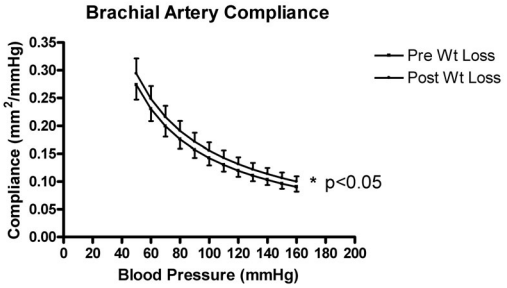
93.5±33.6 mg/dl, p<0.05), and insulin sensitivity (3.3±1.7 vs. 5.4±1.6 μU x 10^{-4} · min^{-1} · ml^{-1}, p<0.0001) following weight loss. Brachial artery compliance (p<0.05) and distensibility (p<0.05) curves over the physiological pressure range improved (figure), whereas endothelial function and intima-media thickness remained unchanged. **Conclusions:** In overweight adults, six months of weight loss resulted in improvements in body composition, insulin sensitivity, lipid profile, and brachial artery compliance and distensibility.



9:45 a.m.

865-6 **Nitric Oxide Synthase Gene Polymorphism (G894T) Influences Arterial Stiffness in Adults: The Bogalusa Heart Study**

Wei Chen, Sathanur R. Srinivasan, M. Gene Bond, Rong Tang, Elaine M. Urbina, Shengxu Li, Eric Boerwinkle, Gerald S. Berenson, Tulane University Health Sciences Center, New Orleans, LA, University of Texas-Houston Health Science Center, Houston, TX

Background: The endothelial nitric oxide synthase (eNOS) gene is known to influence the regulation of blood pressure levels. However, whether the eNOS gene locus influences arterial stiffness, independently of blood pressure, is unknown.
Methods: Arterial stiffness was measured in 118 black and 285 white young adults aged 25-37 years from M-mode ultrasounds of common carotid artery using Peterson's (Ep) and Young's (YEM) elastic modulus.
Results: Blacks displayed a lower frequency of the T allele than whites (0.131 vs 0.321, P<0.001). The T allele was associated with lower systolic blood pressure in blacks (P=0.04), but not in whites. Blacks showed significantly higher values of Ep (i.e. increased stiffness) than whites (49.9 kPa vs 45.5 kPa, P=0.003); whereas no such race difference was found for YEM, a measure of elasticity adjusted for relative wall thickness. After controlling for sex, age, BMI and mean arterial pressure, the T allele was associated with significantly lower values of Ep (P=0.012) and YEM (P=0.034) in blacks. Although similar trends were seen in whites, the genotype effect on Ep and YEM was not significant. In the total sample, including race as an additional covariate, the G894T genotype was associated with Ep (P=0.051) and YEM (P=0.038).
Conclusion: These results suggest that the G894T polymorphism at the eNOS gene locus is associated with lower arterial wall stiffness, adjusting for blood pressure levels, in asymptomatic young adults, especially in blacks.

ORAL CONTRIBUTIONS

867

Vascular Biology: Cell Signalling, Atherosclerosis, and Thromboembolism

Wednesday, March 10, 2004, 8:30 a.m.-10:00 a.m.
Morial Convention Center, Room 243

8:30 a.m.

867-1 **A Functional Role for Inducible Costimulator in Atherosclerosis**

Jacob George, Gad Keren, Tel Aviv Medical Center, Tel Aviv, Israel

Background: Lymphocytes appear to influence atherosclerosis by altering cytokine production. Whereas primary lymphocyte activation requires T cell receptor ligation, costimulatory signals also appear requisite for generation of a functional T cell response. Inducible costimulator (ICOS) is a newly discovered T cell molecule with a dual role in immune mediated disorders. Herein, we tested the importance of ICOS in atherosclerosis.
Methods and results: Atherosclerotic plaques from humans and ApoE-KO mice were studied immunohistochemically for the presence and localization of ICOS and its receptors and its expression in splenocytes. ApoE-KO mice were immunized with human ICOS/Fc-chimera or non-fused Fc and either provided a chow diet for 6 weeks, or a high fat diet for 8 weeks.
ICOS was abundantly expressed within plaques of humans and ApoE-KO mice and colocalized with CD3 cells whereas ICOS ligand was expressed in plaque macrophages. Spleen cells from atherosclerotic mice exhibited lowered constitutive expression of ICOS yet priming with oxLDL enhanced ICOS expression dose-dependently. In mice induced to develop fatty streaks and to generate ICOS blocking antibodies, early atherosclerosis was increased by ~77% whereas upon inducing more advanced lesions, the increase in plaque area upon ICOS blockade group was ~36%. IFN-gamma secretion by oxLDL-primed splenocytes in ICOS-immunized mice increased whereas IL-10 secretion diminished as compared to control animals. A similar trend in cytokine production was evident in the lesion by immunohistochemistry.
Conclusion: ICOS appears as an influential costimulatory pathway in atherosclerosis that may play a protective rather that a proatherogenic role.

8:45 a.m.

867-2 **Simvastatin Augments Rac Activation of Akt Signaling and Inhibits Endothelial Apoptosis by Modifying Subcellular Localization**

David Gregg, Neuza H. Lopes, Pascal J. Goldschmidt, Duke University Medical Center, Durham, NC

Background: HMG Co-A Reductase inhibitors (statins) inhibit atherosclerosis to a greater degree than would be predicted by lipid reduction alone.One focus has been on the ability of statins to block prenylation of the Rho GTPase Rac to inhibit its signaling. We **hypothesized** that rather than completely inhibiting Rac signaling, statins selectively modulate Rac by altering subcellular localization. **Methods and Results**: To test the hypothesis that unprenylated cytosolic Rac may activate signaling pathways unique from cellular membrane localized Rac, we overexpressed a constitutively active RacV12 mutant in human aortic endothelial cells and treated with simvastatin (25μM) or geranylgeranyl transferase inhibitor (GGTI-298 5μM). We found, using fractionation by ultracentrifugation and immunofluorescence, both inhibitors of prenylation altered subcellular localization, shifting RacV12 from 59% membrane localized to 2% and 4.1% with simvastatin and GGTI respectively. Prenylation inhibition had minimal effect on "Rac activity" determined by PAK-PBD affinity precipitation of total lysate and superoxide production but instead strongly shifted activity from the membrane to the cytoplasmic fraction. Untreated Rac V12 activated AKT, but simvastatin and GGTI treatment further increased p-AKT by two fold. Physiologically, this activation was mimicked by a two-fold inhibition of serum starvation induced apoptosis in Rac V12 cells treated with simvastatin or GGTI compared to RacV12 alone (Rac V12 25% decrease relative to CT, RacV12+simvastatin 62%, RacV12+GGTI 55%). In contrast, simvastatin and GGTI had no effect on apoptosis in quiescent adenoviral null control cells where there is minimal Rac activity. **Conclusions**: These data support a novel understanding of Rac signaling, subcellular localization, and its modulation by statins that may better explain the pleotropic effects of statins. Rather than globally inhibiting Rac, prenylation deficient Rac appears to become cytosolically distributed where it remains "active" and has a more potent ability to stimulate endothelial cell survival through AKT phosphorylation that may enhance plaque stability and protect from atherosclerosis.

9:00 a.m.

867-3 **Peroxynitrite Inactivates Akt Pathway and Enhances Tissue Factor Expression in Thrombin Stimulated Endothelial Cells**

Maria Berrozpe, Masato Eto, Hana Joch, Csaba Szabó, Thomas Felix Lüscher, Cardiovascular Research, Institute of Physiology, University of Zurich, Zurich, Switzerland, Inotek Pharmaceuticals Corporation, Beverly, MA

Background: Tissue Factor (TF) plays a pivotal role in thrombus formation in acute coronary syndromes. Peroxynitrite (NOO^-) modifies protein activity by nitration of certain amino acids. However the influence of peroxynitrite in TF expression and activity and the signaling pathways involved in this phenomena remains unclear. **Aim:** To elucidate the effect of NOO^- on TF and the role that Akt has in this effect. **Methods and Results**: Internal mammary artery endothelial cells were incubated with SIN-1 (NOO^- donor), FP15 (NOO^- catalyst), TEMPOL (superoxide scavenger)and wortmmanin (PI3K inhibitor) during 1 hour. Afterwards, thrombin was added. Ten minutes after thrombin stimulation Akt phosphorylation (measured by western blotting) decreased and 5 hours later, TF expression (measured by western blotting) and activity (Measured by American Diagnostica kit) increased (Figure A). Wortmannin and SIN-1 potenciated this effect. Not only potenciation of SIN-1 but also thrombin effect was reversed by coincubation with FP15 and TEMPOL. NOO^- staining by DCF demonstrated a significant increase in thrombin stimulated cells (Figure B, DCF:green, endothelial cell marker CD31: red, nuclei: blue). **Conclusions:** These data demonstrate an inhibitory effect of NOO^- in Akt phosphorilation that potentiates thrombin induced TF expression and activity in human endothelial cells. Thus, oxidative stress with formation of NOO^- from nitric oxide may be thrombogenic via this pathway.