols. Methods: Human StarD4 was His-tag purified and a StarD4 polyclonal antibody generated for Western analysis and immunocytochemistry. Results: Binding assays showed recombinant StarD4 was selective in its binding to cholesterol in a molar ratio of 1:1, but was unable to bind any of the other tested sterols. As we have previously observed with StarD1 and StarD5, Western analysis detected high levels of StarD4 protein in human monocytes/macrophages. Despite prior detection of high mRNA levels within liver tissue, StarD4, like StarD5, was not found in freshly isolated or cultured human hepatocytes. Immunocytochemistry localized StarD4 intracellularly to the macrophage cytoplasm. Cellular fractionation confirmed cytosolic localization of full length (246kDa) StarD4 with a smaller (18kDa) StarD4 degradation band also detected in the mitochondrial fraction. To corroborate cholesterol binding observed with recombinant StarD4, fluorescent labeled cholesterol esters was utilized and it was found that StarD4 was looked for following StarD4 expression in hepatocytes, i.e. cells which do not express detectable StarD4. Interestingly, StarD4 overexpression led to a ~5-fold increase in bile acid synthesis via the CYP27A1 initiated mitochondrial pathway and an increase in cholesterol ester formation. Identification of a cholesterol transporter was also achieved in the absence of any cholesterol efflux across membranes. Conclusion: The presence of StarD4, previously described as ubiquitous to steroid metabolizing tissues, can at this time be ascribed to its localization in tissue macrophages. Furthermore, its selective binding of cholesterol and its ability to mobilize transport cholesterol suggest StarD4 to be an important intracellular sterol transporter in macrophages. Accumulating evidence suggests that StarD1, StarD5, and StarD4 transport and target cholesterol in a selective fashion important to the maintenance of macrophage cholesterol homeostasis.

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A Novel Endothelium-Dependent Organelle Abundantly Present in Cholesterol-Rich Human Macrophages and Expresses Elevated Acyl-Coa: Cholesterol Acytranferase 1 Enzyme Activity
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Under hypopigidemic condition, aortic macrophages continue to internalize modified low-density lipoprotein (LDL), and are transformed into cholesterol-ester-rich foam cells. The modified LDL liberates free cholesterol, which is converted to the enzyme acyty coenzyme A: cholesterol acytranferase (ACAT1). These events occur during early stages of athrogenesis. Using immunoelectron microscopy, we had previously showed that, in human monocyte-derived macrophages grown under normal lipidemic condition, ACAT1 is mainly localized in the endoplasmic reticulum (ER); however, when these cells are overloaded with modified LDL, a significant portion of the ACAT1 signal is present in small, ER derived vesicles. To further pursue this finding, in the current work, we prepared postnuclear cell homogenates from the THP-1 macrophages, and subjected them to subcellular fractionation using Opti-prep ultracentrifugation. Each subcellular fraction was analyzed for ACAT1 protein content and for ACAT1 enzyme activity in vitro. The results showed that, under hypopigidemic condition, the ACAT1 protein is mainly distributed among the middle density fractions characteristic of the ER; the ACAT1 enzyme activity in vitro in each fraction is low. After treating the cells with aggregated LDL, a significant portion of the total ACAT1 protein emerge in a low buoyant density fraction; the ACAT1 enzyme activity in this fraction is much higher than those present in the normal ER fractions. Further purifications by using differential centrifugation and immunosupression procedures disclosed that the low-density, ACAT1 activity rich fraction possesses markers for both the ER and for the trans-Golgi network, but are devoid of the marker for the plasma membrane. Confocal microscopic analysis suggested that, in cells treated with aggregated LDL, the ACAT1 signal significantly colocalizes with the trans-Golgi network signal. Overall, these data suggest that cholesterol loading of macrophages induces the formation of a novel cellular compartment derived from ER, and is rich in cholesterol content. ACAT1 residing in such compartment can efficiently esterifies cholesterol, thus preventing excessive build up of free cholesterol in the ER.
ACE2 Expression in Adipose Tissue Is Regulated by High-fat Diets but Not by Angiotensin II

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**Objectives**: Angiotensin converting enzyme type-2 (ACE2) is a monooxygenase which cleaves both angiotensin (Ang) I and II to produce Ang-I and -II. Recent data suggest that ACE2 functionsally controls the renin-angiotensin system (RAS), metabolizing Ang and regulating blood pressure. ACE2 mRNA is abundantly expressed in mouse adipose tissue, and increases markedly during development of STZ-LDLs. In kidney and aorta, ACE2 is regulated by Ang, ACE inhibitors or AT1 receptor (AT1R) antagonists. The purpose of this study is to define mechanisms for regulation of adipocyte ACE2, focusing on components of the RAS, or high fat (HF) feeding. **Methods and Results**: We examined the effect of Ang, losartan, or an AT2 receptor antagonist on ACE2 mRNA abundance in differentiating 3T3-L1 adipocytes. While ACE2 mRNA increased markedly during adipocyte differentiation, there was no effect of treatments. In adipose tissue from female AT1aR deficient mice, ACE2 mRNA abundance was not altered. To determine the effect of high fat (HF) feeding on adipose ACE2 expression, C57BL/6 mice were fed either normal or high-fat (40% kcal as fat) diets (1 week) or chronically (20 weeks). Female AT1aR-/- mice were also fed normal or HF diets for 20 weeks. ACE2 mRNA expression and activity in adipose tissue were increased within 1 week of HF feeding in C57BL/6 mice. At 20 weeks, body weight (BW) was increased in HF-fed mice, and adipose tissue weight was markedly increased (45% vs 10% of BW, respectively). BF feeding (15% vs 6% of BW, respectively). **Conclusion**: These results demonstrate that adipose ACE2 expression is not regulated the RAS. In contrast, HF feeding markedly increased adipose ACE2. Elevations in adipose expression of ACE2 by HF feeding were accompanied by a reduction in blood pressure. Regulation of ACE2 by specific types of fatty acids may serve as a protective mechanism against obesity-induced cardiovascular disease.

An Insertion Mutation in the Promoter Increased Myosin Light Chain Kinase Expression in Hypertension

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**Introduction**: Blood pressure is a strong genetic trait that is determined by quantitative trait loci of an individual. Many candidate genes responsible for regulating blood pressure have been identified. However, relatively few studies have focused on the genes for smooth muscle contractile proteins despite the fact that smooth muscle contractility and growth are key contributors to vascular function and resistance. Smooth muscle contraction is regulated by the actin-myosin II interaction, which in turn is regulated by phosphorylation and dephosphorylation of regulatory myosin light chain (RLC). The RLC phosphorylation is catalyzed primarily by smooth muscle myosin light chain kinase (smMLCK). Therefore, changes in the expression of smMLCK could elicit changes in blood pressure. **Hypothesis**: We hypothesized that a genetic mutation in the smMLCK promoter could alter smMLCK expression and contribute to the development of high blood pressure. **Methods and Results**: We investigated the regulation of smMLCK expression using spontaneously hypertensive rats (SHR) as an experimental model. Expression of smMLCK in arteries increases during the development of high blood pressure and is always greater in blood vessels from SHR compared to normotensive rats. Analysis of the DNA sequences of the promoters isolated from SHR and normotensive rats revealed that SHR contain a 12 bp insertion. This insertion consists of 6 pairs of CT repeats and is associated with the disease. **Conclusion**: These data provide novel insights into the genetic factors that increase blood pressure and demonstrate the importance of smMLCK expression in the development of hypertension in SHR. These animal studies lay the foundation for studies on human hypertension and possibly provide new insights into diagnosis and treatment of hypertension in humans.

Apolipoprotein B Gene Mutations and Fatty Liver Disease in Japanese Hypobetalipoproteinemia

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**Background**: Familial hypobetalipoproteinemia (FHBL) is an autosomal dominant hereditary disease characterized by decreased plasma levels of low-density lipoprotein cholesterol (LDL-C). FHBL is considered as genetically heterogeneous, and the best-characterized cases are due to mutations of the apolipoprotein B (apoB) gene. Several forms of apoB have been identified in FHBL. In some of these cases, the apoB protein is produced that contains a novel truncation termed apoB-13.7. The apoB-13.7 homolog is asymptomatic despite her extremely low levels of LDL-C (13 mg/dL). Moreover, fat-soluble vitamin levels are normal, possibly due to spared secretion of apoB-48 and increased levels of high-density lipoprotein cholesterol (89 mg/dL). NFALD was observed in 7 of the 14 subjects, including both patients with apoB-82 and apoB-13.7. The parents of the apoB-82 homozygote, also, had fatty liver. We performed liver biopsy in the apoB-13.7 patient, who was diagnosed as severe fatty liver by computed tomography. The liver showed simple steatosis but no diagnostic evidence of NASH. **Conclusions**: Our results demonstrate that apoB gene mutations might not be rare and that NFALD might be frequent in Japanese hypobetalipoproteinemia.

Insulin Inhibits Inflammation in Endothelial Mice in a Phosphatidylinositol 3-Kinase-Dependent Manner

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**Abstract**: Insulin reduces inflammation and morbidity in critically ill patients when used to control pro-inflammatory hyperglycemia. Recent studies indicate that insulin may have glucose-independent anti-inflammatory effects in endotoxemia models. To date, the mechanism by which insulin reduces inflammation has not been elucidated. Insulin is a well known activator of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway. We hypothesized that the PI3K/Akt pathway mediates the protective effects of insulin in endotoxemia models. Using a non-hyperglycemic mouse model of endotoxemia, we continuously administered a very low dose of insulin that did not alter glucose levels. We found that insulin decreased plasma levels of IL-6, TNFα, and sICAM-1, and decreased morbidity and mortality in animals that received either insulin or placebo. Furthermore, the effects of protective effects are mediated by activation of the PI3K/Akt pathway. Using a non-hyperglycemic mouse model of endotoxemia, we continuously administered a very low dose of insulin that did not alter glucose levels. We found that insulin decreased plasma levels of IL-6, TNFα, and sICAM-1, and decreased morbidity and mortality in animals that received either insulin or placebo. Furthermore, the effects of protective effects are mediated by activation of the PI3K/Akt pathway. A significant proportion of patients with acute coronary syndrome (ACS) showed that TF plasma levels, monocyte- and platelet-associated TF are higher than in stable angina (SA) patients. Recently, an alternative spliced form of TF (asTF) has been discovered, which is soluble, circulates in the blood and exhibits procoagulant activity. **Purpose**: To examine TF and asTF mRNA expression in lymphocytes and platelets of patients with acute coronary syndromes (ACS), SA and in control subjects. **Methods**: We studied 16 patients with ACS, 14 patients with SA and 12 healthy subjects. The three groups were matched for age, gender and other clinical variables. Total RNA was extracted from peripheral lymphocytes and from washed platelets free of leukocyte contamination and full length TF as well as asTF mRNA levels were assessed by RT-PCR and real time PCR. **Results**: TF mRNA expression in resting lymphocytes was barely detectable in all subjects. Conversely, a consistent expression of asTF mRNA levels was observed in ACS (rel. exp: 0.38 vs 0.06, p<0.05) compared to SA patients (rel. exp: 0.19 vs 0.04) and controls (rel. exp: 0.12 vs 0.05). In vitro lipopolysaccharide stimulation of lymphocytes upregulated TF mRNA expression in all samples with the highest induction observed in ACS patients (TF rel. exp. vs unstimulated sample: 10.14 ± 24.3 in ACS, 5.78 ± 5.2 in SA and 268 ± 3.4 in control subjects; p<0.05 vs control). By contrast, the asTF induction by lipopolysaccharide was similar in ACS and SA patients and in control subjects (asTF rel. exp. vs unstimulated samples: 38.3 ± 13 in ACS, 54.8 ± 6.7 in SA and 56.7 ± 9.4 in control subjects). Platelet associated TF mRNA levels were significantly higher in ACS patients (rel. exp: 3.11 ± 0.51, p<0.05) compared to SA (rel. exp: 2.5 ± 0.87) and control subjects (rel. exp: 0.7 ± 0.1). No asTF mRNA was detectable in any platelet sample. **Conclusion**: Our results indicate that both TF and asTF mRNAs are detectable in ACS patients with acute coronary syndrome. The presence of asTF mRNA in lymphocytes and platelets of ACS patients can contribute to the hypercoagulability associated with the disease. **Acute Aortic Dissection as an Inflammatory Disease: Acute Phase Protein and Cytokine Changes and Prognostic Implications**

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**Introduction**: Inflammation has been implicated in the pathogenesis of cardiovascular diseases. The role of inflammation in acute aortic dissection, however, has not been thoroughly investigated. **Hypothesis**: We hypothesized that acute aortic dissection (AAD) patients would have characteristic acute phase protein and cytokine responses compared to stable angina (SA) patients. **Methods**: From Jan. 2001 to July 2003, 32 patients presenting with acute aortic dissection to a tertiary medical center were included. Eight patients with complete type A with aortic dissection and six patients with type B aortic dissection comprised the control group. **Results**: The serum was sampled on arrival of the emergency department for measuring high-sensitivity C
reactive protein (hs-CRP), interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α). Comparisons were made between aortic dissection and the control groups, patients with Marfan syndrome and those without, and patients with and without leakage/rupture. The correlations with the survival outcome were also evaluated. Results: Compared to the control, the patients with aortic dissection had significantly higher hs-CRP (1.98 ± 0.66 vs. 0.17 ± 0.11 mg/dl, p < 0.001) and IL-6 (25.26 ± 16.92 vs. 3.1 ± 0.67 mg/dl, p < 0.001). The level in patients with Marfan syndrome was even higher (hs-CRP: 2.26 ± 2.89 mg/dl; IL-6: 30.40 ± 15.36 mg/dl, P < 0.05 and 0.001 vs. control). For Marfan syndrome, only IL-6 was significantly higher than control (12.23 ± 13.94 pg/ml, p < 0.05) and the extent was smaller. The hs-CRP level was significantly different from the control. In patients with evidence of leakage/rupture, the IL-6 was higher than those without (33.04 ± 16.02 vs. 21.55 ± 18.85 pg/ml, p < 0.05). No significant differences were noted in IL-1β and TNF-α. For prognosis, the patients who failed to survive had significantly lower hs-CRP than those who survived (1.72 ± 1.18 vs. 2.33 ± 2.68 mg/dl, p < 0.05). The level was significantly different from the control. Conclusion: Acute aortic dissection is associated with increased IL-6 and hs-CRP. IL-6 may implicate different pathogenic processes and the chance of leakage/rupture. Poor initial hs-CRP response, on the other hand, is correlated with poor survival prognosis.

PKD, a Novel Component in Lysophosphatidylcholine-triggered Signaling Pathway, Controls Egr-1 Expression in Monocytic Cells

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Monocyte activation is an important early event in the development of atherosclerosis and other inflammatory diseases. The nature of monocyte activation is not completely understood. We report here that lysophosphatidylcholine (lysoPC), a prominent component of oxidized low density lipoprotein and arterial material alterations, induces rapid and marked decreases in PKD (PKD) activation in monochylic THP-1 cells. Our data also reveal that PKD activation is required for the activation of both ERK and p38 MAPK. Activation of ERK MAPK, but not p38 MAPK, controls the expression of the early response gene (Egr)-1, which has been reported to be a key pro-inflammatory and anti-apoptotic factor. Our data suggest that PKD activation is critical for the expression of Egr-1. In multiple approaches, including siRNA silencing and dominant negative mutants, we conclude that lysoPC-induced PKD2 activation is required for Egr-1 expression in monocytic THP-1 cells. Our results suggest a role for PKD in the development of atherosclerosis.

MCP-1/CCR2 Involves AT1 Receptor Antagonist Induced Anti-inflammation via PI3K/Akt in SHR

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To understand what is the mechanism involve in the AT1A-induced anti-inflammation, we investigate whether AT1A has the effect on anti-inflammation of monocytes/macrophages. Plasminogen (Plg) facilitates improving the cardiovascular remodeling, especially in high-doses. MCP-1/CCR2 modulates the AT1A-induced anti-inflammation in SHR through PI3K/Akt signaling pathway.

Histone H2B as a Functionally Important Plasminogen Receptor on Macrophages

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Active inflammation has been identified as a defining feature of vulnerable atherosclerosis plaques which rupture and lead to arterial thrombosis and cardiovascular events, such as myocardial infarction, stroke and peripheral occlusive disease. Plasminogen (Pig) facilitates macrophage migration to sites of inflammation and injury. The cellular functions of PIG depend upon its binding to receptors (Pig-Rs), which facilitate its activation to plasmin (Pim), protect Pim from inhibition and harness proteolytic activity to the cell surface. However, the particular Pig receptors (Pig-Rs) that are involved in Pig-mediated macrophage migration during inflammation are not well defined. We have investigated the expression of three previously characterized pig receptors, AT1A, αVβ3, and αVR-1(II), on the surface of two mouse macrophage cell lines (RAW264.7 and J774.A1) and on thiglycolate (TG) induced mouse peritoneal macrophages. In addition, we have also characterized surface expression and function of histone H2B (H2B), a newly identified Pig-R on these cells. Using Fab fragments of anti-H2B, antibody blocking and anti-αVβ3 antibody blocking experiments, we have shown that both AT1A and PIG, the two major receptors, have proteins, we have shown that all of these receptors were contributed to Pig binding to the macrophage cell lines, with H2B playing a particularly prominent role. On TG-induced macrophages, H2B contributed ~45–50% of the Pig binding capacity, whereas αV- integrin, annexin II and p11, the other two receptors, contributed ~<25%. In an in vitro matrigel invasion assay, a function of H2B in the cellular response could also be demonstrated. Interestingly, treatment of mice with anti-H2B Fab led to a marked reduction in macrophage recruitment (~50%) towards TG, whereas Fab to another Plg-R or nonimmune Fab had limited effects. Taken together, these data suggest that multiple Pig-Rs contribute to Pig binding to macrophages, and among these, H2B plays a very prominent role.
Cloning of Human Antibodies to Oxidation-specific Epitopes from Both Innate and Adaptive Immunity
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Oxidation-specific epitopes are present on oxidized LDL (oxLDL), on cells undergoing apoptosis, and in atherosclerotic lesions. Mice have natural germine IgM antibodies (Ab) that recognize oxidation-specific epitopes. These Abs are part of innate immunity and exert important biological functions in atherosclerosis such as ability to bind to apoptotic cells, inhibit uptake of oxLDL and apoptotic cells by macrophages, and confer atheroprotection in mice. Ab titers to oxidation-specific epitopes are found in human adult and umbilical cord blood (uCB) plasma and in atherosclerotic lesions, but it is not known if these Abs are germine. Also, the nature and role of Abs in general to oxidation-specific epitopes in human atherosclerosis is poorly understood. Human uCB contains significant IgM Abs titers to oxidation-specific epitopes. These IgM Abs represent a naive immune repertoire without exposure to exogenous antigens and lack the usual germinal epitopes that hypothetically that humans similar to mice, have germine Abs to oxidation-specific epitopes that play important roles in atherosclerosis. We have generated human Fab (Ab fragment) phage display libraries from five patients with familial hypercholesterolemia and seven uCB samples and screened these libraries against a panel of oxidized lipids. These results show that both innate and adaptive immunity generate Abs against oxidation-specific epitopes and these Abs bind to apoptotic cells and atherosclerotic lesions. Further studies may elucidate the role of these Abs in atherosclerosis and provide new insights to diagnosis and treatment of patients.

Comparison of Fenofibrate Alone or Atorvastatin Alone on Plasma Inflammation, Adhesion, and Oxidation Markers in Type 2 Diabetic Subjects with Moderate Hypertriglyceridemia
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Type 2 diabetes mellitus is associated with elevated plasma triglyceride (TG) levels, low HDL-cholesterol (C) and high incidence of cardiovascular disease. HMGC-CoA reductase inhibitors or fibrates are frequently used in the treatment of diabetic dyslipidemia but their impact on inflammatory, adhesion and oxidation markers in type 2 diabetic subjects with moderate hypertriglyceridemia is not well characterized. The objective of this two-group parallel study was to investigate the differences of atorvastatin 20mg/d alone (M1120) or micronized fenofibrate 200mg/d alone (M119) on inflammation, adhesion and oxidation markers in type 2 diabetic subjects with moderate hypertriglyceridemia. Atorvastatin decreased plasma-C (38.3%, P<0.0001), plasma-TG (-38.3%, P<0.0001), plasma apo B-48 (-41.4%, P<0.0001) and LDL-C (-34.4%, P<0.0001), and increased plasma-HDL-C (+16.5%, P=0.007). Atorvastatin decreased plasma levels of CRP (-22.5%, P<0.004), sICAM-1 (-1.5%, P=0.03), cachexin-1 (-4.4%, P=0.008), s-selectin (-5.7%, P=0.02), MMP-9 (-7.8%, P=0.05), sELAM-1 (-4.8%, P=0.04), sP-selectin (-6.0%, P=0.04) but increased the plasma levels of sP-selectin (+2.5%, P=0.004). Fenofibrate had no significant effect on CRP levels. In conclusion, the results of the present study suggest that atorvastatin is potent to reduce inflammation, oxidation and monocyte adhesion in type 2 diabetic subjects with moderate hypertriglyceridemia while fenofibrate decreased s-selectin levels only and had an unaltered effect on sP-selectin levels.

Effects of Artemisia princeps Pampamini Cultivated Sajabal on LDL Oxidation and Atherosclerosis in LDLR−/− Mice
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Oxidatively modified LDL (oxLDL) plays a key role in the development of atherosclerotic lesions. Many animal and clinical studies have shown the beneficial effects of various antioxidants treatments for reducing atherosclerotic lesions and cardiovascular risk. The purpose of this study was to determine the anti-atherosclerotic effects of Artemisia princeps Pampamini cultivated Sajabal. Jacobson isolated from A. princeps Pamp. cv. Sajabal has an LDL-antioxidant activity. The ethanolic extracts of A. princeps Pamp. cv. Sajabal containing 9.7% (w/v) of the extract reduced atherogenic lesions in LDLR−/− mice. We observed the effect of the jacobsonin on Cu2+-mediated human LDL oxidation. Jacobson showed the potent LDL-antioxidant activity with IC50 values of 3.9 μM in the thiobarbituric acid-reactive substances (TBARS) assay. Jacobson inhibited the formation of conjugated diene during Cu2+-induced LDL oxidation as well as the human macrophage-mediated LDL oxidation. By in vivo study, the effect of the extracts was investigated on the atherosclerotic lesion formation in LDLR−/− mice. LDLR−/− mice (10 weeks old) were randomly divided into two groups (n = 10 per group). The mice were fed a western-type atherogenic diet alone (control group) or an atherogenic diet supplemented with the extracts (1% of the diet). After 9 weeks, the plasma vigilance and macrophage formation accumulation at 37% and 43%, respectively, compared to controls. In aorta, the extracts decreased the transcriptional levels of COX, LOX-1, AOX1, ICAM-1, VCAM-1, TNF-α, IL-1β, COX-2 and INOS and increased the levels of CYP7 family. The extracts not only reduced epididymal fat accumulation (28%) and plasma lipid peroxidation (16%), but also corrected associated hyperlipidemia. These findings indicate that the extracts from A. princeps Pamp. cv. Sajabal effectively ameliorates atherosclerotic lesion formation by inhibiting lipid peroxidation and inflammation.

Phenotypic Switching in Macrophages by Oxidized Phospholipids Involves Toll-like Receptor 2
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Functional heterogeneity is a hallmark of cells of the mononuclear phagocyte system. Macrophages (Mφ) have been ascribed both pro- and anti-inflammatory properties. Classical activation of Mφ via TLR2 and PRR leads to the expression of M1-like, while alternative activation by IL-4 leads to M2-Mφs, that trigger either a Th1 or a Th2 response, respectively. Recently it has been hypothesized that Toll like receptors (TLR) recognize endogenous “danger signals”, such as oxidized phospholipids (oxPL), and their involvement in the development of atherosclerosis has been suggested. Here we investigated that oxidized-1-palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine (oxPAPC) polarizes Mφs towards a unique phenotype, different from the M1 and M2-types. Phenotypic switching of Mφs by oxPL is regulated by upregulation of KC, Mip2, IL1β, COX-2 and HO-1, while the expression of the typical M1 markers TNF α and IL12, as well as the M2 marker arginase 1 are downregulated. This macrophage phenotype exhibits increased survival, reduced migratory capability and facilitated lipoprotein uptake, and is present in atherosclerotic lesions. In order to address the question of involved receptors, we used Mφs from mice lacking TLR2. We show that oxPAPC-induced expression of the chemokines Mip2 and KC on Mφs is dependent on TLR2, while the expression of the oxPL-metabolite 11keto-HO-1 is independent of TLR2. To identify molecular structures in oxPL that determine Mφ polarization by TLR2 we fractionated oxPAPC into long chain (>26:9) and short chain fractions (m/z <782). We could show that TLR2-dependent COX-2 is induced by the long chain fraction of oxPAPC, while TLR2-dependent KC is induced by the short chain fraction of oxPAPC, that contains 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine (POPC), 1-palmitoyl-2-glutaryl-sn-glycero-3-PC (PGPC), and Lyso-PC. Together, we show that oxPAPC via TLR2 induces a unique Mφ phenotype.

Giant-cell Arteritis is a Not So Rare and Not So Easy to Handle Form of Vascular Inflammation: Analysis of 5 Years of Experience
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Introduction: GCA is a rare systemic vasculitis of uncertain, probably T cell driven immunological origin. Early diagnosis is essential in prevention of increased cardiovascular mortality and impaired quality of life. Objective: Identification of patients affected by GCA treated in our hospital in the last 5 years. Analysis of their clinical data in order to improve diagnosis and therapy. Method: analysis of clinical history, diagnostic algorithm, efficacy of therapy especially corticosteroid regimen with a follow up period of 5 years. Results: We analysed the data of 24 identified patients (16M; 77 ±10 years old), which is a population wider than the expected prevalence. 18 patients showed typical cranial, while 6 patients showed large vessel manifestation which are two distinct entities characterized by a different cytokine pattern in the vascular wall. The period of time between the first symptoms and the diagnosis was more than one year that represents the unawareness of this condition. In case of large vessel manifestation vascular ultrasound examination proved to be essential. Despite of a long steroid therapy (mean 19 months) 2 relapses occurred on the average, which is indicative of the limitation of corticosteroid therapy. Complications resulting from corticosteroid therapy, like diabetes, fracture due to osteoporosis, myopathy, infections were prevalent in the examined population. Conclusion: GCA is probably more frequent than generally considered. Diagnostic procedures include clinical observation, vascular ultrasound and histology. Corticosteroid therapy is effective to diminish the symptoms, but in the long run it can be regarded suboptimal, therefore new immunological therapy is needed.
severe CAV (p<0.001). A strong association was found between the need for coronary interventions and the CRP concentration (p=0.017). **Conclusions:** The data suggest that a proinflammatory environment rich in CRP favors early development of severe CAV and increases the need for coronary interventions in heart transplant recipients.

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**Lipoprotein Accumulation and Antigen Presentation in Grossly Normal Human Aorta**

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Earlier we have found that subendothelial intimacies in grossly normal human aorta express HLA-DR and CD1a molecules that are involved in peptide and lipid/carbohydrate antigen presentation, respectively. We hypothesize that low density lipoprotein (LDL) stimulates antigen-presenting function in subendothelial cells. To investigate LDL distribution in grossly normal subendothelial intima antibodies against apoB were used. ApoB was visualized using confocal laser scan microscopy and immunohistochemical methods. We localized apoB both inside the cells and associates with extracellular matrix. Intracellular apoB was found both in CD1a+ and HLA-DR+ cells. CD1a and HLA-DR were expressed not only by typical antigen-presenting monocyte-derived cells but also by resident intimacies (pericytes and smooth muscle cells). HLA-DR and CD1a molecules were associated with membrane antigen-presenting structures and intracellular structures transporting antigen from endoplasmic reticulum to cell surface through antigen-loading vesicles. The size of HLA-DR+ and CD1a+ vesicles were similar and ranged from 0.2 to 1 µm. Intracellular apoB was colocalized with CD1a+ vesicles. In HLA-DR+ cells, the majority of vesicles containing apoB were closely associated with HLA-DR structures but not colocalized. This suggests the difference in antigen processing occurring in HLA-DR+ and CD1a+ compartments. CD1a+ apoB+ cells were stained with anti-galactose-terminated vesicles of different sizes that may reflect the lymphocyte activation in subendothelial intima. High degree of colocalization of antigen-presenting molecules and apoB supports the suggestion that LDL accumulation in subendothelial intima stimulates activation and differentiation of intimal antigen-presenting cells.

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**A Role for Galectin-3 as an Amplifier of Inflammation in Atherosclerotic Plaque Progression**

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Galectins are a family of lectins that are involved in inflammation, cell adhesion, apoptosis and chemotaxis, and can also function as scavenger receptors. We identified Galectin-3 (Gal-3) as a highly abundant transcript in a whole-transcriptome scan of unstable atherosclerotic plaques obtained from carotid endarterectomy specimens. Gal-3 was selected as a candidate therapeutic target and potential biomarker of atherosclerotic plaque progression. We hypothesized that increased levels of soluble Gal-3, found in advanced human and murine atherosclerotic plaques, could exacerbate vascular inflammation by stimulating macrophages to express chemokines and other pro-inflammatory molecules. Gal-3 was found to be up-regulated in unstable plaque regions of carotid endarterectomy specimens compared to stable regions from the same patient (∼2.5-fold), at the mRNA (n=12) and protein level (n=9), as determined by qRT-PCR and western blotting analysis. Gene expression analysis of atherogenic plaques of ApoE-/- mice on a high-fat western-type diet showed that Gal-3 expression also increases with age and lesion size (3.1-fold increase from 8 weeks, n=6, to 16 weeks n=6). In vitro, Gal-3 mediates monocyte chemotraction and causes an up to 10-fold increase in human macrophage expression of pro-inflammatory mediators, such as TNFalpha and RANTES in a dose-dependent manner, as revealed by microarray (Illumina 10x). In vitro, Gal-3 mediates monocyte chemotraction and causes an up to 10-fold increase in human macrophage expression of pro-inflammatory mediators, such as TNFalpha and RANTES in a dose-dependent manner, as revealed by microarray (Illumina 10x). Gene expression analysis of human aortic plaques obtained from carotid endarterectomy specimens revealed that Gal-3 expression is increased more than 10-fold during differentiation while variance in apoB mRNA were less than 1-fold indicating that induction in MTP, not apoB, is responsible for the increased apoB secretion. We used this model system to investigate the molecular basis for the transcriptional induction of MTP. Studying a series of 5’-end truncated human MTP promoter sequences revealed that a 204-bp sequence, conserved during evolution, was sufficient to simulate differentiation-dependent MTP expression. Site-directed mutagenesis of putative transcription factors in 204-bp and co-transfection of candidate transcription factors revealed that HNF1 and DRI elements and HNF4a, HNF1a and HNF1b proteins are involved in the basal expression. For differentiation-dependent expression proximal and distal DRI elements were important. However, differentiation-dependent induction of MTP was not correlated with

with INF-gamma. Production of CRP has been already observed with Immunocytochemical staining, Westernblotting and Immuneoprecipitation. In this study, we examined proinflammatory effects of CRP produced by U937 cells on themselves using the lysate of cells. Cell lysis was prepared by sonication and centrifuge. Cell lysate of U937 cells clearly upregulated expression of inflammation related proteins, such as MCP-1, iNOS, COX2, IL-6 and Oxidized LDL receptor. These effects of lysate were enhanced in cells stimulated with INF-gamma with or without oxidized LDL. On contrary, after only CRP was removed by immuneoprecipitation, these proinflammatory effects were weakened significantly. (Conclusion) U937 cells are one of precious resources of CRP and stimulation with INF-gamma and LDL can upregulate its production. Co-stimulation with INF-gamma and oxidized LDL are synergistic. These results suggest that monocytes and macrophages in atherosclerotic lesions might accelerate vascular wall inflammation by local production of both native and modified CRP.

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**Intracellular Stress Activates BZIP Transcription Factors to Induce the Liver Inflammatory Response**

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Atherosclerosis, the major cause of heart diseases, is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate and propagate disease lesions. Endoplasmic reticulum (ER) stress is known to induce the unfolded protein response (UPR), an intracellular signaling pathway from the endoplasmic reticulum (ER) to nucleus to protect cells from stress caused by accumulation of unfolded or misfolded proteins. Here we found that ER stress, oxidative stress and pro-inflammatory signaling can interact and merge into each other to activate the liver inflammatory response that contributes to atherogenesis. XBP1, ATF6 and CHOP are ER stress-inducible basic leucine zipper (BZIP) transcription factors of CRP/ATF family. ER stress and oxidative stress induced by high-homocysteine diet, inflammatory cytokines TNFa, IL6, IL1b or Lipopolysaccharide (LPS) can induce processing of XBP1, ATF6 and CHOP can interact with each other to activate transcription of the major inflammatory genes encoding C-reactive protein (CRP), Serum Amyloid P-component (SAP) and Serum Amyloid A (SAA). In engineered mice defective in ER stress-transducer molecules IRE1a, XBP1, OASIP and/or ATF6, transcription of the CRP, SAP and SAA3 mRNAs and production of secreted CRP, SAP and SAA3 proteins were significantly reduced in response to LPS that induces ER stress, oxidative stress and inflammation, compared to those of control mice. Our studies suggest that cellular stress and inflammatory stimuli activate BZIP transcription factors XBP1, ATF6 and CHOP to induce oxidative stress, and will be informative to developing novel methods to control inflammatory diseases, particularly atherosclerosis.

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**Increases in apoB-lipoprotein Secretion During Differentiation of Caco-2 Cells due to Enhanced Transcription of MTP**

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Microsomal triglyceride transfer protein (MTP) and apolipoprotein B (apoB) are essential for lipoprotein assembly. In the intestine, the differentiated enterocytes produce apoB-lipoproteins. Caco-2 cells produce apoB-lipoprotein after spontaneously differentiating into enterocyte-like cells in culture. We analyzed changes in apoB secretion and MTP activity in Caco-2 cells cultured on Transwells for 2–3 weeks. ApoB secretion and MTP activity were barely detectable in non-differentiated cells but increased 5 to 6-fold during differentiation. Interestingly, MTP mRNA increased more than 10-fold during differentiation while variance in apoB mRNA were less than 1-fold indicating that induction in MTP, not apoB, is responsible for the increased apoB secretion. We used this model system to investigate the molecular basis for the transcriptional induction of MTP. Studying a series of 5’-end truncated human MTP promoter sequences revealed that a 204-bp sequence, conserved during evolution, was sufficient to simulate differentiation-dependent MTP expression. Site-directed mutagenesis of putative transcription factors in 204-bp and co-transfection of candidate transcription factors revealed that HNF1 and DRI elements and HNF4a, HNF1a and HNF1b proteins are involved in the basal expression. For differentiation-dependent expression proximal and distal DRI elements were important. However, differentiation-dependent induction of MTP was not correlated with
changes in HNF4α, HNF1α and HNF1β. Instead, profiling differentiation-dependent changes in several candidate transcription factors associated with DR1 element unveiled a putative repressor, NR2F1 (also called EAR or COUP-TF1), whose expression was reduced by more than 50% after Caco-2 cell differentiation. Knockdown of NR2F1 by siRNA in undifferentiated Caco-2 cells increased MTP promoter activity. In summary, our data indicate increased transcription of MTP expression in apoB-containing lipoprotein particles. This redistribution of apoB-containing lipoproteins may represent a mechanism to maintain normal MTP expression and function in the absence of nanoparticles.

We previously proposed that the initiation of apolipoprotein apoB particle assembly occurs when δB1 domain of apoB folds into a three-sided lipovitellin-like lipid binding cavity to form the apoB "lipid pocket." We demonstrated, based on experimentally-derived results and molecular modeling, that the N-terminal 1000 amino acid residues (δB1 domain) of apoB (designated apoB:1000) are competent to complete the "lipid pocket" without a structural requirement for microsomal triglyceride transfer protein (MTP) activity, and that this requirement for MTP activity is limited to the secretion of apoB:1000-containing particles. Our results, however, did not rule out a MTP-mediated lipid transfer to apoB:1000. In this study, we investigated the putative role of MTP in the initial lipidation of apoB:1000 by employing metabolic labeling of stable transfectants of MCA-RH7777 cells with [35S]methionine, [14C]oleic acid, and [3H]glycerol in the presence or absence of BMS-197636 and BMS-200150, two inhibitors of MTP lipid transfer activity. BMS-197636 at 0.1 μM and BMS-200150 at 5, 10 and 20 μM had no detectable effect on the synthesis, lipidation, and secretion of apoB:1000-containing particles. At 40 μM BMS-200150, the overall rate of apoB:1000 production was inhibited by 15–20% and the rate of apoB:1000 secretion was inhibited by 90% in the presence of the drug. These results were attributable to the effect of high concentration of this compound generally on hepatic protein synthesis, as reflected in 20–30% inhibition in albumin secretion. In addition, MTP inhibitors had no effect on the lipid composition of secreted apoB:1000-containing particles. Under these experimental conditions, the synthesis, lipidation, and secretion of apoB:1000-containing particles in HepG2 cells were inhibited by 90–97% and secreted particles had a lower content of triglycerides and a higher level of phospholipids. In conclusion, our studies provide compelling evidence that the initial addition of phospholipids to apoB:1000 and the initiation of apoB lipidoprotein assembly occur independently of MTP lipid transfer activity. We propose that a lipid transfer protein other than MTP mediates the formation of the phospholipid-rich primordial apoB particle.

Initiation of Apolipoprotein B-Containing Lipoprotein Assembly Is Independent of Microsomal Triglyceride Transfer Protein Activity

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We previously proposed that the initiation of apolipoprotein apoB particle assembly occurs when δB1 domain of apoB folds into a three-sided lipovitellin-like lipid binding cavity to form the apoB "lipid pocket." We demonstrated, based on experimentally-derived results and molecular modeling, that the N-terminal 1000 amino acid residues (δB1 domain) of apoB (designated apoB:1000) are competent to complete the "lipid pocket" without a structural requirement for microsomal triglyceride transfer protein (MTP) activity, and that this requirement for MTP activity is limited to the secretion of apoB:1000-containing particles. Our results, however, did not rule out a MTP-mediated lipid transfer to apoB:1000. In this study, we investigated the putative role of MTP in the initial lipidation of apoB:1000 by employing metabolic labeling of stable transfectants of MCA-RH7777 cells with [35S]methionine, [14C]oleic acid, and [3H]glycerol in the presence or absence of BMS-197636 and BMS-200150, two inhibitors of MTP lipid transfer activity. BMS-197636 at 0.1 μM and BMS-200150 at 5, 10 and 20 μM had no detectable effect on the synthesis, lipidation, and secretion of apoB:1000-containing particles. At 40 μM BMS-200150, the overall rate of apoB:1000 production was inhibited by 15–20% and the rate of apoB:1000 secretion was inhibited by 90% in the presence of the drug. These results were attributable to the effect of high concentration of this compound generally on hepatic protein synthesis, as reflected in 20–30% inhibition in albumin secretion. In addition, MTP inhibitors had no effect on the lipid composition of secreted apoB:1000-containing particles. Under these experimental conditions, the synthesis, lipidation, and secretion of apoB:1000-containing particles in HepG2 cells were inhibited by 90–97% and secreted particles had a lower content of triglycerides and a higher level of phospholipids. In conclusion, our studies provide compelling evidence that the initial addition of phospholipids to apoB:1000 and the initiation of apoB lipidoprotein assembly occur independently of MTP lipid transfer activity. We propose that a lipid transfer protein other than MTP mediates the formation of the phospholipid-rich primordial apoB particle.

Native 2D Gel Analysis of HDL Subpopulations: A Proteomics Approach

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HDL is a heterogeneous mixture of lipoproteins with a density of 1.063–1.21 g/ml. A powerful method of analyzing HDL subpopulations is the native 2D gel electrophoresis technique of Asztalos and colleagues, which fractionates HDL into its native components by charge in the first dimension and then by size in the second dimension. Traditionally the composition of these particles has been analyzed by Western blotting. However, Western analysis is limited by the need for probing with different antibodies, and unanticipated proteins will not be detected. Here we analyze the composition of HDL subpopulations using a proteomics approach. HDL was isolated from three normolipidemic individuals by sequential density gradient centrifugation and separated by native 2D gel electrophoresis. Gels were stained with Coomassie Blue to visualize protein-containing particles in the HDL fraction. Individual spots were manually picked and trypsinized and the protein composition of each spot was analyzed by LC-MS/MS. As expected, apoA-I was the predominant apolipoprotein in alpha-migrating particles. Alpha-migrating particles also contained apoc-II, alp-1-antitrypsin, SAA4, and PON1; apoE, apoD, and apoJ were seen on certain maps in alpha-migrating particles of higher molecular weight. Additions of an identified low-abundance HDL components, such as haptoglobin and the ryanodine receptor, were also localized to alpha-migrating particles. apoJ was confirmed as the predominant apolipoprotein in pre-alpha-migrating particles. apoJ was also present in particles migrating with pre-alpha mobility. Our results, in conjunction with those of others, are suggestive of considerable heterogeneity in HDL subpopulations. In conclusion, native 2D gel electrophoresis of HDL followed by proteomics analysis is a novel method to characterize high density lipoprotein particle composition. This method may help to elucidate the functional roles of HDL subpopulations.

In Vitro Production of Active ApoJ/Clusterin

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The traditional source for human apoJ/clusterin is pooled human plasma. The purification protocol is tedious, potentially hazardous, and prone to bacterial contamination. Moreover, there is no potential to produce a modified form of apoJ/clusterin. We have utilized an in vitro approach to address these issues. HEK293 cells were stably transfected with an expression construct that contains cDNA for human apoJ/clusterin. We used western blots to identify apoJ/clusterin as a protein that is prominent in the culture supernatant. In a tritiated thymidine uptake assay with A7r5 cells, the same culture supernatant significantly suppresses growth factor stimulated uptake of thymidine in A7r5 cells, as expected. Exogenous apoJ/clusterin has been shown to be an effective suppressor of thymidine uptake in growth factor stimulated A7r5 cells. We used this response as a positive control for responsiveness of the A7r5 cells. We counted that apoJ/clusterin possesses heparin binding domains and we propose to utilize affinity chromatography to purify apoJ/clusterin from the culture supernatant. We anticipate that this in vitro approach will provide a consistent source of apoJ/clusterin that is biologically active. The potential is present to determine on vascular smooth muscle cells not only the receptor(s) but also the domains of the apoJ/clusterin molecule that evoke the protective function of apoJ/clusterin in vascular biology.

Diet Enriched with Oxidized Fatty Acids Lowers Triglyceride Levels in Mice

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We have previously shown through cell culture and animal studies that the intestine efficiently absorbs oxidized linoleic acid and atherogenicity is increased in animals fed a high cholesterol diet in the presence of oxidized linoleic acid. Animals fed non-atherogenic diet did not develop atherosclerosis even in the presence of oxidized fatty acids. Oxidized fatty acids have also been shown to be ligands for PPARs and could induce genes for antioxidant enzymes such as catalase. In the present study, we fed C57BL6 mice normal mouse diet in the presence of oleic or oxidized linoleic acid (13-hydroxyoctadecadienoic acid, 13-HODE) at 50 mg per animal per day for two weeks in alpha-migrating particles of higher molecular weight. Additions of an identified low-abundance HDL components, such as haptoglobin and the ryanodine receptor, were also localized to alpha-migrating particles. apoJ was confirmed as the predominant apolipoprotein in pre-alpha-migrating particles. apoJ was also present in particles migrating with pre-alpha mobility. Our results, in conjunction with those of others, are suggestive of considerable heterogeneity in HDL subpopulations. In conclusion, native 2D gel electrophoresis of HDL followed by proteomics analysis is a novel method to characterize high density lipoprotein particle composition. This method may help to elucidate the functional roles of HDL subpopulations.
Background and aims: Small dense LDL (sdLDL) is an emerging risk factor for coronary heart disease. However, detailed in vivo metabolism of sdLDL has been poorly understood. In order to assess sdLDL metabolism, we performed in vivo kinetic studies utilizing stable isotope-labeled leucine in 10 hypercholesterolemic patients and 5 healthy controls. Effects of statin on sdLDL metabolism were also investigated. Methods: Deuterated leucine was injected and blood samples were collected up to 48 hours. sdLDL was isolated by heparin-magnesium (LIR 44/2193, 2003), followed by ultracentrifugation (d 1.019-1.063g/ml), VLDL, IDL, and LDL were also isolated by sequential ultracentrifugation. ApoB was precipitated by isopropanol method, then hydrolyzed and derivatized to determine tracer/tracer ratios of apoB by gas-chromatography mass spectrometry. Results: Fractional catabolic rate (FCR) was estimated by SAAMII software. The FCR of sdLDL apoB was 0.23 ± 0.06 pools/day, which was 35% lower than that of LDL apoB of 0.35 ± 0.12 pools/day (p < 0.001). Furthermore, statin therapy significantly increased sdLDL apoB FCR by 67% (p < 0.01), in addition to 94% increase in LDL apoB FCR. Conclusions: This is the first in vivo kinetic evidence of the delayed catabolism of sdLDL which was improved by statin therapy. Therefore, the impaired catabolism is translated into the longer residence time, thus supporting the proatherogenic nature of sdLDL.

IRE1α restricts chylomicron production by selectively degrading MTP mRNA


Microsomal triglyceride transfer protein (MTP) is obligatory for the production of intestinal chylomicrons to absorb dietary fat and fat-soluble vitamins as well as very low density and low density cholesterol. Insulin-requiring enzyme 1 (IRE1), a membrane-anchored kinase/ribonuclease, plays a key role in relieving the stress induced by the buildup of misfolded proteins in the endoplasmic reticulum. Although seemingly unrelated, we provide evidence for a cross talk between these two processes to avoid hyperglycemia during postprandial state. IRE1α is expressed ubiquitously, but the intestinal epithelium expresses an additional protein IRE1β. Plasma cholesterol and triglyceride were significantly increased in cholesterol-fed iRed1α–/– mice due to increased chylomicron production. Moreover, the activity and mRNA of MTP were markedly enhanced in the intestine, but not the liver, of these mice. In human hepatoma HepG2 cells, cholestroler enhanced MTP levels but not when IRE1β was expressed. IRE1β specifically decreased MTP mRNA in these cells. IRE1β had no effect on the MTP promoter, but reduced MTP mRNA levels expressed under a heterologous promoter indicating for post-transcriptional degradation. Thus, IRE1β deficiency leads to intestine-specific induction of MTP and increased lipid absorption in response to a high-cholesterol diet. These findings have potentially important mechanistic and therapeutic implications related to the role of ER stress in the production of intestinally derived atherogenic lipoproteins.

ACAT2 and Human Hepatic Cholesterol Metabolism: Identification of Important Gender-related Differences

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Objective ACAT2 is specifically expressed in hepatocytes and plays a role in hepatic cholesterol esterification in humans. To further elucidate its physiologic role in human cholesterol metabolism, liver biopsies from 19 gallstone patients (female/male, 10/9) and 12 gallstone-free patients (female/male, 6/4) were collected, and analyzed for ACAT2 activity and expression. Results Age and BMI were matched between groups and genders, as well as plasma total cholesterol and triglycerides. As expected, HDL cholesterol and Apo A1 were significantly higher in females than in males. Hepatic ACAT2 activity did not differ between gallstone and gallstone-free patients and no correlation was observed between the hepatic ACAT2 activity and the biliary cholesterol content. Interestingly in females, hepatic ACAT2 activity was 1/4 of what was observed in males (7.2 ± 1.2 vs. 29 ± 9.1 pmol/mg/min protein, P < 0.01). This gender-related difference was also seen at protein level, but was not found at mRNA level. Furthermore, the hepatic activity of ACAT2 correlated negatively with serum HDL cholesterol (r = −0.48, P < 0.05) and with Apo AI (r = −0.61, P < 0.05). Conclusion A strong gender-related difference in hepatic ACAT2 activity is present in human liver, and alterations in hepatic ACAT2 activity seems not to underlay the gender of gallstone disease. Furthermore, a new role for ACAT2 in the regulation of HDL cholesterol levels may be hypothesized: when hepatic ACAT2 activity is low, free cholesterol may be preferentially secreted into HDL particles rather than into bile.

Human Macrophage ABCG1 Expression and Function Is Decreased in Patients with Type 2 Diabetes

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Coronary artery disease is the most common cause of death for people with Type 2 diabetes, with diabetic patients being up to four times more likely to develop coronary artery disease than their non-diabetic counterparts. A key early event in the development of atherosclerosis is macrophage foam cell formation. The ABC transporter, ABCG1, plays an important role in macrophage reverse cholesterol transport. Previous work by our lab in mouse models of Type 2 diabetes show a decrease in macrophage ABCG1 protein expression, leading to a decrease in cholesterol efflux to HDL and increased macrophage lipid accumulation. Here, we show similar data in human patients with Type 2 diabetes. Human blood was obtained from consenting patients with and without Type 2 diabetes. Monocytes were isolated and differentiated into macrophages in vitro. Western blot analysis of the human macrophages revealed that ABCG1 protein expression was decreased by approximately 40% in diabetic patients compared to controls, without changes in ABCA1 protein levels. Cholesterol efflux experiments revealed a 30% decrease in cholesterol efflux to HDL by diabetic macrophages compared to controls. Further, we saw no change in efflux to lipid free Apo-A1 by diabetic macrophages, indicating no loss of function of ABCA1. Additionally, macrophages from diabetic patients had significantly more lipid accumulation than non-diabetic patients when challenged with oxidized LDL, as measured by oil red o staining and gas-chromatography. In conclusion, Type 2 diabetes appears to decrease human macrophage ABCG1 expression, resulting in decreased cholesterol efflux to HDL, an important factor in the etiology of atherosclerosis.

The Effect of Simvastatin on Surrogate Markers of Vascular Health in Youth

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Failure to diagnose preclinical CVD in youth misses a major opportunity to prevent the long-term consequences of this disease. We have conducted a pilot study to evaluate surrogate vascular markers (SVMs) that are associated with early arterial injury including flow-mediated vasodilatation, carotid intima media thickness, arterial stiffness, and biomarkers including cell adhesion molecules (ICAM-1 and VCAM-1), asymmetric dimethylarginines (ADMA, symmetric dimethylarginine [SDMA]), and C-reactive protein (C-RP). We hypothesized that one or more of these SVMs which are linked to early pathological vascular changes will identify high-risk youth with early vascular injury compared to a healthy group and that these markers will tend to normalize with risk factor reduction. We further hypothesized that one or more of the markers will correlate with the Pathological Determinants of Atherosclerosis in Youth (PDAY) risk score. Ten subjects without any known risk factors and 22 hypercholesterolemic (HC) youth, aged 10–20 yrs were recruited from the pediatric clinic. The majority of the HC group was also obese. The HC group was randomized to diet + 20 mg of simvastatin vs. placebo for 24 weeks followed by a forced titration to 40 mg vs. placebo for 24 weeks ending with a final evaluation after a 12 week washout period of diet alone. The markers that best distinguished the HC from the control subjects were C-RP (p = 0.03), VCAM-1 (p = 0.05) and SDMA (p = 0.04). With the exception of ICAM-1 and C-RP, the marker values improved in the treatment group more than the placebo group and the relative changes were the largest for the methylarginines—a marker closely tied to insulin resistance. The markers that demonstrated the highest correlation with the number of risk factors were VCAM-1 (r = 0.45, p = 0.05) and SDMA (r = 0.48, p = 0.04). PDAY risk scores were calculated for each subject and SDMA (p = 0.50, p = 0.03) and ApoSDMA (r = 0.50, p = 0.04) again emerged as the most highly correlated SVMs. Consistent with the relatively low PDAY risk scores and a minimally thickened IMT, the data suggest that the majority of subjects do not have advanced atherosclerotic changes. We conclude that SVMs are a useful index of vascular injury in high-risk youth.

Dietary Cholesterol–Mediated Stimulation of Chronic LXRα Activation, Dyslipidemia, Obesity, and Insulin-resistant Diabetes: Evidence for Synergistic Interactions with Dietary Fat and Fructose

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Evidence is presented for an important link between dietary cholesterol, chronic LXRα activation, and development of severe dyslipidemia and insulin resistance. Previously, our...
Naringenin, a citrus flavonoid, potently inhibits the assembly and secretion of apoB100-containing lipoproteins (apoB) from HepG2 and primary mouse hepatocytes. In the present study, we analyzed the impact of high-fat diet on vascular function in transgenic mice overexpressing LOX-1. Methods and Results: In LOX-1 overexpressing mice, bovine LOX-1 transgene was mapped by fluorescence in situ hybridization to chromosome 3 (region 39c–4) of the mouse genome and found to be inserted in multiple copies. Parallel feeding studies performed in Western-fed mice with high fructose and high fat diets supplemented with either varying amounts of cholesterol (0.05% or 0.25%) or the specific LXR agonist T0901317 (25 mg/kg) for 14 days. Interestingly, the lipid and lipoprotein profile of hamsters fed supplemented with high cholesterol (0.25%) were closely similar to those in hamsters treated chronically (2 weeks) with an LXR agonist, indicating that LXR activation may be at least partly responsible for the chronic effects of cholesterol. The data clearly implicates dietary cholesterol, synergistically acting with dietary fat and fructose, as a major determinant of the severity of insulin resistance and dyslipidemia, an effect asayed by both chronic LOX-1 activation.

The Citrus Flavonoid Naringenin Inhibits ApoB100 Secretion and Improves Dyslipidemia and Insulin Resistance in High-fat-Fed LDLR⁻/- Mice

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Naringenin, a citrus flavonoid, potently inhibits the assembly and secretion of apoB100 containing lipoproteins (apoB) from HepG2 and primary mouse hepatocytes. In the present study, we determined if addition of naringenin to a high fat (Western) diet could inhibit hepatic apoB100 overproduction and improve the dyslipidemia, insulin resistance and glucose tolerance in LDL-receptor deficient (LDLR⁻/-) mice. Fed Western diet (high fructose, high fat and 0.05% cholesterol) and Western + 1% or 3% w/w naringenin, were fed ad libitum for 4 weeks (n = 12 per group). The Western diet significantly elevated plasma cholesterol (C) and triglyceride (TG) approximately 3-fold, which were dose-dependently decreased by up to 40% by naringenin. Secretion of TG and apoB100 into plasma, as assessed by the tyrosolase technique were increased 2- and 4-fold in Western-fed mice. Both parameters were significantly decreased (~100% and ~50% respectively) by 3% naringenin, demonstrating that naringenin inhibits hepatic apoB100 secretion in vivo. The Western-fed mice had significantly elevated TG accretion in liver (2-fold), intestine (4-fold) and skeletal muscle (1.3-fold) when compared to Chow, which were corrected by 3% naringenin (~34%, ~40% and ~40% respectively, all P < 0.05). Naringenin inhibited the Western-diet induced weight gain, independent of changes in caloric intake or intestinal TG absorption, suggesting decreased TG synthesis and increased fatty acid (FA) oxidation. The significant increases in hepatic mRNA of acyl-CoA:Acyltransferase (FAT/CD36) and rates of TG synthesis (1.4-fold) in Western-fed mice were completely normalized by 3% naringenin. The expression of hepatic mRNA for acyl-CoA oxidase (ACOX), which is associated with the Lp-PLA2 response, but in opposite directions. Thus, P-OM3-induced dyslipidemia, insulin resistance and tissue TG content in high fat-fed LDLR⁻/- mice, suggesting a novel approach for treatment of the metabolic syndrome.

Investigation of Levels of Serum Lipids in Patients with Periodontitis Compared with Healthy Individuals

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Objective: Periodontal disease is a bacterial infection, which has been classified as a local chronic inflammation. The literature suggested that a link exists between the prevalence of severe periodontitis and several systematic health changes including an altered lipid metabolism. The aim of this study was to investigate the relationship between chronic periodontitis and serum lipids levels. Material and Methods: The level of serum lipids (CHL, TG, HDL and LDL) of total 30 patients with Chronic periodontitis (CPTN score III & IV) ranging in age between 30 to 40 years were examined and compared with data obtained from 30 healthy individuals (control group). The relationship of serum lipids and periodontal disease and CPTN index was tested by means of SPSS software (Ver 13.0). Results: The present of periodontal disease was significantly related with higher total cholesterol in case group (P < 0.05). Triglycerides and HDL were higher in patients but no statistically significant differences were observed with control group. LDL did not show any difference between case and control groups. Conclusion: Analysis of these data revealed that Chronic periodontitis enhances the chance of occurrence of Hyperlipidemia in healthy people. The findings of this research support the reports linking increased prevalence of changing serum lipids among patients with periodontal disease.

Lipoprotein Subfraction Responses Differentially Predict Changes in Lipoprotein-associated Phospholipase A2 During Prescription Omega-3 Therapy

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Lipoprotein-associated phospholipase A2 (LP-PLA2), a secretory product of macrophages, circulates bound to low- (LDL) and high-density lipoprotein (HDL) particles, and is an independent predictor of cardiovascular event risk. The present investigation assessed the influence of triglyceride lowering with prescription omega-3 acid ethyl esters (P-OM3) on LP-PLA2 concentration and lipoprotein subfraction levels (assessed by nuclear magnetic resonance spectroscopy) in men and women with triglycerides 200 to 499 mg/dL while on statin therapy. After 8 weeks on simvastatin 40 mg/d, 256 subjects were randomly assigned to 4 treatment groups: P-OM3 (600 mg/day, n = 65), P-OM3 + simvastatin (40 mg/day, n = 70), simvastatin alone (n = 70), and control (n = 79). All subjects remained on simvastatin for 8 weeks. P-OM3 treatment lowered LP-PLA2 concentration (231 to 200 ng/mL, p = 0.002 vs placebo). The LP-PLA2 response was not significantly related to changes in LDL or HDL cholesterol levels, but was associated with changes in LDL (r = 0.30, p = 0.002) and HDL (r = 0.25, p = 0.01) particle concentrations. In large LDL particle concentration (r = 0.06, p = 0.1) was not associated with the LP-PLA2 response. Changes in small HDL particle concentration (r = 0.06, p = 0.1) did not differ significantly from low-density lipoprotein (LDL) cholesterol. Large HDL particle concentration (r = 0.25, p = 0.01) was independently associated with the LP-PLA2 response, suggesting that LP-PLA2 response may be an independent predictor of cardiovascular outcomes.
changes in Lp-PLA2 are associated with changes in LDL and HDL particle concentrations, and differentially related to changes in subfractions of these lipoproteins. These findings are consistent with those of prior studies showing that Lp-PLA2 is enriched in small, dense, electrophoretically LDL particles and may have implications for understanding how lipid therapies influence Lp-PLA2 and associated cardiovascular risk. Funding provided by Reliant Pharmaceuticals.

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The Lipid Peroxidation Product Malondialdehyde Impairs ABCA1 Cholesterol Export from Cells Through Site-specific Crosslinking of Apolipoprotein A-I

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Objective - LDL extensively modified by malondialdehyde (MDA) is a classic ligand for scavenger receptors that promote macrophage foam cell formation in vitro. Protein-bound MDA has been detected in atherosclerotic lesions by immunohistochemistry, implicating lipid peroxidation of the apoA-I moiety in the pathogenesis of atherothrombosis. Recent studies have shown that MDA-modified HDL (MDA-HDL) lowers HDL-C levels. To MDA have focused on LDL and relied on antibodies that react with unknown epitopes. Remarkably little is known about the specific sites and chemical nature of MDA adducts in proteins. Moreover, the possibility that MDA-modified HDL plays a role in atherogenesis has received little attention. We have investigated the possibility that one important target for MDA might be apolipoprotein A-I (apoA-I), the major HDL protein. Results - Lipid-poor apoA-I promotes efflux of cellular cholesterol from macrophage foam cells by the ABCA1 pathway, apoA-I exposed to increasing concentrations of MDA progressively and dramatically lost its ability to remove cholesterol from cultured VLDL. Total mass spectrometry and LC/MS/MS analysis demonstrated that specific lysine residues (K118, K133, K195, and K226) as well as the N-terminal amino group were modified by MDA in high yield. Importantly, cross-linked lysine residues were identified as the major product of apoA-I exposed to MDA, suggesting that cross-linking between lysine residues confines the structural molecule of the apolipoprotein. One major cross-linking site in apoA-I involved K226, which is located in helix 10 of the protein. This region plays a critical role in triggering apoA-I lipid association and sterol efflux by the ABCA1 pathway. Conclusions - These observations suggest that the high reactivity of MDA with lysine residues could be an important mechanism by which MDA disrupts a key structural element of apoA-I, thereby inhibiting cholesterol efflux by ABCA1. Our observations indicate that MDA may interfere with normal HDL function by site-specifically cross-linking lysine residues in apoA-I, which might play a critical role in atherogenesis by impairing cholesterol removal from arterial wall cells.

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VLDL from Obese Zucker Rats Contain Elevated Eicosanoids and Octadecanoids (Oxylinoids) That Are Released by Lipoprotein Lipase

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Objective: Oxidized fatty acid metabolites (oxylinoids) such as eicosanoids and octadecanoids are found in plasma and their levels are closely related to inflammatory processes. The role of lipoproteins in transport of oxypins is poorly understood. We investigated the effect of hypertriglycerideremia on intravascular lipolyis in VLDL. Total mass spectrometry and LC/MS/MS analysis demonstrated that specific lysine residues (K118, K133, K195, and K226) as well as the N-terminal amino group were modified by MDA in high yield. Importantly, cross-linked lysine residues were identified as the major product of apoA-I exposed to MDA, suggesting that cross-linking between lysine residues confines the structural molecule of the apolipoprotein. One major cross-linking site in apoA-I involved K226, which is located in helix 10 of the protein. This region plays a critical role in triggering apoA-I lipid association and sterol efflux by the ABCA1 pathway. Conclusions - These observations suggest that the high reactivity of MDA with lysine residues could be an important mechanism by which MDA disrupts a key structural element of apoA-I, thereby inhibiting cholesterol efflux by ABCA1. Our observations indicate that MDA may interfere with normal HDL function by site-specifically cross-linking lysine residues in apoA-I, which might play a critical role in atherogenesis by impairing cholesterol removal from arterial wall cells.

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A Novel Role for Apolipoprotein A-V: Association with Intracellular Lipid Droplets

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Accumulating evidence indicates apolipoprotein A-V (apoA-V) is an important regulator of the triacylglycerol (TG) metabolism. Studies with mice and human have shown that a deficiency of apoA-V is associated with increases in plasma TG levels. Given that plasma levels of apoA-V are extremely low, we hypothesized that this protein functions intracellularly by affecting the assembly and/or secretion of apoB-containing particles. Overexpression of apoA-V in Hep3B cells cultured in medium supplemented with oleic acid secreted neither the amount of apoB secreted nor the density distribution of apoB-containing particles. Fluorescence microscopy studies were carried out on oleic acid supplemented Madcke-FH777 cells expressing human apoB100 and apoA-V to determine whether these proteins traffic together in the secretory pathway. Confocal fluorescence microscopy images revealed that apoA-V does not interact with apoB intracellularly. Whereas apoB localized to the endoplasmic reticulum, apoA-V was found in a distinct cellular compartment comprised of a cluster of spherical structures. Nile Red fluorescence staining identified these structures as intracellular lipid droplets. ApoA-V- green fluorescent protein fusion protein localized to the surface of the lipid droplets and co-localized with adipophilin, a protein that interacts with lipid droplet surface protein. The data reveal a unique association of apoA-V with intracellular neutral lipid droplets suggesting a function for this protein in lipid storage and/or mobilization.

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The Influence of Apolipoprotein A-II Introduction on Apolipoprotein A-I Conformation in High-density Lipoproteins

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The metabolism of high density lipoprotein (HDL) is likely influenced by the interaction and conformational change of its two major protein constituents; apolipoprotein (apo) A-I and apoA-II. To understand the impact of apo A-II on apoA-I in HDL we have begun to monitor apoA-I conformational changes on mixed apoA-I-A-II discoidal HDL particles (LpA-I/A-II) compared to apoA-I only HDL particles (LpA-I). Homogeneous, 16 A LpA-I/A-II was reconstituted with apoA-I:apoA-II in a 2:1 ratio and a total of three protein molecules per particle. These particles were subjected to analysis by a cross-linking and mass spectrometry approach previously used to study LpA-I and discoidal apoA-II HDL (Lp-A-II) particles. After cross-linking, delipidation and exhaustive tryptic digestion of LpA-I/A-II were specifically searched for the short and long range apoA-I apoA-II cross-links we previously identified in LpA-I particles. Almost all short-range cross-links (7/8) previously identified in LpA-I particles were present in the LpA-I/A-II particle except that, only 2/9 long range cross-links were present. Furthermore, all short range cross-links (5/5) pertinent to LpA-II were present in Lp-A-II but none of the long range cross-links are found (0/8). These observations strongly suggest that, when present together in a single HDL particle, both apoA-I and apoA-II adopt a conformation that is not consistent with the apoA-II apoA-I cross-links. Our results indicate that apoA-II plays a role in the conformational stability of HDL.
Profound Cardiac Metabolic Changes Caused by Coronary Disease

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We have recently shown that apolipoprotein E knockout (apoE-/-) mice fed a high-fat, Western-style diet for 6 months, develop occlusive coronary lesions and show evidence of myocardial infarction. The same mice fed a normal rodent diet appear to remain healthy, and therefore provide excellent isogeneic controls for the study of cardiac disease. Thus, the aim of this work was to investigate the direct myocardial effects of coronary disease. Male apoE-/- mice were weaned onto a normal rodent diet, and at approximately 8 weeks old, animals were either switched onto a high-fat, Western-type diet (21% fat; 0.15% cholesterol) or were maintained on a normal rodent diet for 6 months. Isolated left ventricles were used to measure cardiac metabolites and function. Isolated myocytes were used to measure mitochondrial flux (NAD+/NADH), contractile function and calcium transients. Mice fed high-fat diet had significantly increased levels of cardiac lactate (from 42 ± 6 to 68 ± 11 mmol/mg protein), decreased glycogen content (from 0.077 ± 0.006 to 0.036 ± 0.005 mg/g wet weight) and decreased levels of ATP (from 16 ± 0.9 to 11 ± 1 mmol/mg protein). Evidence of metabolic stress in diseased hearts was confirmed in isolated perfused myocytes which showed increased NAD+/NADH ratio (from 0.27 ± 0.02 to 0.35 ± 0.02). These metabolic differences did not alter functional characteristics of isolated perfused hearts or myocytes. However, both isolated myocytes and intact hearts from mice with coronary disease that had hearts with increased metabolites were significantly more resistant to cardiac insults than control animals. In conclusion, coronary disease induced by high-fat diet has profound metabolic effects on the myocardium. These changes appear to alter vulnerability to cardiac insults, which may be due to ischemic preconditioning.

Prediction of the Localization of High-Risk Coronary Atherosclerotic Plaques Based on Low Endothelial Shear Stress: A Serial IVUS and Histopathology Natural History Study

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Background: The role of low ESS in the progression of atherosclerotic plaques and evolution of these plaques to thin cap fibroatheromas (TCFA) has not been studied. We investigated the effect of low ESS in the development of TCFA in swine coronary arteries. Methods: In 11 diabetic hyperlipidemic swine, IVUS-based 3D reconstruction of the coronary arteries was performed at baseline (wk 23) and follow up (wk 30). Baseline ESS was calculated using computational fluid dynamics, and plaque-free segments of interest of 3 mm length were identified (n=142). Coronary arteries (n=31) were harvested at follow up, cryosectioned at the subsegments of interest and stained histologically. Intima/media ratio, min cap thickness, lipid deposition and inflammation were quantified and atherosclerotic lesions were histologically classified to minimal (MIN), intermediate (INT) and TCFA. Results: The magnitude of low ESS was significantly associated with larger plaque size, increased lipid deposition, inflammation and cap thinning (p<0.001). Low ESS and hyperlipidemia were independent predictors of the development of TCFA or INT vs. MIN, whereas the differentiation of early lesions to TCFA vs. INT was associated with hyperlipidemia and hyperglycemia (Fig, Table). Conclusion: The magnitude of low baseline ESS determines the severity and heterogeneity of atherosclerotic lesions and, in combination with systemic risk factors, predict the development of TCFA. These findings provide a perspective for the identification of early stages of a high risk plaque, thereby enabling highly selective pro-emptive coronary interventions to prevent adverse coronary events.

The Lysine Binding Site in Kringle IV Type 10 Is Required for Apolipoprotein(a)-Mediated Changes in Vascular Endothelial Cell Phenotype

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Substantial evidence indicates that endothelial dysfunction plays a critical role in atherogenesis. We previously demonstrated that apolipoprotein(a) (apo(a)); the distinguishing protein component of the atherothrombotic risk factor lipoprotein(a) elicits rearrangement of the actin cytoskeleton and intact pericellular endothelial cells (HUVECs), characterized by increased central stress fiber formation and increased cell permeability. These effects are mediated by increased myosin light chain (MLC) phosphorylation via a Rho/Rho kinase-dependent signaling pathway. Apo(a) contains kringle (KV) and KV domains similar to those in plasminogen: apo(a) contains 10 types plasminogen-KV-like sequences, followed by sequences homologous to the plasminogen KV and protease domains. Several of the apo(a) kringle domains contain lysine-binding sites (LBS) that have been proposed to contribute to the pathogenicity of apo(a). Here, we tested receptor engagement by apo(a) [r- apo(a)]-variant containing both aminoterminal sequences of the molecule, and found that the effect of apo(a) on increases in MLC phosphorylation and HUVEC permeability can be attributed to the kringle IV type 10 (KV10) domain, within the carboxy-terminal half of the apo(a) molecule. Accordingly, 17K r- apo(a) full length apo(a) species with a mutation in the strong LBS in KV10 does not elicit these effects, nor does 17K r- apo(a) in the presence of the lysine analog epsilon-ammoniac acid. In keeping with our previous observations, the effects of apo(a) on MLC phosphorylation and EC permeability were abrogated by Rho/ Rho kinase inhibitors as well as the MLC kinase inhibitor ML-7. We have shown that 17K r- apo(a) treatment of HUVECs enhances the Rho kinase-mediated phosphorylation of MLC in vitro. Thus, our findings indicate that the strong LBS in apo(a) KV10 mediates all of our observed effects of apo(a) on EC phenotype. Studies are ongoing to further dissect the molecular basis of these findings.

CCR7 Is Functionally Required for Atherosclerosis Regression and Is Activated in Vivo by LXR

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We have developed a mouse model of atherosclerosis regression by changing, after plaque formation, the plasma environment of the plaque from hyperlipidemia to normolipidemia by transferring aortic segments from apoE-/- mice to wild type recipients with normolipidemic environment. As a control, mice with hyperlipidemia were also transplant recipients to continue the progression environment. In this model, we reported emigration of plaque macrophages to regional and systemic lymph nodes after 3 days in the regression environment. During regression, the foam cells had features of dendritic cells (DCs). Because DCs require the chemokine receptor CCR7 for migration, we measured its mRNA and protein, and found foam cell expression of this factor to be induced, but only during regression. Further experiments using blocking antibodies to CCR7 demonstrated a functional requirement for it in regression. We have recently reported that LXR mRNA increases in foam cells during regression and were interested in the present study using a murine model of DCs. Using a murine model of immature DCs, we found that CCR7 expression is increased 8-9 fold upon LXR activation by the agonist T0901317. Importantly, this increase is dependent on CCR7 gene transcription, because pretreatment with actinomycin D abolished the observed response. To extend the results in vivo, we treated western diet-fed apoE-/- mice with LXR agonist. This also induced CCR7 expression in foam cells. Foam cell content and lesion area decreased by 21% and 24%, respectively, consistent with a report that a LXR agonist promoted regression of atherosclerosis in LDLR-/- mice. The dependence of the agonist’s effects on CCR7 was demonstrated by treatment of apoE-/- mice with LXR agonist: the increase in CCR7 was significant, but only in LXR agonist mice. This also induced CCR7 expression in foam cells. These findings provide a perspective for the identification of early stages of a high risk plaque, thereby enabling highly selective pre-emptive coronary interventions to prevent adverse coronary events.

Fibroblast and Aortic Smooth Muscle Cell Activation Is Regulated by Niemann-Pick Type C2 Protein

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Fibroblast and smooth muscle cell (SMC) phenotypic change represents a major underlying cause in several pathologies, including maladaptive vessel wall remodeling induced by hypertension, atherosclerosis, and angiospasm. Niemann-Pick type C2 (NPC2) gene has recently been identified as the second gene in the autosomal recessive cholesteryl storage disorder, the NPC disease. It encodes a secretory, 151 amino acid protein with unknown function. This report identifies NPC2 protein as a novel autocrine/paracrine factor that negatively regulates fibroblast and SMC activation, thus playing a major role in regulation of aortic tissue remodeling. Using a transgenic NPC2 knock-out mouse model, we found that NPC2 protein in primary human dermal fibroblasts resulted in their activation. This was reflected by a significant up-regulation of the SMC markers expression, growth factors and their receptors, as well as by induction of the collagen and inflammatory cytokine genes. The latter correlated strongly with the increased activity of NF-κB (~2.5-fold). Second, silencing of NPC2 gene in aortic SMC through siRNA transfection resulted in stimulation of the cell migration toward PDGF by ~2.5-fold. Third, NPC2-deficient cells displayed constitutive activation of the ERK and FGF receptor tyrosine kinase (RTK) signaling, which is consistent with the NPC2 primary function as a suppressor of the RTK activation/transactivation. These findings could provide a solid basis for the development of novel therapeutics to treat the coronary artery and cerebral vascular diseases.

The Receptor for Advanced Glycation Endproducts Induces Cellular Migration via Binding of Its Intracellular Domain to Diaphanous-1: Implications for Vascular Biology and Disease

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Ligand binding to the Receptor for Advanced Glycation Endproducts (RAGE) activates a cascade of intracellular signaling events that lead to vascular cell migration. The short cytoplasmic domain of RAGE is essential to activate these pathways, and hence the understanding of how
this domain functions in the intracellular environment is significant. In this study, we identified using the yeast two-hybrid assay that the RAGE cytoplasmic tail directly binds Diaphanous-1 (Dia-1); a molecule that mediates intracellular signaling and cellular motility. Evidence supporting this interaction is as follows: First, co-immunoprecipitation (IP) and binding of epitope tagged RAGE tail and Dia-1 was confirmed in transfected cells. Second, RAGE/Dia-1 was co-immunoprecipitated from RAGE over-expressing cells, but not from control cells. Third, by confocal microscopy in intact cells, RAGE and Dia-1 co-localized after RAGE/ligand stimulation, with significantly less co-localization observed with DN-RAGE and Dia-1 expressing cells. To demonstrate the interaction between RAGE and Dia-1 was direct, in vitro GST-RAGE tail pull-down bound Diaphanous-1 alone. Next, we developed in silico distinct domains of Dia-1 to delineate the precise domain of Dia-1 that binds the RAGE tail. Experiments revealed the RAGE tail interacted with the Formin Homology domain (FH) of Dia-1, but not the Rho GTPase binding domain. The vascularity of this interaction was identified by immunoprecipitation and expression analysis, and its co-localization in RAGE in aortic arch ESs and coronary, and monocytederived cells. To test the biological relevance of the RAGE / Dia-1 interaction, functional studies were performed using siRNA to knockdown Dia-1 expression or scramble siRNA as control. Compared to siRNA scramble control, Dia-1 siRNA blocked RAGE ligand stimulated cellular migration and invasion. In conclusion, we have identified a novel signal transduction mechanism linking the intracellular function of RAGE to Diaphanous-1. We propose that blockade of RAGE/Dia-1 interaction represent a novel target for therapeutic intervention in vascular disease.

Multiplexed Immunoassays for Simultaneous Quantification of Soluble Cardiovascular and Metabolic Biomarkers in Mouse Blood

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Mouse is a model in cardiovascular research. Limited blood volume has increased the difficulty of measuring multiple biomarkers necessary for understanding the biological processes associated with cardiovascular disorders (e.g. atherosclerosis, inflammation and arterial thrombosis). Using the xMAP system, we developed multiplexed immunoassay panels requiring small sample volume for simultaneous quantification of multiple circulating biomarkers (e.g. apolipoproteins, adhesion molecules, acute phase proteins, and proinflammatory cytokines, etc) in mouse samples. Based on analyte compatibility, four separate multiplexed immunoassay panels were developed (Panel 1: sE-selectin, sICAM-1, sVCAM-1; Panel 2: Adiponectin, Apolipoprotein A1, Apolipoprotein E, and Fibrinogen; Panel 3: 22 cytokines and chemokines; and CRP single-plex assay). Samples were incubated overnight at 4 °C in a 96-well microtiter filter plate containing a mixed population of polyethylene beads with unique fluoroscent signature and covalently immobilized with various specific capture antibodies. After washing, captured antibodies on beads were incubated for 1 h at RT with a cocktail of biotinylated detection antibodies. Following subsequent incubation with streptavidin-phycocerythrin, fluorescent signals on beads were quantified using a LumineX Reader. Each antibody pair targeting an individual analyte is highly specific, with no or negligible cross-reactivity to other analytes within the panel. The assay robustness is demonstrated by acceptable precisions (CV: 15% for inter-assay variations; CV: 10% for intra-assay variations), and accuracy (100 ± 30%) for serum or plasma samples. According to analyte concentrations, serum or plasma samples require no dilutions (Panel 3), dilutions of approximately 1:100 (Panel 1), 1:200 (CRP, or 1:5000 (Panel 2). Thus, the total serum/plasma volume required for measuring all analytes is less than 55 μl for duplicate measurement. The assays may be used for other sample types (e.g. cell culture supernatant, tissue/cell lysate). The small sample assay protocol represents a reproducible and economic tool for simultaneous quantification of multiple CVD and metabolic biomarkers in mouse samples.

The Apolipoprotein(a) Present in the Carotid Artery Plaques of Subjects with High Lipoprotein(a) Reacts Against an Antibody Specific for Oxidized Phosphatidylcholine, Which Is Endowed with a Proinflammatory Potential

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Introduction-In previous studies in human macrophage cultures we showed that human apolipoprotein(a), apo(a), has pro-inflammatory properties attributable to the presence of oxidized phospholipid (ox-PC) chemically linked to lysine residues in apo(a), kringle V. Hypothesis-Based on these observations and that circulating apo(a) is found in human carotid artery plaques, we set out to determine whether plaque apo(a) also contains ox-PC adducts, shown previously in vitro to have pro-inflammatory properties. We speculate that in concentrations of apo(a). We show that plaque apo(a) can undergo cleavage, an expression of the oxidative stress and inflammation processes associated with cardiovascular disorders (e.g. atherosclerosis, inflammation and arterial thrombosis). We have previously shown that PDGF-BB enhances monocyte chemoattractant protein-1 (MCP-1) mRNA stability in SMCs by down-regulating a ribonuclease activity. To identify downstream targets of the PDGF-RK pathway, we transiently transfected human smooth muscle cells with 5 μM of actinomycin D (Act D) and PDGF-BB (5ng/ml) for 3 hrs in the presence of inhibitors of PDGF-mediated signaling pathways, and MCP-1 mRNA levels were measured by RT-PCR. 15αM of PD2 (Src inhibitor), PP98059 (MAP kinase MEK, inhibitor), wortmannin (PI3-kinase inhibitor) or DSP (NABP inhibitor) blocked the effect of PDGF, whereas U73122, a phospholipase C (PLC) inhibitor completely blocked the effect. We next identified subjects with high plasma Lp(a), the apo(a) complexed to ox-PC may contribute to the formation and progression of the carotid plaques.
downstream targets of PLC, Go 6850 (an inhibitor of PKCcζ, ε, γ), 6851 (an inhibitor of PKCε, γ), and 6852 blocked the effect of PDGF whereas Ros320432 (an inhibitor of PKCe, ε, γ) and 6853 blocked the effect of PDGF. Because of similar results, we used the single PKCε, e, ε inhibitor or BAPTA (a Ca2+ chelator) did not. To further elucidate the particular PKC isozyme(s) involved in the mRNA stabilizing effect, siRNA to PKCc, ε, and γ/ε were transfected into SMC at a ratio of 60 pmol siRNA/3 million cells. The efficacy of each siRNA was more than 70% as determined by immunoblotting. Reduction of PKCe blocked the stabilizing effect of PDGF-BB on MCP-1 mRNA, whereas reduction of PKCe ε or ε did not. The above studies suggest a novel role for PKCe in mediating the effect of PDGF on mRNA stability. Further elucidation of the signaling pathways through which PDGF-BB enhances MCP-1 mRNA stability may provide new approaches for inhibiting vascular inflammation and atherosclerosis.

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**Gene Expression and Pathway Analyses of Atherosclerotic Aorta Reveals Significant Dysregulation of Calcium Signaling Pathway**

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Atherosclerosis is a complex disease resulting from the interactions of genetic and environmental risk factors leading to heart failure and stroke. We hypothesize that risk factors interact to alter expression of genes and pathways in the aortic wall and hence render it susceptible to atherosclerosis. Using an atherosclerotic mouse model (Ldlr−/−Apoe−/− designated as LDLr), we performed microarray gene expression analysis to investigate candidate genes and pathways which are perturbed by the following risk factors: genetics (control C57BL/6 vs. LDLr mice), eaher stress (lesion-prone vs. lesion-resistant regions in LDLr mice), diet (chow vs. high fat fed LDLr mice) and age (2-month-old vs. 8-month-old LDLr mice). Male C57BL/6 and LDLr mice (n=16/group) were fed on either a chow or a high fat diet, sacrificed at 2- and 8-months-old, and RNA was isolated from the aortic lesion-prone and -resistant segments. Using Affymetrix Murine 430 2.0 chips (n=64), we profiled differentially expressed genes with the following criteria: p-value of \( < 0.05 \), fold change \( > 2.0 \). Then, normalized using two normalization methods the invariant probe sets (dChip) and the quantile normalization (known as RMA), the statistical analysis was performed using t-test and ANOVA and pathway analyses (Pathway Express by Wayne State) were performed. The results revealed altered biological pathways. Both statistical analyses ranked calcium signaling as the most perturbed pathway followed by focal adhesion, MAPK, complement and coagulation, cytokine-cytokine receptor, apoptosis, circadian rhythm, P53 signaling, Toll-like receptor, Wnt signaling and so on. When we stratified the data by age, MAPK pathway was perturbed at 2-month of age, whereas Calcium signaling pathway was most perturbed in the 8-month-old mice. In summary, our analyses demonstrated that genes in MAPK pathway might play a significant role in the onset of atherosclerotic disease, which is augmented to perturb Calcium signaling pathways which are perturbed by the following risk factors: genetics, environment stress, age and diet. These results may serve as important targets for future therapeutic intervention.

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**Ultrasonic Confocal Imaging of C-Reactive Protein Binding to Fcγ-Receptors**

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**Background:** C-reactive protein (CRP), the prototype human acute phase protein, is widely regarded as a key player in cardiovascular disease. Fcγ-receptors have been controversially discussed since 1999 to be receptors for CRP. Applying of FACS analysis and anti-CRP antibodies or radioactive labeled CRP to resolve the CRP binding led to false positive results. Aim of the studies: Definition of CRP-binding to Fcγ-receptors applying highly sensitive technique overwhelming methodological problems leading to artifacts. Methods: Using ultrasonic confocal imaging analysis and gently labeled CRP we were able to overcome that problems. The technique enables us to perform observations and incubations on single native cells and precisely association and equilibrium analysis. Results: We have, therefore, generated an ApoE−/− mouse model genetically deficient in COX-2 (DKO), to address the role of COX-2 in early and late atherosclerosis. COX-2−/− mice were crossed with ApoE−/− mice to generate ApoE−/−/COX-2−/− double knockout (DKO). ApoE−/−/COX-2−/−, and ApoE−/−/COX-2+/− mice. Mice (n=3 per group) were fed a 1% cholesterol diet for 8 or 20 weeks. Serum cholesterol levels did not differ between groups at either time point, nor did serum triglyceride, and plasma high density lipoprotein cholesterol levels change between groups. At 8 weeks, there was no difference in atherosclerotic lesion burden in the aortic arch or descending aorta of ApoE−/−/COX-2−/−, ApoE−/−/COX-2+/− or ApoE−/−/COX-2+/+ mice. Following 20 weeks cholesterol feeding, however, plaque burden was significantly increased in the aortic arch of the ApoE−/−/COX-2−/− compared with the ApoE−/−/COX-2+/+ (1.9±0.1 vs. 33.8±5%, P<0.01), and was increased to an intermediate level in the ApoE−/−/COX-2−/− (52.9±1.2%, P<0.05). En face analysis of the descending aorta revealed that, although there was a trend towards a modest increase in plaque burden in the DKO (21±5.3% vs. 15±6.2%), this did not reach statistical significance. The discrepancy between aortic arch and descending aorta atherosclerosis may reflect differing rates of plaque formation at different areas of the aortic tree, as has been reported elsewhere (Moore, 2005). In conclusion, these data imply an anti-atherosclerotic function for COX-2 in late atherosclerosis, and its absence may accelerate advanced atherosclerotic plaque development at some lesion prone sites of the aortic tree.

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**COX-2 Gene Deletion Increases Advanced Aortic Arch Atherosclerosis in the ApoE−/− Mouse Model**

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Cyclooxygenase-2 (COX-2) catalyses the formation of prostaglandins from arachidonic acid. COX-2 expression is induced in the early phase of the inflammatory response, and again during the late phase, when it has a role in the resolution of inflammation. Atherosclerosis has a chronic inflammatory component, associated with increased COX-2 expression and COX-2 derived prostaglandin production. The role of COX-2 in atherosclerosis is unclear. We have shown that selective COX-2 inhibition does not affect early atherosclerosis in the ApoE−/− (Belton, 2003). To date, however, the effect of COX-2 gene deletion has not been investigated.

We have, therefore, generated an ApoE−/− mouse model genetically deficient in COX-2 (DKO), to address the role of COX-2 in early and late atherosclerosis. COX-2−/− mice were crossed with ApoE−/− mice to generate ApoE−/−/COX-2−/− double knockout (DKO). ApoE−/−/COX-2−/−, and ApoE−/−/COX-2+/− mice. Mice (n=3 per group) were fed a 1% cholesterol diet for 8 or 20 weeks. Serum cholesterol levels did not differ between groups at either time point, nor did serum triglyceride, and plasma high density lipoprotein cholesterol levels change between groups. At 8 weeks, there was no difference in atherosclerotic lesion burden in the aortic arch or descending aorta of ApoE−/−/COX-2−/−, ApoE−/−/COX-2+/− or ApoE−/−/COX-2+/+ mice. Following 20 weeks cholesterol feeding, however, plaque burden was significantly increased in the aortic arch of the ApoE−/−/COX-2−/− compared with the ApoE−/−/COX-2+/+ (1.9±0.1 vs. 33.8±5%, P<0.01), and was increased to an intermediate level in the ApoE−/−/COX-2−/− (52.9±1.2%, P<0.05). En face analysis of the descending aorta revealed that, although there was a trend towards a modest increase in plaque burden in the DKO (21±5.3% vs. 15±6.2%), this did not reach statistical significance. The discrepancy between aortic arch and descending aorta atherosclerosis may reflect differing rates of plaque formation at different areas of the aortic tree, as has been reported elsewhere (Moore, 2005). In conclusion, these data imply an anti-atherosclerotic function for COX-2 in late atherosclerosis, and its absence may accelerate advanced atherosclerotic plaque development at some lesion prone sites of the aortic tree.

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**Passive Immunization with IK-17, a Human Oxidation-specific Antibody, Reduces Progression of Atherosclerosis in LDLr−/− mice**

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Introduction Experimental evidence suggests that immunization strategies targeting oxidation-specific epitopes may be atheroprotective. IK-17 is a human Fab antibody that binds both maldialdehyde/MDA-LDL and copper-oxidized LDL (oxLDL) and also inhibits uptake of oxLDL by macrophages. We therefore hypothesized that passive immunization of LDLr−/− mice with IK-17 will reduce the progression of atherosclerosis. Methods and Results 24 male LDLr−/− mice (6 weeks old) were placed on a 1.25% cholesterol/21% milkfat diet for 2 weeks to increase total cholesterol (TC) levels to ~1500 mg/dl and to initiate atherosclerosis. They were then switched to a 0.5% cholesterol diet and reached TC levels of ~600 mg/dl. The mice were then randomized to treatment with IK-17 (2.5 mg/kg/d) or PBS control intraperitoneally 3 times per week. After 14 weeks of treatment, atherosclerosis was quantitated as the % of the aortic arch lesion area and as the aortic root area. There was significant reduction in % atherosclerotic surface area lesion size in the IK-17 group compared to the control group (4.9±0.8 vs. 6.9±1.6%, P<0.001), whereas there was only a trend for reduction in the aortic root area with IK-17 treatment (1.98 ± 0.57 μm2 vs. 2.25±0.28 μm2, P<0.17). The diet led to significant and progressive increases in autoantibody titers and titers of immune complexes to oxLDL in both groups. There was a significant increase in anti-human antibodies in the IK-17 group but not in the control group. Conclusion Passive immunization with human oxidation-specific antibody K17 in LDLr−/− mice led to a reduction in atherosclerosis in the entire aorta and a trend in the aortic root. This study suggests that human antibodies to oxLDL may prevent progression of atherosclerosis.
Glycemia, Triglycerides, and Disease Severity Are Best Associated with Higher Platelet Activity in Patients with Coronary Artery Disease

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Background: Although higher platelet activity has been described in patients with acute coronary syndromes consistently by many authors, consistent findings are reported about the relation of platelet activity to disease severity in stable patients with chronic coronary artery disease. Nonetheless, most reports studied only very small groups of patients. The aim of our study was to assess the relation of platelet activity to disease severity in sufficient number of patients with chronic coronary artery disease. Methods: One hundred and sixty stable patients with chronic coronary artery disease were studied (25 with single-, 63 with double- and 72 with triple-vessel disease). 95% of them were on aspirin, 70% on clopidogrel medication. All patients suffered from acute coronary syndrome. Platelet activity was determined as membrane expression of antigens CD62P (P-selectin, as % of positive cells) and CD41 (part of GpIIb/IIIa integrin, as mean fluorescence intensity) by flow cytometry. Platelet aggregability was measured by light ADP aggregation (LADP, compared by Student's t-test, correlation by Spearman test). Data are shown as mean±SD. Results: Membrane CD62P expression correlated with vessel severity (p<0.001, Kruskal-Wallis test). Patients with triple-vessel disease had the highest CD62P expression (1.81±0.19) followed by patients with double-vessel (1.64±0.22) and single-vessel (0.69±0.09) disease. Positive correlation was found between CD62P expression with triglycerides (r=0.49, p<0.05) and CD41 with fasting glucose (r=0.05). No differences in ADP aggregability were found between groups. Conclusion: Higher platelet activity is present in patients with more severe coronary artery disease. More aggressive antiplatelet treatment in these patients should be considered, especially when metabolic syndrome is simultaneously present.

Human Prostacyclin Receptor Polymorphisms and Atherosclerosis

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Selectivity inhibitors of cyclooxygenase 2 (COX-2) confer a small but absolute risk of myocardial infarction and stroke presumably through inhibition of COX-2-dependent prostacyclin (PGI2). PGlu acts as a general renant on endogenous stimuli to platelet activation, vascular proliferation, and remodeling, hypertension, atherosclerosis, and cardiac function risk. Development of genetic biomarkers will be useful to identify the patients uniquely susceptible at developing risk of cardiovascular complications by selective inhibition of COX-2. We aimed to investigate the association between 31 distinct single nucleotide polymorphisms (SNPs) in the human PGI2 receptor (IP) and intima-media thickness(IMT) of the common carotid arteries, a surrogate measure for systemic vessel disease, in 40 individuals with a previous objectively confirmed deep vein thrombosis (DVT) and 21 controls, i.e. individuals without DVT but with comparable cardiovascular risk factors (cigarette smoking, diabetes, hypertensive status, lipid levels, body mass and systemic inflammatory status index, i.e CRP, fibrinogen). We identified 5 SNPs which were not statistically significant different between the 2 groups (using Chi square test). Three were synonymous (no alteration in the coding amino acid sequence), V53V(DVT, 40% versus controls, 13%), V196 (DVT, 2.5% versus controls, 0%), S282S (DVT, 60% versus controls, 57%), and 2 were nonsynonymous (change in the coding amino acid sequence), P226T (DVT, 2.5% versus controls, 0%), and R212C (DVT, 7.5% versus controls, 5%). Interestingly, R212C polymorphism is associated with functional deficiencies. In DVT and controls, IMT values were not significantly different (1.12±0.37 in DVT vs. 1.08±0.37 in controls, p>0.05). No differences in ADP aggregability were found between groups. Conclusion: Higher platelet activity is present in patients with more severe coronary artery disease. More aggressive antiplatelet treatment in these patients should be considered, especially when metabolic syndrome is simultaneously present.

Prevalence of PAD decreases with increasing total bilirubin level

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Background: Endogenous protective mechanisms that lessen susceptibility to peripheral arterial disease (PAD) are unknown. Bilirubin, a potent antioxidant and cytoprotectant, is a strong candidate for atheroprotection. We hypothesized that higher bilirubin levels would diminish the likelihood of developing PAD. Methods and Results: We examined the association of serum total bilirubin level with PAD in the National Health and Nutrition Examination Survey, 1999–2004. PAD was defined as an ankle brachial index of < 0.9. A total of 7074 adults had complete data for analysis. The overall prevalence of PAD was 5.8%. PAD prevalence inversely associated with bilirubin level (Figure). A 1 mg/dl increase in bilirubin was associated with a 48% reduced likelihood of PAD (0.52 [0.33; 0.83]) after adjustment for age, gender, race/ethnicity, BMI, smoking status, diabetes, hypertension, hypercholesterolemia, coronary kidney disease, CRP, and homocysteine. Additional adjustment for liver disease and alcohol intake did not affect this finding. Using logistic regression models (P<0.05) we found that HDL-C and LDL-C were associated with PAD (OR of 1.05 per 1 mg/dl increase). Conclusions: Higher platelet activity is present in patients with more severe coronary artery disease. More aggressive antiplatelet treatment in these patients should be considered, especially when metabolic syndrome is simultaneously present.

The known antioxidant and cytoprotectant properties of bilirubin and suggest that bilirubin lessens susceptibility to PAD.

Association of A-290G Polymorphism of CYP3A4 Gene in Response to Atorvastatin Therapy in Coronary Artery Disease

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The P450 cytochromes are super family of hemeproteins involved in the metabolism of various drugs and the isoenzymes show wide variation influencing both drug responses. Cytochrome 3A4 (CYP3A4) is the primary enzyme in the metabolism of Atorvastatin (lipid lowering drugs) and activity varies 10-folds in different ethnic populations. In the present study, we have examined the association of the functional variant in CYP3A4 gene in response to Atorvastatin in coronary artery disease (CAD) patients in North Indian population. It was a case-control study consisting of 101 CAD patients & 102 controls. We studied the single nucleotide polymorphism in the promoter region (A-290G) of CYP3A4 gene by Polymerase chain reaction - restriction fragment length polymorphism. The genotype frequencies of CYP3A4 gene were, AA: 33.67, AG: 39.60% and GG: 26.73% in patients group and in controls the frequencies were, AA: 59.60%, AG: 28.43 & GG: 11.78%. The frequency of homozygous mutant genotype GG was significantly higher in patients as compared to controls (p=0.008 and a significant reduction was seen in the levels after treatment with statin (p = Standard error

Chronic Intermittent Hypoxia Induces Atherosclerosis in C57BL/6J Mice

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Introduction: Obstructive sleep apnea (OSA), a condition leading to chronic intermittent hypoxia (OIHX), is associated with hyperlipidemia, atherosclerosis and a high cardiovascular risk. A causal link between OSA and atherosclerosis has not been established. Hypothesis: CHI may induces atherosclerosis in C57BL/6J mice. Methods: Forty male C57BL/6J mice, 6 – 8 weeks of age, were fed either a high cholesterol diet (HCD) or a regular chow diet (RD) and subjected either to CIH or intermittent air (IA) for 12 weeks. During exposure to CIH, FIO2 was reduced from 20.9 to 4.9 ± 0.1 % over a 30 s period and then rapidly reoxygenated to room air levels in the subsequent 30 s period. The CHI and IA states were induced during the light phase alternating with 12 h of constant room air during the dark phase. After the exposure, animals were sacrificed. The heart and proximal aorta were embedded in OCT and cross-sections were examined by Oil Red O staining. The descending aorta was examined in en face preparation stained with Sudan IV. Results: Nine out of ten mice simultaneously exposed to CHI and HCD developed atherosclerotic lesions in the aortic origin and descending aorta. In contrast, atherosclerosis was not observed in mice exposed to IA and HCD or in mice exposed to CHI and RD. HCD resulted in significant increases in serum total and LDL cholesterol (LDL-C) levels and a decrease in HDL cholesterol. Comparing to mice exposed to IA and HCD, combined exposure to CHI and HCD resulted in marked progression of dyslipidemia with further increases in serum total cholesterol (206 ± 8 mg/dl vs. 172 ± 12 mg/dl, respectively, p< 0.005) and LDL-C (124 ± 4 mg/dl vs. 106 ± 6 mg/dl, p < 0.05), a z-fold increase in serum lipid peroxidation (malondialdehyde level of 1.33 ± 0.11 μM vs. 0.61 ± 0.05 μM, p< 0.05), and up-regulation of an important hepatic enzyme of lipoprotein secretion, stearoyl coenzyme A desaturase 1. Conclusions: CHI causes atherosclerosis in the presence of diet-induced dyslipidemia.

Deficiency of Herp, an ER Stress Protein, Suppresses Atherosclerosis in apoE-deficient Mice

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Introduction: Herp is ER stress protein originally found in vascular endothelial cells. It is not expressed in atherogenesis. Deficiency of Herp, an ER stress protein, suppresses atherosclerosis in apoE-deficient mice. Methods: Twelve-week-old C57BL/6J mice were divided into four groups: wild-type control (C), ApoE−/− (Def), and ApoE−/− on a high-cholesterol diet (HCD). Results: Herp mRNA and protein were detected in the aorta. Atherosclerotic lesion was significantly smaller

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In Herp-/-;apoE-/- mice than in apoE-/- mice, while there was no significant difference in serum cholesterol, TG and FFA levels. ER stress induced IL-1b in cultured macrophages, and Herp deficiency decreased the expression. Amounts of mRNA for IL-1b and VCAM1 were significantly lower in Herp-/-;apoE-/- mice than in apoE-/- mice. Conclusions: Herp deficiency suppresses atherosclerosis in apoE-/- mice. It is partly attributed to decrease in ER-stress-induced IL-1b production by acrylamide and decreased VCAM-1 expression in the artery.

Leptin Induces C-reactive Protein Expression in Vascular Endothelial Cells

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There is increasing evidence of an association between leptin and increased cardiovascular risk. Several studies have shown an independent interaction between high leptin levels and atherosclerosis, myocardial infarction, stroke, and coronary artery intima-media thickness, suggesting that high leptin levels imply increased vascular risk. Thus, mechanisms underlying the association of leptin with poor cardiovascular outcomes are not well understood. C-reactive protein (CRP) elicits proatherogenic effects in the vascular endothelium and positively correlates with leptin levels in humans. There have been no studies investigating the role of leptin in regulating CRP expression in vascular endothelial cells. We tested the hypothesis that leptin induces CRP expression in human coronary artery endothelial cells (HCAEC) and sought to determine the signaling pathways involved. We confirmed the presence of both long and short isoforms of the leptin receptor in HCAEC. Incubation of HCAEC with leptin (0–400 ng/ml) caused a dose-dependent increase of CRP mRNA and protein. This leptin-induced increased CRP expression was attenuated in the presence of anti-leptin receptor antibodies and also by inhibition of extracellular signal-regulated kinases1/2 (ERK1/2) by PD98059 (20–40 μM). Time (0–60 min) and leptin concentrations (0–200 ng/ml) dependence of ERK1/2 phosphorylation were evident in response to leptin treatment. Leptin (100 ng/ml) also elicited reactive oxygen species (ROS) generation. Inhibition of ROS by catalase (400 μg/ml) prevented ERK1/2 phosphorylation. Thus, leptin induces CRP expression in HCAEC via activation of the leptin receptor, increased ROS production and phosphorylation of ERK1/2. The local expression of CRP in HCAEC likely plays an important role in the development and progression of atherosclerotic lesions. In conclusion, these studies suggest a mechanism for the proatherogenic effects of leptin. Leptin may provide a target for the development of preventive and therapeutic strategies against inflammatory mechanisms predisposing to cardiovascular disease.

Environmental and Lipid Oxidation–Derived Aldehydes Induce Unfolded Protein Response in Endothelial Cells

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Accumulating evidence suggest that endoplasmic reticulum (ER) stress and unfolded protein response (UPR) contribute to several disease process including atherosclerosis. Increased expression of UPR target genes activating transcription factor 3(ATF3) and ATF4 has been reported in human atherosclerotic lesions. Staining for the marker of the unfolded protein in cells with antibodies that recognize the aldehydic epitopes of oxidized LDL, suggests that lipid derived aldehydes could be involved in mediating ER stress and UPR. In the present study, we examined the role of endogenous aldehydes generated during phospholipid oxidation (e.g., 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) and 4-hydroxy, trans-2-nonenal (HNE) and acrolein, an environmental aldehyde in the induction of ER stress in human umbilical vein endothelial cells (HUVEC) and human aortic endothelial cells (HAECE). Our data suggest, that these aldehydes (50 μM) cause the activation of double stranded RNA-activated protein kinase-like ER kinase (PERK) by 1.5–3.0 fold in endothelial cells. Moreover, HNE (10–50 μM) and POVPC (10–25 μM) also cause the activation of its downstream effectors eIF2α (eukaryotic initiation factor-2α) by 1.5–5.5 fold, ATF4 by 1.5–3.0 fold and ATF3 by 2–10 fold in these cells. POVPC (10–25 μM) also cause the activation of mitogen activated protein (MAP) kinase-ERK, p38 and JNK by (3–6 fold) in endothelial cells. Reduction of POVPC to its phosphatase-3

Regulation of Homocysteine Transport in Vascular Cells

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Increased levels of plasma homocysteine is an independent risk factor for cardiovascular disease and has cell-type distinct proatherosclerotic effects on vascular cells. In this study, we characterized L- homocysteine transport in cultured human aortic endothelial and aortic smooth muscle cells. L-homocysteine was transported into vascular cells in a time-dependent fashion, L-homocysteine transport activity was about 2-fold higher in aortic smooth muscle cells. In addition, L-homocysteine transport in both cell types was mediated by sodium-dependent and independent carrier systems. Competition studies revealed that the neutral amino acids cysteine, glycine, serine, tyrosine, alanine, leucine, and methionine, and inhibitors of these transport systems inhibited L-homocysteine uptake in both cell types, but the inhibition was greater in endothelial cells. Edetate-DEPENDENT PLAYS A ROLES IN THE UPTAKE OF L-HOMOCYSTEINE IN ENDOTHELIAL CELLS. Moreover, L-homocysteine transport in endothelial cells was partially inhibited by lysosomal inhibitors. Our studies indicate that L-homocysteine shares transporter systems with cysteine and can be inhibited for transport by multiple neutral amino acids in vascular cells, and that L-homocysteine transport involves both sodium-dependent and sodium-independent transporters in endothelial cells. The specific lysosomal feature of L-homocysteine transport in endothelial cells may contribute to cell-type specific growth inhibition effects and therefore play a role in homocysteine atherogenic potential.

Activation of the Endothelial S1P1 Receptor Inhibits Erk1/2 Phosphorylation in Type 1 Nonobese Diabetic Mice Through Induction of MAP Kinase Phosphatase-3

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Endothelial activation is a key early event in vascular complications of Type 1 diabetes. The non-obese diabetic (NOD) mouse is a well-characterized model of Type 1 diabetes. We recently
found that diabetic NOD mice have increased endothelial activation, with increased production of MCP-1 and IL-6, and a 30% induction of surface VCAM-1 expression. Using freshly isolated aorta in an ex vivo adhesion assay, we found that diabetic NOD mouse aorta versus 71.9+9 monocytes bind in diabetic NOD mouse aorta, p<0.0001). Further, the sphingolipid sphingosine-1-phosphate (S1P) prevents monocytic endothelial interactions in these diabetic NOD mice. Previously, we reported that S1P treatment of diabetic NOD endothelial cells (EC) reduced Erk1/2 phosphorylation by 90%, with no significant changes in total Erk1/2 protein. To further investigate the mechanism causing the dramatic downregulation of Erk1/2 phosphorylation by S1P, we examined expression of mitogen-activated kinase phosphatase-3 (MKP-3), a cytoplasmic phosphatase expressed in EC that dephosphorylates Erk1/2 to prevent mobilization to the nucleus for gene transcription. S1P caused a significant 3-fold increase in MKP-3 expression in EC. To mimic the S1P induction of MKP-3 in diabetic NOD EC, we performed time-course phosphorescence spectroscopy in EC. Downregulation of MKP-3 in EC decreased Erk1/2 phosphorylation. We next incubated EC with either 100 nM S1P, 1µM S2EW2871, a S1P receptor-specific agonist or with 10µM VPC 23019, a S1P1/3 receptor antagonist. S1P and S2EW2871 significantly increased expression of MKP-3 and reduced Erk1/2 phosphorylation, but VPC 23019 decreased the expression of MKP-3, both results supporting a role for S1P1 in MKP-3 regulation. We also found that U0126 (10µM), a novel MEK1/2 inhibitor, blocked the anti-inflammatory action of S1P, resulting in an 80% increase in monocyte adhesion. Correspondingly, there was a 4-fold decrease in MKP-3 levels suggesting that U0126 regulates MKP-3 expression. Thus, a primary mechanism for the anti-inflammatory action of S1P in diabetic NOD EC is inhibition of Erk1/2 phosphorylation through induction of MKP-3 expression via the S1P1 receptor. SEW2871 significantly increased expression of MKP-3 and reduced Erk1/2 phosphorylation, but VPC 23019 decreased the expression of MKP-3, both results supporting a role for S1P1 in MKP-3 regulation. We also found that U0126 (10µM), a novel MEK1/2 inhibitor, blocked the anti-inflammatory action of S1P, resulting in an 80% increase in monocyte adhesion. Correspondingly, there was a 4-fold decrease in MKP-3 levels suggesting that U0126 regulates MKP-3 expression. Thus, a primary mechanism for the anti-inflammatory action of S1P in diabetic NOD EC is inhibition of Erk1/2 phosphorylation through induction of MKP-3 expression via the S1P1 receptor.

Acute Changes in Shear Stress Induce Expression of Inflammatory Proteins in a Mouse Coarctation Model

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Blood flow has been identified as an important factor in the pathogenesis of atherosclerosis. Under disturbed flow conditions, wall shear stress (WSS) is transduced by the endothelium into a biological signal that produces pro-atherosclerotic responses. There is experimental data from cell culture studies in artificial hemodynamic environments. Therefore, we developed a novel mouse aortic coarctation model to alter the hemodynamic environment in vivo. This model utilizes shape memory polymer clips to provide a high degree of control over aortic diameter and subsequently WSS. We employed this model to test the hypothesis that acute changes in WSS with on-treatment LDL-C (r = 0.39, p = 0.002). Change in NWI and on-treatment lipid levels were correlated for TC (r = 0.39, p = 0.03). TG (r = 0.36, p = 0.04) and Apo B (r = 0.39, p = 0.03). Change in % of LRNC correlated with on-treatment LDL-C (r = 0.35, p = 0.05). Conclusion: Greater lipid response to RSV therapy was associated with a reduction in plaque size. Individuals with less response to lipid-lowering therapy continued to have plaque progression. These findings support the idea of very intensive lipid lowering to achieve reduction in atherosclerosis.

Significance of Focal Coronary Calcification Adjoining Noncalcified Plaques Evaluated by Multislice Computed Tomography

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Purpose To determine the significance of focal coronary calcified plaques (PC) adjoining noncalcified plaques (NCP) using multislice computed tomography (CT). Materials and Methods We evaluated the coronary arteries of 348 subjects using ECG-gated multislice CT. We classified subjects into the following four groups: (1) with focal PC adjoining NCP, (2) without focal PC but NCP occurring adjacent to the PC, (3) with PC but no NCP, and (4) neither NCP nor CP. Groups were compared as to age, sex, coronary risk factors (RFs) and incidence of RFs between Groups 2 and 3. Comparing Groups 1 and 2, the incidence of RFs (p < 0.01), and smoking (p < 0.01). The incidence of obesity was higher in Group 1 (44%) than in Group 3 (28%, p < 0.01). The incidence of HL was higher in Group 1 (62%) than in Group 2 (40%, p < 0.01) and 4 (34%, p < 0.01). There was no significant difference among the other RFs between Groups 1 and 4. Comparing Groups 2 and 3, the incidence of RFs (p < 0.01), and smoking (p < 0.01) were significantly higher in Group 1 than in Group 2. Variables were used in logistic regression models with the presence of focal PC adjoining NCPs as the dependent variable in subjects with NCPs (sum of Groups 1 and 2), HD and obesity (relative risks 2.40 and 2.52 [95% CIs: 1.02–5.65 and 1.03–6.18, respectively] were associated with increased incidence of focal PC adjoining NCPs. Conclusion Coronary RFs were more frequent in subjects with focal PC adjoining NCP than in other groups. Focal PC adjoining NCP may indicate the most advanced coronary atherosclerosis and may be important in the treatment of coronary artery disease.

Association of On-treatment Lipid Levels and Carotid Plaque Progression Assessed by Magnetic Resonance Imaging

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Background: Lowering of low-density lipoprotein cholesterol (LDL-C) via statin therapy has been associated with regression in coronary atherosclerosis. We examined the association between lipid-lowering therapy and changes to carotid plaques as identified by magnetic resonance imaging (MRI) after 2 yrs of on-treatment (–1.18 mg/dL) treatment. Methods: Fourteen male subjects (70% men; mean age 65 yrs) with LDL-C ≥100 and <250 mg/dL and 16%-79% carotid stenosis by ultrasound were administered daily RSV (low dose [5 mg] or high dose [40/80 mg]) for 2 yrs. Multi-sequence, high-resolution carotid MRI at 1.5T was done at baseline and after 2 yrs of RSV treatment. Subjects were compared as to age, sex, hypertension (HT), diabetes mellitus (DM), hyperlipidemia (HL), smoking history, and obesity). Significantly younger than the other groups, but there was no significant difference among the other RFs between Groups 2 and 3. Comparing Groups 1 and 2, the incidence of RFs (p < 0.01), and smoking (p < 0.01). The incidence of obesity was higher in Group 1 (44%) than in Group 3 (28%, p < 0.01). The incidence of HL was higher in Group 1 (62%) than in Group 2 (40%, p < 0.01) and 4 (34%, p < 0.01). There was no significant difference among the other RFs between Groups 1 and 4. Comparing Groups 2 and 3, the incidence of RFs (p < 0.01), and smoking (p < 0.01) were significantly higher in Group 1 than in Group 2. Variables were used in logistic regression models with the presence of focal PC adjoining NCPs as the dependent variable in subjects with NCPs (sum of Groups 1 and 2), HD and obesity (relative risks 2.40 and 2.52 [95% CIs: 1.02–5.65 and 1.03–6.18, respectively] were associated with increased incidence of focal PC adjoining NCPs. Conclusion Coronary RFs were more frequent in subjects with focal PC adjoining NCP than in other groups. Focal PC adjoining NCP may indicate the most advanced coronary atherosclerosis and may be important in the treatment of coronary artery disease.
network length (AU) | 4222 ± 163 vs 2550 ± 367 and 4222 ± 163 vs 1830 ± 489 for Notch 1 and 4, respectively. Moreover, inhibition of endogenous Notch mediated CBF1/RBP-JK regulated gene expression using Epstein-Barr virus encoded RPMs-1 resulted in a significant decrease in ethanol-induced network formation; network length (AU) | 2220 ± 380 vs 1090 ± 263 for control EDS vs RPMs-1 EDS. These data demonstrate that ethanol, at a level consistent with moderate consumption, regulates the expression of Notch receptors and downstream target genes in endothelial cells. Moreover, ethanol-stimulated EC angiogenic activity is mediated via a Notch/CBF-1/RBP-JK dependent pathway. These actions of ethanol may be relevant to the effects of alcohol consumption and disease progression.

Important Role of Erythropoietin Receptor in Promoting Vascular Endothelial Growth Factor Expression and Angiogenesis in Peripheral Inflammation in Mice

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Background: Prognosis of patients with severe peripheral artery disease (PAD) still remains poor when there are no indications of recanalization therapies such as bypass surgery or percutaneous transluminal angioplasty. It has been demonstrated that erythropoietin (Epo) promotes angiogenesis. The role of Epo in solid tumors, however, is not fully clarified. Furthermore, it has been demonstrated that erythropoietin (Epo) can stimulate angiogenesis in ischemic tissues. We have also recently demonstrated that endogenous Epo/Epo receptor (EpoR) system plays an important protective role in hypoxia-induced pulmonary hypertension. In this study, we examined the role of Epo/EpoR system in ischemia-injured angiogenesis in EpoR-/- rescued mice that lack EpoR in most organs except erythropoietin-lineage cells. Methods and Results: Two weeks after femoral artery ligation, blood flow recovery, activation of vascular endothelial growth factor (VEGF)/VEGFR receptor system, and mobilization of endothelial progenitor cells (EPCs) were all impaired in EpoR-/- rescued mice compared with wild-type (WT) mice. Bone marrow (BM) transplantation of WT-BM into EpoR-/- rescued mice partially but significantly improved blood flow recovery after hindlimb ischemia. The extent of VEGF up-regulation and the number of BM-derived cells in ischemic tissue were significantly less in EpoR-/- rescued mice compared with WT mice even after BM reconstitution with WT-BM cells. Similarly, the recovery of blood flow was significantly impaired in recipient EpoR-/- rescued mice that had been transplanted with WT-BM or EpoR+/−-rescued-BM as compared with recipient WT mice. Furthermore, the Matrigel implantation assay and aortic ring assay showed that microvessel growth in vitro was significantly reduced in EpoR-/- rescued mice as compared with WT mice. Conclusions: These results indicate that vascular Epo system, in addition to Epo, plays an important role in angiogenesis in response to hindlimb ischemia through up-regulation of VEGF/VEGFR receptor system, both directly by enhancing neovascularization and indirectly by recruiting EPCs or BM-derived angiogenic cells. Therefore, vascular Epo system may be a new therapeutic target for the treatment of ischemic cardiovascular diseases, including PAD.

Unfolded Protein Response Mediates Induction of VEGF in Endothelial Cells by Oxidized Phospholipids

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Previous work in our group identified angiogenic activity of oxidized phospholipids (OxPLs) in several in vitro and in vivo models. Furthermore, we have shown that effects of OxPLs are mediated by upregulation of VEGF, IL-8 and COX-2-derived prostanoids, which stimulated endothelial cell growth (ECs) in a dose dependent manner. OxPLs mediates upregulation of VEGF, IL-8, COX-2 and upregulation of EpoR. In present study we used oxPLs under high glucose conditions to study the mechanism of action of these factors in endothelial cells. We identified two distinct patterns of gene expression, blood flow and microvessel formation in oxPLs treated ECs. These differences in oxPLs treated ECs, in addition to Epo, play an important role in angiogenesis in response to hindlimb ischemia through up-regulation of VEGF/VEGFR receptor system. Furthermore, these differences in oxPLs treated ECs, in addition to Epo, play an important role in angiogenesis in response to hindlimb ischemia through up-regulation of VEGF/VEGFR receptor system, both directly by enhancing neovascularization and indirectly by recruiting EPCs or BM-derived angiogenic cells. Therefore, vascular Epo system may be a new therapeutic target for the treatment of ischemic cardiovascular diseases, including PAD.

Fractalkine Induces Angiogenesis by Stimulating Vascular Endothelial Growth Factor-A Production by Human Vascular Endothelial Cells and Improves Hind Limb Ischemia

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Background : Angiogenesis facilitates the process of inflammation or improves ischemic condition. The present study investigated the detailed mechanism by which fractalkine (Fkn), a CXC3 chemokine, induces angiogenesis in vivo. Methods and Results : Fkn induced vessel sprouting from excised rat aorta and angiogenesis on chick’s chorioallantoic membrane (CAM) through CX3CR1 activation. Immunoblotting analysis and EMSA showed that Fkn upregulated hypoxia–inducible factor 1 alpha (HIF-1α) by cultured human aortic endothelial cells (HAEcs), which in turn induced mRNA and protein productions of VEGF-A, a dominant isoform of vascular endothelial growth factor (VEGF), through pathways involving p42/44 MAPK, not through RhoA. Fkn potently induced angiogenesis on CAM and ears of C57/B16 female mice in vivo, which was blocked by a specific Rho inhibitor and a VEGF receptor 2 (KDR) blocker. The condition of hindlimb ischemia induced by obliterating rats’ left common femoral artery using a metal coil through remote access increased expression of Fkn and VEGF-A in the ischemic tissue, and the blood flow to the ischemic hindlimb was improved by injection of rat-specific whole-length Fkn protein. Conclusions : Fkn-induced angiogenesis involves two sequential steps; the induction of HIF-1α and VEGF-A gene expression through CX3CR1 activation, and the subsequent VEGF-A/VEGFR-2-induced angiogenesis through RhoA-dependent pathway. The development of new microvessels by Fkn may contribute to the growth of plaques and neoplasms during the process of atherogenesis. On the other hand, the potent induction of angiogenesis by Fkn can be used as therapeutic strategy for peripheral ischemia.

Oxidized Low-Density Lipoprotein Induces Endothelial Progenitor Cell Apoptosis by Inhibiting PI3 Kinase/Akt Pathway via Tyrosine Nitration

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Objective: The mechanisms by which risk factors such as hypercholesterolemia reduce the number of circulating endothelial progenitor cells (EPCs) in patients with critical ischemic vascular disease (CAD) are incompletely understood. We tested the hypothesis that oxidized low density lipoprotein (oxLDL) induces EPC apoptosis by inactivating PI3 kinase/Akt cell survival pathway. Methods and Results: In EPCs harvested from wt/rm (wt mice, oxLDL induced apoptosis and inhibition of Akt phosphorylation dependent manner. oxLDL rescued Akt phosphorylation and rescued apoptosis induced by oxLDL. Interestingly, oxLDL also increased p38 phosphorylation in cultured EPC in a dose-dependent manner. Treatment with the pharmacologic inhibitor of p38, SB203580, further increased oxLDL induced Akt inhibition and EPC apoptosis, suggesting an interaction between Akt and p38 MAPK pathways. Lastly, oxLDL induced hypercholesterolemic hyperphosphoprotein EPCs deficient in LDL receptor (ApoE−/Ldr−/− mice) showed a higher rate of apoptosis than EPCs from wt mice. Conclusions: oxLDL inhibits the PI3 kinase pathway and induces apoptosis by causing tyrosine nitration of PI3 kinase and inhibiting Akt mediated cell survival signaling in cultured EPCs, and may explain the enhanced apoptosis displayed by EPC harvested from spontaneously hypercholesterolemic ApoE−/Ldr−/− mice. Key words: oxidized low density lipoprotein, endothelial progenitor cell, apoptosis, tyrosine nitration, PI3 kinase, Akt, p38

Cellular and Molecular Mechanisms Mediating Blood Flow Recovery After Acute and Gradual Femoral Artery Occlusion Are Distinct in the Mouse

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Introduction: Critical limb ischemia in humans results from gradual arterial occlusion caused by atherosclerosis. Current mouse models of hindlimb ischemia use acute arterial occlusion that does not accurately mimic the pathogenesis of human chronic critical ischemia. We therefore developed the first mouse model of gradual arterial occlusion. Methods: Gradual arterial occlusion was induced by placing ameroid constrictors on the proximal and distal femoral artery, and ligating the femoral artery branches (n=34). Arterial occlusion was accomplished by excising the left femoral artery (n=34). Blood flow recovery, ischemia-induced changes in gene expression, and immunohistochemical analysis were performed for each animal model. Results: We identified two distinct patterns of gene expression, blood flow recovery, SDF-1 expression, and macrophage and hemangiocyte recruitment after gradual versus acute femoral arterial occlusion. Hypoxia-related genes increased significantly in the calf (p<0.05), but not in the thigh (p<0.05), after gradual versus acute femoral arterial occlusion. Shear-stress dependent genes and inflammatory genes were upregulated immediately in the thigh only after acute femoral arterial occlusion (p<0.05). These differences in gene expression were consistent with increased SDF-1a expression, recruitment of macrophages and hemangiocytes, and higher blood flow recovery after acute arterial occlusion compared to gradual arterial occlusion (p<0.05). Conclusion: This is the first study to show that the molecular and cellular mechanisms that regulate collateral artery enlargement and blood flow recovery are critically dependent on the rate of arterial occlusion. Overall, after gradual arterial occlusion, shear stress dependent and inflammatory genes were not upregulated. Also, macrophages and hemangiocytes recruitment were lower than after acute occlusion. Models of critical chronic limb ischemia, that more closely resemble the human condition, may provide more accurate mechanistic insights with the objective of creating novel molecular therapies.

Carotid 3-dimensional Ultrasound Atherosclerotic Plaque and Vessel Wall Thickness Maps: Location-specific Changes After Intensive Statin Treatment

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Currently, carotid atherosclerosis progression and regression is typically measured using plasma surrogates and regionally by measurement of intima media thickness from 2D ultrasound. We previously studied 38 subjects, those treated with atorvastatin for 3 months displayed a mean 14% decrease in 3DUS total plaque volume and no change in the placebo treatment group. We have subsequently developed a software analysis tool to create 2-dimensional (2D) maps of vessel wall and plaque thickness from the measured volumetric data of the intima media thickness. These maps will show location-specific changes in plaque and wall thickness in the atorvastatin group.
Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the pentose phosphate pathway, where it metabolizes glucose 6-phosphate to 6-phosphogluconolactone and generates NADPH. In endothelial cells, NADPH is an essential cofactor for dihydrolipoyl reductase, which generates tetrahydrobiopterin. Because tetrahydrobiopterin is an essential cofactor for endothelial nitric oxide synthase (eNOS), G6PD is very important for endothelial function. Increases in blood flow activate eNOS. We hypothesized that G6PD plays an essential role in flow-mediated eNOS activation. Using a core and plate viscometer, we showed that flow activated G6PD within 2 minutes. Small interference RNA knockdown of G6PD significantly inhibited flow-mediated eNOS phosphorylation. In contrast, overexpression of G6PD increased flow-mediated eNOS phosphorylation, while a G6PD mutant lacking enzymatic activity did not alter eNOS phosphorylation. Our hypothesis was further supported by data that G6PD expression increased significantly in cells exposed to laminar flow for 24 hours. In the Pretsch mouse, which has much lower G6PD activity due to decreased G6PD protein expression, aortic eNOS phosphorylation was dramatically decreased compared to wild type mice. These findings demonstrate a novel role for G6PD in vascular homeostasis, by regulating eNOS function.

**WITHDRAWN**

**Omega-3 Fish Oil Plus Ginkgo Biloba Impairs Alzheimer Nanoplaque Formation and Size in Vitro**

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The relationship between Alzheimer’s disease and coronary artery disease is striking; Alzheimer plaques and arteriosclerotic plaques are basically very similar in their chemical composition. The principal constituents of Alzheimer plaques are proteoglycans, lipoproteins (preferentially VLDL and IDL), beta-amyloid and calcium, thus nearly extended by the omega-3 fatty acids. In the present study, we tested the effect of omega-3 fish oil plus ginkgo biloba (Probrin®; SevenSeas, Hull, England) on Alzheimer nanoplaques (J. Colloid Inter. Sci. 276, 503–506 (2004) and measured in vitro their formation and size by ellipsometric techniques, a laser-based physicochemical procedure. For the study with omega-3 fish oil plus ginkgo biloba (Probrin®; SevenSeas, Hull, England), VLDL apoE4/E4 from a cardiovascularly and stroke-endangered high-risk patient was used as lipoprotein. Furthermore, human A-beta42 (0.1 g/L) which inclines strongly to aggregation and fibrillogensis, as well as EPA (21.9 mg/L), DHA (15.4 mg/L) and Ginkgo biloba (0.28 mg/L) were applied in a concentration as could be expected in the blood of proband after the intake of one capsule. The VLDL apoE4/E4 plasma fraction (30 mg/dL) showed beginning Alzheimer nanoplaque formation already at a normal blood Ca2+ concentration. Fish oil plus ginkgo applied acutely in the experiment, markedly slowed down this process of quaternary aggregational nanoplaque complex at all Ca2+ concentrations used. In a normal blood Ca2+ concentration of 2.52 mmol/L, the omega-3 + ginkgo-induced reduction of nanoplaque formation and molecular size amounted to 10.1 ± 1.3% (p < 0.03) and 14.3 ± 2.9% (p < 0.02), respectively, as compared to the controls. From these results, we concluded that the combination of omega-3 fish oil plus ginkgo hampered the docking of VLDL apoE4/E4 to the proteoglycan receptor and of A-beta42 to VLDL apoE4/E4, and that altogether Alzheimer nanoplaque formation is diminished as compared to control experiments without these substances. These in vitro experiments provide a mechanistic explanation for a possible beneficial mode of action of fish oil and ginkgo in Alzheimer, vascular or age-related cognitive decline.

**Salt Inactivates eNOS: A Mechanism of How Salt Contributes to Hypertension**

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The contribution of a high salt diet to hypertension has been debated for decades. One pertinent study, the EP 0 946 876 model (preferentially VLDL and IDL), beta-amyloid and calcium, thus nearly extended by the omega-3 fatty acids. In the present study, we tested the effect of omega-3 fish oil plus ginkgo biloba (Probrin®; SevenSeas, Hull, England), VLDL apoE4/E4 from a cardiovascularly and stroke-endangered high-risk patient was used as lipoprotein. Furthermore, human A-beta42 (0.1 g/L) which inclines strongly to aggregation and fibrillogensis, as well as EPA (21.9 mg/L), DHA (15.4 mg/L) and Ginkgo biloba (0.28 mg/L) were applied in a concentration as could be expected in the blood of proband after the intake of one capsule. The VLDL apoE4/E4 plasma fraction (30 mg/dL) showed beginning Alzheimer nanoplaque formation already at a normal blood Ca2+ concentration. Fish oil plus ginkgo applied acutely in the experiment, markedly slowed down this process of quaternary aggregational nanoplaque complex at all Ca2+ concentrations used. In a normal blood Ca2+ concentration of 2.52 mmol/L, the omega-3 + ginkgo-induced reduction of nanoplaque formation and molecular size amounted to 10.1 ± 1.3% (p < 0.03) and 14.3 ± 2.9% (p < 0.02), respectively, as compared to the controls. From these results, we concluded that the combination of omega-3 fish oil plus ginkgo hampered the docking of VLDL apoE4/E4 to the proteoglycan receptor and of A-beta42 to VLDL apoE4/E4, and that altogether Alzheimer nanoplaque formation is diminished as compared to control experiments without these substances. These in vitro experiments provide a mechanistic explanation for a possible beneficial mode of action of fish oil and ginkgo in Alzheimer, vascular or age-related cognitive decline.

**Glucose 6-phosphate Dehydrogenase Regulates Laminar Flow-induced Endothelial Nitric Oxide Synthase Activation**

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Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the pentose phosphate pathway, where it metabolizes glucose 6-phosphate to 6-phosphogluconolactone and generates NADPH. In endothelial cells, NADPH is an essential cofactor for dihydrolipoyl reductase, which generates tetrahydrobiopterin. Because tetrahydrobiopterin is an essential cofactor for endothelial nitric oxide synthase (eNOS), G6PD is very important for endothelial function. Increases in blood flow activate eNOS. We hypothesized that G6PD plays an essential role in flow-mediated eNOS activation. Using a core and plate viscometer, we showed that flow activated G6PD within 2 minutes. Small interference RNA knockdown of G6PD significantly inhibited flow-mediated eNOS phosphorylation. In contrast, overexpression of G6PD increased flow-mediated eNOS phosphorylation, while a G6PD mutant lacking enzymatic activity did not alter eNOS phosphorylation. Our hypothesis was further supported by data that G6PD expression increased significantly in cells exposed to laminar flow for 24 hours. In the Pretsch mouse, which has much lower G6PD activity due to decreased G6PD protein expression, aortic eNOS phosphorylation was dramatically decreased compared to wild type mice. These findings demonstrate a novel role for G6PD in vascular homeostasis, by regulating eNOS function.
exposure, cells were labeled with anti-ICAM-1, -E-selectin, and -VCAM-1 antibodies for flow cytometry analysis. Monocyte adhesion studies were performed using calcein-AM labeled mononuclear cells added to the permeate for 45 min. Expression of E-selectin, VCAM-1, and ICAM-1 was upregulated on cytokine-stimulated HSEC after exposure to both VF (2.5, 1.1 and 1.9 fold) and non vascular bed-specific CAM (1.6, 1.5 and 2.5 fold). In marked contrast, expression of ICAM-1 and VCAM-1 to vascular bed-specific CAM was decreased in downregulation of E-selectin and VCAM-1 expression when compared to expression levels under static conditions. Whereas VF had no impact on ICAM-1 expression on cytokine-stimulated HCAEC, CAM significantly increased ICAM-1 expression on HCAEC above the levels measured in HLEC (mean fluorescence intensity of 1052 ± 129, 814 ± 129, respectively, p<0.0005). To examine the functional relevance of our findings we quantified adhesion of mononuclear blood cells to both EC types. Adhesion to cytokine-stimulated HSAEC significantly exceeded adhesion to TNF-α-stimulated HCAEC when exposed to CA (arbitrary fluorescence units, 1.92 ± 0.02 and 1.70 ± 0.07, respectively). These results may give rise to the notion of vascular bed specific endothelial molecular responsiveness: intrinsic differences in the vascular beds that serve as the source for different EC may account for the different effects of flow observed.

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Recovery of Endothelial Barrier Function by Kinin B1 Receptor-induced Activation of Inducible Nitric Oxide Synthase
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Impaired endothelial barrier function can result from the release of inflammatory mediators in the cardiovascular system. We previously showed that human lung microvascular endothelial cells (HLMEC) treated with cytokines (20 ng/ml interleukin-1β + 200 U/ml interferon-γ for 16 h) express kinin B1 receptors (B1Rs) and iNOS. Measurement of NO output in real time with a porphyrinic electrode showed that 1 mM Arg alone generated prolonged (80 min) iNOS-dependent “high output” NO (maximum = 295 ± 22 nM NO at 40–60 min) whereas addition of a B1 agonist, 100 nM des-Arg10-kallidin (DAKD), produced “super-high output” NO (maximum: 745 ± 27 nM NO at 80–90 min) which was markedly higher than NO generated by Arg alone. To investigate the hypothesis that B1 activation of iNOS-dependent NO production affects endothelial permeability, HMVEC barrier function was continuously assessed by electric cell-substrate impedance sensing. Cytokine treatment of HMVEC as above markedly decreased resistance to half the control value. Addition of 1 mM L-Arg to stimulate basal iNOS activity resulted in partial recovery of cell resistance (25% increase from the post-cytokine level) whereas addition of a B1 agonist 100 nM DAKD to acutely activate iNOS resulted in a significantly greater recovery (80% increase). ACE inhibitor enalaprilat (100 nM) also activated B1Rs and gave a response similar to that of DAKD. This effect fully developed by 40–60 min after agonist treatment, lasted for at least 2 h and was blocked by B1 antagonist des-Arg9-Lys9-kallidin or by iNOS specific inhibitor N-(1-iminoethyl)-L-lysine. The role of NO was explored using the NO donor DETA-NONOate. Doses of DETA-NONOate (1 - 50 µM) that generated NO in the same range as B1 agonist stimulation resulted in a ~65% increase in resistance whereas doses caused no change (100 µM) or a further decrease (0.5 mM - 5 mM) in resistance. Thus, B1 activation of iNOS restores endothelial barrier function and could represent a novel target for repair of endothelial damage in inflammation. This mechanism could also underlie some of the beneficial therapeutic effects of angiotensin converting enzyme (ACE) inhibitors as these compounds are also direct agonists of B1Rs.

P382
A Critical Role for NF-κB during FFA Impairment of Nitric Oxide Production in Endothelial Cells
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There is substantial evidence that endothelial vasodilatation mediated by nitric oxide (NO) production is impaired in animal models of diabetes and in humans with type 2 diabetes. Increased free fatty acid (FFA) levels are often found in type 2 diabetes and are associated with endothelial dysfunction. We have previously shown that excess FFA, glucose, or TNF-α inhibits NO production in endothelial cells. FFA activates IKKα, a regulatory kinase in the NF-κB inflammatory activation pathway and IKKα is both necessary and sufficient to impair insulin-mediated NO production. Objective: We hypothesized that NF-κB activation is necessary for FFA mediated impairment of endothelial insulin signaling. Results: Endothelial cells were exposed to 100 µM palmitate/BSA for 3 h and exhibited impaired insulin signaling and NO production compared to the BSA treated control condition. FFA treatment resulted in activation of both IκBα and NF-κB dependent IKKα activation and IκBα and IκBβ production. We used a reporter construct expressing NF-κB and NF-κB dependent transcriptional reporter activity. We further validated whether NF-κB dependent transcriptional activity is necessary for FFA-mediated inhibition of endothelial insulin signaling, we used a phospho-specific NF-κB reporter (pNF-κB-Luc) and NF-κB reporter construct expressing NF-κB super repressor) which blocks activation of NF-κB without affecting FFA-mediated IKKα activation. The luciferase phospho resistant construct was cloned into pBAM-IREs-puro retroviral vector and retrovirus was generated for transduction of HMEC. Overexpression of NF-κB was confirmed by Western blot analysis. Control endothelial cells were transduced with a retroviral GP construct. FFA treatment of HMEC transduced with the phospho-resistance resistant IκBα construct did not impair insulin signaling or insulin-mediated NO production when compared to the control GFP transduced cells. These experiments suggest that FFA-mediated impairment of NO production is dependent on NF-κB activation.

P383
Adiponectin and Its Receptors Increased Cholesterol Efflux in HEK293T Cells
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A decrease in adiponectin secretion causes the early stages of atherosclerosis. High-density lipoprotein (HDL) takes up cholesterol through ATP binding cassette A1 (ABCA1) as reverse cholesterol transport (RCT). Recently, a new therapeutic strategy, reconstituted (r)HDL, has been shown to enhance RCT. Therefore, we hypothesized that adiponectin increases the uptake associated with ABCA1 and also enhances HDL-induced uptake in human kidney cells (HEK293T), which endogenously expressed ABCA1. We transfected adiponectin receptor 1 and 2 (AdipoR1 and AdipoR2) cDNA to HEK293T cells. The transfected cells were labeled with [3H]-cholesterol following cholesterol loading with or without adiponectin for 24 hours. The levels of cholesterol efflux were assayed using [3H]-cholesterol uptake, and these results may give rise to the notion of vascular bed specific endothelial molecular responsiveness: intrinsic differences in the vascular beds that serve as the source for different EC may account for the different effects of flow observed.

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Specificity in Interactions Between Matrix GLA Protein and Bone Morphogenetic Proteins
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Arterial calcification is ubiquitous in vascular disease, and contributes significantly to morbidity and mortality. Prevention of calcification is mediated in part by the activity of matrix GLA protein (MGP), an inhibitor of bone morphogenetic protein (BMP). MGP is characterized by the presence of propeptide-exonated glutamic acid-rich scaffold that is responsible for its inhibitory action on BMPs. However, it is not clear whether MGP inhibits multiple BMPs involved in vascular biology, and what mechanism is used to bind BMP. We compared the effect of MGP on four different BMPs that were expressed in bovine aortic endothelial cells (BAEC). BMP-2, -4, -6, and -7. All four BMPs were similarly bound and inhibited by MGP as determined by a BMP-sensitive luciferase reporter gene assay and co-immunoprecipitation, although MGP appeared to have the highest affinity for BMP-2 as determined by an ELISA-based assay. We studied the binding mechanism of MGP using mutagenesis of a FLAG-tagged MGP construct. The various mutations were expressed at similar levels in BAEC, and were analyzed using luciferase reporter gene assays, co-immunoprecipitation, and calcium binding. The results showed that binding of BMP by MGP was dependent on a specific proline residue in the MGP protein localized in the center of the GLA-rich region, which has been associated with BMP-binding. In addition, at least two GLA-residues were required for BMP binding, one on each side of the proline residue. Mutagenesis of the GLA-residues, but not the proline-residue, was associated with changes in calcium binding. Together, our results suggest that MGP can interfere in multiple processes in vascular biology where the tested BMPs are present. In addition, the results suggest that MGP uses a BMP-binding mechanism that is in part similar to that of the BMP receptor and Noggin, another BMP-inhibitor. These results may be important in providing guidance as to appropriate directions for development of therapeutic interventions for arterial calcification.

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In Vivo Expression of Human Group II Secretory Phospholipase A2 Is Associated with Increased Production of Biglycan and Macrophage Colony Stimulating Factor
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Secretory phospholipase A2 (sPLA2) digestion of lipoproteins leads to the formation of lysophosphatidylcholine (lysPC), a molecule that we previously have shown stimulates the synthesis of biglycan and the proteoglycan form of M-CSF (PG-MCSF) by arterial smooth muscle cells. These matrix molecules have pro-atherogenic effects. To test whether increased lysPC generation in vivo is associated with increased synthesis of biglycan and PG-MCSF we used transgenic mice overexpressing human sPLA2-IIA (sPLA2-IA). These mice have increased susceptibility to LPS-induced shock and to atherosclerosis when fed a high cholesterol diet. Female hsPLA2-IIA mice were maintained for 4 weeks on (i) control diet, (ii) control diet with 200 U/ml interferon-γ for 48hrs prior to sacrifice, or (iii) western diet supplemented with 2% (w/v) LPS injection 48hrs prior to sacrifice, or (iii) western diet. Female hsPLA2-IIA mice were maintained for 4 weeks on (i) control diet, (ii) control diet with 200 U/ml interferon-γ for 48hrs prior to sacrifice, or (iii) western diet supplemented with 2% (w/v) LPS injection 48hrs prior to sacrifice, or (iii) western diet.要不然,在西方国家的饮食中，大麦草的消化作用对整体健康有重要意义。
hypothosis that siPLA2-mediated production of hspOC can influence synthesis of the extracellular matrix molecules biglycan and PG-MSGF, with potential pro-atherosclerotic consequences.

Inhibition of Chemokine Receptor CCR2 in Bone Marrow Cells by siRNA

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Introduction: Abdominal aortic aneurysms (AAA) form a relatively common vascular disorder among elderly men and can be lethal if untreated. Treatment consists mostly of surgery when the expansion of the aorta has reached a certain diameter (5.5 cm). Before the aneurysms become this large, treatment is scarcely available. It has been shown that chemokine receptor CCR2 facilitates aneurysm formation in a murine AAA model, indicating that CCR2 gene recruitment plays a role in AAA. Hypothesis: Methods to block macrophage recruitment by manipulation of CCR2 may lead to novel therapeutic approaches to prevent aneurysm growth. Method: siRNA-mediated gene silencing of CCR2 was used to decrease aneurysm formation. For delivery of the CCR2 siRNA to leukocytes, a bone marrow transplantation experiment was performed in Apoe-deficient (Apoe−/−) male mice. Bone marrow cells were harvested from donor Apoe−/− mice and transduced with concentrated lentiviral supernatant containing the CCR2 siRNA vector (siCCR2) or control vector. Lentiviral-transduced cells (1x10⁶) were injected into irradiated recipient mice (single dose of 9 Gy X-ray total body irradiation). After 6 weeks the mice were fed a Western-type diet. During the last 4 weeks of diet, angiotensin(1-4 mg/kg/day) was given to induce AAA. Results: We observed 30% (3/10) premature death after the onset of AngII perfusion in the control mice and 18% (2/11) in the siCCR2 mice. When large and small aneurysms are counted, the control mice contained 2.7 aneurysms per aorta, while the siCCR2 mice had a significantly decreased number of 1.4 aneurysms per aorta (p < 0.04). Conclusion: Genes silencing resulting in decreased aneurysm growth, showing that silencing of CCR2 by siRNA lentiviral vectors forms a therapeutic approach to inhibit aneurysm formation.

Role of Pericytes and PDGF-B in the Antileakage and Vascular Remodeling Actions of Angiopoietin-1

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Angiopoietin-1 (Ang1) is a clinically relevant mitogen for endothelial cells with important anti-leakage and remodeling actions: it transforms distal capillaries of the microvasculature into enlarged vessels that are leakage-resistant in the setting of acute inflammation. In addition, Ang1 stabilizes blood vessels by promoting recruitment of pericytes during development. Recently, the anti-leakage effect of Ang1 was suggested to be due to its ability to reduce gap formation at intercellular junctions. However, little is known about the possible role of pericytes in this process. Abdominal coverage of blood vessels by pericytes is requisite for microvascular stability and is regulated by platelet-derived growth factor B (PDGF-B), a chemotactic factor promoting recruitment of pericytes to the microvasculature. To investigate the role of pericytes in the anti-leakage effect of Ang1, we tested the ability of Ang1 to reduce inflammatory-induced microvascular leakage in the mouse trachea in the presence of a PDGF-B inhibitor, a small molecule which blocks the PDGF-B receptor on pericytes. Significantly, pericyte coverage in the microvasculature after Ang1 treatment, despite a 40% reduction in leakage. PDGF-B inhibition did not affect vascular leakage, even though pericyte coverage was reduced by 31%. Interestingly, PDGF-B expression was increased in combination with Ang1 treatment resulted in a substantial increase in leakage by 61%, and a 55% reduction in pericyte coverage. Thus, Ang1 and the PDGF-B inhibitor had additive effects on pericyte coverage and the anti-leakage effect of Ang1 was completely reversed by inhibition of PDGF-B. The results suggest that pericytes are involved in the anti-leakage actions of Ang1 and that a threshold level of pericyte coverage may be necessary for vascular stability. They also indicate that crosstalk between Ang1 and PDGF-B pathways exists.

Anticonnective Tissue Growth Factor Antibody Attenuates Vascular Fibrosis and Prevents Passive Arterial Stiffness

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Connective tissue growth factor (CTGF) mediates production of extracellular matrix (ECM) and vascular remodeling that facilitates development of atherosclerosis, arteriosclerosis and hypertension. We hypothesized that inhibition of CTGF might reduce vascular fibrosis and the stiffness of vessel wall. Vascular fibrosis was induced by IV-silico-l-arginine methyl ester (L-NAME; 40 mg/kg/d in drinking water containing 1% NaCl) and Angiotension II (120 ng/kg/min s.c.) for 3 weeks in SD rats. CTGF was inhibited by a human full-anti-CTGF antibody (FG-3019, 10 mg/kg, 3x/week i.p.) for 3 weeks starting simultaneously with administration of L-NAME/AngII. Purified human IgG (H6g), 10 mg/kg, 3x/week i.p. and losartan (10 mg/kg p.o.) were used for negative and positive controls respectively. The thoracic aorta was collected for evaluation of ECM proteins by Western blot, and carotid artery was dissected and instrumented for measurement of passive arterial stiffness (PAS). The PAS, as assessed by pressure/diameter curves was significantly increased with L-NAME/AngII treatment and there was clear trend towards reducing the enhanced PAS by FG-3019 or losartan (Figure 1). L-NAME/AngII induced an over production of fibronectin in the vessel wall and FG-3019 significantly prevented the enhancement of this protein (58 ± 0.257 g/ml vs 17 ± 0.134 g/ml; P < 0.001). Data demonstrate that FG-3019 attenuates L-NAME/AngII-mediated vascular fibrosis and prevents fibrosis-related passive arterial stiffness, suggesting inhibition of CTGF is a potential therapeutic strategy for treatment of vascular remodeling associated with arteriosclerosis and hypertension.

Circulating Inactive Human Matrix Gla Protein as a Biomarker for Cardiovascular Disease

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Matrix Gla protein (MGP) is a vitamin K-dependent protein and a strong inhibitor of vascular calcification. In both animals and humans MGP deficiency results in extensive arterial calcification. MGP activity depends on vitamin K-dependent carboxylation, hence vitamin K-deficiency leads to undercarboxylated MGP (ucMGP) which is inactive. Recently it was shown that ucMGP is strongly upregulated in calcified arteries, while in contrast local GaMP expression was very low. Therefore, we developed an ELISA-based assay with which ucMGP can be detected in the circulation. Serum ucMGP levels were measured in controls (n = 54) and in 3 patient populations, those with aortic stenosis (n = 25), chronic kidney disease (CKD SD) (n = 40) and calcipolyuria (n = 10). Neither age nor gender influenced the circulating ucMGP concentrations. As compared to age- and sex-matched controls, all patient groups had significantly decreased ucMGP levels. However, in calcification-prone patients with renal failure virtually all patients had ucMGP levels below the control range. The renal patients underwent MSQL (multi-slice computed tomography) scanning of the coronary arteries. After straining the renal patients for territories of coronary calcification, ucMGP levels showed a significant decrease with increasing amount of vascular calcification: lowest tertile: 218 (79), intermediate tertile:
Role of Lipid Rafts/Caveolae in Nongenomic C-Src Signaling by Aldosterone in Vascular Smooth Muscle Cells

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Objective: We demonstrated c-Src-regulated p38 MAP kinase activation and NAD(P)H oxidase-inducible generation of superoxide anion (O2−) as novel nongenomic signaling pathways for aldosterone in vascular smooth muscle cells (VSMC). These effects were inhibited by eplerenone, mineralocorticoid receptor (MR) antagonist. c-Src is identified as lipid raft-associated protein. Whether non-genomic c-Src-dependent signaling by aldosterone involves the rafts and specialized caveolin-enriched membrane microdomains remains unclear. Here we tested the hypothesis that aldosterone induces c-Src trafficking into caveolae/lipid rafts. We also assessed whether these interaction influence NAD(P)H oxidase-derived O2−. Methods and results: VSMCs from WKY rats, caveolin 1 (Cav 1)−/− mice and wild-type Cav 1+1 were studied. siRNA was used for Cav 1 gene knockdown in mice cells. Membrane cholesterol depletion/sequestration was achieved with 10 mM methyl-β-cyclodextrin and nystatin. Cholesterol-rich fractions from VSMC were obtained by sucrose-gradient ultracentrifugation and identified by filipillin-2 expression. Membrane-β-cyclodextrin and nystatin abolished 0.1 μM aldosterone-induced c-Src phosphorylation and O2− generation in VSMCs. Aldosterone significantly increased c-Src trafficking and phosphorilation into (by 1–2-fold, p < 0.05) lipid rafts/caveolae. Aldosterone also stimulates p47phox translocation into these cholesterol-rich fractions, indicating the activation of NAD(P)H oxidase (1 fold, p < 0.05). Knockdown of Cav 1 inhibits aldosterone-induced c-Src and contactin phosphorylation without abolishing this effect. Similar results were observed in Cav 1 deficient cells. Aldosterone induced c-Src translocation into cholesterol-rich domains in Cav 1+1 and Cav 1−/− VSMCs. Membrane-β-cyclodextrin and nystatin localized to cholesterol-enriched fractions, indicating lipid raft association. Conclusions: We provided evidence for the functional and spatial importance of lipid raft/caveolae in MR-mediated aldosterone induced c-Src phosphorylation, which is required for redox signaling in VSMCs. These findings highlight the importance of lipid rafts in nongenomic signaling by aldosterone in VSMCs.

Oxidative Stress Induces Vascular Smooth Muscle Cell Calcification via AKT Signaling

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Oxidative stress plays a critical role in the pathogenesis of atherosclerosis, including the development of atherothrombotic plaques, a prominent feature of this disease. Enhanced osteogenic differentiation of vascular smooth muscle cells (VSMC) is associated with oxidative stress in vitro. Further, Akt phosphorylation has been associated with human VSMC calcification in culture. Thus, we hypothesized that increased oxidative stress induces VSMC calcification through Akt signaling pathways. VSMC were explanted from the aorta of C57BL/6 mice and sorted by flow cytometry with smooth muscle specific α-actin antibody. We found that a model oxidant, H2O2, induced VSMC mineralization in a dose-dependent manner (fold increase at 0.05 mM: 1.5 ± 0.7, 0.2 mM: 6.0 ± 1.4, and 0.5 mM: 16.0 ± 1.4 compared with control, n = 4 for each, p < 0.05). H2O2 increased intracellular peroxide production as determined by dichlorofluorescein diacetate (DCFH-DA). H2O2 increased intracellular peroxide production as determined by dichlorofluorescein diacetate (DCFH-DA). H2O2 induced Akt phosphorylation (n = 4, p < 0.05). Taken together, these data suggest that oxidative stress induces a phenotypic transition of VSMC into osteogenic cells. Furthermore, we identified that H2O2 induced Akt phosphorylation (n = 4). Akt, a pharmacologic inhibitor of Akt, inhibited VSMC calcification and the expression of bone-associated proteins in a dose-dependent manner. These data demonstrate an important role of Akt in mediating oxidative stress-induced VSMC calcification. In summary, we found that oxidative stress induced translocation of VSMC into osteogenic cells by increasing bone-associated proteins and decreasing SMC α-actin. Increased phosphorylation of AKT contributes to the osteogenic differentiation of VSMC. Our results provide important insights into understanding the molecular mechanisms of oxidative stress induced vascular calcification.

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Thymidine Phosphorylase Induces Intermediate Conductance, Ca2+−Activated Potassium Channel Expression in Rat Vascular Smooth Muscle Cells

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The intermediate conductance Ca2+−activated potassium channel (KCNMB4) has been reported to promote neointimal vascular smooth muscle cell (VSMC) proliferation, and its blockade could prevent restenosis after angioplasty. We recently reported that thymidine phosphorylase (TP) inhibited VSMC proliferation and migration. Here, we examined whether the effect of TP on VSMC was via regulating KCNMB4 expression. Phagocytic vectors encoding human TP cDNA were transfected into rat VSMC. TP overexpressing clones (C2 and C4) proliferated more slowly than VSMC transfected with empty vector (PC) or parent VSMC. KCNMB4 mRNA and protein expression was significantly higher in C2 and C4 compared to PC cells and parent VSMC (p < 0.001). KCNMB4 mRNA expression was similar in all cells. KCNMB4, 3 and S6 genes were not expressed in VSMC. Patch clamping confirmed increased functional KCNMB4 expression in the plasma membranes of the C2 and C4 clones (Figure). Intracellular Ca2+ ([Ca2+]i) was higher in C2 and C4 cells at baseline and after ATP stimulation. The KCNMB4 specific inhibitor clotrimazole (CLZ) and Tram 34 decreased [Ca2+]i in C2 and C4 to the level in PC and VSMC cells, but did not affect PC or C4 proliferation. Collectively, these results indicate that TP inhibits VSMC proliferation by increasing KCNMB4 expression in VSMC. This study suggests that gene therapy with TP for vascular disease may be benefit by preventing neointimal VSMC proliferation and regulating vascular tone.
shown that, infusion of AngII (1,000 ng/kg/min) into male C57BL/6 mice decreased LRP protein abundance in the abdominal, but not the thoracic aortic region. We also demonstrated that AngII decreased the abundance of LRP and its intracellular chaperone, RAP in ex vivo aortic tissue in the abdomen, but not in the thorax. SMCs cultured from the abdominal region retained the property of AngII-induced reductions of both LRP and RAP (p < 0.05), which was attenuated by the AT1 receptor antagonist, losartan. AngII also reduced cell association and degradation of the LRP ligand, 125I-labeled alpha-2 macroglubulin, in cultured abdominal SMCs, but not in thoracic SMCs. This reduction was attenuated by the AT1 receptor antagonist, losartan. Real-time PCR demonstrated that AngII reduced abundance of RAP, but not LRP mRNA. In addition, AngII also reduced RAP protein and mRNA abundance in abdominal SMCs deficient in LRP. To investigate if AngII-induced reductions in RAP causes premature posttraumatic degradation of LRP, experiments were conducted with cyclohexammine (protein synthesis inhibitor) and a combination of cyclohexammine and MG-132 (proteasomal inhibitor). AngII reduced LRP protein in the presence of cyclohexammine, but not in the presence of MG-132, whereas RAP protein expression was inhibited even in the presence of MG-132 (p < 0.05). Conclusion: AngII decreased expression of LRP in abdominal aortic SMCs in both ex vivo tissues and cultured cells in a AT1 receptor dependent manner. AngII reduced RAP independently of LRP, and caused degradation of LRP but not RAP, in a proteasomal dependent pathway. These data are consistent with AngII inhibiting RAP transcription to indirectly decrease cell surface expression of LRP.

Matrix Metalloproteinase Transactivation of Epidermal Growth Factor Receptor Regulates Mitochondrial ATP Synthesis in Vascular Smooth Muscle

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Introduction: G-protein coupled receptors (GPCR) modulate vascular tone, at least in part by matrix metalloproteinase (MMP)-dependent epidermal growth factor receptor (EGFR) transactivation. We previously have reported that vascular alpha-1 adrenoceptor mediated activation of MMPs, such as MMP-7, and the EGFR engages mitochondrial redox processes to maintain vascular tone. In the present study we investigated the hypothesis that maintenance of adrenergic vascular tone by the MMP-EGFR pathway involves mitochondrial ATP synthesis. Methods and Results: In rat vascular smooth muscle cells (VSMcs), stimulation of alpha-1 adrenoceptors with phenylephrine triggered ATP synthesis in a concentration and time dependent manner. The increase in ATP synthesis was blocked by inhibitors of mitochondrial ATP synthesis (oligomycin), MMPs (GM 6010), EGFR (AG1478) and phosphoinositide-3-kinase (PI3K)- dependent Akt phosphorylation (wortmannin and LY 29402). Further, inhibition of MMPs or silencing EGFR expression blunted the phosphorylation of Akt and reduced GLUT4 translocalization, effects that were also observed with PD98059 inhibitors. In small rat mesenteric arteries, exogenous ATP promoted activation of MMP-7, which was dependent on PKx but not PK2 purinergic receptors. Downstream of alpha-1 adrenoceptors, the activation of MMP-7 was blocked by inhibitors of ATP synthesis (oligomycin) or its bioavailability (aprase). Moreover, blockade of PI3K or ATP synthase down-dependently inhibited adrenergic vascular tone. Conclusion: Modulation of mitochondrial ATP synthesis by MMPs and promotion of MMP activity by ATP are two new regulatory events in the signaling pathway of alpha-1 adrenoceptors. We suggest that metabolic and growth pathways merge to modulate MMP activity and thereby impact vascular tone. Supported by an operating grant from the CIHR to CPP, PRN was supported by a program grant from the HSF and the MSFHR, Canada.

Novel Effects of Lovastatin on Extracellular Matrix Gene Expression in Vascular Smooth Muscle Cells

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HMCoA reduce inhibitors (statins) have been shown to reduce adverse cardiovascular events. Moreover, the cardioprotective effects of these agents do not appear to be limited to their lipid lowering capabilities. Other putative beneficial effects include inhibition of smooth muscle cell proliferation and migration. Piae stability is another critical component of coronary and peripheral vascular disease. We examined the effects of a common HMCoA reduce inhibitor, lovastatin, on the expression of plaque stabilizing genes including collagen Ia1 (COLI), collagen III (COL III), lysyl oxidase (LOX), thrombospondin-1 (TSP-1), and tropoelastin (TROPO), in human umbilical artery smooth muscle cells (UASMC). In brief, UASMCs were treated with 0.5 mM lovastatin, TROPO, and TSP-1 were seen following treatment with simvastatin and exposure to the MEX inhibitor, PD98059. Simultaneous exposure to both lovastatin and PD98059 produced a synergistic effect and TSP-1 and TROPO were reduced to 67% and 82%, respectively. Real-time PCR demonstrated that AngII reduced abundance of RAP, but not LRP mRNA. In addition, AngII also reduced RAP protein and mRNA abundance in abdominal SMCs deficient in LRP. To investigate if AngII-induced reductions in LRP causes premature posttraumatic degradation of LRP, experiments were conducted with cyclohexammine (protein synthesis inhibitor) and a combination of cyclohexammine and MG-132 (proteasomal inhibitor). AngII reduced LRP protein in the presence of cyclohexammine, but not in the presence of MG-132, whereas RAP protein expression was inhibited even in the presence of MG-132 (p < 0.05). Conclusion: AngII decreased expression of LRP in abdominal aortic SMCs in both ex vivo tissues and cultured cells in a AT1 receptor dependent manner. AngII reduced RAP independently of LRP, and caused degradation of LRP but not RAP, in a proteasomal dependent pathway. These data are consistent with AngII inhibiting RAP transcription to indirectly decrease cell surface expression of LRP.
TRP7 and Its Kinase-sensitive Substrate Annexin-1 Are Differentially Regulated by Stretch and Vasoactive Agents in Vascular Smooth Muscle Cells

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Introduction: Transient receptor potential melastatin cation channel 7 (TRPM7) is important in vascular smooth muscle cell (VSMC) function. Mechanisms regulating TRPM7 are unclear.

Hypothesis: Mechanical stretch and vasoactive agents, important in regulating vascular tone and growth, modulate TRPM7 in VSMCs. Methods: Rat mesenteric VSMCs were studied. Expression of TRPM7 mRNA and protein was assessed by RT-PCR and immunoblotting. Cellular localization of TRPM7 was evaluated by immunofluorescence microscopy. Activation of annexin-1, a TRPM7 kinase-sensitive substrate, was assessed by cytosol-to-membrane translocation. TRPM7 was downregulated by siRNA. Cells were stimulated with vasoactive agonists, e.g. ang II (10^-6 10^-6 mol/L), a vasocostructor, or bradykinin (10^-6 10^-6 mol/L), a vasodilator, or exposed to cyclic stretch using a PheaxCell system. Results: Immunofluorescence confocal microscopy demonstrated TRPM7 distribution along the cell membrane and co-localization with flotillin-2, marker of lipid rafts, in VSMCs. In the basal state TRPM7 was completely inhibited by pre-incubation with an intracellular calcium chelator, BAPTA. The siol in 1,4,5-triphosphoric (IP3) receptor blocker, 2-aminophosphoryl borate and xestospongin C, both significantly inhibited the VSMC migration, while the ryanoide receptor blocker, ryanodine (10^-6 mol/L), a Ca^2+ ionophore, and thePKG inhibitor, BG-154, did not. Conclusion: These results indicate that VSMC migration is accelerated in response to the increase in mean pressure, pulse rate, and pulse pressure, for which increased release of intracellular calcium from sarcoplasmic reticulum via IP3 receptor may play an important role in the migration. They also suggest that the anti-atherogenic actions of CCs are mediated not only by their blood pressure lowering effects but also by their inhibitory effects on VSMC migration.

Intravascular Delivery of Rapamycin with Molecularly Targeted Nanoparticles Inhibits Stenosis Without Delaying Endothelial Healing After Angioplasty

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Drug eluting stents have demonstrated the anti-restenotic benefit of local anti proliferative drug delivery following angioplasty. However, delayed re-endothelialization creates a risk for late in-stent thrombosis. Furthermore, small vessels may not be amenable for stent placement. We have shown that eGFP-encoded targeted fluorescent nanoparticles deliver rapamycin into the arterial wall and reduce stenosis after angioplasty. The objective of this study was to determine whether intramurally targeted rapamycin nanoparticles impair endothelial repair. Femoral arteries of NZW rabbits fed an atherogenic diet for 4 months were subjected to balloon stretch injury. Vascular smooth muscle cells were observed en face with human CD34+ targeted nanoparticles to measure plaque area observed en face. A paracrine effect of nanoparticles was observed in the intimal plaque area observed en face. 

CD34+ Cells Stimulate Vascularization, Inhibit Inflammation, and Increase Cell Viability in Implantable Bioartificial Devices

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For successful engraftment of allogenic cells using a bioartificial device, the device must allow for the growth of cells, be immuno-invisible, exhibit good bulk transport characteristics, and be rapidly vascularized. Current devices are not vascularized quickly enough to maintain adequate cell viability and bulk transport, and device encapsulation is problematic. To address these issues, we tested devices consisting of a pro-angiogenic scaffold surrounding a cell-encapsulating alginate core in mice. Our goal was to prevent device encapsulation while stimulating vascularization. We found that despite their pro-angiogenic nature, the scaffolds acted as a physical barrier, inhibiting the approach of vessels to the alginate core. CD34+ peripheral blood cells promote vascular growth in diabetic mice, so we also examined their ability to create new blood vessels from circulating progenitor cells. Our results demonstrated that CD34+ cells in mice promote device vascularization and significantly alter the tissue response to the device. This suggests that CD34+ cells may serve as a potential source of cells for future bioartificial devices.

Cytokines and Anti-inflammatory Mediators in Human Coronary Endothelial Cells

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Background: Levels of pro-inflammatory cytokines and anti-inflammatory mediators in human coronary endothelial cells (ECs) are up-regulated by TNFalpha and IL1beta. We hypothesized that these cytokines may alter the expression of anti-inflammatory mediators in human coronary ECs.

Methods: Human coronary ECs were cultured in serum-free endothelial basal medium and stimulated with TNFalpha and IL1beta. Protein expression of TNFalpha and IL1beta and anti-inflammatory mediators (IL10, PGE2, TGF-beta) was determined by Western blotting.

Results: Treatment with TNFalpha and IL1beta decreased protein expression of TNFalpha and IL1beta in human coronary ECs. Treatment with TNFalpha and IL1beta increased protein expression of IL10 and TGF-beta in human coronary ECs.

Conclusion: TNFalpha and IL1beta decreased protein expression of TNFalpha and IL1beta and increased protein expression of IL10 and TGF-beta in human coronary ECs.

Progenitor Cells and Secreted Paracrine Factors Induce Regional Cardiomyocyte Hypertrophy Following Therapy for Acute Myocardial Infarction

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Administration of circulating progenitor cells (Pb) is a promising therapy for post-infarction cardiac repair. However, the mechanisms that underlie apparent beneficial effects on myocardial remodeling are unclear. We investigated the therapeutic effects of peripheral blood progenitor cells, and the therapeutic effects of paracrine factors secreted by these cells, on cardiac hypertrophy following myocardial infarction.}

ASS Nonresponder Rate Using Multiple Aggregation Tests Has High Prevalence in CABB Patients

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Platelet Function Analyzer-100 (PFA-100). ASA 300mg was ad-ministered intravenously by 40 patients on 7–9 post day after CABG, Non compliance administration was eliminated. Blood was drawn immediately before, 60 minutes and 24 hours after ASA application. ASA Non-Responsiveness (NR) was defined as max-imal AA-induced PA below 20mm, corresponding to at least 66% inhibition. Results: ASA NR was observed in 9 patients (22%) 1hr and 12 patients (30%) 24hrs after ASA application. Before ASA injection, patients had significant greater A values than 60 healthy controls (p < 0.001) still PA determination was not different to control values. Most ADP and Coll-induced PA and WA values were not reduced 1hour and 24 hours after ASA injection. Conclusion: One third of the patients after CABG are non responders to ASA therapy. Screening for ASA resistance and optimised antiplatelet therapy may help to further improve potency of bypass grafts.


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To prevent the loss of blood following a break in blood vessels, components in blood and vessel wall interact rapidly to form a thrombus to limit hemorrhage. This hemostatic response is rapid and regulated, since excessive and inappropriate clotting reduces the potency of blood flow. During the last century many of the components involved in the hemostasis have been identified including proteins and cellular components in blood, elements in the vessel wall and hydrodynamic factors. Direct numerical simulation (DNS) is being used as a new tool to study biological processes. The aim of this study is to develop a meaningful computational model of clot development taking into account both physiological, biochemical and mechanical factors, which usually occur at different length scales. This involves the development of a Multiscale Computational Toolkit for Modeling Thrombus Development (MMTD). The proposed Toolkit MMTD builds upon a combination of FronTier, a software framework using a front tracking approach for the simulation of multiphase flows (developed by Dr. Gilim at SUNY at Stony Brook), and a Cellular Potts Model-based (CPM) modeling environment, CompCell3D (developed by Dr. Alber). The prediction of the thrombus mass is tested by high resolution confocal monitoring of thrombus development following laser injury of the mesenteric vasculature. The model permits tracking platelet accumulation, fibrin deposition, leukocyte incorporation and hydrodynamic parameters. The comparison between predictions of the computational model and results of the experimental system permits refinement of the Toolkit MMTD. Initial studies suggest the importance of hydrodynamic parameters in the heterogeneous domain structure of a developing thrombus. The development of a computational model incorporating physiologically important parameters of thrombus development will enable one to identify rate limiting, critically important, regulatory parameters for which small perturbations result in large effects on thrombus development. Such improved understanding will make significant contributions in the development of therapeutic strategies to treat thrombosis and hemorrhage.

Molecular Characteristics of Inactivated Human Tissue Plasminogen Activator Association with Fibrin Implicate the Nature and Sites of the Binding Interactions

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Introduction. Recombinant tissue plasminogen activator (tPA) is the predominant thrombolytic agent employed for clinical treatment of myocardial infarction and ischemic stroke. The self-regulating nature of the thrombolytic process is mediated by two discrete fibrin-binding sites in the tPA molecule, a higher affinity site in the finger domain and a lower affinity site in the urokinase domain structure of a developing thrombus. The development of a computational model incorporating physiologically important parameters of thrombus development will enable one to test the hypotheses that determination of thermodynamic parameters associated with tPA-fibrin binding could provide information about the molecular nature of the associations and that binding characteristics at 37°C would be retained in serum. Methods. Testing of the latter hypothesis required that we use Activase that had been inactivated using D-phe-pro-arg-chloromethylketone (PPACK). Fibrin pads were prepared in 96-well microplates. After blocking exposed binding sites with an albumin solution, various tPA/PPACK concentrations in buffer or rabbit serum were incubated in wells to equilibrium at three temperatures. The contents of wells were removed and assayed for tPA using a sandwich ELISA. From the free tPA fractions thus measured, Scatchard determination showed the thrombolytic and fibrin-binding properties. Purpose. To test the hypotheses that determination of thermodynamic parameters associated with tPA-fibrin binding could provide information about the molecular nature of the associations and that binding characteristics at 37°C would be retained in serum.

Polymorphism of Factor XIII and Gender-related Differences in Risk of Cardiac Events Among Postinfarction Patients

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Background. There is limited data on the effect of the FXIII V34L polymorphism in patients with coronary artery disease. Methods. We evaluated the effect of the Leu34 genotype of FXIII on risk of recurrent cardiac events (cardiac death, nonfatal MI or unstable angina) in a cohort of 1012 post-infarction (MI) patients: 219 with the Leu34 genotype and 383 without the Leu34 genotype. There were 760 men and 252 women; gene frequencies were similar across the genders (p = 0.73). Results. In total population, the Leu34 genotype was not associated with increased risk of event (adjusted HR = 1.18, p = 0.20). The Leu34 genotype was associated with higher rate of events among men, but not among women (Figure 1). After adjustment for clinical covariates, the Leu34 genotype remained risk factor for recurrent events in men (HR = 1.40, p = 0.03) but not in women (HR = 0.81, p = 0.38). Conclusions. The V34L polymorphism of FXIII gene is an independent risk factor for recurrent events among post-MI men but not among women.
Hematopoietic Cell–Derived Tissue Factor Accelerates Thrombosis in C-Reactive Protein Transgenic Mice

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Elevated plasma levels of C-reactive protein (CRP) are associated with increased risk of myocardial infarction, and transgenic mice expressing human CRP demonstrate accelerated thrombosis after vascular injury. However, the mechanisms underlying the prothrombotic effects of CRP are poorly defined. We tested the hypothesis that CRP promotes thrombosis via effects on TF expression by circulating cells of bone marrow origin. We irradiated male CRP-transgenic (CRP-Tg) mice and transplanted them with bone marrow cells (BMC) from wild-type (WT) mice or completely deficient in mouse TF, but expressing a human TF transgene at a very low level (approximately 1% normal human TF levels, termed “low-TF” mice). Six weeks after transplant we subjected mice to photochemical carotid artery injury and measured the time required to form an occlusive thrombus. Mean time to occlusion was 13.2 ± 2.3 min in CRP-Tg mice transplanted with WT BMC (n=10) vs. 18.1 ± 2.3 min in CRP-Tg transplanted with low-TF BMC (n=7; p<0.08; additional transplant experiments are in progress). Pulse TF activity was significantly higher in CRP-Tg mice transplanted with WT BMC (n=3) vs. CRP-Tg mice transplanted with low-TF BMC (n=4) (2.74 ± 0.19 µM vs. 1.35 ± 0.18 µM, respectively, p<0.05). In addition, expression of WT TF in vivo in CRP-Tg mice markedly enhanced the aggregation induced by other agonists including collagen and/or flow cytometry.

Methods. CRP-Tg mice were backcrossed seven times to C57BL/6J before transplantation. Adult male mice were anesthetized and intra-carotid photocoagulation was performed. After 30 min, the mice were killed and the carotid arteries were removed and imbedded in paraffin, sectioned, and stained with Masson’s trichrome. TF positive cells were quantitated by blinded researchers. Data are presented as mean ±SD. Statistical analysis was performed with the non-parametric Mann-Whitney test. Overall survival was determined using Kaplan-Meier survival analysis.

Introduction: The association between CRP and atherosclerosis is well established. However, the specific mechanisms underlying this effect are not well understood. While CRP is a well-known pro-inflammatory and pro-thrombotic factor, its role in thrombosis in the setting of atherosclerosis is not well characterized. In this study, we hypothesized that CRP promotes atherosclerotic lesion formation in the carotid arteries of male and female CRP-Tg mice by enhancing TF expression and consequently accelerating thrombosis.

Conclusion: This study provides evidence for an inflammatory mechanism of pro-thrombotic action of CRP. CRP promotes TF expression by circulating cells of bone marrow origin and accelerates thrombosis after vascular injury. These findings have potential implications for both atherothrombotic disease and acute coronary syndrome.

Platelet Thromboassay Assay Is a Good Predictor of Aspirin Response

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Introduction: Persistent normal platelet function despite aspirin therapy, referred to as aspirin resistance, has been associated with a threefold increased risk of major cardiovascular events. Hypothesis: We hypothesised that newer point-of care aspirin resistance assays, such as the platelet function analyzer (PFA) -100, may be a less sensitive and specific measure of cyclooxygenase pathway inhibition when compared with laboratory assays of aspirin response. Methods: Patients with unstable coronary disease, who were on aspirin therapy for a minimum of 1 week prior to procedure were enrolled. Platelet function was assessed using the PFA-100 (collagen/epinephrine) cartridge. Twenty five aspirin-resistant and 25 matched aspirin-sensitive patients were recalled for further analysis. Assays of serum thromboxane (TX) B2, platelet TXB2 generation in response to exogenous arachidonic acid (AA) (platelet TX) and cellular TX, platelet aggregation to AA (1.6 mM), epinephrine (0.5ug/ml) and (TRAP) (5µM) were performed. Results: Fifteen percent of patients were found aspirin resistant, failing to prolong the PFA-100 closure times (>193 seconds). The PFA-100 correlated with weak but not strong platelet agonists or TX generation (Table 1). The platelet TX assay was the most sensitive measure of aggregation response to both weak and strong platelet agonists. Conclusion: In a stable cardiovascular population, the PFA-100 point-of-care assay correlated with weak but not strong platelet agonists nor serum thromboxane B2 generation. Platelet thromboassay generation correlated closely with most assays of platelet function and may represent a sensitive assay of platelet cyclooxygenase inhibition and thus aspirin resistance.

TABLE 1. ASPIRIN ASSAY CORRELATION: RELATIVE RISK (95% CI): P-VALUE* FROM FISHER’S EXACT TEST.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Serum TX</th>
<th>Platelet TX</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFA-100 (≤ 193 sec)</td>
<td>Serum TX</td>
<td>Platelet TX</td>
</tr>
<tr>
<td>Serum TX (µg/ml)</td>
<td>1.19 (0.34 to 4.17)</td>
<td>3.69 (1.02 to 13.34)</td>
</tr>
<tr>
<td>Platelet TX (&gt;90ng/ml)</td>
<td>0.0083</td>
<td>3.00 (0.68 to 13.27)</td>
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<tr>
<td>Urinary TX (&gt;130 pg/mg)</td>
<td>0.0001</td>
<td>3.94 (1.24 to 12.54)</td>
</tr>
<tr>
<td>AA Aggregation (1.6mM) (&gt;90%)</td>
<td>0.0001</td>
<td>1.61 (3.76 to 8.29)</td>
</tr>
<tr>
<td>Epinephrine Aggregation (5µm) (&gt;50%)</td>
<td>0.0002</td>
<td>4.5 (1.53 to 13.23)</td>
</tr>
<tr>
<td>Collagen Aggregation (0.5µg/ml) (&gt;20%)</td>
<td>0.0021</td>
<td>1.75 (0.81 to 4.37)</td>
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Conclusion: The PFA-100 point-of-care assay is a good predictor of aspirin response. Further studies are needed to validate the PFA-100 as a point-of-care aspirin resistance assay.

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Higher Platelet Activity Is Present in Patients with Restenosis After Percutaneous Coronary Intervention but Not in Patients with an Occlusion of Coronary Artery Bypass Graft

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Background: platelet activity plays an important role in acute coronary syndromes as well as in the progression of atherosclerosis. The aim of the study was to compare platelet activity in patients with restenosis after PCE and in patients with an occlusion of coronary arteries. A group with an occlusion of bypass graft was included, with an occlusion of bypass graft after coronary artery bypass grafting surgery (CABG) and with a restenosis after percutaneous coronary intervention (PCI). Methods: Forty-five patients with coronary artery disease were studied in a cross-sectional designed study. Fifteen of them were patients with worst bypass graft patency from Prague-4 control (study protocol- controlled coronary angiography) which was performed within 14 days after PCI and if it was performed. No patients was at dual platelet antagonist treatment at the time of blood sampling. Platelet activity was determined by membrane expression of platelet antigen CD62P (P-selectin, % of positive cells) by flow cytometry, aggregability by ADP-aggregometry. Data are expressed as mean ±SEM. Results: All patients were included in the analysis. The similarity of patient groups in some variables, such as age, gender, the history of diabetes mellitus. No patient suffered from acute coronary syndrome. Membrane expression of CD62P antigen was significantly higher in the patients with restenosis compared to patients with occluded or patent bypass grafts (1.96 ±0.07 vs. 0.77 ±0.03 vs. 0.57 ±0.03, p<0.001, Kruskal-Wallis test). CD62P expression was not different between patients with occluded vs. patent grafts. ADP-aggregometry was not different between groups (55.5 ±1.1 vs. 56.1 ±0.8).
Mechanism of Paradoxical Platelet Activation Induced by Blockers of Platelet Integrin αIIbβ3 (GPⅡb/Ⅲa)

Nicole Baslier, Baker Heart Rech Institute, Melbourne, Australia; Christoph Leoffler, Univ Hosp, Freiburg, Germany; Pierre Mangin, Yaping Yuan, Monash Univ, Melbourne, Australia; Meike Schwarz, Univ Hosp, Freiburg, Germany; Christof Hagemeyer, Steffen U Eisenhardt, Ingo Ahrens, Baker Heart Rech Institute, Melbourne, Australia; Christoph Bode, Univ Hosp, Freiburg, Germany; Shaun P Jackson, Monash Univ, Melbourne, Australia; Karthikeya Peter, Baker Heart Rech Institute, Melbourne, Australia.

Introduction: αIIbβ3 blockers provide benefits when applied intravenously (although with limitations) but failed as oral drugs. We developed a model describing the current concept of ligand-mimetic integrin blockade as potential reason for paradoxical platelet activation.

Methods: Platelet activation was determined by flow cytometry, immunofluorescence microscopy and Ca2+ measurements. Results: As an experimental model, we show activation of platelets in solution induced by ligand-mimetic αIIbβ3 blockers consisting of 3 components: 1. Pre-stimulation (ADP 1 μM). 2. Induction of ligand-bound conformation of αIIbβ3 (binding of αIIbβ3 blockers, RGD-peptides and anti-LiBS antibodies) and 3. αIIbβ3 clustering (via antibodies). Platelet adhesion on collagen represents an in vivo correlate of platelet pre-stimulation and receptor clustering, in which the presence of ligand-mimetic αIIbβ3 blockers results in platelet activation as detected by P-selectin expression (mean fluorescence ± SEM: no addition 6.13±0.49 vs. epothilone 18.25±1.02, p<0.001) CD63 and CD40L expression as well as by measuring Ca2+ (Ca2+ [μM] ± SEM: no addition 18.46±2.45 vs. epothilone 54.30±10.62, p<0.05). This paradoxical platelet activation can be inhibited by ADP (P2Y12) receptor blockers (e.g. clopidogrel). Conclusion: We describe a mechanism of αIIbβ3 blocker-induced paradoxical platelet activation, which may explain major limitations of αIIbβ3 blockers. These findings suggest co-medication with ADP-receptor blockers and moving beyond the initial approach of ligand-mimetic blockade towards the development of allosteric or activation-specific integrin integrin.

Assays Evaluating Platelet Inhibition Provided by Clopidogrel Yield Significantly Different Results and Correlate Poorly Among Themselves

Chantal Pharan, Marie Lordkipanidze, Donald A Palisaitis, Hopital Sacré–Coeur, Montreal, Canada; Jacques Turgeon, Universite de Montreal, Montreal, Canada; Erick Schampaert, Jean G Diodati; Hopital Sacré–Coeur, Montreal, Canada; Pierre Lordkipanidze, Steffen U Eisenhardt, Ingo Ahrens, Baker Heart Rech Institute, Melbourne, Australia; Christoph Bode, Univ Hosp, Freiburg, Germany.

Background: The level of inhibition of platelet aggregation provided by clopidogrel is subject to important inter-individual variations; however, it has been reported in various populations, using different platelet function tests, thus making the results difficult to compare. Hypothesis: We assessed the hypothesis that inhibition of platelet aggregation by clopidogrel is subject to variability, with respect to the platelet function test used in its evaluation. Methods: One hundred and twenty patients suffering from stable coronary artery disease were recruited prior to diagnostic angiography. They received clopidogrel for 1 to 7 days before the procedure. Blood samples were obtained before clopidogrel initiation and at the time of diagnostic coronary angiography using the following platelet function tests: optical aggregometry (LTA: adenosine diphosphate [ADP] 5 and 20 μM as the agonist), electrical impedance in whole blood (WBI; ADP 10 μM), drop in platelet count (ADP 5 and 20 μM), PFA-100® (ADP cartridge) and VerifyNow® P2Y12. Results: The correlation between different platelet function tests was generally low. However, the same test performed at different concentrations of ADP yielded highly correlated results. Conclusion: Platelet function tests are not equally effective in measuring clopidogrel's antiplatelet effect. When compared to the current gold standard (LTA-ADP), aggregation in whole blood shows moderate correlation, while point-of-care assays correlate poorly. Thus, platelet function assays should not be used interchangeably.

TABLE: PEARSON'S CORRELATION COEFFICIENTS

<table>
<thead>
<tr>
<th>TESTS</th>
<th>LTA, ADP 5 μM</th>
<th>WBL, ADP 5 μM</th>
<th>WBL, ADP 20 μM</th>
<th>Platelet count drop, ADP 5 μM</th>
<th>Platelet count drop, ADP 20 μM</th>
<th>PFA-100® VerifyNow® P2Y12</th>
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<tbody>
<tr>
<td>LTA, ADP 5 μM</td>
<td>0.94*</td>
<td>0.37*</td>
<td>0.47*</td>
<td>0.51*</td>
<td>0.53*</td>
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<tr>
<td>LTA, ADP 20 μM</td>
<td>0.36*</td>
<td>0.48*</td>
<td>0.40</td>
<td>0.53*</td>
<td>0.30</td>
<td>0.37</td>
</tr>
<tr>
<td>WBL, ADP 5 μM</td>
<td>0.92*</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.29</td>
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<tr>
<td>WBL, ADP 20 μM</td>
<td>0.11</td>
<td>0.06</td>
<td>0.27</td>
<td>0.31</td>
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P417, P418, P419

Prolonged Prophylactic Administration of High-dose Clopidogrel Before Elective Percutaneous Coronary Intervention Is More Effective Than the Standard 300-mg Bolus in Inhibiting Platelet Aggregation

Chantal Pharan, Thuy Anh Nguyen, Marie Lordkipanidze, Donald A Palisaitis, Hopital Sacré-Coeur, Montreal, Canada; Jacques Turgeon, Universite de Montreal, Montreal, Canada; Erick Schampaert, Jean G Diodati; Hopital Sacré-Coeur, Montreal, Canada.

Background: Effective platelet inhibition at the time of PCI reduces the risk of periprocedural thrombosis. In patients with stable angina who undergo elective PCI, the issue of optimal loading dose and time of clopidogrel administration remains controversial. Hypothesis: We assessed the hypothesis that increasing the cumulative clopidogrel dose administered before PCI would result in better inhibition of platelet aggregation. Method: One hundred and twenty patients were prospectively randomized in a double-blind, placebo-controlled fashion into one of 4 groups of clopidogrel dosing regimens 1 week prior to PCI (300 mg on the day prior to PCI, 650 mg on the day prior to PCI, 300 mg followed by 75 mg daily for 7 days starting on the day prior to PCI, and 300 mg followed by 150 mg daily started one week before PCI). Platelet function was assessed at baseline, at the time of diagnostic coronary angiography, and 2 hours after stenting by optical aggregometry (LTA) induced by 20 μM of ADP. Results: All regimens significantly reduced platelet aggregation at the time of angiography, as well as 2 hours following stenting when compared to baseline (p<0.0001; Figure). The 300 mg bolus followed by 150 mg daily showed the greatest inhibition of platelet aggregation, while a single 300 mg bolus resulted in the least inhibition, an absolute difference of 30% at the time of angiography (p=0.007), which increased to 36% 2 hours post-stenting (p=0.007). Conclusion: In choosing a clopidogrel regimen, it is important to effectively block the surge in platelet activity induced by the PCI. The 300-mg bolus and 150-mg daily regimen seemed most effective in achieving and maintaining such a level of platelet inhibition.

Altered Reactive Oxygen Species Generation and Scavenging in Platelets from Patients with Heart Failure

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Heart failure (HF) is characterised by increased oxidative stress, which results in a reduction in bioactive nitric oxide (NO) as well as increased platelet activation leading to increased thrombotic events. Platelets have the capacity both to produce and to scavenge reactive oxygen species (ROS). However, the production and scavenging of ROS by platelets in HF has not been well characterised. We therefore sought to determine whether platelets from patients with HF have higher ROS production and/or defective ROS scavenging capacity. Twenty HF patients (mean age 58.3 ± 3.3 years; 20 male, 5 female; 7/9/4/5 patients in NYHA classes I/II/III/IV) and 20 healthy controls of similar age and sex distribution (mean age 57.1 ± 2.9 years; 12 male, 7 female) were studied. ROS production was measured in gel-filtered platelets by pholisin chemiluminescence, in the presence of horseradish peroxidase, both at baseline and for 30 minutes after stimulation with collagen (8 μg/ml). Responses were expressed as arbitrary light units/10^5 platelets. In other experiments, the decrease in light signal over 10 minutes was measured following the addition of gel-filtered platelets to Tyrode solution, as a measure of ROS-scavenging capacity, and this was expressed as percentage decrease in luminescence. All data were expressed as mean ± SEM, and were analyzed by paired or unpaired Student’s t test as appropriate, with p<0.05 (two tailed) taken as significant. Platelets from HF subjects exhibited greater ROS production than did those from controls, both basally (13320 ± 1665 vs. 8062 ± 1293 light units/10^5 platelets respectively, p=0.023) and after stimulation with collagen (36020 ± 9863 vs. 11120 ± 2176 light units/10^5 platelets respectively, p=0.036). On the other hand, the reduction in pholisin luminescence by platelets was less for HF subjects than for controls (65.4 ± 4.8 vs. 88.2 ± 2.3 % reduction in light signal respectively, p<0.007).
The potential for vascular endothelial cell injury caused by hemodynamic stress during physical activity in humans is unknown, but may provide insight into the flow stimulus that improves endothelial cell function following exercise training. The purpose of the present study was to assess the hypothesis that different muscle contraction-produced shear forces could stimulate the release of von Willebrand factor (vWF), a marker of endothelial cell injury. Methods: Eight healthy young men [25.6 ± 3.1 (SD) y] performed 20 min of single-leg knee extension exercise at two contraction rates: fast (FR, 11% duty cycle) and slow (SR, 50% duty cycle). To control for metabolic demand of blood flow, work rate was held constant (15.25 W) between FR and SR. Common femoral artery blood flow was measured at rest and during knee extension exercise using Doppler ultrasound. Rocket immuno-electrophoresis was used to measure plasma levels of vWF from venous blood collected by venipuncture before immediately after, and 60 min following exercise. High-intensity cycling exercise was performed on a separate day as a control condition since previous studies have shown post-exercise increases in vWF with this mode of exercise. Results: The FR and SR exercise protocols produced significantly different blood flow patterns with the FR resulting in a larger retrograde flow component (>100 m/min) than the SR which consisted solely of antegrade flow. As a result, the magnitude of blood velocity oscillations experienced by the vascular endothelium was different between contraction rates (FR: 154.5 ± 34.1 vs. SR: 112.7 ± 21.6 cm/s, P < 0.05) with the average calculated shear rate being greater for FR than SR (383.1 ± 147.7 vs. 357.6 ± 127.3 s⁻¹, P < 0.01). Since workrate was held constant, mean blood flow was similar between FR (1890.7 ± 127.3 s⁻¹) and SR (1374.4 ± 798.1 s⁻¹), Plasma levels of vWF were similar between FR and SR at rest and failed to change following exercise. In contrast, vWF increased by 16% (P < 0.05) in plasma collected at 60 min following cycling. Conclusion: The different levels of hemodynamic stress that accompany lower limb exercise, at least during moderate intensity, do not induce endothelial cell injury as assessed by plasma levels of vWF.

**Physiological Testosterone Stimulates Tissue Factor Pathway Inhibitor Expression in Human Umbilical Vein Endothelial Cells Via the Androgen Receptor**

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**Aims:** The aim of this study was to evaluate the effect of testosterone with varied concentrations on antigen and mRNA levels of tissue factor pathway inhibitor (TFPI) released by human umbilical vein endothelial cells (HUVECs). The purpose of this study was to investigate the mechanism of this regulation. Methods: HUVECs within 2–3 passages were cultured in 96-well plates and 25 mm flasks. The cells were incubated in the presence or absence of testosterone (3, 30, 300, 3000 nmol/L) for 48 h. After the incubation TFPI levels of media were measured by UHIMBD Total Elisa kit. And RT-PCR was carried out to compare each group's TFPI mRNA level. Then experiments were repeated with HUVEC incubated in androgen receptor antagonist (flutamide 10 μmol/L) for 3 h previously. Results: Testosterone at physiologic concentrations (3.30 nmol/L) stimulated the secretion of TFPI significantly (P < 0.05). However, TFPI antigen and mRNA levels were markedly reduced at a larger dose (3000nmol/L). Flutamide attenuated testosterone's effects (P < 0.05). Conclusion: Our results demonstrated that testosterone, at physiological concentrations, has beneficial influence on hemostatic system by enhancing the anticoagulant activity through stimulating the TFPI levels secreted by the endothelium, and that the vascular androgen receptor is involved in the processes. Figure 1 Testosterone's effect on TFPI gene expression in absence or presence of flutamide (x = 5, n = 4). (A) M, marker; 1, control; Lane 2, 3nM T; Lane 3, 30nM T; Lane 4, 300nM T; Lane 5, 3000nM T; Lane 6, 6000nM T; Lane 7, F and 3nM T; Lane 8, 6 and 30nM T; Lane 9, F and 300nM T; Lane 10, F and 3000nM T. (B): testosterone F: flutamide.
randomized controlled trials, this finding may reflect treatment of more complex lesions with DES as opposed to BMS.

P426
Glutathione Peroxidase Activity in Cerebral Vasospasm After Subarachnoid Hemorrhage
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An oxidizing environment has long been implicated in the etiology of cerebral vasospasm (CV) after subarachnoid hemorrhage (SAH). This environment has been variously postulated to be generated by reactive oxygen, lipid, and nitrosative radicals. Using human CSF we have previously shown that in CVS human cortex and serum thiocyanate concentrations are increased. We sought to determine the glutathione (GSH) redox status in SAH patients with CV, and from non-reactive hydrocephalus patients (control CSF). A commercially available GSH activity kit (ZapetMetalX Corp.) was used to assess activity, mononuclear human anti-Gpx was obtained from AbCam for the western blot analysis and other chemicals were obtained from Sigma. GSH from vasospastic patients (CSFs, n=5), non-vasospastic patients (CSFs, n=7) and non-hemorrhagic patients (Control, n=10) were subjected to the following analyses: GSH, GSH protein levels, copper levels and iron levels. Hemoglobin, bilirubin and lipid peroxidation levels had previously been determined for the same patient samples. GSH activity levels (uU) were 32 ± 2.9, 98 ± 9.1 and 341 ± 29.7 for Control, CSFv and CSFh, respectively. We then performed western blot analysis to determine whether the difference in the GSH levels was due to increased levels of GSH protein. We found no significant difference in band intensity between the three groups. Hemoglobin concentrations did not differ significantly between hemorrhagic CSF groups. Both copper and iron can act as pseudoperoxidases (non-enzymatic peroxidation). Copper(I) concentrations (expressed as µg copper per g hemoglobin) did not differ significantly between CSFv and CSFh (58.50 ± 3.6 vs. 40.78 ± 5.28). Similarly, Fe(I) concentrations (µM) were not significantly different in both SAH CSF groups (CSFv, 29.7 ± 9.8 CF, 18 ± 29.6). These results suggest an increase in Gpx activity that is indicative of CV after SAH. Further studies will reveal if this observation can be predictive, or if Gpx could be a viable therapeutic angle for this serious complication.

P427
Flow-mediated Changes in Pulse Wave Velocity in Patients with Hypertension
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Background: Flow mediated changes in brachial artery diameter are widely used to assess endothelial dysfunction. Recently, a new measure of endothelial function was proposed using hyperemic pulse wave velocity (PWV). Objective: In this study, we wanted to compare changes in PWV upon hyperemia in healthy hypertensive subjects (HTN). Method: We measured flow-mediated changes in PWV in 16 healthy and 10 HTN subjects. Baseline blood pressures (BP) and arterial stiffness (augmentation index-AI and PWV) by applanation tonometry were measured. A right upper arm Hokenson’s cuff was inflated 50 mm Hg above the systolic BP for 4 min. Post 1 min deflation and 2 min intervals, reactive hyperemic response was measured by PWV. Results: Healthy subjects were younger (40 ± 17.3 vs 54.4 ± 10.5), BPs and HR were similar to the 2 groups. Baseline arterial stiffness parameters including AI (22.1 ± 17.4 % vs 23.6 ± 10.4 %, p < .01; and PWV (6.6 ± 1.4 m/s vs 7.1 ± 1.4 m/s) did not differ significantly between the 2 groups. In both subjects, flow-mediated hyperemic response was measured in PWV. Results: Healthy subjects were younger (40 ± 17.3 vs 54.4 ± 10.5). These results suggest an increase in Gpx activity that is indicative of CV after SAH. Further studies will reveal if this observation can be predictive, or if Gpx could be a viable therapeutic angle for this serious complication.

P428
Combining Paclitaxel and Sirolimus Favorably Improves Endothelial Cytotoxicity and Platelet Proaggregatory Effect of Either Agent Alone
Chanwit Roongsritong, Sandra Rodriguez, Jan Simoni, Ashwani Kumar; Texas Tech Univ HSC, Lubbock, TX

Drug eluting stents (DES) have over the past few years emerged as a mainstay of percutaneous coronary intervention (PCI) due to its superior patency rate over bare metal stents. With widespread utilization of DES, late thrombotic risk associated with these stents has increasingly become a public concern. Paclitaxel and sirolimus are the two agents used in the currently available DES in the US. Our previous research has shown significant differences between the effects of paclitaxel and sirolimus on human coronary artery endothelial cells (HCAEC) and human platelets. Given that these two components have been found to independently elicit endothelial damage in vivo, we hypothesized that combining these agents might lead to more desirable overall effects than either agent alone for DES. Methods In this study the platelet anti- and pro-aggregatory effects were tested using different dose combinations of both drugs. The cytotoxic effects were evaluated using the RBC fragility model. Results We found that the platelet pro-aggregatory effect of sirolimus can be attenuated with paclitaxel. Furthermore, the cytotoxic effect of paclitaxel was diminished in the presence of sirolimus. The most desirable effect in terms of platelet aggregation and endothelial cytotoxicity was observed at a 500 to 1 paclitaxel to sirolimus molar ratio. Conclusion This study suggests that combination therapy could be effective in ameliorating pathological platelet pathway or sirolimus alone. However, the effect of paclitaxel and sirolimus combination on smooth muscle cell proliferation in comparison to either agent alone is not yet known. Further study to explore this novel concept is warranted.

P429
Heme Oxygenase-1: A Novel Key Player in the Development of Tolerance to Response to Organic Nitrates
Philip Wenzel, Il Medizinische Klinik, Johannes-Gutenberg-Univ Mainz, Mainz, Germany; Matthias Oelze, Meike Coldewey, Labor I Molekularle Kardiologie, Mainz, Germany; Dirk Stalleiken, Activis Deutschland GmbH, Langenfeld, Germany; Thomas Munzel, Il Medizinische Klinik, Johannes-Gutenberg-Univ Mainz, Mainz, Germany; Andreas Daiber; Labor I Molekularle Kardiologie, Mainz, Germany

Objective-Nitrate tolerance is likely due to an increased production of reactive oxygen species (ROS) leading to an inhibition of the mitochondrial aldehyde dehydrogenase (ALDH-2), representing the nitrite reductase enzyme, and to impaired nitric oxide bioactivity and signaling. We tested whether differences in heme oxygenase-1 (HO-1) induction might explain why PENTX but not GTN therapy is devoid of nitrate and cross-tolerance. Methods and Results-Wister rats were treated with PENTX or GTN (10.5 or 8.64A2±23.8A2/kg/min for 4d). In contrast to GTN, PENTX did not induce nitrate or cross-tolerance as assessed by isometric tension recordings in isolated aortic rings. Vascular protein and mRNA expression of HO-1 and ferritin were increased in response to PENTX but not GTN. In contrast to GTN therapy, NO signaling, ROS formation (as determined by chemiluminescence) and the activity of ALDH-2 (as assessed by an HPLC based method) were not significantly inhibited by PENTX. Inhibition of HO-1 expression by apigenin induced tolerance to PENTX whereas HO-1 gene induction by hemin prevented tolerance in GTN treated rats. Conclusions-HO-1 expression and activity appear to play a key role in the development of nitrate tolerance and might represent an intrinsic antioxidative mechanism of therapeutic interest.

P430
Adequately Treated Type 2 Diabetes Is Associated with Lower Wall Shear Rate of the Common Carotid Artery
Eva Chytliova, Zdeslava Kasalova, Jan Malik, Radka Dolcezalova, Tomas Stulc, Richard Ceska; General Faculty Hosp, Prague, Czech Republic

Introduction: Arterial sites with low wall shear stress (WSS) are more prone to the development of atherosclerotic plaques, as was observed in carotid arteries in subjects with atherosclerosis risk factors. Diabetes mellitus, a strong risk factor, could be modified by statins and angiotensin-converting enzyme inhibitors (ACEI). The aim of our study was to discover if type 2 diabetes mellitus (DM) subjects compensated by metformin, with established statin and ACEI therapy, still have lower WSS in common carotid arteries than healthy controls. Methods: We enrolled 26 compensated DM subjects aged 60 ± 10 years, treated by metformin, statins and ACEI for more than 6 months, and 16 age-matched healthy controls. Ultrasound examination was targeted to distal 1 cm of common carotid arteries, where maximal and mean velocities were measured. Internal diameter (ID) and intima-media thickness (IMT) were analyzed by an expert using a special machine. Wall shear stress (WSS) is defined as a measure of WSS, calculated according to the following formula: WSR = 4x velocity/ID 2. Differences between groups were analyzed by unpaired t-test. Results: Diabetic subjects had significantly lower WSR, because of both thinner lumen and slower blood flow velocities. Lower WSR was accompanied by higher IMT. Conclusion Adequately treated subjects with compensated DM still have atherogenic hemodynamic profile.
Prediction equations thus developed were applied on validation data set (30%). RESULTS: The simplest equation for predicting %BF included age, gender, BMI, tripole skinfold and waist circumference \( R^2 = 84.4\% \). Replacing BMI with weight and height reduced the overall variance \( R^2 = 86.4\% \). BMI showed a strong correlation with all abdominal fat subcomponents, but it best correlated with SCAT \( R = 0.79 \). The most precise predictive equation for estimating IAA included age, gender, BMI, hip circumference and waist circumference \( R^2 = 52.1\% \). Waist circumference was the strongest predictor of IAA \( R = 0.69 \) and hip circumference of SCAT \( R = 0.80 \), irrespective of age and gender. BMI and hip circumference explained 66.6% variability in SCAT. CONCLUSION: The following predictive equations would be clinically relevant for fat estimation: age, gender, BMI, hip circumference and waist circumference.

Diet-Induced Obesity in Male Apolipoprotein E-Deficient Mice Increases Adipose Serum Amyloid A Expression and Atherosclerosis

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The current epidemics of obesity and related comorbidities such as cardiovascular disease is increasing at alarming rates in the U.S. and worldwide. Changes in dietary habits and lifestyle have been implicated in the development of obesity. The mechanisms linking obesity to accelerated atherosclerosis are not fully understood. In part due to a paucity of models of obesity that closely emulate human obesity, the direct impact of obesity and its components, including dietary factors, on inflammation and arterial remodeling is largely unexplored. AIM/HYPOTHESIS: We hypothesized that diet induced obesity accelerated atherosclerosis in male apoE-/- mice, which may in part be mediated by increased adipose SAA synthesis.

Methods

Male apoE-/- mice were fed either chow diet containing 10% or 60% fat from 10 weeks of age. Following 10 weeks, both groups were fed a chow diet containing 60% fat for 5.5 high fat diet fed mice had increased body weight gain (3.3 fold, P<0.001) and plasma total cholesterol levels (17%, P<0.05) compared to mice fed the low fat diet. Atherosclerosis was increased in high fat fed mice compared to low fat fed mice (0.473±0.084 vs. 0.370±0.062 mm2, P<0.001). Mice obtained from fat fed mice had increased SAA and IL-6 levels throughout the 18 hours period, up to 5.5 mEq glucose, after 18 hours primary growth at 16 mm. Integrons or collagens are identified by immunoprecipitation, quantified with an ELISA, adhesion, migration or proliferation measured on HuVSMCs. Results: Adhesion decreases collagen I, II, and IV, but increases to type VI after post-CM Glucose treatment. Equimolar sorbitol has no effect. Antibody to TNFa reduces adhesion to type I collagen. Post-CM Glucose decreases the expression of SCAT and IL-6. Inhibitors expression, elevated systemic SAA concentrations (2.6 fold, P<0.05). Moreover, SAA was associated with VLDL, LDL and HDL in mice fed the high fat diet compared to those fed the low fat diet, in which SAA was primarily associated with HDL. Thus diets enriched in saturated fat induce obesity accelerated atherosclerosis in male apoE-/- mice, which may in part be mediated by increased adipose SAA synthesis.

Role of Postconditioned Medium from Glucose Primed Endothelial Cells on Human Umbilical Vasculature

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Objective: Glucose is considered to be implicated in the development of cardio-vascular disease in diabetes. Changes interactions between endothelial and human arterial smooth muscle cells (HuVSMCs) are not fully understood, in part due to a paucity of models of obesity and accelerated atherosclerosis. Similar to humans, apolipoprotein E deficient (apoE-/-) mice spontaneously develop atherosclerosis over their lifetime. Therefore, we sought to determine if diet induced obesity accelerated atherosclerosis in apoE-/- mice. Eight week old male apoE-/- mice were fed either chow diet containing 10% or 60% fat from 10 weeks of age. Following 10 weeks, both groups were fed a chow diet containing 60% fat for 5.5 high fat diet fed mice had increased body weight gain (3.3 fold, P<0.001) and plasma total cholesterol levels (17%, P<0.05) compared to mice fed the low fat diet. Atherosclerosis was increased in high fat fed mice compared to low fat fed mice (0.473±0.084 vs. 0.370±0.062 mm2, P<0.001). Mice obtained from fat fed mice had increased SAA and IL-6 levels throughout the 18 hours period, up to 5.5 mEq glucose, after 18 hours primary growth at 16 mm. Integrons or collagens are identified by immunoprecipitation, quantified with an ELISA, adhesion, migration or proliferation measured on HuVSMCs. Results: Adhesion decreases collagen I, II, and IV, but increases to type VI after post-CM Glucose treatment. Equimolar sorbitol has no effect. Antibody to TNFa reduces adhesion to type I collagen. Post-CM Glucose decreases the expression of SCAT and IL-6. Inhibitors expression, elevated systemic SAA concentrations (2.6 fold, P<0.05). Moreover, SAA was associated with VLDL, LDL and HDL in mice fed the high fat diet compared to those fed the low fat diet, in which SAA was primarily associated with HDL. Thus diets enriched in saturated fat induce obesity accelerated atherosclerosis in male apoE-/- mice, which may in part be mediated by increased adipose SAA synthesis.

Are Fats Created Equal in Obese and Lean Mice?

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INTRO: Obesity is defined as the excessive accumulation of lipids in adipocytes. These lipid-filled cells were considered inert blocks until a discovery in 1995 revealed that they contain metabolism-regulating proteins which have profound effects on the development of diabetes. Fatty acids compose 65% of adipocytes, and triglycerides make up 98%. Yet the major component of adipocytes and its role in obesity is largely unexplored.

In this study, we assessed the hypothesis that a significant discrepancy exists between the fatty acid composition of obese and lean mice. METHOD: We obtained epididymal fat from 10 adult ob/db mice and 10 control C57Bl/6j mice. We extracted fatty acids with chlorform and added heptadecanoic acid as an internal standard. We then quantified fatty acid composition by gas chromatography equipped with an omega wax 250 capillary column. RESULTS: We found that levels of palmitic and stearic acid were significantly higher in the epididymal fat of obese mice, while the amounts of linoleic acid were lower compared to that of lean mice (refer to table below). CONCLUSION: In this study, we found that the adipose tissue of obese mice contains significant more saturated fatty acids and less unsaturated fatty acids than that of lean mice. PROSPECTIVE: The diverse types of fatty acids play important roles. Our study indicates a possible connection between fatty acid functions and obesity.
decisions. For men, a single WC (%>102cm) had both the highest area under ROC curve (0.76±0.06, 95%CI 0.64–0.88) and highest diagnostic accuracy, 78% with sensitivity 73%, specificity 80% and OR 10.4±6.6, 95%CI 3.1–36.1. For women, a single WC (%>99cm) had both highest area under ROC curve (0.81±0.05, 95%CI 0.79–0.91) and highest diagnostic accuracy, 86% with sensitivity 86%, specificity 77% and OR 19.2±13.5, 95%CI 4.7–78.9. Lipid and glucose parameters above and below WC cut offs of greatest risk are shown below. In conclusion for African Americans the WC which best predicts insulin resistance, dyslipidemia and glucose intolerance were WC (%>102cm for men and WC %>99cm for women. Therefore in African American women the WC which best identifies risk associated with central obesity may be higher than is currently recommended by the NCEP-ATPIII guidelines.

### Lipid and Glucose Parameters According to the WC of Greatest Risk (Total 68M, 63W)

<table>
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<tr>
<th>WC&lt;102</th>
<th>WC&gt;102</th>
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<td>48.1±19</td>
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<tr>
<td>CHOL (mg/dL)</td>
<td>162.2±32</td>
<td>194.4±44 **</td>
<td>161.3±34</td>
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<tr>
<td>HDL (mg/dL)</td>
<td>47.1±11</td>
<td>45.8±5</td>
<td>55.1±11</td>
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<tr>
<td>HDL size (nm)</td>
<td>8.9±0.1</td>
<td>8.6±0.24 **</td>
<td>9.36±0.35</td>
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<tr>
<td>LDL (mg/dL)</td>
<td>99±29</td>
<td>130±41 **</td>
<td>97±29</td>
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<tr>
<td>LDL particle no.</td>
<td>1194±420</td>
<td>1482±503 **</td>
<td>1393±309</td>
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<tr>
<td>Glucose Intolerant</td>
<td>12%</td>
<td>44%**</td>
<td>6%</td>
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Note: *P<0.05, **P<0.01, ***P<0.001

### Population-based Response Estimates for Extended-release Nicotinic Acetylcholine Receptor Antagonist Therapy in Patients with the Metabolic Syndrome

Eric J Stanek, Kos Pharmaceuticals, Cranbury, NJ; Ralph A Quimbo, Kos Pharmaceuticals, Cranbury, NJ

**Background:** The atherogenic dyslipidemia of the metabolic syndrome (MetSyn) is characterized by low HDL-C, elevated triglycerides (TG), and elevated non-HDL-C. This dyslipidemia may be treated with statins, niacin, or the combination of statin plus niacin. We compared the effects of extended-release nicotinic acetylcholine receptor (ERN/S) with other high-potency lipid altering agents in a MetSyn population model. **Methods:** Patients were selected from a 2.1 million record managed care plan database if they had a baseline lipid panel between 1/1/00–12/31/01, no concomitant lipid therapy, and continuous plan eligibility for 24 months. Patients with MetSyn were identified by ICD-9 code (277.7) or by the presence of 3 or more clinical criteria for the MetSyn. Percent achieving optimal values for LDL-C, HDL-C, TG, non-HDL-C, TG/HDL-C and combined LDL-C, HDL-C, and TG were modeled from individual patient baseline values using current product labeling (assuming additive effect for ERN/S). **Results:** ERN/S 2g/40mg (ERN/S 2/40) was compared to atorvastatin 80mg (A80), simvastatin 80mg (S80), simvastatin 80mg/ezetimibe 10mg (S/E 80/10), and rosuvastatin 40mg (R40). **Conclusions:** We analyzed 23,773 MetSyn patients. Mean (SD) age was 64±13 years: 12,236 (51%) female; 16,788 (71%) HTN; 13,018 (51%) diabetes mellitus (24%); and 11,191 (47%) CHD or risk equivalent. Modeled treatment effects are provided in the table. **Conclusions:** In this MetSyn population model, ERN/S achieved greater overall projected optimal lipid value achievement compared to other high-potency lipid-altering therapy.
Conclusions: Caveolin-1 and combination II did not affect export of cholesterol from the macrophages in vivo. Appearance of labeled cholesterol in blood, liver, fecal bile acids and steroids was measured after 24 h. Combination I and to a lesser extend ABCA1 alone caused elevation of cholesterol export from macrophages to plasma, liver and feces. ABCA1, caveolin-1 and combination I did not affect export of cholesterol from the macrophages in vivo. ABCA1 null mice show high cholesterol content in their liver and it was accelerated in ABC/A/ABCA1-/- model of cholesterol efflux. Mouse model of cholesterol efflux to apoA-I is consistent with our findings and suggests a role for ABCA1 in macrophage foam cells in vivo.

P443
LCAT Deficiency Accelerates Cholesterol Accumulation of Liver in ABCA1 Null Mice
M Anwar Hossain, Nobukatsu Akita, Fumimiko Kobayashi, Shinji Yokoyama, Masi Tsujiita; Nagoya City Univ, Nagoya, Japan

Aim: To reveal the cholesterol homeostasis on hepato/procollagernia in mice, both 1) the diffusion mediated ‘efflux’ pathway which accelerate by LCAT reaction and 2) apoA1/ABCA1 mediated cellular cholesterol releasing pathway were abolished in mutant mice. Method: Six genotypes, wild, LCAT(-), ABCA1(-), ABCA1(LCAT(-))/ABCA1(-) and LCAT(-)/ABCA1(-), of 22-week old mice were examined. Euthanized mouse were perfused with PBS with subsequent fixation of liver samples. Stereoscopic hematoxylin content of tissue was measured by enzymatic and color detection method (Kyowa Med.). Results: Tissue TC of liver (43.3±8.7 μg/mg) from female mice were increased in LCAT(-) (134.7±17.0 μg/mg), ABCA1(-) (79.8±7.4 μg/mg) and in LCAT(-)/ABCA1(-) (197.2±16.3 μg/mg). Liver TC from female mice (44.7±6.6 μg/mg) were also modified in LCAT(-) (80.12±5.5 μg/mg), ABCA1(-) (62.58-24.4 μg/mg) and in LCAT(-)/ABCA1(-) (80.13±15.4 μg/mg). On the other hand, CE content in steroidogenic tissue was decreased in LCAT(-)/ABCA1(-). TC level of spleen and brain were also significantly decreased in all of ABCA1-/- back ground mice. No significant differences of TC contents were observed between ABCA1-/- and Wt mouse. Cholesterol accumulation within liver in ABCA1 null mice show high cholesterol content in their liver and it was accelerated in LCAT/ABCA1 double deficient mice. This result indicates that one of cellular non-specific pathway was also highly involved to maintained cholesterol homestasis in liver. Involvement of ABCG1 in this phenomenon will also be examined.

P444
Triglyceride Alters Cholesterol Metabolism and ER-Stress Pathways in Cholesterol Ester–Laden Macrophage Foam Cells
Jody C Ullery, Jerod S Denton, Brian E Cox, W G Jerome; Vanderbilt Univ Sch of Medicine, Nashville, TN

Macrophage foam cells are prominent in atherosclerotic lesions. In late stage disease, much of the cholesterol accumulation in these foam cells is found in large, swollen, lysosomes. Tissue culture models using human macrophages incubated with various modified LDLs indicate that accumulation of lipids within lysosomes can disrupt lysosome function leading to foam cells with significant lysosomal free and esterified cholesterol, similar to cells found in atherosclerotic lesions. The cholesterol is trapped and not accessible for efflux, even in the presence of strong efflux promoters. In the artery wall, however, the foam cells are bathed not only modified LDLs but other lipid particles as well, including triglyceride-rich particles (TRP), such as VLDL. Little is known about how metabolism of these TRP might affect cholesterol metabolism and, specifically, the formation of cholesterol-rich macrophage foam cells. Our studies explore the effect of TRP on intracellular cholesterol metabolism. Results show that triglyceride (TG), delivered to the cell as a complex of VLDL or TG-rich lipoprotein, reduces cholesteryl ester (CE) accumulation by 50% in THP-1 macrophage foam cells. Reduced CE accumulation occurs in response to increased TG levels within the cell particularly within lysosomes. TG, delivered to the cell as a component of TRP, decreases the volume of lysosomes providing further evidence of increased lysosomal cholesterol clearance. Cholesterol accumulation in lysosomes inhibits acidification of lysosomes but lysosomal TG reduced this inhibition and maintained lysosome acidity. The maintenance of an acidic lysosomal environment would enhance the degradation and clearance of internalized CE, facilitating the movement of cholesterol out of the lysosome, to the cell surface, to be released by the efflux pathway. However, the presence of excess TG within the macrophage also activates ER-stress proteins, such as CHOP and Gp78, and induces the phosphorylation of eIF2A, indicating the activation of the unfolded protein response. Our results show that excess TG in CE-laden foam cells has multiple effects, the balance of which would influence the atherogenic potential of the foam cell.

P445
The Absence of abcg1 in Alveolar Macrophages Triggers Pulmonary Inflammation
Allison J Wojcik, Susseela Srinivasan, Borna Mehrad, Catherine C Hedrick; Univ of Virginia, Charlottesville, VA

The ATP-binding cassette transporter G1 (abcg1) effluxes cholesterol from macrophages and plays an important role in pulmonary lipid homeostasis. Deletion of abcg1 in mice results in pulmonary lipids, consisting of accumulation of lipid-filled type 2 pneumocytes containing altered surfactant composition, and changes in lipid metabolism genes. We hypothesize that alveolar and monocyte-macrophages contribute to pulmonary lipidosis in these mice by triggering inflammation in the lung. To study the early development of pulmonary lipidosis in abcg1-/- mice, alveolar and monocyte-macrophages and dendritic cells were isolated from lungs of 10-week old mice by bronchoalveolar lavage (BAL). BAL was used for RNA expression analysis, cytokine bio-plex arrays or flow cytometry. Alveolar macrophages isolated from lungs of 10-week old abcg1-/- mice showed 5-fold or greater increased expression of proinflammatory cytokines MIP-2, IL-6, and IL-1beta compared to wildtype mice. Flow cytometry analysis of abc1-/- BAL lymphocytes showed a 10-fold increase (2,725 abc1-/-/- monocyte-macrophages, 265 wt) in the number of newly recruited monocyte-macrophages (CD11c+low CD11b+high MHCIIClow) compared to wild type prior to phenotypic onset of lipidosis. When abc1-/- mice received an intraperitoneal injection of 2mg/kg of LPS, macrophages isolated in BAL 4 hours showed increased expression of proinflammatory cytokines TNFalpha, IL-6, KC and increased expression of COX-2 and its secretion into BAL fluid was measured using a Bio-plex suspension array and KC and compared to wild type. These data suggest that alveolar macrophages contribute to the pulmonary disease of abcg1-/- mice by secreting more proinflammatory cytokines and recruited monocyte-macrophages to the inflamed lung in the very early stages of lipidosis. Thus, we provide a novel link between macrophage abcg1 function and regulation of inflammation. Understanding the role of abcg1 in alveolar macrophages and its link to inflammation will aid in developing therapies for various pulmonary diseases and other inflammatory diseases.
Whole Genome Expression Profiling Reveals a Significant Role for Immune Function in Human Abdominal Aortic Aneurysms

Guy M Lenk, Gerald Tromp, Shantel Weinsheimer, Wayne State Univ, Detroit, MI; Zoran Gatalica, Creighton Univ, Omaha, NE; Ramon Berguer, Univ of Michigan, Ann Arbor, MI; Helena Kuvinari; Wayne State Univ, Detroit, MI

Abdominal aortic aneurysm (AAA) is a common disorder of the aged population, with approximately 10% of those 65 years or older harboring an AAA. Most AAs are asymptomatic until aneurysm rupture occurs, often leading to sudden death. Global collaborative observation of a pressure-sensitive endoluminal prosthesis (n = 7) and control abdominal aorta (n = 7) was carried out using two distinct microarray platforms; Illium Sentrix-6 and Affymetrix U133plus2.0. The analysis of the data included differential expression and a determination of enrichment in the Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathways as well as Gene Ontology (GO) categories to provide information about possible functional changes in AAA tissue samples. After a correction for multiple testing using False Discovery Rate (FDR), there were 3,274 distinct genes that had an FDR < 0.05. The top 10 enriched KEGG pathways included six immunity-related pathways including adaptive immunity (rs20458; p-value: 8.06e-07) and leukocyte transendothelial migration (hsa04670; p-value: 8.06e-07) pathways, and four that were general biological pathways. The top 10 GO categories included six immune-related and four general cell signaling categories. Taken together, the gene expression patterns strongly indicated an involvement of the adaptive immune system in AAA. A more detailed follow-up of changes in gene expression in the two most enriched KEGG pathways implicates these immune-related biological processes in the pathogenesis of AAA.

A Polymorphism in the Protease-like Domain of Apolipoprotein(A) Is Associated with Severe Coronary Artery Disease

May M Luke, Celeria, Alameda, CA; John P Kane, Univ of California, San Francisco, San Francisco, CA; Dongming Liu, M.D., Charles M Rowland, Dov Shiffman, Celeria, Alameda, CA; June Cassano, Cleveland Clinic Foundation, Cleveland, OH; Joseph J Catanese, Celeria, Alameda, CA; Olve R Pullinger, Univ of California, San Francisco, San Francisco, CA; Diane U Leong, Andre R Arellano, Carmen H Tong, Celeria, Alameda, CA; Inna Movsessyan, Josephine-Jane Vigne, M.D., San Francisco, San Francisco, CA; Curtis Noonhof, Nicole T Feric, Queen’s University, Kingston, Canada; Mary J Mailly, Univ of California, San Francisco, San Francisco, CA; Eric J Topol, Cleveland Clinic Foundation, Cleveland, OH; Mary E Koshinsky, Queen’s University, Kingston, Canada; James J Devlin, Celeria, Alameda, CA; Stephen G Ellis, Cleveland Clinic Foundation, Cleveland, OH

Genetic variants reproducibly associated with severe coronary artery disease (CAD) could improve risk stratification and shed light on disease mechanism. In case-control studies of white subjects whose severity of CAD had been assessed by angiography, we previously tested 12,077 single nucleotide polymorphisms (SNPs) and found 5 SNPs that were nominally associated with severe CAD in 2 studies: Study-1 (781 cases, 603 controls) and Study-2 (471 cases, 296 controls). We have now tested the hypothesis that the risk alleles of these 5 SNPs may be associated with severe CAD in a third study (654 cases, 373 controls). We found that the risk allele of one of these 5 SNPs, LPA Ile4399Met (rs3798220), was associated with severe CAD (2.7% of controls had an adjusted odds ratio for severe CAD of 3.14 [P = 0.005]). This association remained significant after correcting for multiple testing. Carriers had higher plasma lipoprotein(a) levels (P = 0.003) and smaller apolipoprotein(a) isomers (P < 0.001). After adjusting for apolipoprotein(a) size, the association of LPA Ile4399Met with severe CAD and lipoprotein(a) levels remained significant. In conclusion, the LPA 4399 Met allele is associated with elevated lipoprotein(a) levels and increased risk of severe CAD.

Variation at the PCSK9 and LDLR Loci, LDL Levels, and Heart Disease in PROSPER

Eliana Polisacchi, Tufts Univ, Boston, MA; Hind Musallam, Nebyo Maeda, Univ of North Carolina, Chapel Hill, NC; Stella Trompet, Wouter Jukema, Leiden Univ Med Cntr, Leiden, Netherlands Antilles; Alex McMahon, Michele Robertson, Ian Ford, Univ of Glasgow, Glasgow, United Kingdom; Gerard Blauw, Leiden Univ Med Cntr, Leiden, Netherlands Antilles; Michael McCarthy, Univ College, Cork, Ireland; James Shepherd, Univ of Glasgow, Glasgow, United Kingdom; Ernst Schafer; Tufts Univ, Boston, MA

Genetic variation at the low density lipoprotein (LDL) receptor (LDLR) and the proprotein convertase subtilisin/kexin type 9 (PCSK9) genes loci have been reported to affect LDL cholesterol (C) levels and coronary heart disease (CHD) risk. Both PCSK9 and LDLR gene products are regulated by intracellular cholesterol levels and affect LDL clearance. In order to analyse the association between variation at these gene loci, LDL C levels, and CHD, we examined 4070 cases and 4070 controls. Genotyping was performed from the mouse data from 1291 cases, 1291 controls, and 3042 cases who were from the mouse data. The findings indicate that genetic variation at the LDLR and the PCSK9 gene loci can affect LDL C levels and CHD risk.

Temporal Gene Expression of Prosthetic Graft Neointima Isolated by Laser Capture Microdissection

Junaid Y Malek, Thomas S Monahan, Nicholas D Andersen, Sheng-Gian Wu, Somwya Senani, Mauricio A Contreras, Frank W LoGerfo; Beth Israel Deaconess Med Cntr, Boston, MA

Temporal gene expression of neointima, for microarray analysis to define gene expression changes induced by pros-thetic arterial grafting. Continued analysis of identified gene expression patterns will provide insight into the cellular pathways responsible for the formation of AH and aid in the identification of targets for therapy.
Increased Interferon- Gamma Production in Response to Beta-2GPI Immunization in B Cell-Deficient Apeo -/- Mouse
Nicole A Braun, Adam C Morgan, Amy S Major; Vanderbilt Univ Med Ctr, Nashville, TN

B cells have been shown to be protective in atherosclerosis. Our laboratory and others have demonstrated that absence of B cells increases atherosclerosis in LDL(-/-) and apoE(-/-) mice. Immunization of atherosclerosis-susceptible animals with oxidized LDL elicits antibodies against modified lipoprotein and protects against atherosclerosis. Conversely, immunization of mice with another atherosclerosis-associated antigen, β2-glycoprotein I (β2-GPI) exacerbates atherosclerosis in a T helper cell-dependent manner. Because B cells have been shown to regulate T cell responses to specific antigen, we hypothesized that immune responses to β2-GPI by T helper cells could be regulated by B cells. To test this hypothesis, we compared the T helper cell immune response to β2-GPI in apoE(-/-) mice to apoE(-/-) mice deficient for B cells (μMT apoE(-/-)). In these experiments, we immunized 10 apoE(-/-) and 10 μMT apoE(-/-) mice with 10 μg of purified human β2-GPI with adjuvant (TiterMax Gold) intraperitoneally. Controls received adjuvant only. Two weeks following the initial immunization, mice were boosted using the same protocol. Six weeks following the antigen boost, flow cytometry demonstrated no difference in absolute numbers of CD4+ T helper cells among the groups. However, compared to β2-GPI immunized μMT apoE(-/-) mice, immunization of β2-GPI in apoE(-/-) mice decreased the percentage of CD4+ (20% ± 3% vs 6% ± 1) and CD8+ (20% ± 3% vs 9% ± 2) T cells expressing the activation marker CD69. In vitro restimulation of splenocytes with 10 μg/ml of β2-GPI demonstrated that μMT apoE(-/-) splenocytes had increased production of IFN-γ compared to apoE(-/-) splenocytes (3.49 ± 1.75 vs. 10.53 ± 1.869 pg/ml). Proliferation of splenocytes in response to β2-GPI was not different among the groups. These data suggest that B cells may function to modulate CD4+ T cell responses to atherosclerosis antigens such as β2-GPI and that in the absence of B cells, CD4+ T cells increase production of pro-inflammatory cytokines.

RELATIVE LUCIFERASE ACTIVITY ±SD OF FOUR DIFFERENT SNP HAPLOTYPES OF HCRP

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P461
Aldose Reductase Expression and Activity Are Induced in Human Monocyte–Derived Macrophages by Oxidized LDL
Christian A Gleissner, Univ of Virginia, Charlottesville, VA; Hyung-Jun Cho, Univ of Korea, Seoul, Republic of Korea; Dane M Dunson, Klaus Ley; Univ of Virginia, Charlottesville, VA

Aldose reductase (AR) is the rate-limiting enzyme of the polyol pathway, which utilizes excess glucose and reduces it to sorbitol and fructose. AR activity has been associated with retinopathy, nephropathy, and neuropathy in diabetic patients. Overexpression of human AR has been shown to increase atherosclerotic lesions in LDL receptor-knockout mice. Using 22 Atheromac mouse models, we measured gene expression in human peripheral blood mononuclear cells (PBMC) and monocytes/macrophage-derived macrophages as well as foam cells induced by native LDL, minimally modified LDL (mmdLDL) or oxidized LDL (oxLDL). Statistical analysis was performed using a linear model and ANOVA. Using a cluster analysis approach, we identified a set of genes that were differentially expressed in response to oxLDL. These genes included genes involved in the polyol pathway, as well as genes involved in lipid metabolism. In conclusion, we have identified a set of genes that are induced by oxLDL, which could be potential targets for future investigation.

P462
Plasmid Activation of MMP-9 Regulates Peritoneal Macrophage Recruitment by Preventing Cellular Accumulation in the Mesothelium
Yangong Gong, Erika Hart, Jane Hoover-Plow; Cleveland Clinic, Cleveland, OH

Inflammation plays a critical role in the initiation of cardiovascular, respiratory and gastrointestinal diseases. Based on studies using deficient mice, plasminogen (Plg) has been shown to play a major role in inflammatory cell recruitment; however, the mechanism underlying its partici-
Immunodominant and Atheroprotective MDA-derived Epitopes

Karsten Hartvigsen, Univ of California, San Diego, La Jolla, CA; Christopher J Binder, Med Univ of Vienna, Vienna, Austria; Apais Rafia, Richard Harkewicz, Lotte F Hansen, Joseph L Witztum; Univ of California, San Diego, La Jolla, CA

Immunization of atherosclerosis-prone animals with malondialdehyde (MDA)-modified homologous LDL is atheroprotective. Previously, we showed that MDA-LDL immunization leads to a MDA-LDL-specific Th2 biased cellular and humoral response. The mechanisms as well as the chemotactic activity of the MDA-derived epitopes are largely unknown. MDA-modification of biomolecules results in diverse molecular structures, both ‘simple’ and ‘complex’, with and without inter- or intra-molecular cross-linking. Our goal is to define immunodominant epitopes in MDA-LDL that lead to atheroprotective immunity. Here, we immunized mice with candidate anti-inflammatory Th2 cytokines and selected optimal immunization doses and impact on atherosclerosis. Modified mouse serum albumin (MSA) was used as a homologous protein carrier of the MDA-derived adducts in order to test whether LDL-tissue or apoB are essential for atheroprotection. Male LDLR-/- mice were divided into 7 groups (n=15) from 6 mice immunized with Freund’s adjuvant (FA) and the 7th with PBS. Mice immunized with MDA-LDL, two preparations of MDA-modified MSA (with ‘simple’ or ‘complex’ MDA-derived adducts), and propanal-modified (PA)-MSA (carrier-adduct control) were immunized with Freunds adjuvant (FA) and the 7th with PBS. Mice immunized with MDA-LDL, and propanal-modified (PA)-MSA (carrier-adduct control) showed elevated IgM and IgG1 titers to their respective immunogens, suggesting Th2 biased responses. Antigen-specific immunoglobulin G (IgG), IgG2a, and IgG2b titers were to their respective immunogens, suggesting Th2 biased responses. Antigen-specific immunoglobulin G (IgG), IgG2a, and IgG2b titers were to their respective immunogens, suggesting Th2 biased responses. Antigen-specific immunoglobulin G (IgG), IgG2a, and IgG2b titers were to their respective immunogens, suggesting Th2 biased responses. Antigen-specific immunoglobulin G (IgG), IgG2a, and IgG2b titers were to their respective immunogens, suggesting Th2 biased responses.

Resistin Gene Variation Is Associated with Plasma Resistin Levels and Inflammatory Markers but Not Atherosclerosis or Metabolic Syndrome in Nonobese Italians

Atif N Gasim, Thomas Metkus, Mahlet Tadesse, Stephanie Rostine, Megan Wolfe, Daniel Rader, Muredach P Reilly; Univ of Pennsylvania, Philadelphia, PA

Introduction Resistin is an adipokine which has been linked to inflammation, insulin resistance and atherosclerosis in mice, however a similar role in humans has been debated. We have shown previously that plasma resistin levels are associated modestly with markers of inflammation and atherosclerosis but not with metabolic syndrome or insulin resistance. Aging has been shown to have a role in the dynamics of resistin promoter polymorphisms. We recently performed a genome-wide association study testing for association of resistin levels, TNF alpha and CRP, as well as lipids, NCEP-defined metabolic syndrome and atherosclerosis in humans. We have shown previously that plasma resistin levels are associated modestly with markers of inflammation and atherosclerosis but not with metabolic syndrome or insulin resistance. Aging has been shown to have a role in the dynamics of resistin promoter polymorphisms. We recently performed a genome-wide association study testing for association of resistin levels, TNF alpha and CRP, as well as lipids, NCEP-defined metabolic syndrome and atherosclerosis in humans.

Materials and Methods: A total of 840 non-obese (BMI < 30) healthy Italian men who had previously been genotyped for 500,000 SNPs were studied. Atherosclerosis was assessed by magnetic resonance angiography and coronary artery calcium scanning. CRP and resistin measurements were taken. Statistical analysis was performed using multivariate regression models. The -420C to T SNP was associated with resistin levels (p = 0.033) and CRP (p = 0.017) levels. The -420C to T SNP was associated with resistin levels (p = 0.033) and CRP (p = 0.017) levels. The -420C to T SNP was associated with resistin levels (p = 0.033) and CRP (p = 0.017) levels. The -420C to T SNP was associated with resistin levels (p = 0.033) and CRP (p = 0.017) levels. The -420C to T SNP was associated with resistin levels (p = 0.033) and CRP (p = 0.017) levels. The -420C to T SNP was associated with resistin levels (p = 0.033) and CRP (p = 0.017) levels.

Increased Presence of Macrophages Secreting Resistin in Epicardial Fat of Patients with Acute Coronary Syndrome

Giacomo Rutgersio, Silvia Langheim, San Raffaele Scientific Institute, Milan, Italy; Lorenza Dreas, Ospedali Riuniti, Trieste, Italy; Lorenzo Vescini, Francesco Maisano, Chiara Fogliani, San Raffaele Scientific Institute, Milanano, Italy; Bartolo Zingone, Ospedali Riuniti, Trieste, Italy; Mauro Arfelli, Elisabetta Fantoni, Ali Masert, San Raffaele Scientific Institute, Milan, Italy

Aim of the present study was to evaluate the expression of several adipocytokines in epicardial fat of patients undergoing CABG surgery for acute coronary syndrome (ACS, n=23) as compared to that of age- and BMI-matched patients with chronic stable angina (CSA, n=26). Patients undergoing cardiac surgery for valvular defects, but with angiographically normal coronary arteries served as reference group (n=20). The local expression and protein secretion (24h, cultured medium) of several adipocytokines from epicardial fat was measured by: 1) antibodies directed against Real-Time PCR (adiponectin, leptin, resistin, visfatin, IL-6, IL-8, IL-10, CRP, MCP-1, PAI-1, MIF), and multiplexed fluorescent immunosassay (adiponectin, resistin, leptin, IL-6, PAI-1, MCP-1), respectively. Immunohistochemical stainings of epicardial fat slides were also performed to show the presence of inflammatory cells (lymphocytes, macrophages, mast cells). The 24h medium from epicardial adipose tissue culture was also tested in experiments of endothelial...
Leukocyte Core 2 1–6–N-glycosaminlytransferase-I (C2GnT-NaC-T) Deletion Decreases Neointimal Formation After Arterial Injury in Apolipoprotein E-deficient Mice

Huan Wang, Rong Tang, Univ of Minnesota, Minneapolis, MN; Jamey D Marth, Univ of California, La Jolla, CA; Klaus Ley, Univ of Virginia, Charlottesville, VA; Yueling Hou; Univ of Minnesota, Minneapolis, MN

Vascular inflammation after arterial injury causes neointimal hyperplasia, which is one of major reasons for restenosis after percutaneous transluminal coronary angioplasty (PTCA). Neointima formation involves the interactions between endothelial cells and platelets, and their receptors are critically involved in the interactions of leukocytes and platelets with injured vessel wall. Leukocyte C2GnT-NaC-T is an enzyme modifying several molecules including P-selectin glycoprotein ligand-1 (PSGL-1), CD43, CD44 and CD45. It modulates PSGL-1 binding activity, leukocyte rolling, adhesion of activated T-cells and NaK-Lewis x-molecules, which is crucial for PSGL-1 optimal binding to P-select. In this study, we investigated whether and how leukocyte C2GnT-NaC-T influences neointimal formation using a mouse carotid artery wire injury model. We crossed C2GnT-NaC-T deficient mice with apoE deficient (apoE−/−) mice to generate double knock out mice. Their neointima formation and their littor control were fed a western diet for 2 weeks, followed by wire injury on their left carotid arteries. Three, 5 and 7 days after arterial injury, immunostaining was performed to examine accumulation of leukocytes and platelets to injured arteries and Evans blue staining was conducted to assess endothelial repair. Four weeks after arterial injury, injured arteries was collected and Movat’s pentachrome was used to stain cross-sections of arteries for overall features of neointimal hyperplasia. We found that C2GnT-NaC-T deletion in apoE−/− mice suppressed neutrophil and platelet adhesion to injured arteries and markedly benefited artery endothelialization, resulting in a significant decrease in the size of neointima after arterial injury. This study demonstrates that leukocyte C2GnT-NaC-T plays an important role in the formation of arterial neointima and suggests that inhibition of leukocyte C2GnT-NaC-T may be a novel approach in the treatment of restenosis after PTCA.

Overexpression of Glutathione Peroxidase 4 Reduces Atherosclerosis in Apolipoprotein E-deficient Mice

Lichun Zhou, Zhongmiao Guo, Hong Yang; Meharry Med College, Nashville, TN

Accumulation of oxidized lipids in the arterial wall is believed to give rise to atherosclerosis. Glutathione peroxidase 4 (GPx4) is a peroxide scavenger that removes oxidative modifications from lipids, e.g., free fatty acids, cholesterol and phospholipids. The primary goal of this study is to assess the effect of overexpressing or downexpressing GPx4 on atherosclerosis in apolipoprotein E-deficient (ApoE−/−) mice. Our data demonstrated that the atherosclerotic lesions in the aortic tree and the aortic sinus of the ApoE−/− mice overexpressing GPx4 (hGPx4Tg Apoe−/−) were significantly smaller than those of the ApoE−/− control mice. Almost all of the ApoE−/− control mice at 4–5 months of age developed both early stages of atherosclerotic lesions (e.g., foam cells and free lipids) and advanced lesions (e.g., fibrous caps and calcific areas) in the aortic sinus. In contrast, only about two thirds of the hGPx4Tg Apoe−/− developed acellular areas in the atherosclerotic lesions. We also observed that overexpression of GPx4 reduced the aorta F2-isoprostane levels in Apoe−/− mice, attenuated 7-ketocholesterol (7-Kc)-induced apoptosis and lysophosphatidylcholine-induced necrotic death to macrophages. Our data suggest that overexpression of GPx4 inhibits atherogenesis, and that reducing lipid oxidation and suppressing the sensitivity of vascular cells

CD4+ T-Helper Cell Distributions and Associations with Atherosclerosis: Results from the Multi-Ethnic Study of Atherosclerosis (MESA)

Russell P Tracy, Nancy Swords Jenny, Margaret F Doyle, Sally A Huber; Univ of Vermont College of Medicine, Colchester, VT; Bruce M Psaty, Richard A Kronmal; Univ of Washington, Seattle, WA

Introduction: CD4+ T Helper (Th) cells are key effectors of adaptive immunity and important in atherosclerosis development in mice. Less is known about their role in human atherosclerosis. Methods: We examined this association in 524 white, black, Hispanic and Chinese MESA participants. Mean age was 59 years (range 44–84), 56% were women. Fresh blood samples were shipped overnight to the Central Laboratory. CD4+ cells in peripheral blood mononuclear cell preparations were stimulated with ionomycin/phorbol myristate acetate. Th1 cells were defined by flow cytometry as CD4+ IFNγ+ and Th2 cells were defined as CD4+ IL-4+ and/or IL-10+. Total CD4+ cells were expressed as percentage of lymphocytes. Results: The mean (standard deviation) for %CD4+ Th1 and Th2 cells were 43.5% (13.4%), 14.5% (7.6%) and 7.0% (0.8%), respectively. Neither %CD4+ nor %Th1 cells were significantly correlated with inflammation markers interleukin-6 and C-reactive protein. In age, sex and ethnicity adjusted regression models, %CD4+ Th1 cells were higher with older age (1.4%/10yrs), female sex (4%), and white ethnicity (2.6–9.6% higher than others). However, %Th1 cells were lower with age (1%/10yrs) and female sex (1.2%). %CD4+ cells were associated with cytomegalovirus (CMV) serology markers (p < 0.05). Th1 cells were associated with markers for CMV and hepatitis A (both p < 0.05). In stepwise regressions of coronary calcification (modeled as In-transformed Agatston score in those with a positive score, n = 237) with age, sex, ethnicity, cardiovascular disease risk factors and T cell indices, %Th1 remained significantly positively associated with degree of coronary calcification (p < 0.001). Conclusions: In a multi-ethnic population of men and women, a Th cell distribution and associations with atherosclerosis:

Natural Killer T-Cells Influence Both Atherosclerosis and Plasma Lipid Levels in Low-Density Lipoprotein Receptor–Deficient Mice

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Natural killer T (NKT) cells are a subset of T-lymphocytes that respond to lipid antigens presented in the context of CD1 molecules and have been shown to be proatherogenic in a number of mouse models through either exogenous stimulation or genetic deficiency. To present NKT cells in the context of CD1 molecules and have been shown to be proatherogenic in a murine model of atherosclerosis, the low density lipoprotein receptor (LDLR) deficient mouse was used. Wild type C57BL6 mice and ApoE−/− E−/− knock-out mice at the age of 7 weeks were subjected to quantification of monocyte adhesion to the thoracic aorta by new En face method for optimal observation of endothelial surface (NEMOes) (Azuza et al 2006 ATVB) using Mac2 as a marker of activated macrophage and conventional histological evaluation. Results: In wild type mice, NEMOes occasionally identified mac-2 positive cells on endothelial cell surface at the atherosclerosis prone sites, such as branching of intercostal artery or intimal thickening area. The macrophages were with many protruberances and were mostly on endothelial surface. Surprisingly, alpha-smooth muscle actin positive particles whose diameters are approximately 1 to 3 micrometers were also found at the atherosclerosis prone sites. Most of these particles were located just above the fenestrated intra elastica lamina. A few particles were found on the endothelial cell surface. Surrounding the particles on the endothelial surface, we frequently found activated macrophages. In addition, the smaller alpha-smooth muscle actin positive particles were observed around the macrophages. These smaller particles seem to be the debris of large particles. These features are reminiscence of interaction between these particles and macrophages. In apo E knock-out mice, we found profoundly more adherent macrophages. In addition, we found more particles around the macrophages. Conclusion: Our data suggest the pathological role of alpha-smooth muscle actin positive particles. The interaction between these particles and macrophages seems to be involved in the lesional initiation of the progression of atherosclerosis.

Overexpression of Glutathione Peroxidase 4 Reduces Atherosclerosis in Apolipoprotein E-deficient Mice

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Accumulation of oxidized lipids in the arterial wall is believed to give rise to atherosclerosis. Glutathione peroxidase 4 (GPx4) is a peroxide scavenger that removes oxidative modifications from lipids, e.g., free fatty acids, cholesterol and phospholipids. The primary goal of this study is to assess the effect of overexpressing or downexpressing GPx4 on atherosclerosis in apolipoprotein E-deficient (ApoE−/−) mice. Our data demonstrated that the atherosclerotic lesions in the aortic tree and the aortic sinus of the ApoE−/− mice overexpressing GPx4 (hGPx4Tg Apoe−/−) were significantly smaller than those of the ApoE−/− control mice. Almost all of the ApoE−/− control mice at 4–5 months of age developed both early stages of atherosclerotic lesions (e.g., foam cells and free lipids) and advanced lesions (e.g., fibrous caps and calcific areas) in the aortic sinus. In contrast, only about two thirds of the hGPx4Tg Apoe−/− developed acellular areas in the atherosclerotic lesions. We also observed that overexpression of GPx4 reduced the aorta F2-isoprostane levels in Apoe−/− mice, attenuated 7-ketocholesterol (7-Kc)-induced apoptosis and lysophosphatidylcholine-induced necrotic death to macrophages.
to oxidized lipids are the mechanisms by which Gp4A inhibits atherosclerosis. Unexpectedly, heterogeneous mutation to Gp4X did not increase atherothrombotic lesions and oxidized lipids in ApoE−/− mice.

Mac-1 Mediates CD40L-induced Atherosclerosis

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To probe different regions of this 343 amino acid protein, 4 single Trp apoA-V variants were generated. The variant with a Trp at position 325, distal to the tetra-proline sequence at residues 290–309, resulted in Mac-1-dependent adhesion and migration of inflammatory cells as well as myeloperoxidase release in vitro. Furthermore, mice deficient in CD40L show significantly reduced thyloglobulin-elicted accumulation of inflammatory cells in the peritoneal cavity compared to mice deficient in CD40L and wild-type controls. Inhibition of Mac-1 in LDLR−/− mice attenuated lesion development and delayed the accumulation. These observations identify the interaction of CD40L and Mac-1 as an alternative pathway for CD40L-mediated inflammation. This novel mechanism explains understanding of inflammatory signaling during atherosclerosis and has implications regarding novel anti-inflammatory therapies.

Structure-Function of Apolipoprotein A-I-Milano and Related Variants


Strong evidence supports a role for CD40L as marker and mediator of inflammatory diseases such as atherosclerosis. Despite extensive characterization of CD40, the classical receptor for CD40L, in immune defense, its role in inflammatory diseases remains uncertain. This study aimed to characterize the contribution of CD40L signaling to atherosclerosis. Surprisingly, mice deficient in both CD40L and the low-density lipoprotein-receptor (LDLR) do not develop smaller lesions in the aortic arch, root, and thoraco-abdominal aorta compared to LDLR−/− mice. However, lesions in these two groups of mice also had similar composition. Based on previous reports demonstrating functional binding of Gp4B to CD40L in thrombosis, we investigated other integrins as potential alternative receptors for CD40L. We demonstrated that CD40L interacts with the integrin Mac-1 on human monocyte/macrophages (using flow cytometry, radio binding assays, and immunoprecipitation), resulting in Mac-1-dependent adhesion and migration of inflammatory cells as well as myeloperoxidase release in vitro. Furthermore, mice deficient in CD40L show significantly reduced thyloglobulin-elicted accumulation of inflammatory cells in the peritoneal cavity compared to mice deficient in CD40L and wild-type controls. Inhibition of Mac-1 in LDLR−/− mice attenuated lesion development and delayed the accumulation. These observations identify the interaction of CD40L and Mac-1 as an alternative pathway for CD40L-mediated inflammation. This novel mechanism explains understanding of inflammatory signaling during atherosclerosis and has implications regarding novel anti-inflammatory therapies.

A-C-Terminal Truncated Apolipoprotein A-V Displays Unique Structural and Lipid-Binding Properties

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The interphalangeal sequence between Helices 7 and 8 of Human Apolipoprotein A-I Influences High Density Lipoprotein Subclass Association and Generation

Ronald Camenisch, Andrew Dzianio, Catherine A Reardon, The Univ of Chicago, Chicago, IL; Jiajun Wang, Wayne State Univ, Detroit, MI; Godfrey S Getz; The Univ of Chicago, Chicago, IL

ApoE−/− mice expressing R173S apoA-I or apoA-IMilano had reduced apoA-I levels and HDL cholesterol levels compared to those expressing WT apoA-I. Mice expressing WT apoA-I had plasma HDL cholesterol concentrations of 80 ± 3.9 mg/dl. In summary, the altered structure and function of apoA-IMilano is due both because of a superior ability of A-IM HDL to protect the endothelium. HDL-C levels, A-IM carriers do not display features of endothelial dysfunction, such as the plasma concentration of antiatherogenic HDL but do not present with preclinical atherosclerosis and premature CHD. Aim of the present study was to investigate endothelial function in A-IM carriers, since low HDL-C levels have been associated with features of endothelial dysfunction. Plasma concentrations of soluble cell adhesion molecules (sCAMs) and forearm arterial compliance (FAC) during reactive hyperemia were evaluated in 21 A-IM carriers, 21 healthy subjects with low HDL-C, and 42 controls. Low HDL-C subjects had significantly higher plasma sCAM levels than controls (sVCAM-1: 656.3 ± 49.3 vs 502.6 ± 25.7 mg/dl, p<0.03); non-HDL cholesterol (37.8 ± 9.7 mg/dl, p<0.03) beyond the reductions observed by the expression of Trp-325 apoA-V alone. A specifically novel antithrombotic pathway for CD40L in thrombosis, we investigated other integrins as potential alternative receptors for CD40L. We demonstrated that CD40L interacts with the integrin Mac-1 on human monocyte/macrophages (using flow cytometry, radio binding assays, and immunoprecipitation), resulting in Mac-1-dependent adhesion and migration of inflammatory cells as well as myeloperoxidase release in vitro. Furthermore, mice deficient in CD40L show significantly reduced thyloglobulin-elicted accumulation of inflammatory cells in the peritoneal cavity compared to mice deficient in CD40L and wild-type controls. Inhibition of Mac-1 in LDLR−/− mice attenuated lesion development and delayed the accumulation. These observations identify the interaction of CD40L and Mac-1 as an alternative pathway for CD40L-mediated inflammation. This novel mechanism explains understanding of inflammatory signaling during atherosclerosis and has implications regarding novel anti-inflammatory therapies.

Normal Endothelial Function in Carriers of the Apolipoprotein A-I-Milano Mutation Despite Low HDL-cholesterol Levels

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Carriers of the apolipoprotein A-I-Milano (apoA-IM) mutant show severe reductions in the plasma concentration of antiatherogenic HDL but do not present with proclivity atherosclerosis and premature CHD. Aim of the present study was to investigate endothelial function in A-IM carriers, since low HDL-C levels have been associated with features of endothelial dysfunction. Plasma concentrations of soluble cell adhesion molecules (sCAMs) and forearm arterial compliance (FAC) during reactive hyperemia were evaluated in 21 A-IM carriers, 21 healthy subjects with low HDL-C, and 42 controls. Low HDL-C subjects had significantly higher plasma sCAM levels than controls (sVCAM-1: 656.3 ± 49.3 vs 502.6 ± 25.7 mg/dl, p<0.03); non-HDL cholesterol (37.8 ± 9.7 mg/dl, p<0.03) beyond the reductions observed by the expression of Trp-325 apoA-V alone. A specifically novel antithrombotic pathway for CD40L in thrombosis, we investigated other integrins as potential alternative receptors for CD40L. We demonstrated that CD40L interacts with the integrin Mac-1 on human monocyte/macrophages (using flow cytometry, radio binding assays, and immunoprecipitation), resulting in Mac-1-dependent adhesion and migration of inflammatory cells as well as myeloperoxidase release in vitro. Furthermore, mice deficient in CD40L show significantly reduced thyloglobulin-elicted accumulation of inflammatory cells in the peritoneal cavity compared to mice deficient in CD40L and wild-type controls. Inhibition of Mac-1 in LDLR−/− mice attenuated lesion development and delayed the accumulation. These observations identify the interaction of CD40L and Mac-1 as an alternative pathway for CD40L-mediated inflammation. This novel mechanism explains understanding of inflammatory signaling during atherosclerosis and has implications regarding novel anti-inflammatory therapies.

The interphalangeal sequence between Helices 7 and 8 of Human Apolipoprotein A-I Influences High Density Lipoprotein Subclass Association and Generation

Ronald Camenisch, Andrew Dzianio, Catherine A Reardon, The Univ of Chicago, Chicago, IL; Jiajun Wang, Wayne State Univ, Detroit, MI; Godfrey S Getz; The Univ of Chicago, Chicago, IL

ApoE−/− mice expressing R173S apoA-I or apoA-IMilano had reduced apoA-I levels and HDL cholesterol levels compared to mice expressing WT apoA-I. However, mice expressing either R173S apoA-I or apoA-IMilano had reduced apoA-I levels and HDL cholesterol levels compared to WT apoA-I. Mice expressing WT apoA-I had plasma HDL cholesterol concentrations of 80 ± 3.9 mg/dl while mice expressing R173S apoA-I had only 31 ± 2 mg/dl. Interestingly, mice expressing R173S apoA-I displayed an intermediate phenotype with plasma HDL cholesterol levels of 48 ± 5 mg/dl. In summary, the altered structure and function of apoA-IMilano is due both because of a superior ability of A-IM HDL to protect the endothelium. HDL-C levels, A-IM carriers do not display features of endothelial dysfunction, such as the plasma concentration of antiatherogenic HDL but do not present with preclinical atherosclerosis and premature CHD. Aim of the present study was to investigate endothelial function in A-IM carriers, since low HDL-C levels have been associated with features of endothelial dysfunction. Plasma concentrations of soluble cell adhesion molecules (sCAMs) and forearm arterial compliance (FAC) during reactive hyperemia were evaluated in 21 A-IM carriers, 21 healthy subjects with low HDL-C, and 42 controls. Low HDL-C subjects had significantly higher plasma sCAM levels than controls (sVCAM-1: 656.3 ± 49.3 vs 502.6 ± 25.7 mg/dl, p<0.03); non-HDL cholesterol (37.8 ± 9.7 mg/dl, p<0.03) beyond the reductions observed by the expression of Trp-325 apoA-V alone. A specifically novel antithrombotic pathway for CD40L in thrombosis, we investigated other integrins as potential alternative receptors for CD40L. We demonstrated that CD40L interacts with the integrin Mac-1 on human monocyte/macrophages (using flow cytometry, radio binding assays, and immunoprecipitation), resulting in Mac-1-dependent adhesion and migration of inflammatory cells as well as myeloperoxidase release in vitro. Furthermore, mice deficient in CD40L show significantly reduced thyloglobulin-elicted accumulation of inflammatory cells in the peritoneal cavity compared to mice deficient in CD40L and wild-type controls. Inhibition of Mac-1 in LDLR−/− mice attenuated lesion development and delayed the accumulation. These observations identify the interaction of CD40L and Mac-1 as an alternative pathway for CD40L-mediated inflammation. This novel mechanism explains understanding of inflammatory signaling during atherosclerosis and has implications regarding novel anti-inflammatory therapies.

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Humans exhibit a heterogeneous HDL profile (i.e., HDL1, HDL2, and HDL3) in contrast to other species (e.g. mouse) that display a monophosphatic profile. HDL is thought to be more atheroprotective
than HDL3. Human apoA-I is a major determinant of the biphasic distribution of HDL. ApoA-I is made up of 10 repeating amphipathic α-helices (11 or 22 a.a.) interrupted in most cases by proline residues, except between helices 7 and 8. We generated apoA-I mutants in which the interhelical sequence (IHS) between helices 7/8 (7 residues) was substituted with each of the remaining IHS found in huA-I. The mutant and wild-type (wt) apoA-I proteins did not significantly differ in their ability to support post-translational functional properties examined. We used reverse commutant huA-I and HDL subclasses, we observed that wt-huA-I has a near equal affinity for HDL3 (1.42 μM) and HDL1 (1.63 μM). In contrast, huA-I mutants with IHS3/4 or IHS9/10 replacing IHS7/8 preferentially associated with HDL2, and displayed higher Kd association values for HDL (0.49 μM and 0.27 μM respectively) versus HDL3 (1.22 μM and 4.6 μM respectively). The huA-I mutant in which IHS4/5 replaced IHS7/8 preferentially associated with HDL2 and displayed a higher Kd association constant for HDL2 (0.63 μM) versus HDL3 (5.4 μM). To examine if the mutant huA-I proteins generate nascent HDL particles of different densities, we labeled individual rat hepatocytes expressing wt- and huA-I proteins with a biotin-expressing probe. We were able to express comparable levels of huA-I protein and species-specific huA-I antibodies, we found that wt-huA-I forms two major nascent HDL particles with peaks at 1.0901 g/mL and 1.1557 g/mL. In contrast, huA-I (IHS7/8IHS3/4) generates a single nascent HDL between 1.1037–1.1191 g/mL. The peak of the nascent HDL formed by huA-I (IHS7/3IHS4/5) mutant is 1.3692 g/mL, but also appears to have a shoulder at a lower density. The distribution of the endogenous rat apoA-I on the nascent HDL followed that of the huA-I protein in each case. In conclusion, the IHS placed between helices 7 and 6 in huA-I can influence affinity for HDL subclasses and can modify the type of HDL profile secreted from stably transfected rat hepatoma cells. This focuses new attention on the interhelical sequences in controlling the properties of apoA-I.

Effect of Oxidation on Rate of Apolipoprotein A-I–HDL Exchange

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In the atherosclerotic plaque, reverse cholesterol transport begins with ABCA1 transporter-mediated transfer of cholesterol from cholesterol-laden macrophages to lipid-poor apolipoprotein A-I apoA-I. Lipid-associated apoA-I is a very poor substrate for cholesterol uptake from ABCA1; interestingly, the vast majority of circulating apoA-I is lipid associated on spherical HDL. Curtiss et al. have proposed that cholesterol efflux to HDL is facilitated primarily by the release of lipid-poor apoA-I from spherical HDL and as a result may be a key event in the mobilization of cholesterol from the atherosclerotic plaque. Oxidized HDL (specifically apoA-I) is observable in patients with established atherosclerosis and in vitro severely impairs cholesterol efflux by ABCA1. We hypothesized that oxidation of apoA-I impairs its ability to exchange between lipid-free and lipid-bound states, which leads to depletion of lipid-poor apoA-I as substrate for ABCA1. The rate of apoA-I - HDL exchange was measured, using a fluorescent apoA-I construct, which exhibited fluorescence resonance energy transfer (FRET) only in the lipid-poor state and was used to estimate the rates of exchange ranging from 7 to 8 nm HDL particles. Rates of exchange were monitored by addition of excess lipid free apoA-I, which displaces fluorescent apoA-I off HDL composed of lipid and fluorescent apoA-I construct. The rate of AEDANS fluorescence intensity increase is a measure of the apoA-I - HDL exchange. We found that the bound fluorescent-apoA-I exchanged into the lipid-poor state. The rates of 7.8 nm and 9.6 nm HDL exchange require 50 seconds and 18 minutes to achieve 50% transfer, respectively, indicating that apoA-I - HDL exchange rates on 9.6 nm HDL are at least 18-fold slower than on 7.8 nm HDL. These results confirm that our assay is sensitive enough to measure biologically relevant differences in HDL exchange rate properties. When myeloperoxidase oxidized apoA-I was used to displace the fluorescent apoA-I variant from HDL, we found that oxidized apoA-I - HDL exchange rates were reduced. While this result requires further validation, it is an indication that impairment of apoA-I exchangeability by oxidation may be a key molecular mechanism for oxidaition in atherogenesis.

Electronegative LDL Circulating in Smokers Impairs Endothelial Progenitor Cell Differentiation by Inhibiting Akt Phosphorylation via the LOX-1

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Patients with type 2 diabetes have high levels of triglyceride-rich lipoproteins (TRL), including apolipoprotein (apo) B-48-containing TRL of intestinal origin but the mechanism leading to overaccumulation of these lipoproteins remains to be fully elucidated. Therefore, the objective of this study was to examine the in vivo kinetics of TRL apoB-48 in type 2 diabetic subjects (N=13) and in controls (N=11) using a primed-constant infusion of [5,5,5-2H3]-triolein for 12 hours in the fed state. Diabetic subjects had significantly higher fasting glucose (7.7 ± 2.0 g/L, P = 0.03), higher plasma TG (4.6 ± 1.73 g/L, P = 0.001) and lower HDL-C levels (0.58 ± 0.16 vs. 1.19 ± 0.24 mmol/L, P = 0.0009) than controls. As compared with controls, diabetic subjects had a higher cholesterol and ApoB-48 postprandial peak size (12.6 ± 39.2 ps, P = 0.001), a lower LDL cholesterol at 0.4 ± 0.8 mmol/L (P < 0.0001), and lower HDL-C levels (0.27 ± 0.18 mmol/L, P = 0.001) as compared with controls. Reduced TRL apoB-48 fractional catabolic rate (5.8 ± 1.6 vs. 7.8 ± 2.0 pools/day, P = 0.01) and elevated TRL apoB-48 production rate (PR) (11.0 ± 5.2 vs. 3.0 ± 1.9 mg/kg/d, P = 0.04) further. Moreover, multiple linear regression analyses revealed that the diabetic/control status was an independent predictor of TRL apoB-48 fractional catabolic rate (p = 0.01) and production rate (p = 0.03). These results suggest that overaccumulation of apoB-48-containing TRL seen in patients with type 2 diabetes is not due to decreased catabolism of TRL apoB-48 but also to increased production rate of these lipoproteins.

Electroengative LDL Circulating in Smokers Impairs Endothelial Progenitor Cell Differentiation by Inhibiting Akt Phosphorylation via the LOX-1 Receptor

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Endothelial progenitor cells (EPCs) play an important role in endothelial regeneration and vasculogenesis. Chronic smoking is associated with reduced EPCs, but the mechanism was unclear. We previously examined cigarette smoke and found increased LDL oxidation in cigarette smokers. We hypothesized that pro-oxidant bioactivity of circulating electronegative low-density lipoprotein (LDL) using anion-exchange chromatography, we purified the LDL of chronic smokers into 5 subfractions, L1-L5, where L5 is the most electronegative. L5 was not present in matched, nonsmoking subjects. Under normal conditions, human circulating monocytes expressed CD31 and KDR on day 3 and differentiated into mature EPCs by day 21 in culture. L5 inhibited CD31 and KDR expression and EPC differentiation, whereas L1-L4 had no effect. L5 also inhibited telomerase activity to accelerate EPC senescence, as demonstrated by enhanced cystosin acid β-galactosidase activity. These effects inversely correlated with reduced Akt phosphorylation. Transfection of EPCs at day 3 with dominant-negative Akt constructs (Akt-DN) mimicked the effects of exposure to L5 by inhibiting CD31 and KDR expression, stalling EPC differentiation, and promoting early senescence. In contrast, transfection with constitutively-active Akt (Akt-CA) reduced the EPCs resistant to exposure, allowing for normal maturation. Moreover, L5 upregulated the lectin-like oxidized low-density lipoprotein receptor (LOX-1) expression in differentiated EPCs with JTX20, a LOX-1 neutralizing antibody, prevented L5-mediated inhibition of EPC differentiation. Furthermore, internalization of DiI labeled LDL (DiI-L5) at the plasma membrane was blocked by JTX20. These findings suggest that cigarette smoke is associated with the formation of L5, which inhibits EPC differentiation by impairing Akt phosphorylation via the LOX-1 receptor.

Intensive Statin Therapy from Vulnerable Plaque to Prevention of Progression of CAD in Patients with High Levels of Lp(a)

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Many cardiovascular risk factors for CAD show familiar clustering, in particular we have studied a family from Jordany where 9 people showed a severe increase of Lp(a) >40 mg/dl with an high recurrence of multivessel CAD, stroke, and lower limbs vascular disease; all of them showed a genetic variant of gene (6262.2-7), consisting on a polymorphism C/T, presence of allele 8, and presence of small isoforms, which are clearly linked to a increased synthesis of Lp(a). It has been demonstrated that Lp(a) levels greater than 30 mg/dl are responsible for about 15% of CAD, for this reason it seems to constitute an independent risk factor of athero-thrombotic disease. Notoriously there are not drugs that can lower the levels of Lp(a), except rapamycin, high dosed probucol, and aspiragene, for this reason we have used high doses of rosuvastatin (40mg/die). Statins, other than inhibiting HMGCoA reductase, present a pleiotropic effect. In fact, they inhibit the synthesis of isoprenoid units (farnesol, and geraniol) with an anti-proliferative effect. they also inhibit MMP-9, they inhibit TF, increase e-NOS synthesis, and they have an anti-oxidant effect. Statins are also characterised by an anti-inflammatory property, as they reduce level of VCAM-1, ICAM-1, E-selectin, IL-6, PCR. Among all our patients (aged between 9 and 54 years) two showed an anamnesis of recurrent angina pectoris and two artery bypass operations. Among them, with the reduction of plasma concentration of Lp(a) of 24%, with a significant decrease of PCR (>35%), MMP-9, MMP-12 and elastase; all these effects appeared to be independent to the LDL-cholesterol lowering effects (38 %). These findings appear to be very suggestive for the treatment of these very problematic patients and for the control of coronary stenosis progression.
Antisense Inhibition of Apolipoprotein B-100 Significantly Reduces LDL Cholesterol and Glucose Levels in Ob/Ob Mice

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In previous studies, we demonstrated that intraperitoneal (i.p.) administration of a mouse-specific apolipoprotein B-100 (apoB-100) antisense oligonucleotide (ASO), ISIS 147764, produced dose- and time-dependent reductions in hepatic apoB mRNA and protein and serum apoB-100 levels in hypertriglyceridemic mice. The in vivo pharmacological effects have been confirmed in multiple animal models and most importantly, in humans during Phase 1 and 2 clinical trials. As a large number of individuals with diabetes also suffer from dyslipidemia and coronary artery disease, we administered ISIS 147764 to ob/ob mice on a high-fat/cholesterol diet in order to determine the effects of inhibiting apoB-100 in insulin-resistant mice. Treatment of these animals with 50 mg/kg/wk of ISIS 147764 for 6 weeks resulted in a 65% reduction in hepatic apoB mRNA with a commensurate reduction in total cholesterol (34%), VLDL-C (61%), LDL-C (65%) and serum triglycerides (21%). Serum glucose levels were also reduced by 33% when compared with controls. As observed in other murine hyperlipidemic models, reductions in serum apoB-100 did not cause hepatic steatosis as determined by Oil Red O staining of liver and quantitation of liver triglyceride levels. Furthermore, ASO administration in these mice was well tolerated. These and other data from this study support the clinical feasibility of inhibiting apoB-100 in various dyslipidemic states.

The N-Terminal 50 Amino Acid Residues of the $\beta$ Domain of Apolipoprotein B Induce Instability in Lipoprotein Particle Assembly

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ApoB-100, essentially the only protein component of the atherogenic LDL, has a pentameric structure, $\text{N}\text{H}_2$-$\text{H}2$-$\text{H}3$-$\text{H}4$-$\text{H}5$. The $\beta$ domain contains multiple amphipathic $\beta$ strands and the $\alpha$ domains contain multiple amphipathic $\alpha$ helices. The $\beta_1$ domain, like lampry lipovitellin (L), is a globular composite of $\alpha$-helices and $\beta$-sheets. We previously proposed that the apoB particle assembly is initiated when the $\beta_1$ domain, comprising of the first 1000 amino acid residues of apoB (designated apoB1000), folds into a $\text{L}1$-like lipidic pocket to form the apo $\text{L}1$ lipid pocket. We demonstrated that in stable transformants of MCA-RH7777 cells, apoB1000 is secreted as a stable monodisperse phospholipid-rich particle. We also showed that apoB1200, containing 200 residues of the $\beta_1$ domain, of apoB is secreted predominantly as a lipid-poor particle with only a fraction of the protein as a relatively lipid-rich particle. To map the effect of each domain of amphipathic $\beta$ strands within the N-terminal region of the $\beta_1$ domain, on the relative degrees of secretion of large versus small particles, we made sequential truncations of region between apoB1200 and apoB1000 to produce apoB1050, apoB1100, and apoB1150. Characterization of the secreted particles by metabolic labeling of stable transformants of MCA-RH7777 cells with [$\text{H}3$]glycerol and their isolation by non-denaturing gradient gel electrophoresis, demonstrated that the presence of only 50 amino acid residues of the N-terminal region of the $\beta_1$ domain causes instability in the particle. Thus, in contrast to apoB1000, apoB1050 was secreted in two forms, a large lipidated particle and a small lipid-poor particle. Inclusion of residues 1050 to 1100 caused further destabilization in the particle. ApoB1100 appeared to form at least four particles, suggesting that the domain between residues 1050 and 1100 might be more destabilizing than the previous 50 residues. ApoB1150 formed particles that were similar to those formed by apoB1050, suggesting that the domain between residues 1100 and 1150 might partially restore particle stability. In conclusion, our results suggest that not all the sequences in the N-terminal 200 residues of the $\beta_1$ domain are equal in their effects on particle stability.

ABC1A and SR-BI Are Significant Contributors to in Vivo Reverse Cholesterol Transport from Macrophages

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BACKGROUND: Cholesterol transporters, ABC1A, ABCG1 and SR-BI, are key mediators of cholesterol efflux to apoA-I and HDL, which represents the first step of reverse cholesterol transport (RCT) in vivo. However, the individual contributions of each transporter to in vivo RCT, defined here as transport from macrophages to liver and further into bile have not been thoroughly measured. Here we first measured the in vivo RCT from cholesterol labeled macrophages with either ABC1A or SR-BI deficiency. METHODS AND RESULTS: Bone marrow derived macrophages from ABC1A(-/-) or SR-BI(-/-) or control mice were labeled with 3H-cholesterol-ACLDL or 3H-cholesterol-LDL and injected into control mice. After injection, return of 3H-cholesterol from labeled acLDL(-/-) or -SR-BI(-/-) macrophages to serum, liver, bile and feces, was measured. ABC1A deficiency in macrophages reduced cholesterol return from macrophages by 65% and -SR-BI by 55% as compared with control mice, by up to 50% from cholesterol-LDL, dependent on the readout (plasma, liver or bile radioactivity). SR-BI deficiency only reduced cholesterol return from LDL labeled macrophages, but not from acLDL labeled macrophages, consistent with the role of SR-BI in efflux to HDL and not to apoA-I. CONCLUSION: These results indicate that both ABC1A and SR-BI play significant roles in efflux and in vivo RCT, consistent with their protective functions against development of atherosclerotic lesions.

Identification and Molecular Characterization of New PCSK9 Missense Mutations Associated with Familial Hypercholesterolemia

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Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) is a bona fide inhibitor of the LDL-receptor. In humans, PCSK9 gain of function mutations are associated with FH, whereas mutations inactivating PCSK9 are associated with reduced plasma LDL and cardiovascular events. Characterization of the naturally occurring mutations reported to date has provided some insights into PCSK9 mechanisms of action but it has not been possible to distinguish the phenotypic effect of some gain of function from some loss of function mutations based on their autophagic cleavage and secretion pattern. In the present study, we analysed the PCSK9 exons and intronic junctions of FH patients found to be non LDL-receptor or apolipoprotein B100 mutation carriers. The previously reported S127R French mutation was found in a South-African family, whereas new heterozygous missense mutations D129G and A168E were found in two families from New Zealand. Except for the A168E, these mutations modify a highly conserved residue. Segregation with the FH phenotype was also incomplete in the A168E family. PCSK9 overexpression studies in HuH7 hepatoma cells shows that both S127R and D129G missense PCSK9 mutants have 75% reduced autocatalytic activity compared to wild type, whereas the A168E mutant is processed normally. The S127R and D129G mutants were compared with those overexpressing wild-type PCSK9. Overexpression of the A168E mutant did not cause hepatic steatosis as determined by Oil Red O staining of livers and quantitation of liver triglyceride levels. Furthermore, A50G mutation in these mice was well tolerated. These and other data from this study support the clinical feasibility of inhibiting PCSK9 in various dyslipidemic states.
Diurnal Transcriptional Regulation of Microsomal Triglyceride Transfer Protein and Plasma Lipid Levels

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Several cardiovascular diseases such as atherosclerosis and coronary heart disease exhibit circadian variations. High plasma lipid levels are risk factors for these diseases and plasma lipids also show diurnal variations. Our aim was to identify molecular mechanisms controlling diurnal variations in plasma lipid levels in rats and mice maintained in a 12-h photoperiod with free access to chow diet. Plasma triglyceride and cholesterol levels were high in the dark than in the light phase in these animals. These variations were mainly due to changes in apoB-48-proteins, as HDL levels did not show circadian rhythm. Intestinal lipoprotein production studies revealed that the amount of [3H]triolein or [3H]cholesterol was high at 24:00 h and at 12:00 h. These observations were in line with recent studies showing that intestinal triglyceride transfer, produced in the liver, is high in the midnight than at midday. To examine tissue specificity, we also measured MTP in the intestinal mucosa at 12:00 and 24:00 h. The MTP mRNA levels exhibited diurnal variations. These studies show that the intestinal and liver MTP expression undergoes diurnal regulation at the translational level and suggest that changes in MTP contribute to diurnal variations in plasma lipid levels.

Acquisition of Triacylglycerol Transfer Activity by Microsomal Triglyceride Transfer Protein During Evolution

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Microsomal triglyceride (TG) transfer protein (MTP) is essential for the assembly of neutral lipid rich apolipoprotein B (apoB)-lipoproteins. Previously we reported that the Drosophila MTP does not transfer TG but transfers phospholipids. In contrast, human MTP transfers both lipids. To explore the acquisition of TG transfer activity by MTP during evolution, we obtained MTP from species sequenced from birds, amphibians and insects that were homologous to human MTP. Sequence comparison revealed a MTP specific sequence. Using this sequence we identified homologous proteins in nematodes. Based on phylogenetic analysis, we divided these proteins into four groups (mammals, fish, insects, and nematodes). Structural analysis demonstrated that all MTP possess similar secondary and tertiary structures. In addition to their structural similarities, MTP homologous were found to share several biochemical properties. Expression of candidate proteins from each group as FLAG chimera showed that all proteins were associated with protein disulfide isomerase in the endoplasmic reticulum as well as in the Golgi, and supported assembly and secretion of apoB-lipoproteins. In vitro lipid transfer assays revealed that invertebrate MTP (nematode and insect) unable to transfer TG in vitro. However these studies demonstrated that TG transfer activity first appeared in fish, matured during the evolution of amphibians and birds, and was conserved in mammals. We concluded that TG transfer activity was acquired during a transition from invertebrates to vertebrates coincident with the use of apoB-lipoproteins capable of carrying large amounts of neutral lipids. We speculate that this acquisition might have provided a significant advantage to the evolution of larger and more complex organisms.
h.mg/dl) were determined for each of the four postprandial studies. Fasting lipids were similar after 5 weeks of a DAG or TAG diet. We found no statistically significant acute or chronic effects of DAG in our two primary comparisons. Thus, neither DAG PPTG on chronic TAG (AUC: 503 ± 439) nor TAG PPTG on chronic DAG (AUC: 517 ± 638) was significantly different from TAG PPTG on chronic TAG (AUC: 565 ± 362). In a subgroup-analysis, subjects with fasting TG levels lower than 200mg/dl had significantly smaller AUCs during DAG PPTG compared with TAG PPTG on a TAG background diet (p = 0.02). However, no significant difference in AUCs was found in the subjects with fasting TG greater than 200mg/dl. Conclusion. We conclude that a DAG enriched diet had no acute or chronic effects on PPTG in the overall group of insulin resistant subjects, but we did observe an acute PPTG-lowering effect in subjects with fasting TG less than 200mg/dl. Further studies are necessary to understand the absence of DAG effects on lipid metabolism in individuals with high fasting TG.

Measurement of Cholesterol Efflux and Global Reverse Cholesterol Transport Rates In Vivo with Stable Isotopes

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RCT, the transport of cholesterol (C) from peripheral tissues and out of the body, is the only route for reducing C burden in tissues, including vascular wall macrophages. RCT is the leading explanation for the cardioprotective effects of HDLC. Accordingly, interventions that stimulate flux through the RCT pathway represent a therapeutic target. There had not previously been a method for measuring the first arm of the pathway (efflux of C from tissues) or measuring RCT rates in humans. We have developed an in vivo method for quantifying efflux rate and flux through the global RCT pathway. The method involves non-radioactive (stable) isotopes, is non-invasive and can be used in humans. Efflux is measured by the isotope dilution principle, through a constant IV infusion of [1-14C]-cholesterol. A mono-exponential rise-to-plateau in free C was observed in humans and rats. Efflux rates were 7.2 ± 1.3 mg/kg/hr in healthy humans, with a rapidly exchanging free C pool size of 7.6 /+ 2.0 g. Intra-individual variability during repeated studies was ~10%. Efflux rates in rats were 15 ± 2 mg/kg/hr. Administration of an LXR agonist for 14 days (T0901317, 10–20 mg/kg) increased efflux rates, and ezetimibe significantly increased efflux. By multiplying efflux rates by the fractional recovery of label in the stool over 4–7 days, global RCT (flux from tissue cholesterol to fecal steroids) was determined. Administration of an LXR agonist to rats increased global RCT flux two-fold. Ezetimibe treatment also increased global RCT in rats. Measurements of de novo cholesterol synthesis rates in tissues with [1-14C]-O2 were consistent with increases in RCT. In summary, efflux rates of C from tissues in humans are ~12 g/day, representing a much greater quantitative value than whole body C balances (~1 g/d) or apoprotein-mediated transport of C (~1 g/d). Anti-atherogenic interventions after RCT fluxes in animals. Application of this technique in rodents and humans could provide new insights into the physiological and pharmacological control of C efflux and RCT.

Effects of Reconstituted HDL on Cholesterol Efflux from Tissues in Vivo

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The first step in reverse cholesterol transport (RCT) is efflux of free cholesterol from peripheral tissues into the bloodstream. Plasma HDL concentrations do not accurately reflect efflux, so a direct, in vivo, measurement is required. We have developed an in vivo method for quantifying the efflux of cholesterol from peripheral tissues into the rapidly exchanging cholesterol pool in blood and liver. The efflux is measured by the constant isotope dilution technique during an IV infusion of 13C-cholesterol. In rodent studies, IV infusions of human reconstituted HDL (HDL) resulted in an immediate decrease in free cholesterol enrichment and an increase in cholesterol efflux. Subsequent studies demonstrated that prolonged continuous infusion of HDL increased cholesterol efflux in a dose-dependent manner. A 7 hour infusion of HDL (1mg/kg/hr, 135mg/kg total) increased cholesterol efflux from 15mg/kg/hr to 20mg/kg/hour. Similarly, a 24 hour infusion of HDL (12mg/kg/hour, 290mg/kg total) increased efflux from 15mg/kg/hr to 23mg/kg/hour. Consistent with increased efflux rates, plasma cholesterol concentrations increased dramatically during HDL infusions. Results of these studies support the model of rapid-turnover pool in communication with a large store of extra hepatic cholesterol. Moreover they demonstrate that cholesterol acceptor capacity (HDL), not the activity of transporters (ABCA1, etc.), is rate limiting for whole body cholesterol efflux in rats. Application of this technique in both rodents and humans could provide new insight into the effects of HDL treatment on cholesterol efflux and RCT.

Beneficial Effects of Pioglitazone on Lipoprotein Profiles in Non-diabetic Patients: Initial Clinical Observations

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Background: Pioglitazone (PIO) favorably affects lipid patterns in patients (pts) with diabetes and the metabolic syndrome, but the effects on non diabetic pts is unknown with limited clinical trials. We evaluated the short term effects of PIO on the lipoprotein profile of non-diabetic patients already on aggressive medical therapy. Methods: Retrospective chart review was used to identify non-dietetic pts with dyslipidemia who failed to achieve goals despite treatment. Pts were started on PIO 15mg while other lipid therapies remained constant. Paired t-tests were used for statistical analysis. Results: 16 pts fulfilled the study criteria. Pts were male with a mean age of 61 yrs and a mean weight of 221 lbs. At baseline, 100% were treated with both a statin and ezetimibe and 75% were treated with niacin. After mean follow up of 1.2 years, 44% (7/16) of pts shifted their pattern size from type B (small, dense) to type A (large, buoyant). Mean HDL increased by 13% while LDL particle number decreased by 15%, and LDL particle size increased by 2% (see figure). There was no significant change in TC, LDL, and TG. No side effects were reported. Conclusions: The addition of PIO to non diabetic pts already on niacin, statins, and ezetimibe resulted in a significant increase in HDL and a modest decrease in LDL particle number and an increase in LDL particle size. Larger randomized clinical trials are needed to confirm these findings, and if confirmed, to assess the therapeutic benefits.

Antibody Response to Several Different Autoantibodies Is Strongly Associated with Acute Coronary Syndromes

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Over the last several years, the idea that immune processes play a key role in atherosclerosis and its complications, acute coronary syndromes (ACS), has received attention. We assessed...
deficiency was found, suggesting that S1P does not affect basal CD36 mRNA and protein. Additionally, S1P inhibited oxLDL-mediated reduction in basal CD36 mRNA and protein. Incubation of B6 macrophages with S1P for 24h showed approximately a 50% CD36 mRNA or protein after 4h or 12h treatments with OxLDL and/or S1P. However, incubation of C57BL/6J (C57) macrophages with oxLDL also showed a 50% reduction in CD36 mRNA or protein after 4h or 12h treatments with OxLDL alone. These results are consistent with AT1a receptors having a profound effect on atherogenesis promoted by both hypercholesterolemia and sidestream cigarette smoke.

Conclusions: These results are consistent with AT1a receptors having a profound effect on atherogenesis promoted by both hypercholesterolemia and sidestream cigarette smoke.

Ps05

Absence of CD36 and SR-A/Ii Protects Against Atherosclerosis in ApoE Knockout Mice: Protection Is Equivalent to Absence of CD36 Alone

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Objective: The role of scavenger receptors in atherogenesis has become controversial as a result of conflicting reports and a recent proposal that suggested that scavenger receptor absence would enhance the pro-inflammatory, pro-atherogenic effects of oxLDL. The purpose of this study was to determine the effect of combined absence of scavenger receptors CD36 and SR-AI in atherosclerosis development in the apoE0 mouse model. Methods: We created background matched strains of apol, SRα0/apoE0, CD360/apoE0 and CD360/SRα0/apoE0 that were greater than 99% C57Bl/6 mice. These mice were fed a Western diet at 4 weeks of age for 16 weeks. Results: In DKO mice, lesion size in the aorta was decreased by 50% compared with Sp1E0 mice that fed the same diet. Conclusions: These studies suggest that Id3 has a protective effect on the vasculature. Further studies will be needed to determine the mechanism for the observed difference in atherosclerosis lesion formation.

Ps06

Sphingosine 1-Phosphate Reduces Oxidized LDL-Mediated Upregulation of Macrophage CD36

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CD36 is highly expressed on macrophages where it recognizes oxidized lipids that are present in apoptotic cells and in oxidized LDL (oxLDL). CD36 mediates lipid accumulation and macrophage foam cell formation associated with atherosclerosis. Macrophages from C57BL/6J (C57) mice were isolated by peritoneal lavage, and incubated with oxLDL (50μg/ml), S1P (500nm) or oxLDL plus S1P for 4, 12 and 24h. CD36 mRNA and protein levels were measured. We observed that the timecourse of macrophage CD36 mRNA expression is sigmoidally shaped compared to control macrophages in vitro exposed to oxLDL. The CD36 mRNA or protein after 4h or 12h treatments with oxLDL and/or S1P. However, incubation of B6 macrophages with R=120 Macrophage cultures were grown in 24-well plates. The CD36 expression was determined using a monoclonal antibody to CD36. The results were consistent with a novel regulator of macrophage function in atherosclerosis by promoting macrophage cell survival and controlling macrophage apoptotic cell uptake and foam cell formation by CD36.

Ps07

Contribution of Myocardial Small Arteries in the Development of Human Coronary Atherosclerosis

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Background: The solution of IHD problem is hidden in its prevention. Do we know all risk factors? Aim. The purpose of the study was to look for IHD risk factors in myocardial microcirculatory bed. Material and methods. The study was carried out on heart of 80 people (42 healthy in age of 24–87 years died of the violent reasons and 38 died from the first acute myocardial infarction in age of 50–89 years). Methods of autopsy, morphometry, x-ray microscopic analysis, histology, histochemistry have been used. The statistics have been analyzed with the method of volume density of intramural coronary arterial bed of the left ventricle wall (Vart) on histological slides was measured. The stage and intensity of atherosclerotic lesion in main coronary arteries, the degree of stenosis were evaluated. Results. In hearts prepared for angiography the index Vart, exceeded in 9 times the value of a similar index for the hearts not subjected to injection. In average the value of Vart, of hearts with a myocardial infarction was 28 %
below of healthy hearts index. There was a significant variation of the value of Vvart. both among healthy people and patients. The distribution of indices Vvart. was different. In group of healthy young and middle-aged persons the indices Vvart. were distributed with comparatively regular intervals between extremes. The Vvart. of elderly and senile healthy persons was piled up mainly at the top borders of the scale. In group of myocardial infarction the indices Vvart. were accumulated near to bottom border of scale. Significant negative correlation between value of Vvart. and coronary arteriosclerosis intensity was found out both in healthy people and at died of myocardial infarction. Conclusion. Due to quantification of morphological changes and anatomical features of heart an earlier unknown risk factor of IH was disclosed. It has two negative influences. At low value of volume density of heart intramural arterial bed both intramyocardial blood supply suffers and development of coronary arteriosclerosis is accelerated. Combined using of radiological (MRI, CT, ultrasound) and developed morphological methods could reveal correlation between results of two methods. Afterwards with only non-invasive methods indicated risk factor of IH in once lifetime can be exposed.

Dietary 7-ketocholesterol Does Not Alter the Extent of Atherosclerosis in Male LDL Receptor–Deficient Mice Despite a Decrease in Hepatic Paraoxonase-1 Expression

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Dietary oxysterols have been implicated in the pathogenesis of arteriosclerosis. Diet enriched in oxysterols decrease activity of paraoxonase-1 (PON-1), a circulating atheroprotective protein, and lead to enhanced arteriosclerosis despite decreased plasma cholesterol levels. Whether individual oxysterols have similar effects is unknown. The most abundant dietary oxysterol is 7-ketocholesterol (7KC). Culturing AML12 mouse hepatocytes for 24 hours with 10μM of 7KC, but not unsaturated cholesterol or other oxysterols, decreased PON-1 and apolipoprotein A-I expression and increased the inflammatory protein, serum amyloid A (SAA). These changes were mediated by NF-κB activation and might decrease the atheroprotective effects of HDL. We hypothesized that adding 7KC to an atherogenic diet would lead to similar changes in the hepatic expression of these proteins in vivo, and increase arteriosclerosis. To test this hypothesis, we replaced 10% of the cholesterol of 7KC in an atherogenic diet (21%; saturated fat and 0.15% cholesterol) fed to male LDLR-/- mice for 12 weeks and measured hepatic apoA-I, PON-1 and SAA mRNA, plasma lipids and arteriosclerosis. At the end of the study, those mice fed 7KC-feeding exhibited significantly higher plasma cholesterol levels than the control group. This phenomenon is presumably due to defective lipoprotein excretion. In conclusion, 18-MEF reduced atherosclerotic lesion and improved inflammatory changes observed in vitro.

Native C-Reactive Protein Does Not Increase Atherosclerotic Lesion Formation in Apolipoprotein E-Deficient Mice

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Background: Elevated C-reactive protein (CRP) levels are associated with endothelial activation and development of arteriosclerosis. However, the conclusive proof of this association in vivo remains lacking. Some researchers reported a pro-atherosclerotic effect of CRP in ApoE-deficient mice (apoE-/-) while others did not. We assessed the hypothesis that continuous administration of azide- and endotoxin-free human native-CRP (n-CRP) to apoE-/- mice would increase arteriosclerotic lesion formation. Methods and Results: Twelve-week old male apoE-/- mice (n = 22) were used. Half of the animals received a continuous infusion of human n-CRP (20.4 μg/mice/day) for four weeks using osmotic pumps; the other half received vehicle alone. After four weeks, the mice were killed and the thoracic aorta was removed. Atherosclerotic lesion area in the aortic sinus was not different between the two groups. We conclude that despite altering hepatic production of apoA-I, PON-1 and SAA in vitro, 7KC, when added to an atherogenic diet, had similar effects on hepatic PON-1 expression, but did not increase atherosclerosis in male LDLR-/- mice. The lack of a difference in aortic lesion area may in part be explained by the adverse effect on PON-1 being offset by a reduction in plasma cholesterol. Higher doses of 7-ketocholesterol may be required to achieve the type of inflammatory changes observed in vitro.
production was also higher in patients compared to controls (74±27 vs. 76±27, p=0.03) and correlated with coronary endothelial dysfunction (r=0.5, p<0.005). CRP net production was not significantly different between the groups. Conclusion: Early coronary atherosclerosis in humans is characterized by local production of LP-PLA2. Local coronary generation of lysoPC, a product of LP-PLA2, is then associated with coronary endothelial dysfunction. These results support the role for LP-PLA2 in the mechanism of regional vascular inflammation and early atherosclerosis in humans.

The Effect of Sphingomyelin Biosynthesis Inhibition on Lipid Absorption in Mice

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Serine palmitoyltransferase (SPT) is the rate-limiting enzyme in the sphingomyelin (SM) biosynthesis pathway. Mammalian SPT is composed of 2 different subunits, Sptl and Splt2. Our previous studies showed that Sptl mice are smaller than wild-type mice with myosin, a specific SPT inhibitor, reduces SM, cholesterol, and triglycerides levels in the circulation, while intraperitoneal treatment reduces only SM levels. Since oral administration of myosin has a direct effect on the gastrointestinal tract, as suggested by a previous report, we hypothesized that the decrease of cholesterol and TG levels in the circulation might be due to a reduction in lipid absorption. We carried out cholesterol absorption studies on wild-type and apoE knockout (KO) mice, with or without oral myosin administration, using the conventional fecal dual-isotope ratio method. The approach involved the gavage of a single bolus of 0.1 uCi [14C] cholesterol and 0.2 uCi [3H] sitosterol, together with 1 mg cold cholesterol in 30 uI olive oil. We observed that, after myosin treatment, wild-type or apoE KO mice absorbed significantly less radioactive cholesterol than their controls (40% and 61%, respectively). The amount of radiolabeled cholesterol assimilated into the circulation was significantly reduced in these mice, compared to their controls (63% and 82%, respectively). Moreover, myosin treated mice absorbed significantly less radiolabeled triglyceride than their controls (56% and 49%, respectively). More importantly, we found that heterozygous Sptlc2 KO mice, with less than 50% SPT activity in the small intestine, absorbed significantly less cholesterol (44%, p<0.01) and contained significantly less radiolabeled cholesterol and [3H]glycerolipids in the circulation (46% and 51%, respectively) than control mice. These results indicate that SPT is involved in the intestinal absorption of cholesterol and triglycerides. Manipulation of SPT activity by either specific inhibitors or gene therapy might provide a novel alternative treatment for dyslipidemia.

Spontaneous Atherosclerosis in Old LPL-Deficient Mice with Severe Hypertriglyceridemia on a Normal Chow Diet

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Whether severe hypertriglyceridemia (HTG) in lipoprotein lipase (LPL)-deficiency is pro- or anti-atherogenic remains an unresolved question. To investigate this issue we pursued a long term observation of development of atherosclerotic lesions in a unique LPL gene deficient mouse model, and compared with those in wild type counterparts fed normal rodent chow diet. At 4 month of age homogenous LPL-/- mice absorbed significantly less HTG HTG than any sign of any atherosclerosis. However, these mice developed foam cell-rich lesions at the aortic root at age over 15 months, while wild-type and heterozygous (LPL+/-) mice were lesion-free at the same age. In searching for causes of such unexpected development of spontaneous atherosclerosis in LPL deficient mice, we observed that mice with less than 50% SPT activity in the small intestine, absorbed significantly less cholesterol (44%, p<0.01) and contained significantly less radiolabeled cholesterol and [3H]glycerolipids in the circulation (46% and 51%, respectively) than control mice. These results indicate that SPT is involved in the intestinal absorption of cholesterol and triglycerides. Manipulation of SPT activity by either specific inhibitors or gene therapy might provide a novel alternative treatment for dyslipidemia.

Dexamethasone Induces MCP-1 mRNA Destabilizing Activity in Vivo in Rat Aortas

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Introduction: Monocyte chemottractant protein-1 (MCP-1) plays an important role in the pathogenesis of atherosclerosis. We have previously shown that glucocorticoids specifically destabilize MCP-1 mRNA in cultured vascular smooth muscle cells (SMC) by a glucocorticoid receptor (GR)-dependent mechanism. The purpose of this study was to define whether this phenomenon also occurs in vivo. Methods: Sprague-Dawley rats were treated with Dexamethasone (Dex) 1mg/kg i.P. 24hrs and 1hr before sacrifice. Aortic media were isolated and S-100 cytoplasmic extracts were obtained as done for cell culture. Extracts were incubated with in vitro-transcribed and radiolabeled MCP-1 mRNA and analyzed on 4% polyacrylamide gels. Protein content was determined by Bio-Rad assay. Results: Extracts from Dex-treated rats induced a more rapid degradation of radiolabeled MCP-1 mRNA (t1/2= 10min) as compared to extracts containing equal amount of proteins from untreated rats (t1/2= 60min). Experiments were done to determine whether the Dex-sensitive activity of aorta was specific to this region described in cell culture. We previously demonstrated that the initial 224 nucleotides of the MCP-1 mRNA contain the Dex-sensitive region. Similarly, extracts from Dex-treated rats degraded the 1–224 region, but not other Dex-insensitive regions. Like in cell culture, the Dex sensitive degradation activity was heat unstable and sensitive to protease K. We have previously shown that addition of exogenous GR blocked the degradative activity of Dex-treated cultured VSMC. Similarly, addition of GR blocked the degradation of MCP-1 mRNA in aortic extracts in a concentration-dependent fashion. Conclusion: These results show that Dex treatment induces an MCP-1 mRNA degradative activity in rat aorta in vivo. Further elucidation of this mechanism may provide novel approaches to regulate vascular MCP-1 expression.

Changes of Renin Angiotensin System in Visceral Adipose Tissues in Rats with Metabolic Syndrome

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Recent studies showed that renin angiotensin system (RAS) in adipose tissue play an unique role in the regulation of insulin sensitivity and adipocyte differentiation. It is unclear the changes of RAS in abdominal fat in metabolic syndrome (MS). This study aims to investigate the RAS of visceral adipose tissue in rats with MS and effect of angiotensin II on adipocyte differentiation. Methods: thirty male Wistar rats were divided into rats on normal chow or high-fat diet. The rats on high-fat diet for 24 weeks developed the MS compared with rats on normal diet. The mRNA and protein expression of RAS in mesenteric fat tissue were measured by RT-PCR and Western blot. Lipid droplet in 3T3-L1 preadipocytes and mature adipocytes were observed using oil-red staining. Cystolic free calcium level was measured by the fluorescence technique. Results: The mRNA expressions of angiotensinogen, angiotensin-converting enzyme I, angiotensin II type 1 receptor in mesenteric fat were significantly increased in rats with MS compared with those in rats on normal diet (P<0.05). After administration of angiotensin II, less lipid droplets in mature adipocytes were observed in vitro. In contrast, dominant lipid droplets in mature adipocyte was found after administration of captopril and candesartan. It suggested that angiotensin II metabolism the increase of cystolic free calcium level in mature adipocyte (0.21±0.04 for captopril, 0.51±0.03 for candesartan). Conclusions: it concluded that RAS in the visceral adipose tissues was activated in rats with MS, and antagonizing of RAS can recover the lipogenesis of adipocyte, which may be associated with diminishing of ectopic lipid distribution and improving the insulin sensitivity.

Increased Oxidative Stress Promodes Leukflet Resitance in Early Aortic Valve Disease

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Oxidative stress is evident in atherosclerotic plaques, but its role in calcific aortic valve stenosis (AVS) is unknown. We tested the hypothesis that oxidative stress is increased in human valves with calcific aortic valve stenosis (AVS), and that increases in oxidative stress precede valve dysfunction in a mouse model of AVS. Dihydroethidium and dichlorofluorescein fluorescence indicated that both superoxide and hydrogen peroxide levels are markedly increased near the calcified regions of explanted aortic valves from humans with AVS (n = 7). Quantification of real-time RT-PCR showed no changes in Nox1, Nox2, or Nox4 expression in calcified regions versus noncalcified regions. In contrast, expression of extracellular superoxide dismutase (SOD) (p < 0.05), copper zinc SOD (p < 0.05) and catalase (p < 0.05) were reduced in regions with high oxidative stress near calcium deposits. We recently reported that Lp-PLA2/ ApoA1(IIb)hI mice fed a normal chow diet develop AVS at ~18–22 months of age (Circulation, 2006; 114: 2065–9), and that superoxide levels were elevated in the valves of mice with severe AVS. Here, hypercholesterolemic male Ldlr-/ApoE(-/-)hI/Mtp/H2Cre(+)SOD(+) mice fed a high fat (HF) diet for 18 months showed increased superoxide levels in the aortic valve versus noncalcified aortic tissue control mice given pI:pC at 1 month (Tempol-inhibitable fraction of DHE: control = 6 ± 2 RLU (Mean ± SD), HF = 14 ± 4 RLU, p < 0.05), despite no significant reduction in aortic valve opening (leaflet separation measured by echocardiography: control = 1.0 ± 0.1 mm, HF = 0.9 ± 0.1 mm). However, MRI assessment of aortic valve function showed increased aortic valve regurgitation in 75% of the fed-mice (versus none of the noncalcified controls), indicating aortic valve sclerosis/dysfunction. Histological examination of the valves revealed small but significant increases in leaflet calcification (Alizarin Red: control = 0.2 ± 0.7%, HF = 1.8 ± 0.7%, p < 0.05) and marked lipid deposition (Oil Red O: control = 0 ± 4%, HF = 18 ± 5%). Collectively, these data suggest that oxidative stress occurs early in aortic valve disease. We speculate that reducing oxidative stress is a potential therapeutic strategy to slow progression of calcific aortic valve stenosis.
developing rat brain, was found to be upregulated by approximately 10 fold in the aorta of db/db and by 2 fold in the aorta of high fat diet fed mice. The neuronatin protein was selectively increased in the aortic endothelium of db/db mice, but expression in other tissues (plutary, brain, heart, kidney) was not altered in diabetic mice. Infection of human aortic endothelial cells with a rat enzating adenovirus resulted in increased expression of a panel of NF-κB regulated inflammatory cytokines, chemokines, and adhesion molecule genes. Neuronatin expression also augmented Tnf-α induced IL-6 production in endothelial cells. Neuronatin expression activated Erk kinase in endothelial cells, which may be a mechanism by which neuronatin activates the NF-κB program of inflammation. In summary, we have discovered that neuronatin expression leads to a stimulation of specific indicative genes in endothelial cells that have direct relevance to the pathogenesis of atherosclerosis. The upregulation of neuronatin in the vasculature of diabetic mice may be a mechanism by which diabetes accelerates athero-

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Activated Factor XII Type A Is an Independent Predictor of All-cause Mortality in Patients Admitted with Chest Pain

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Background: Activated Factor XII (XIIa) is a predictor of recurrent coronary ischemic events in patients following a myocardial infarction (MI). Recently, novel in vivo types of XIIa have been described. We assessed the relation between admission levels of activated factor XII type A (XIIaA) and long-term all-cause mortality in a large, consecutive cohort of patients admitted with chest pain. Methods: Blood samples for XIIaA determination were obtained immediately following admission in 871 patients admitted with chest pain suspected of having a MI. Plasma XIIaA concentrations were determined by ELISA at admission. All cause mortality within each quartile of XIIaA was compared for a 24-month follow-up period. Results: After a follow-up period of 24 months, 130 patients (14.9%) had died. The unadjusted risk ratio for death of patients with XIIaA in the highest quartile (Q4) was 2.92 (95% CI 1.72–4.95; p < 0.0001) added prognostic information for all-cause mortality additional to patients with XIIaA in the highest quartile (Q4) was 2.92 (95% CI 1.72–4.95; p < 0.01) added prognostic information for all-cause mortality additional to...

**WITHDRAWN**

### Structural Transition from Pentamer to Monomer: A Mechanism That Finely Regulates the Reactivity and Behavior C-Reactive Protein Exerts in the Inflammatory Process of Atherosclerosis

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### MMP-9 Deficiency Does Not Influence Atherosclerosis but Promotes Abdominal Aortic Aneurysms in Both Hypercholesterolemic and Angiotensin II-Infused Mice

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**Objective:** The role of matrix metalloproteinase-9 (MMP-9) in the development of atherosclerosis remains to be clarified. However, MMP-9 has been implicated in the development of experimental abdominal aortic aneurysms (AAAs). The purpose of this study was to define the role of MMP-9 deficiency on hypercholesterolemia- and AngII-induced atherosclerosis and AAA formation in Apoe-/- mice.

**Methods and Results:** ApoE-/- mice were developed that were either wild type or deficient in MMP-9, with all comparisons being performed between littermates. Mice were infused with either saline or AngII (1,000 ng/kg/min) via osmotic pumps for 28 days and fed a normal laboratory diet. MMP-9 deficiency had no effect on plasma cholesterol concentrations, lipoprotein-cholesterol distributions, or systolic blood pressure during saline or AngII infusion. MMP-9 deficiency had no effect on the size of atherosclerotic lesions during either saline or AngII infusion. Unexpectedly, AAAs were observed in the supra-renal aorta (P < 0.027) of saline-infused MMP-9 -/- mice. Furthermore, infusion of AngII led to significantly increased AAA formation (P < 0.002) and increased death due to rupture of the abdominal aorta (P < 0.004). To determine whether MMP-9 deficiency led to structural changes, the AAA prone region was studied for cellular content and matrix integrity. We also determined whether MMP-9 deficiency led to functional defects by performing contractility studies in aortic rings. No structural or functional changes were discernable in the AAA prone region that could account for the exacerbated disease.


### Cilostazol and Atorvastatin Have Synergistic Effects on Endothelial Nitric Oxide Synthase Phosphorylation and Protection Against Ischemia-Reperfusion Injury

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**Background:** Cilostazol (ATV) limits myocardial infarct size (IS) by upregulating Akt and protein kinase A (PKA) which activate endothelial nitric oxide synthase (eNOS) via phosphorylation of eNOS Ser1177. However, when given orally, high doses of ATV (10 mg/kg/d) are needed to achieve maximal protective effect. Cilostazol (CIL) is a phosphodiesterase III inhibitor, used for treating patients with peripheral arterial disease. Several studies have suggested that CIL stimulates NO production by activating PKA. Hypothesis: CIL and ATV may have synergistic effects on eNOS phosphorylation and IS limitation. Methods: Sprague-Dawley rats received 3-day oral: 1) water; 2) ATV 2mg/kg/d; 3) CIL 20mg/kg/d; 4) ATV + CIL. Rats underwent 30min coronary artery occlusion and 4h reperfusion (n=10 in each group), or hearts were explanted for immunoblotting without being subjected to ischemia. Area at risk (AR) was assessed by blue dye and IS by triphenyltetrazolium chloride (TTC). Results: Body weight and the size of AR were comparable among groups. There were no significant differences among groups in mean blood pressure and heart rate. CIL, but not ATV, reduced IS. The ATV+CIL group had IS that was significantly smaller than in the other three groups (P<0.001 for each comparison) (Figure). ATV, CIL and their combination did not affect total eNOS expression. ATV at 2 mg/kg/d did not affect Ser1177 P-eNOS levels, whereas CIL increased it (258±15%). Myocardial P-eNOS levels were higher in the ATV+CIL group (408±7%). Conclusions: ATV and CIL have synergistic effect on eNOS phosphorylation and in IS limitation. By increased activation of eNOS, CIL may augment the pleiotropic effects of statins.

### Sirolimus Attenuates Angiostatin II Plus Diet-accelerated Atherosogenesis and Abdominal Aortic Aneurysm Formation in ApoE-deficient Mice


**Background:** Sirolimus is a macrolide inhibitor of mTOR kinase that markedly attenuates transplant vasculopathy and neointimal hyperplasia in animal models and humans. The effects of sirolimus on atherosclerotic lesions and abdominal aortic aneurysm (AAA) formation require further characterization. **Objective:** We investigated the effects of sirolimus on atherosclerotic lesion development and AAA formation in apolipoprotein E deficient (apoE-/-) mice on a high lipid diet receiving an angiotensin II (angII)-intoxication to accelerate vascular pathology. **Methods:** Male apoE-/- mice were placed on a proatherogenic diet for 4 wks and given a continuous infusion of angII (1 μg/kg/min) via osmotic minipump. Sirolimus (0.5, 1.0, 4.0 mg/kg, iv) or vehicle were administered once daily for 4 wks. In a second study, apoE-/- mice received an angII infusion and proatherogenic diet for 8 wks. After 4 wks of infusion, sirolimus (1.0 mg/kg, ip) or vehicle were given once daily for the remaining 4 wks to assess the potential for lesion regression. Atherosclerotic lesion area and histology, AAA characterization and plasma cytokine levels were assessed. **Results:** Daily ip injection of sirolimus significantly reduced atherosclerotic plaque area (en face analysis) in a dose-dependent manner at 4 wks (sirolimus: 3.1 ± 0.4 mg/kg vs vehicle: 12.9 ± 1.8 mg/kg, P < 0.0005) and attenuated progression of established lesions at 8 wks (sirolimus: 18.4 ± 2.3% vs vehicle: 37.6 ± 2.7%, P < 0.0001). Sirolimus
markedly reduced the incidence (sirolimus: 0 %, vs vehicle: 32 %, P < 0.005) and severity of AAA as determined by aortic diameter (sirolimus: 0.74 ± 0.02 mm vs vehicle: 1.38 ± 0.25 mm, P < 0.005). Sirolimus prevented elastin disruption and reduced CD68+ inflammatory cell infiltration in aneurysmal tissue. These effects were associated with an altered Th1/Th2 cytokine profile in vivo. Conclusion: Our data suggest that sirolimus reduces the development of atherosclerotic lesions and AAA in ang-inflamed apoE- mice and may prove to be beneficial in modulating the severity of inflammatory vascular diseases.

Quantitative Annual Effect of Atorvastatin on Size and Content of Noncalcified Plaques of Coronary Arteries Following Atorvastatin Treatment by Multislice CT

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Background: Intensive lipid-lowering treatment with Atorvastatin reduced progression of coronary atherosclerosis, confirmed by NUS. To quantify the annual effect of Atorvastatin on size and content of non calcified coronary plaques (NCP) using MSCT and comparing LDL cholesterol levels. Methods: 21 subjects (16 males, 35–79 years, median 69) with NCP by MSCT (Light Speed Ultra 16) were enrolled. All were asymptomatic to differentiate thrombi from NCP in coronary arteries. Following LDL measurements, all were given 10mg of Atorvastatin (2 were 5mg as LDL levels were already lower than 70mg/dl for 1 year, and MSCT and LDL measurements were repeated. One remarkable NCP was selected in each subject and evaluated as representative of effect of Atorvastatin. The area and CT values of NCP, excluding calcified portions, were manually measured from axial or multiplanar reconstruction images under the same conditions. Results: 21 NCP (18 LAD, 2 LCx, and 1 RCA) were evaluated. The mean LDL level was 122 mg/dl at the first scan and significantly decreased to 96 mg/ml at the second scan (P<0.05). The areas of NCP were 2–31 mm2 (mean 11.8) at first-scan, and 2–32 mm2 (mean 12.6mm2 at second-scan. The mean areas of NCP were not significantly different between the both-scans (11.8 at first and 12.6mm2 at second scan). The averages of CT values were 55HU at first scan and 62HU at second scan and the mean of SDs of CT values were 40HU at first and 45HU at second scan and both were significantly higher in the second scan (P<0.05). There was a significant positive correlation between ratios (%) of annual change in area to baseline area at first scan of NCP (y) and LDL cholesterol levels (x) after one year of Atorvastatin treatment (y = 0.0106x−0.2765, R2 = 0.1514, P<0.05). Conclusion: Using MSCT, we could quantify the effect of Atorvastatin to the size and content of NCP and compare those with LDL cholesterol levels. Atorvastatin may decrease area of NCP if LDL levels are sufficiently decreased. Also, it may increase CT values, which could suggest a change in NCP components. LDL levels may be an important factor in decreasing the area of NCP. Further studies are needed using 64-slice MSCT in a larger population with sufficient decreases in LDL levels.

Cyclic Bending Is a Major Contributor to Critical Stress Conditions Leading to Coronary Plaque Rupture: 3D MRI-Based FSI Models and Mechanical Image Analysis

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Introduction Mechanical forces play an important role in the complicated process of atherosclerotic plaque rupture. We have developed MRI-based 3D multi-component models with complex flow interactions (FSI) in order to perform stress/strain analysis for atherosclerotic plaques and to identify possible mechanical and morphological indices for accurate plaque vulnerability assessment. Hypothesis Cyclic bending of coronary arteries caused by heart motion may be a major contributor to critical stress conditions of atherosclerotic plaque leading to increased plaque rupture. Method A 3D multi-component FSI model was introduced to evaluate the effects of cyclic bending on stress/strain distributions in coronary plaques using geometry re-constructed from a 3D ex vivo high resolution MRI data set (36 slices) acquired from a human coronary plaque. Blood flow was assumed to be laminar, Newtonian, and incompressible. Both vessel and plaque component materials were assumed to be hyper-elastic and isotropic. Cyclic arterial bending secondary to cardiac motion was introduced into the computational model by specifying a region of asymmetric repeat displacement. The displacement was adjusted to achieve desirable curvature changes. In vitro flow experiments using hydrogel stenotic tubes with cyclic bending were conducted to validate our models. Results Computational simulations were conducted using the hydrogel stenosis model and the coronary plaque sample under a 70–130 mmHg physiological pulsating stress. Pressure behaviors tracked at selected critical sites (thin cap and major stress sites) showed that cyclic bending causing 100–400% higher stress variations. Multi-component plaque structure and cyclic bending led to nonuniform compression/expansion and higher stress variations in the plaque. Conclusions Our initial study indicates that cyclic bending affects stress variations in coronary plaques to the extent that it plays at least as important a role as blood pressure does and must be included in coronary models for accurate mechanical analysis and stress-based plaque vulnerability assessment. Additional studies using this new model are warranted.

Postischemic Neovascularization Is Modulated by the A1 Adenosine Receptor

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Atherosclerosis and angiogenesis play a critical role in cardiovascular development and in adaptive responses to tissue ischemia associated with arterial stenosis. Peripheral arterial disease (PAD) affects over 7 million Americans and claims the limbs of over 100,000 of these annually. Adenosine is a purine nucleoside metabolite of ATP degradation released by all ischemic tissues, where it acts to limit tissue injury via multiple mechanisms, including angiogenesis. Many of the effects of adenosine are mediated through its interaction with four subtypes of G protein-coupled cell surface receptors: A1, A2A, A2B, and A3. Proangiogenic effects mediated by A1 adenosine receptors (AR) have been described, but the role of the A1, AR is unexplored. The aim of our study was to identify the role of the A1, AR in neovascularization of the ischemic hindlimb. We compared neovascular responses between wild type (WT) mice and mice with gene-targeted A1, AR-deficiency (A1 KO) following femoral transection. While this technique does not result in distal tissue necrosis in WT mice, 50% of the A1 KO animals developed digital gangrene on the ischemic side. Laser doppler perfusion imaging (LDP) of the distal extremity on post-operative day 7 demonstrated a 40% reduction in perfusion index (ischemic/non-ischemic flow ratio) in the A1 KO (29±4%, n=7) versus WT mice (52±8%, n=8, p=0.03). These observations were supported using endothelial staining in histologic sections of gastrocnemius muscle, in which we observed 55% fewer capillaries in A1 KO sections than in WT (p=0.03). Delivery of A1 AR antagonist (WS15771) in WT mice following ischemia, resulted in a 30% attenuation in LDP index (control 42±11%, n=4 vs. WS1 31±2%, n=5) and a 33% decrease in capillary number, suggesting that impaired neovascularization in A1 KO mice is the result of receptor deficiency and not artifact from manipulation of the targeted locus. Further support comes from contrast-enhanced ultrasound imaging in the proximal hindlimb day 3 following ischemia showing a 42% lower perfusion index in A1 KO (41±4%, n=4) versus WT (71±4%, n=4, p=0.05) muscle. We conclude that the A1, AR plays a significant role in stimulation of ischemia-mediated neovascularization and is a potential therapeutic target for PAD.

Peroxisome Proliferator-Activated Receptors and Angiogenesis: Direct Comparative Analysis of Selective Agonists

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Objective: In recent years, there has been increasing appreciation of the fact that peroxisome proliferator-activated receptors (PPARs) might be important modulators of angiogenesis. However, whether PPARα and PPARγ, the two main members of the PPAR family, act as inhibitors or inducers of the molecular mechanisms that underlie angiogenesis still remains a controversial issue. Importantly, all the studies investigating the effects of these receptors on angiogenesis have been carried out with molecules such as thiazolidinediones, endogenous prostaglandins, and fibrates, that, in addition to act as PPAR ligands, are also able to activate a number of PPAR-independent pathways. In this study, we used highly-selective PPARα and PPARγ agonists and performed a direct comparative analysis of their in vitro and in vivo angiogenic properties. Methods and Results: For the in vitro study, a co-culture model of human endothelial cells and intestinal cells was used. For the in vivo studies, the murine corneal model of angiogenesis was utilized. We found that WT14663 and GW15129, two selective agonists of PPARα and PPARγ, respectively, had the ability to induce tube formation in vitro and neangiogenesis in vivo. PPARα- and PPARγ-induced angiogenesis was associated with increased expression of vascular endothelial growth factor (VEGF) and did not differ in extent and morphology from that induced by VEGF. Conclusions: This is the first study performing a direct comparison of the angiogenic properties of highly-selective PPAR agonists. Our findings suggest that a careful revision of data obtained by using non-selective and non-specific ligands of PPARα and PPARγ and indicating an anti-angiogenic effect of these receptors is needed.
Bone Marrow–Derived Stem Cells Engineered to Overexpress Prostaglandin Synthase Survive Under Hypoxia Condition and Enhanced Capillary Assembly Around Homing Site in Hind Limb Ischemia Model

Yasashi Numaguchi, Masakazu Iishi, Ryoji Kubota, Chintatsu Hattori, Toyoko Murohara; Nagoya Univ, Nagoya, Japan

Mesenchymal stem cell (MSC) delivery contributes to collateral formation through cell incorporation into vessels and secretion of angiogenic cytokines like HGF, VEGF, and MCP-1 in a paracrine manner. Prostaglandin is a proangiogenic protein which has a multifactorial function such as antiapoptosis and antiaggregation in endothelial cells. To test hypothesized that cell therapy with MSC overexpressed PGIS would enhance proangiogenic effect to lead to accelerated recovery from hindlimb ischemia to the delivery of MSC alone. Methods and Results: Murine MSC was homed by flushing femurs and transplanted with adenoviral vector encoding GFP alone or GFP and PGIS (AdGFP and AdBiG: P = 10^5 M0i each). Cell cycle analysis assessed by FACS revealed that, under hypoxia condition (5%O2), apoptosis rate was reduced by 68% in GFP + PGIS-transfected MSCs and promoted cell proliferation by 52% in accordance with flow cytometry of bcl-2, bax, and bcl-x/Caspase-3. Protein expression confirmed by western blotting. C57BL/6 mice received gene or MSC injection to the adductor muscle 1 day after femoral artery ligation. Mice were divided into 4 groups (n = 8 each) following injected content; vehicle, C57BL6/J mice received gene or MSC injection to the adductor muscle 1 day after femoral artery ligation. Mice were divided into 4 groups (n = 8 each) following injected content; vehicle, AdGFP (Ad-GFP+I, 4×10^9 PFU), MSC with AdGFP (MG), and MSC with AdBiG + PGIS (MG). Mice were fed a phytoestrogen diet (caryophyllaceae) seeds. Segetalins are phytoestrogens used in Chinese pharmacopoeia for anti-bone loss and anti-tumor effects. Here we evaluated these phytoestrogens for their potential to inhibit arthritis and tumor angiogenesis. Segetalins have been reported to inhibit VEGF secretion. Hypothesis: Segetalin molecules could inhibit angiogenic process and tumour growth by inhibiting VEGF secretion from breast cancer cells. Methods and results: Natural Segetalin (SA) and synthetic (SA1) analog were synthesized in our laboratory. SA1 was obtained from the natural compound by an alanine > tryptophan substitution. 1) Cell proliferation tests were performed using Human Umbilical Endothelial Cells (HUVEC), and breast cancer cells lines MCF7 and MDA-MB-231. No difference between SA or SA1 treated cells and vehicle treated cells was observed. 2) At 10^-7 M and 10^-5 M, SA1 only inhibits tube formation in a matrigel assay (an in vitro angiogenesis model). 3) Using cell culture supernatants, we observed that SA and SA1 (10^-7 M and 10^-5 M) decreased by 60% VEGF production in MCF7 and MDA-MB-231 vs vehicle treated cells (p < 0.008) (VEGF ELISA). Conclusion: SA and SA1 do not modify HUVEC, MCF7 and MDA-MB-231 proliferation but inhibit tumour angiogenesis by decreasing VEGF production from MCF7 and MDA-MB-231. SA1 does not inhibit endothelial cell differentiation.

Type 2 Diabetes Impairs Collateral Artery Enlargement More than Type 1 Diabetes in Response to Hind Limb Ischemia in Mice

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Objective: Diabetes is an important major risk factor for peripheral arterial disease. Whether Type 1 or Type 2 diabetes has similar effects on the vascular response to limb ischemia is unknown. We investigated the effects of type 1 versus type 2 diabetes on blood flow recovery, arteriogenesis, and angiogenesis after induction of hindlimb ischemia. Methods: Ischemia was induced in streptozotocin (STZ)-treated (Type 1 diabetes), db/db (Type 2 diabetes), and wild type C57BL/6 mice (control). Laser Doppler perfusion studies followed by angiography were performed in all three groups to determine blood flow recovery, and the number of collateral arteries. Histological analysis of muscle was also performed to determine capillary density, collateral diameter and fat infiltration. Results: Blood flow recovery after hindlimb ischemia was less complete in both Type 1 and 2 diabetes than in wild type mice (p < 0.05). Both types of diabetes showed significantly fewer collateral arteries and smaller diameters than did controls (p < 0.05). However, Type 2 diabetic mice showed a greater impairment in blood flow recovery than Type 1 diabetic mice (p < 0.05). This difference was not due to fewer numbers of collateral arteries or lower capillary density (p = NS), despite lower pre-ischemic capillary density in type 2 diabetic mice (p < 0.05). Rather, Type 2 diabetic mice had collateral arteries of significantly smaller diameters than Type 1 diabetic mice (p < 0.05). Despite very low perfusion in the ischemic limb, no gangrene was observed in type 2 diabetic mice. In contrast, muscle weight was decreased in type 2 diabetic mice due to extensive fat infiltration unlike in type 1 diabetic or control mice. Conclusions: Type 2 diabetic mice displayed a distinctly different response to hindlimb ischemia than Type 1 diabetic mice. Type 2 diabetic mice had a greater impairment in blood flow recovery than Type 1 diabetic mice because of less collateral artery enlargement. The extensive fatty infiltration of the ischemic muscle of type 2 diabetic mice has not been documented in any other disease model. The molecular mechanisms responsible for these differences in blood flow recovery and collateral artery enlargement between Type 1 & 2 diabetes remain the focus of ongoing studies.

In Vivo Imaging of Murine Vasodynamics by Fourier Domain Optical Coherence Tomography

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In vivo imaging of vessels can provide new insights in the regulation of vasodynamics. In contrast to established isometric force measurements, fourier domain optical coherence tomography (OCT) imaging allows the investigation of vasorelaxation and vasodilatation in the anatomical context of the vessel without preparation trauma. The murine saphenous artery is a suitable model for the in vivo evaluation of novel compounds that are designed to increase blood flow. OCT is well-suited to assess the significance of the blood vessel to vasomotor stimuli and the advantageous anatomical position for transluminal imaging. Male C57BL/6 mice (8 weeks of age) were fed a control and a high-fat diet for 10 weeks. Changes in blood vessel diameter were induced by dermal application of the vasodilator antinmipridine (PBN) and the vasoconstrictor potassium. OCT images were acquired with a two-axis galvonometer scanner head which operated in 2D or 3D mode. Three dimensional image stacks were used to determine the morphology of the saphenous artery, vein and nerve. Time series (3 frames per second, 300×512 pixel per frame) of cross-sectional images were analysed with image processing software measuring the time course of the vessel lumen dynamics. The results of this feasibility study are summarized in the table (n = 4, mean ± SEM). In conclusion, fourier domain optical coherence tomography allows the imaging of the vasodynamics of murine vessels in vivo. Further studies using high-fat diet in arteriosclerosis-sensitive mice models will extend our knowledge about diet-specific changes in vasodynamics.
markers for endothelial injury and plasma cholesterol was not independently studied. Therefore, we analyzed in patients with no interfering coronary disease risk factors the impact of high (HC) as opposed to normal cholesterol on EMP release, circulating levels respectively the correlation with plasma cholesterol level and low-density lipoprotein (LDL). Methods: High and normal cholesterol participants, age (<45 years) and gender matched, exhibited no significant difference. EMP quantification in fresh blood samples was assayed by flow cytometry using anti-CD31, 42b, 62E antibodies. Coronary artery endothelial cells (CAEC) were cultured and treated with plasma from each group. All methods employed were previously reported. Statistical analysis was performed considering a p < 0.05 of significance. Results (see table): Cholesterol and LDL were significantly higher in the HC group (each p < 0.01). Circulating levels of CD31 +/42b− EMP but not CD 62E + were increased with HC as opposed to control (p = 0.001). CD 31 +/42b− release from CAEC in culture was induced with HC plasma compared to normal cholesterol and negative control (each p < 0.01). There was no effect on CD 62E + induction (p = 0.62). Correlation analysis between cholesterol, LDL levels and circulating EMP count revealed a consistently positive association only with CD 31 +/42b− and not with CD 62E + phenotype. Conclusion: Cholesterol rich plasma affects EC and induces select phenotype EMP release. HC, respectively LDL is independently and directly associated with increased CD 31 +/42b− EMP phenotype. Therefore, elevated levels, in normotension and development EMP and phenotype reflect the endothelial injury pattern associated with HC.

STUDY RESULTS TABLE

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Excess Plasma Cholesterol in Patients with Single Cardiovascular Risk Factor, Increases Select Phenotype Endothelial Microparticles Expression and Directly Correlates With Elevated Circulating Levels in Blood

Marian T Calfa, Lucia M Mauro, Alexander C Ferreira, Hannah J Dodson, Wenche Jy, Yeon S Ahn, Eduardo De Marchena, Joaquin J Jimenez; Univ of Miami, Miami, FL

Background: In response to injury, endothelial cells (EC) were shown to release membrane-derived microparticles CD 31 +/42b− and CD 62E + (EMP). Elevated circulating EMP levels in blood were documented with multiple cardiac risk factors. Nonetheless, the link between EMP,

Excess Plasma Cholesterol in Patients with Single Cardiovascular Risk Factor, Increases Select Phenotype Endothelial Microparticles Expression and Directly Correlates With Elevated Circulating Levels in Blood

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examined. Aim: We examined whether TNF-α activates Mst1 in EC. Results: Western blot analysis for Mst1 showed that TNF-α-induced cleavage of Mst1 in a time- and dose-dependent manner, which indicated the activation of Mst1 kinase activity. TNF-α-induced Mst1 activation was significantly attenuated by pretreatment with Z-DEVD-FMK, a caspase3 inhibitor, confirming the previous report showing that Mst1 is activated by caspase 3. However, downregulation of Mst1 by siRNA did not affect TNF-α-induced activation of caspase 3, which is not consistent with the previous data that Mst1 and caspase 3 constitute a positive feedback loop. Inhibitors for mitogen activated protein kinases (ERK, p38MAPK and JNK) did not affect TNF-α-induced Mst1 activation. Diphenylethionamide, a NADPH oxidase inhibitor, and N-acetylcycteine, an antioxidant, did not affect TNF-α-induced activation of Mst1 and caspase3, suggesting a role of reactive oxygen species in the activation of Mst1. Nuclear staining with Hoescht33258 and fluorescence activated cell sorting (FACS) analysis showed that TNF-α and overexpression of Mst1 induced apoptosis of ECs. TNF-α-induced EC apoptosis was blocked by introduction of siRNA for Mst1 but not by scramble RNA. Conclusion: These results suggest that TNF-α induces Mst1 activation through caspase3 and oxidative stress, and that Mst1 plays an important role in the induction of TNF-α-induced apoptosis of ECs. Therefore, inhibition of Mst1 in unstable plaque may reduce apoptosis of ECs and stabilize vulnerable plaque.

Flow Differentially Regulates the mTOR Pathway in Cocultured Endothelial and Smooth Muscle Cells

Carla Olive, Marina Santacana, MIT, Cambridge, MA; Angelo A Cardoso, Univ of Indiana, Indianapolis, IN; Mercedes Balciunas, Elazer R Edelman; MIT, Cambridge, MA

Hemodynamic forces are powerful regulators of vascular endothelial cell (EC) and smooth muscle cell (SMC) biology and phenotype. Endothelial stents, in particular drug-eluting, are routinely used for atherosclerotic obstructive disease and yet the biology of local delivery has not been fully elucidated. We hypothesized that stent deployment alters local hemodynamics eliciting profound effects on the response of underlying SMC and recovery of damaged endothelium. Single cultures of EC or SMC, and sequentially layered SMC/EC co-cultures seeded on silicone tubes were exposed to coronary artery-like flow for 20 min or 24 h. Pertussis consisted of cytokine-containing media (VEGF, FGF-2, IGF-1 and EGF), in the presence and absence of the rapamycin analogue CCI-779 (10 nM). After flow exposure, cells were immediately harvested and fixed for analysis. mTOR signalling was evaluated by flow cytometric detection of phosphorylated S6 ribosomal protein (P-S6RP). Bare metal stents were expanded within tubes before exposure to flow. Endothelial recovery after stenting was assessed using microscopic examinations. Experiments were carried out in triplicate. P-SP6K expression in EC increased 2-fold by flow exposure, but not in SMC. Mean intensity fluorescence (MF) of isolated EC cultures under static conditions was 535.0 ± 21, and after flow exposure increased to 1054 ± 42 (p < 0.001). Interestingly, no differences in mTOR activation were observed for SMC. In the sequentially layered SMC/EC co-cultures, the expression of P-SP6K markedly increased in C301 + EC under static (MF 980 ± 14 < p < 0.001 vs single cultures of EC) and flow (1578 ± 66, p < 0.002 vs single culture of EC). CCI-779, abrogated the effect of flow on mTOR signalling reducing P-SP6K to below basal level both with (105 ± 16, p < 0.001 vs flow basal level), and without flow (151 ± 6, p < 0.001 vs static basal level). Stent endothelialization under flow in stents with inhibitor was 4.6-fold lower (4.8 ± 0.9 cells·10³/µm²) than in stents without inhibitor (22.2 ± 6.7 cells·10³/µm²; p < 0.05). Our findings suggest that activation of the mTOR pathway on EC and SMC is differentially regulated by flow, extending our understanding of the integration of flow and autocrine/paracrine regulation of vascular repair.

In Vivo Human Lower-extremity Saphenous Vein Bypass Grafts Manifest Flow-mediated Vasodilation

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Endothelial-derived NO is an important mediator of vascular function. Under normal conditions, eNOS generates low levels of NO and is strictly regulated by a variety of mechanisms. Exposure of endothelial cells to inflammatory mediators induces expression of iNOS and the kinin B1 receptor (B1R). Once expressed, iNOS is thought to constitutively produce high output NO until it is degraded. Here we show that iNOS activity can be acutely upregulated by activation of B1R in human endothelial cells or transformed HEK293 cells to generate 2.5 to 3-fold higher NO. To test the hypothesis that B1R agonists induce activation of extracellular signal-regulated kinase (ERK) which then phosphorylates and activates iNOS, we cotransfected HEK cells with B1R and iNOS. B1R agonist des-Arg10-kallidin (DAKD; 100 nM) stimulated ERK activation, phosphorylation of iNOS on serine and generation of "super-high" output NO. ERK activation inhibitor P208359 greatly diminished NO production and also inhibited phosphorylation of iNOS. Ser547 in iNOS was identified as the critical residue phosphorylated by ERK using an in vitro kinase assay and MALDI-TOF mass spectrometry of tryptic peptides from iNOS immunoprecipitated from transfected HEK293 cells. A peak corresponding to the phosphorylated form of the tryptic peptide iNOS[544-551]PSSR at m/z 1197.526 was detected in iNOS treated in vitro with activated ERK or iNOS from cells stimulated with 100 nM DAKD, but not in iNOS from untreated cells or treated cells transfected with iNOS mutated at Ser547. iNOS and ERK were colocalized in subcellular domains as determined by confocal imaging and also co-immunoprecipitated, indicating that they interact. Transfection of cells with S745A mutant iNOS eliminated phosphorylation and abolished the ability of B1R signaling to generate iNOS-dependent NO, but did not inhibit basal iNOS activity. In contrast, the S745D mutant (mimics phosphorylation) had much higher basal activity, but was not activated by DAKD. This is the first demonstration that iNOS can be acutely activated by receptor-dependent ERK signaling and reveals a previously unappreciated level of complexity in its regulation. This novel pathway may play an important role in inflammatory vascular disease.
Temperature and Blood Pressure Following Amlodipine Overdose: A Test of the Thermoregulatory-Vascular Remodeling Hypothesis

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BACKGROUND: The cardiovascular system participates in both blood pressure (BP) and temperature regulation. The thermoregulatory-vascular remodeling (TVR) hypothesis postulates that temperature homeostasis has precedence over BP homeostasis. As a result, salt ingestion creates conflict between BP and temperature homeostasis, for prompt vasodilatation to lower the BP would increase cutaneous blood flow, thereby accelerating heat loss and lowering the core body temperature. In order to avoid heat loss, salt ingestion elevates the BP until the kidneys excrete the excess salt. During these transient elevations in BP, peripheral resistance increases due to vascular remodeling. Since vascular remodeling is irreversible, the baseline peripheral resistance increases incrementally following an ingestion of salt. After numerous episodes of salt ingestion, the baseline BP also rises. OBJECTIVE: A case of amlodipine overdose offered an opportunity to test one of the predictions of the TVR hypothesis: that vasodilators increase cutaneous blood flow, thereby accelerating heat loss. Consequently, one would expect either a drop in body temperature and/or an increase in the metabolic rate. METHODS: Following the ingestion of 1000 mg of amlodipine, the temperature and BP of a single patient were monitored following presentation for emergency care, during the initial 24 hours of hospitalization, and during the final 24 hours of hospitalization. Temperature readings were available beginning 7 hours post ingestion, and then only intermittently, varying from once every hour to once every four hours. RESULTS: The BP dropped markedly between the 5th and 7th hours post ingestion, but then the BP rose steadily and normalized by 28 hours post ingestion. The temperature was normal at 7 hours post ingestion, declined gradually between the 7th and 26th hours post ingestion, stabilized between the 26th and 31st hours post ingestion, then began to rise. CONCLUSIONS: During this single case of amlodipine overdose, a modest temperature decline lagged behind a marked BP decline. The BP normalized following medical therapy. As the BP rose, the temperature also rose, but lagged behind the BP increases. These results are consistent with the predictions of the TVR hypothesis.

Angiotensin II Infusion Results in Region-Specific Aortic Hypertrophy and Hyperplasia Requiring AT1a Receptor Activation of p47^{phox} and ID3 in a Pressure-independent Manner

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Objective: We previously demonstrated that angiotensin II (AngII) infusion leads to an AT1a receptor (AT1aR)-mediated smooth muscle cell hyperplasia as well as a medially hypertrophic response in the thoracic and abdominal regions; independent of blood pressure. The objective of this study was to determine the mediators of this AngII-mediated regional change. Furthermore, we assessed the mechanism responsible for AngII-mediated aortic hyperplasia. METHODS AND RESULTS: Continuous AngII-infusion led to progressive aortic root hyperplasia. Mice were assigned to 3 groups at 28 days: Group 1 with AngII-infusion (1000 ng/kg/min) were used as control. Group 2 was treated with AngII and 50 ug/ml of the AT1a receptor inhibitor, losartan. Group 3 was treated with AngII and 50 ug/ml of the AT1a receptor inhibitor, losartan and 10 ug/ml of the p47^{phox} inhibitor. Whole mount and immunohistochemical staining revealed that p47^{phox} was significantly increased in AngII-infused mice, whereas the AngII-infused mice treated with losartan or losartan and p47^{phox} inhibitor showed reductions in p47^{phox} expression. Furthermore, the p47^{phox} inhibitor reduced the AngII-mediated aortic root hyperplasia as well as the AngII-mediated increase in p47^{phox} expression. Id3 is a helix-loop-helix factor that induces AngII-mediated SMC proliferation. Id3^-/- mice were infused with saline (n=6) and AngII (n=7) to ascertain if this mitogenic protein leads to the aortic arch hyperplasia. Id3 deficiency did not alter the AngII-mediated medial thickening, however, the AngII-induced medial hyperplasia was attenuated in the ascending arch. Conclusion: The increase in aortic medial thickness, induced by AngII infusion, is mediated via AT1aR leading to the activation of the p47^{phox} subunit of NADPH oxidase, in a pressure independent manner. Furthermore, attenuation of the AngII-mediated aortic arch hyperplasia in Id3^-/- mice suggests that this protein is responsible for the mitogenic effect in this region.

Continuous Angiotensin II Infusion Promotes Progressive Expansion and Vascular Remodeling of Abdominal Aortic Aneurysms in Apolipoprotein E-/- Mice

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Objective: Chronic subcutaneous angiotensin II (AngII) infusion into mice leads to rapid initiation of abdominal aortic aneurysms (AAA). The objective of this study was to determine whether proangiogenic AngII infusion led to AngII-induced progression and changes in pathogenesis. METHODS AND RESULTS: Male Apoe^-/- mice were infused with AngII (1000 ng/kg/min) by Atzet mini-osmotic pump for 28 days. Lumen diameters of the abdominal aortas were measured by high frequency (40 MHz) ultrasound. Mice with AAs were assigned to 3 groups at 28 days. Group 1 was terminated (n=15); Group 2 was infused for a further 56 days with AngII (n=15); Group 3 was infused for a further 56 days with saline (n=13). An additional Group 4, without any infusion, was observed at 84 days. Infusion of AngII increased systolic blood pressure (SBP) by ~30 mmHg throughout the infusion period in Group 2. However, the removal of AngII led to an immediate return to baseline SBP in Group 3. Groups 1–3 exhibited increased aortic lumen dimensions at 28 days of infusion. Lumen dimensions were further increased during continued AngII infusion in Group 2, but remained at 28 days value in saline-infused Group 3 mice. At 28 days of AngII infusion, the expanded lumen was frequently associated with adventitial thickening, thrombus and macrophage infiltration (CD68+ cells). At 84 days of AngII infusion, profoundly dilated aortas were associated with extensive wall thinning and neomedialed areas comprised of CD68+ cells. Thrombus was not evident in AAs from mice infused with 84 days of AngII. Group 3 mice infused with saline also had thinned aortic walls with reduced thrombus; however, CD68+ cells were less prevalent. No abnormal pathology was noted in abdominal aortas of mice infused with saline for 84 days. Conclusion: Continuous AngII-infusion led to progressive expansion of the aortic lumen. These dilated AAs exhibited different features, including thinned walls, and profound macrophage infiltration than normal vessels.
In the present study we sought to determine whether Nox1 plays a role in the activation of redox-sensitive pathways leading to development of hypertension and cardiac hypertrophy in a model in which the endogenous renin-angiotensin system is chronically upregulated. The role of other Noxisoforms, such as Nox1, in chronic Ang II-dependent hypertension is unknown.

**Objective:** In this study we aimed to determine whether Nox1 contains NAD(P)H oxidase may not be important in hypertension and cardiac hypertrophy in a model in which the endogenous renin-angiotensin system is chronically upregulated. The role of other Nox isoforms, such as Nox1, in chronic Ang II-dependent hypertension is unknown.

**Methods and results:** Nox1-deficient mice and transgenic mice expressing Nox1 in the liver (TTHRN, Nox1-1 deficient) and TTHRN transgenic Nox1-1 deficient (TTHRN/Nox1-1). Blood pressure, cardiac mass, and cardiac fibrosis were increased in TTHRN/Nox1-1 versus controls. This was accompanied with increased activation of redox-sensitive signaling pathways leading to development of hypertension or cardiac hypertrophy in TTHRN/mice and p47phox translocation was no altered. Expression of Nox1 homologues, Nox2 and Nox4 was not different between groups. Phosphorylation of redox-sensitive growth signaling molecules such as Akt, p38MAPK, SAPK/JNK, ERK 1/2 and ERK5 was increased 2–3-fold in TTHRN mice. Akt, p38MAPK and SAPK/JNK phosphorylation was significantly reduced in TTHRN/Nox1-1 versus TTHRN mice (p < 0.05). Activation of the non-receptor tyrosine kinase c-Src was increased in TTHRN mice (2.5-fold) and attenuated in TTHRN/Nox1-1 mice. Conclusion: Our findings demonstrate that in TTHRN/Nox1-1 deficient mice activation of NA/P/H oxidase and phosphorylation of redox-sensitive signaling molecules are reduced. However, these effects are not associated with reduction of blood pressure and cardiac hypertrophy. These data suggest that Nox1-containing NA/P/H oxidase may not be important in hypertension and cardiac hypertrophy in a model in which the endogenous renin-angiotensin system is chronically upregulated.
and pDES implantation. Gene expression profiles were generated from five male donor LIMaS divided into three parts: non-stented control, BMS, or paclitaxel DES. Less than 10% of the probe sets were differentially expressed in the BMS and pDES groups, compared to control, and in pDES compared to the BMS. No more than 3% of all genes were differentially regulated among the three groups. Genes involved in cell growth were up-regulated in both BMS and pDES. However, paclitaxel DES displayed a pronounced effect in the cell cycle, with up-regulation of genes promoting cell cycle arrest. This suggests that paclitaxel DES may have a more profound effect on cell cycle arrest than BMS.

**Enos Gene Expression by Adenosine Prevents Angiostasis II-induced Vascular Smooth Muscle Cell Hypertrophy Through Selective Inhibition of the Rho/Rho-Kinase Pathway**

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Enhanced angiostasis II (AngII) actions are frequently associated with endothelial dysfunction, which is characterized as decreased nitric oxide (NO) availability. Although endothelial NO synthase (eNOS) is believed to antagonize vascular remodeling induced by the AT1 receptor, the exact signaling mechanism remains controversial. Therefore, we investigated a possible signal cross talk between eNOS and AT1 along with their impacts on vascular hypertrophy by using vascular smooth muscle cells (VSMCs) infected with adenovirus encoding the eNOS gene.

In VSMCs infected with eNOS adenovirus, basal G kinase activity was enhanced as detected by enhanced VASP Ser239 phosphorylation. AngII-activated VSMC hypertrophy as judged by protein synthesis as well as by cell volume was again markedly inhibited by eNOS adenovirus. These effects are accompanied with selective inhibition of the Rho/Rho-Kinase (ROCK) cascade. The downstream effectors of Rho and ROCK are involved in the regulation of cytoskeletal rearrangements and cell shape, which are known to contribute to the development of vascular hypertension. ROCK inhibitors have been shown to reduce vascular hypertrophy in experimental models. These studies demonstrate that eNOS adenovirus infection inhibited AngII-induced VSMC hypertrophy through selective inhibition of the Rho/ROCK pathway. These findings support the involvement of the eNOS/eNOS signaling pathway in the regulation of vascular hypertrophy.

**The Reversible Oral P2Y12 Antagonist AZD6140 Inhibits ADP-induced Contractions in Mouse Aorta in Addition to Established Inhibitory Effects on Platelet Aggregation**

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**Introduction.** Platelet P2Y12 receptor has long been used as a target for antithrombotic drugs of the thienopyridine family such as clopidogrel and ticlopidine, which are prodrugs whose bioactive metabolites bind irreversibly to P2Y12 receptor. AZD6140, a reversible oral P2Y12 antagonist with no requirement for metabolic activation, is being studied for its potential to prevent thrombotic events in patients with acute coronary syndromes (ACS). Clinically, AZD6140 showed greater inhibitory effects on platelet aggregation than clopidogrel, with a similar incidence of total bleeding events. P2Y12 receptors have also been shown on vascular smooth muscle cells (VSMC), where they mediate a contractile function after stimulation by ADP; this could contribute to local vasospasm and poorer outcomes in ACS. **Objective.** To elucidate if AZD6140, in contrast to clopidogrel, can act on VSMC and thereby inhibit ADP-mediated contractions. **Methods.** Nine female mice were used: 5 were pretreated with clopidogrel 50 mg/kg the day before, and 2 hours before the experiment; 4 were not pretreated. Thoracic aorta sections obtained from all mice were dissected from connective tissue and denuded. Ring segments (7 or 8 per mouse) were mounted into temperature-controlled tissue baths with physiological Krebs buffer. AZD6140 10 μM or DMSO 1:1000 as control was added; after 20 minutes, segments were precontracted with 10 nM norepinephrine, followed by the P2Y12 agonist 2-MeSAMP (10 μM). **Results.** Mean 2-MeSAMP-induced contraction (% maximal contraction induced by 60 mM K+ in clopidogrel-treated and untreated DMSO control groups was 64% and 59%, respectively; this was decreased to 32% (P<0.002) and 33% (P<0.015), respectively, with AZD6140. **Conclusion.** AZD6140 blocked ADP-induced vasoconstriction mediated by P2Y12 receptors in denuded mouse aortic rings, regardless of in vivo pretreatment with clopidogrel. This effect of AZD6140 could potentially modulate vasoactivity mediated by locally concentrated levels of ADP in vivo. Further investigation is required to examine this question.
Abundance and Plasticity of Circulating Stem Cells in Patients with Abdominal Aortic Aneurysm

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Background: Abdominal aortic aneurysms (AAAs) are characterised by progressive degenerative changes in the wall of the aorta, leading to a structurally weaker vessel which is at risk of potentially fatal rupture. Recently, we reported that aneurysmal rupture correlated with ablative changes in the wall of the aorta, leading to a structurally weaker vessel which is at risk of potentially fatal rupture.

Methods: Venous blood samples are collected from patients undergoing endovascular repair of AAA. CD133+ cells are isolated from peripheral blood mononuclear cells (PBMCs) using magnetic cell sorting of CD133 antibody labelled cells (Miltenyi Biotech). CD133+/34+ cells are plated onto Permanox coated Teflakides, for subsequent analysis. PBMC-conditioned medium was HUVEC MEM harvested, spun, filtered and aliquoted for further use. Plasma was collected and ELISA analysis performed using an R+ D System Sandwich ELISA kit (Quantikine).

Results: CD133+/34+ cell numbers are increased in patients in AAA compared to normals (2.43 versus 1.25P, p=0.008). Plasma VEGF concentration is increased compared to normals (33.2±0.36 vs 18.6±0.14, p<0.002).

Conclusions: The number of circulating CD133+/34+ stem cells are increased in patients with AAA. We have found that PBMC-conditioned medium optimises the growth conditions of CD133+ enriched cells. Mediator substances such as growth factors and cytokines secreted by the PBMCs promotes cultivation of these rare progenitor cells in preference to endothelial cell growth factor alone.