Program Overview
The 32nd annual Vascular Research Initiatives Conference (VRIC), presented by the Society for Vascular Surgery®, is designed to encourage interaction and collaboration between vascular surgeon investigators and scientists from other vascular biology-related disciplines. An additional objective is to stimulate interest in research among trainees who are aspiring academic vascular surgeons. The VRIC is a one day conference preceding the main Arteriosclerosis, Thrombosis, and Vascular Biology (ATVB) Scientific Sessions on May 10-12, 2018.

Learning Objectives
At the end of the following sessions, participants should be able to:

Session I: Stem Cells and Regeneration
1. Discuss the current use of animal, cellular, and mathematical models elucidating the pathophysiology of stem cells and regeneration.
2. Identify new areas of basic and methodological research in stem cells and tissue engineering.
3. Describe new venues of current clinical and translational research in stem cells and regeneration.

Session II: Peripheral Arterial Disease
1. Discuss the current use of animal models elucidating the pathophysiology of peripheral arterial disease.
2. Identify new areas of basic and methodological research in peripheral arterial disease.
3. Describe new venues of current clinical and translational research in peripheral arterial disease.

Session III: Vascular Endothelium and Thrombosis
1. Discuss the current use of animal models elucidating the pathophysiology of thrombosis in both arterial and venous disease.
2. Identify new areas of basic and methodological research in thrombosis in both arterial and venous disease.
3. Describe new venues of current clinical and translational research in thrombosis in both arterial and venous disease.

Session IV: Vascular Inflammation and Injury
1. Discuss the current use of animal models elucidating the pathophysiology of vascular inflammation and injury.
2. Identify new areas of basic and methodological research in vascular inflammation and injury.
3. Describe new venues of current clinical and translational research in vascular inflammation and injury.

Target Audience
The Vascular Research Initiative Conference brings together vascular surgeons, vascular biologists, physicians with an interest in vascular problems, vascular surgery trainees, research trainees in vascular surgery and vascular biology, and industry personnel with an interest in vascular disease.

Accreditation
The Society for Vascular Surgery is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.
Designation of Credit
The Society for Vascular Surgery designates this live activity for a maximum of 7 AMA PRA Category 1 Credits™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.
VASCULAR RESEARCH INITIATIVES CONFERENCE:
ROAD TO INNOVATION, INVENTION AND ENTERPRISE

WEDNESDAY, MAY 9, 2018
8:00AM - 5:15PM

7:00 AM  REGISTRATION AND CONTINENTAL BREAKFAST

8:00 AM  INTRODUCTORY REMARKS
- Edith Tzeng, MD – Chair, Research and Education Committee
- Michel S. Makaroun, MD – President-Elect, Society for Vascular Surgery
- Alan Dardik, MD PhD - Chair, SVS Research Council

ABSTRACT SESSION I: STEM CELLS AND REGENERATION
Moderator: Katherine Gallagher, MD
Moderator: Philip S. Tsao, PhD FAHA, ATVB

8:15 am  Palmitate Regulates Diabetic Macrophage Inflammation via the Epigenetic Enzyme JMJD3
*Frank M Davis, Andrew Kimball, Amrita Joshi, Anna Boniakowski, Matthew Schaller, Aaron DenDekker, Steven Kunkell, Bethany Moore, Katherine Gallagher
Univ of Michigan, Ann Arbor, MI

8:27 am  Oral Nitrite Supplementation Improves Rates of Wound Healing in Diabetic Mice
*Karim M Salem, Nandan Nath, Ankur Aggarwal, Edith Tzeng
VA Medical Ctr and Univ of Pittsburgh, Pittsburgh, PA

8:39 am  Fatty Acid Binding Protein 4, FABP4, Causes Impaired Wound Healing in Diabetes
Anna Boniakowski, Andrew Kimball, Frank Davis, Amrita Joshi, Matt Schaller,
Aaron denDekker, Steve Kunkel, Katherine Gallagher
Univ of Michigan, Ann Arbor, MI

8:51 am  Defining the Mechanisms of Autologous Bone Marrow Cell Therapy in Critical Limb Ischemia
Ashley Gutwein, Bianca Kenyon, S. Keisin Wang, Linden Green, Michael Murphy
Indiana Univ Sch of Med, Indianapolis, IN

9:03 am  Muscle Ischemia Induces Nlrp3 Inflammasome Activation in Platelets via Tir4,
Promoting Platelet Aggregation and Interfering With Perfusion Recovery
Sebastian Vogel, Pranav Murthy, Xiangdong Cui, Bowen Xie, Ulka Sachdev
UPMC, Pittsburgh, PA
9:15 am  **Hyperglycemia Enhances Pro-inflammatory Properties Of Macrophage-derived Exosomes to Drive Hematopoiesis in Apolipoprotein E-deficient Mouse.**
Laura Bouchareychas, Allen Chung, David K Wong, Phat Duong, Robert L Raffai
Div of Vascular and Endovascular Surgery, Dept of Surgery, Univ of California San Francisco & VA Medical Ctr, San Francisco, CA

**SPECIAL SESSION I: ALEXANDER W. CLOYES DISTINGUISHED LECTURE**

9:30 am  Introduction: Ronald M. Fairman, MD, Chair, SVS Foundation
Moderator: John Curci, MD

Speaker: Alan Daugherty, PhD, DSc, FAHA

“Angiotensin II and cellular complexity of the aorta: A recipe for aneurysmal location”

10:10 AM  COFFEE BREAK

**ABSTRACT SESSION II: PERIPHERAL ARTERIAL DISEASE**

Moderator: Sherene Shalhub, MD
Moderator: Peter K. Henke, MD, ATVB

10:30 am  **Hydrogen Sulfide Limits the Development of Intimal Hyperplasia in a Mouse Model of Femoral Wire Injury and In Human Veins**
Alban Longchamp, Natsumi Yamagushi, Martine Lambelet, Celine Dubuis, Francois Saucy, Jean-Marc Corpataux, Florent Allagnat, Sebastien Déglise
CHUV, Lausanne, Switzerland

10:42 am  **Short-term Oral Supplementation with a Novel Marine Oil Fraction Alters Resolution Phenotype in Healthy Subjects and Patients with Peripheral Arterial Disease**
Melinda S Schaller¹, Mian Chen¹, Romain A Colas³, Thomas A Somentino¹, S Marlene Grenon¹, Esmond Dalli², Michael S Conte¹
¹UCSF, San Francisco, CA; ²William Harvey Res Inst, Queen Mary Univ, London, United Kingdom

10:54 am  **Role of PDE10A in Arterial Calcification**
Yujun Cai, Xue-lin Wang, Tonghui Lin, Raul J Guzman
Beth Israel Deaconess Medical Center, Boston, MA

11:06 am  **Genome Wide Association Study in the Million Veteran Program Identifies a Novel Role for Thrombosis in the Pathogenesis of Peripheral Artery Disease**
Derek Klarin¹, Julee Lynch², Krishna Aragam³, Tim Assimes³, Kyung Lee⁴, Qing Shao⁴, Mark Chaffin⁵, Pradeep Natarajan⁶, Shipra Arya³, Aeron Small⁶, Yan V Sun⁷, Jennifer S Lee⁸, Donald Miller⁹, Peter Reaven⁹, Scott DuVall¹⁰, William Boden¹¹, J. Michael Gaziano¹², John Concato¹³, Sekar Kathiresan¹, J. Rader¹⁴, Kelly Cho¹²,
Peter W Wilson7, Kyong-Mi Chang14, Christopher J O’Donnell22, Phil S Tsao3, Scott M Damrauer14

11:18 am  Characterization of Peripheral Artery Disease Severity by Near-Infrared Spectroscopy
Matthew Fuglestad1, Hernan Hernandez1, Henamari Ybay2, Molly Schieber1, 2, Katya Brunette1, Yue Gao1, Mahdi Hassan2, Sara Myers2, George Casale1, Iraklis Pipinos3
1Univ of NE Medical Ctr, Department of Surgery, Omaha, NE; 2Univ of NE Omaha, Department of Biomechanics, Omaha NE

11:30 am  Circulating Exosomes from PAD Patients Modulate Vascular Repair and Inflammation
Thomas A Sorrentino1, Phat Duong1, Laura Bouchareychas1, Brian E Sansbury2, Pete Mitchell2, Mian Chen1, Allen Chung1, Melinda Schaller1, Matthew Spite2, Robert L Raffai1, Michael S Conte1
1Univ of California, San Francisco, San Francisco, CA; 2Brigham and Women’s Hosp, Boston, MA

NOON  LUNCH

1:00 PM  SVS FOUNDATION UPDATE AND AWARDS CEREMONY
Ronald Fairman, MD, SVS Foundation Chair

SVS Foundation VRIC Trainee Travel Scholarship Awards
Frank M Davis
Catherine Go
Omar Saffaf
Karim M Salem

SPECIAL SESSION II

1:10 PM  SVS FOUNDATION MENTORED CLINICAL SCIENTIST RESEARCH CAREER DEVELOPMENT AWARD (K08) – 2016 RECIPIENT
Karen Ho, MD
Northwestern University Feinberg School of Medicine
Project Title: The Role of Gut Microbiota in Neointimal Hyperplasia after Vascular Injury
ABSTRACT SESSION III: VASCULAR ENDOTHELIUM AND THROMBOSIS

Moderator: Joseph Raffetto, MD
Moderator: Naomi Hamburg, MD MS FACC, ATVB

1:20 PM  
PARP-1 Silencing Upregulates FOSL1 Transcription, Enhances Angiogenesis and Accelerates Ischemic-Diabetic Wound Healing  
Jaideep Banerjee, Divya Cheedu, Raul Sebastian, Robyn Mascata, Anton Sidawy, Lopa Mishra, Bao Nguyen,  
George Washington Univ, Washington, DC

1:32 PM  
Clinical and Genetic Determinants of Varicose Veins: A Prospective, Community-Based Study of ~500,000 Individuals  
Alyssa M Flores1, Eri Fukaya1, Daniel Lindholm2, Stefan Gustafsson2, Daniela Zanetti1, Erik Ingelsson1, Nicholas J. Leeper1  
1Stanford Univ Sch of Med, Stanford, CA; 2Uppsala Univ, Uppsala, Sweden

1:44 PM  
Fenofibrate Induces Endothelial Cell Tubule Formation Independent of Phospholipogenesis  
*Omar Saffaf, Larisa Belaygorod, Khalid Saffaf, Clay F. Semenkovich, Mohamed Zayed  
Washington Univ Sch of Med, St Louis, MO

1:56 PM  
Real-time Modulation of Platelet Phenotype and Vein Wall Biology in Patients with Chronic Venous Insufficiency  
Doran S Mix, Zane Young, Sandra Toth, Rachel Schmidt, Adam J Doyle, Jennifer L Ellis, Michael C Stoner, Igor Gosev, Sunil Prasad, Peter Knight, Sara Ture, Craig Morrell, Scott J Cameron  
Univ of Rochester Medical Ctr, Rochester, NY

2:08 PM  
Vascular Injury-Induced ATP release leads to Inflammation and Endothelial Dysfunction  
Joyce Cheung-Flynn, Weifeng Luo, Christy M Guth, Padmini Komalavilas, Colleen M Brophy  
Vanderbilt, Nashville, TN

2:20 PM  
Application of a Cryogel-Coated Prosthetic Vascular Graft Material for Delivery of Targeted Gene Therapies in a Rabbit Model  
Cindy Huynh1, Ting Shih2, Mauricio Contreras1, David Mooney2, Leena Pradhan-Nabzdyk1, Frank LoGerfo1  
1Beth Israel Deaconess Medical Ctr, Boston, MA; 2Harvard Univ, Cambridge, MA

TRANSLATIONAL PANEL: ROAD TO ENTREPRENEURSHIP

2:32 pm  
Moderator: Michel S. Makaroun, MD
Moderator: Luke Brewster, MD
- Speaker: Colleen M. Brophy, MD “Transmogrifying Scientific Knowledge into Technologic Advancements”
- Speaker: Mary Albertson, “In the Middle, Between Science and Development”
- Speaker: Geoffrey C. Gurtner, MD, FACS, “Other People’s Money: Getting the Resources to Do Stuff”

3:40 PM          COFFEE BREAK

ABSTRACT SESSION IV:  VASCULAR INFLAMMATION AND INJURY

Moderator: Mohamed Zayed, MD
Moderator: Lars Maegdefessel, MD PhD, ATVB

4:00 PM  Efficacy and Mechanisms of Metformin Therapy in Established Experimental Abdominal Aortic Aneurysms
Baohui Xu1, Gang Li1, Fanru Shen1, Trevor Weden1, Anna Cabot1, Hongping Deng1, Xiaofeng Chen2, Ronald L Dalman1
1Stanford University School of Medic, Stanford, CA; 2Whenzhou Medical Univ Taizhou Hosp, Linhai, China

4:12 PM  Retrograde Hemorrhage and Malperfusion Injury after REBOA in a Porcine Model of Uncontrolled Aortic Injury
*Catherine Go, Partha Thirumala, Jenna Kuhn, Yanfei Chen, Youngjae Chun, Bryan Tillman
Univ of Pittsburgh Med Ctr, Pittsburgh, PA

4:24 PM  Cell Mimetic Liposomal Nanocarriers Tailored for Vascular Smooth Muscle Cell Molecular Therapeutics
Samuel I Mattem-Schain1, Richard K Fisher2, Lauren B Grimsley2, Stacy S Kirkpatrick2, Oscar H Grandas2, Michael D Best1, Deidra J Mountain1
1Univ of Tennessee, Knoxville, TN; 2UT Graduate School of Medicine, Knoxville, TN

4:36 PM  Increased Plasma Sulfide in Vascular Surgery Patients Correlates with Reduced Post-Operative Mortality
Kaspar Trocha1,2, Alban Longchamp3, Christopher Hine4, Michael MacArthur2, Janine Ganah5, Peter Kip1, Ming Tao1, Peter Nagy6, C. Keith Ozaki1, James R. Mitchell5
1Brigham & Women's Hosp, Boston, MA; 2Harvard Sch of Public Health, Boston, MA; 3Dept of Vascular Surgery, Lab of Experimental Med, Ctr Hospier Univire Vaudois, Lausanne, Switzerland; 4Cleveland Clinic, Cleveland, OH; 5Molecular Immunology and Toxicology, Natl Inst of Oncology, Budapest, Hungary

4:48 PM  Parthenolide Inhibits Inflammatory Dysfunction of Human Aortic Endothelial Cells and Proliferation of Smooth Muscle Cells in vitro and Restenosis in a Rat Model
Bowen Wang1, Mengxue Zhang1, Xudong Shi2, Lian-Wang Guo1, Craig Kent3
1The Ohio State Univ, Columbus, OH; 2Univ of Wisconsin-Madison, Madison, WI

5:00 PM  Targeted Nanotherapy for the Treatment of Atherosclerosis
Neel A Mansukhani1, Miranda So1, Mazen S Albaghdadi1, Erica B Peters2, Zheng Wang1, Samuel I Stupp1, Melina R Kibbe1, 2
1Northwestern Univ, Chicago, IL; 2Univ of North Carolina at Chapel Hill, Chapel Hill, NC

5:15 PM  RECEPTION AND POSTERS

7:00 PM  VRIC CLOSE

* SVS Foundation Vascular Research Initiatives Trainee Travel Scholarship Awardee
Course Director

Edith Tzeng, MD
*University of Pittsburgh, Pittsburgh, PA*

Faculty

Mary Albertson, CLP, RTTP
*Stanford University, Stanford, CA*

Luke P. Brewster, MD, PhD
*Emory University, Atlanta, GA*

Colleen M. Brophy, MD
*Vanderbilt University Medical Center, Nashville, TN*

John Curci, MD
*Vanderbilt University Medical Center, Nashville, TN*

Alan Dardik, MD, PhD
*Yale University, New Haven, CT*

Allan Daugherty, PhD, DSc, FAHA
*University of Kentucky, Lexington, KY*

Ronald M. Fairman, MD
*University of Pennsylvania Health System Program, Philadelphia, PA*

Katherine Gallagher, MD
*University of Michigan, Northville, MI*

Geoffrey C. Gurtner, MD, FACS
*Sandford University, Sandford, CA*

Peter Henke, MD
*University of Michigan, Ann Arbor, MI*

Naomi Hamburg, MD, MS, FACC
*Boston University, Boston, MA*

Karen J. Ho, MD
*Northwestern University, Chicago, IL*

Lars Maegdefessel, MD, PhD
*Karolinska Institute, Stockholm, Sweden*

Michel S. Makaroun, MD
*University of Pittsburgh, Pittsburgh, PA*

Joseph Raffetto, MD
*VA Boston Health Care System, West Roxbury, MA*

Sherene Shalhub, MD
*University of Washington, Seattle, WA*

Philip S. Tsao, PhD
Stanford University School of Medicine, Palo Alto, CA
Mohamed Zayed, MD
Washington University School of Medicine, St. Louis, MO
2018 SVS – Vascular Research Initiatives Conference

FINANCIAL DISCLOSURES
As an accredited sponsor of the Accreditation Council for Continuing Medical Education (ACCME), SVS must ensure balance, independence, objectivity and scientific rigor in all its individually sponsored educational activities. In accordance with ACCME guidelines and standards, all faculty participating in an accredited activities must disclose to the audience any significant financial interest or other relationship with (1) the manufacturer(s) of any commercial product(s) and/or provider(s) of commercial services discussed in an educational presentation and (2) any commercial supporters of the activity. (Significant financial interest or other relationships can include such things as grants or research support, employment, consultant, major stockholder, member of speakers’ bureau, etc.) Listed below are the disclosures provided by the faculty for this meeting.

<table>
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<th>Financial Relationship</th>
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<tr>
<td>Mary Albertson, CLP, RTTP</td>
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<td>Luke P. Brewster, MD, PhD</td>
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<td>Colleen M. Brophy, MD</td>
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<td>John Curci, MD</td>
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<td>Alan Dardik, MD, PhD</td>
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<td>Allan Daugherty, PhD, DSc, FAHA</td>
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<td>Ronald M. Fairman, MD</td>
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<td>Katherine Gallagher, MD</td>
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<td>Geoffrey C. Gurtner, MD, FACS</td>
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<td>Peter Henke, MD</td>
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<td>Naomi Hamburg, MD, MS, FACC</td>
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<td>Karen J. Ho, MD</td>
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<td>Michel S. Makaroun, MD</td>
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<td>Mahmoud Malas, MD</td>
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<td>Gale L. Tang, MD</td>
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The Education Council has oversight of all SVS programs for continuing education for practicing vascular surgeons.

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<td>Rabih Chaer, MD</td>
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<td>Ravi Rajani, MD</td>
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<td>Amy Reed, MD</td>
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<tr>
<td>Megan Mathy</td>
<td>CME Staff</td>
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Chair
Edith Tzeng, MD
*University of Pittsburgh, Pittsburgh, PA*

Members

Shipra Arya, MD
*Emory University School of Medicine, Atlanta, GA*

Luke P. Brewster, MD, PhD
*Emory University School of Medicine, Atlanta, GA*

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Katherine Gallagher, MD
*University of Michigan, Northville, MI*

Karen J. Ho, MD
*Northwestern, Chicago, IL*

Linda Le, MD
*Houston Methodist, Houston, TX*

Louis Nguyen, MD
*Brigham and Women’s Hospital, Boston, MA*

Mahmoud Malas, MD
*John Hopkins University, Baltimore, MD*

Joseph D. Raffetto, MD
*VA Boston Health Care System Vascular Surgery, West Roxbury, MA*

Sherene Shalhub, MD
*University of Washington, Seattle, WA*

Gale L. Tang, MD
*University of Washington School of Medicine, Seattle, WA*

Mohammed Zayed, MD
*Washington University School of Medicine, St. Louis, MO*
ABSTRACT SESSION I:
STEM CELLS AND REGENERATION
Palmitate Regulates Diabetic Macrophage Inflammation via the Epigenetic Enzyme JMJD3

Frank M Davis, Andrew Kimball, Amrita Joshi, Anna Boniakowski, Matthew Schaller, Aaron DenDekker, Steven Kunkell, Bethany Moore, Katherine Gallagher, Univ of Michigan, Ann Arbor, MI

Macrophage (Mφ) plasticity, allowing for transition of Mφs from an inflammatory to a reparative phenotype, is critical for normal wound healing. In pathologic conditions, such as type 2 diabetes (T2D), wounds fail to heal due to impaired resolution of inflammation. The mechanism(s) responsible for the persistent inflammatory phenotype in T2D wounds are unclear. Prior studies have shown that the Toll-like receptor (TLR) 4 pathway regulates Mφ-mediated inflammation in tissues. Growing evidence indicates that TLR4 is a versatile receptor binding a spectrum of ligands including non-microbial ligands such as saturated fatty acids (SFAs). Given the excess SFAs in T2D, the purpose of this study was to examine the role of the SFA palmitate on TLR4 signaling and Mφ phenotype in diabetic wound healing. We have previously shown that Mφs isolated from wounds in a murine model of glucose intolerance (diet-induced obesity; DIO) maintained on a 60% high fat diet (HFD) for 12-18 weeks, display increased levels of inflammatory cytokines (IL-1β, IL-12, and TNFα) at both a gene expression and protein level. In the current study, we found that blood monocytes and wound Mφs from DIO mice display increased TLR4 receptors compared to control blood and wounds. To determine if altered metabolites in the diabetic environment impact Mφ phenotype, bone marrow derived macrophages (BMDMs) were incubated in serum isolated from DIO or control mice. BMDMs incubated with DIO serum displayed a hyperinflammatory response following LPS stimulation. Further, stimulation with the metabolite palmitate produced significantly increased IL-1β expression in DIO BMDMs compared to controls suggesting that DIO BMDMs are programmed toward an inflammatory response. To determine the mechanism, we examined several epigenetic enzymes known to affect Mφ polarization and found that palmitate stimulated expression of JMJD3, a histone demethylase, which increases inflammatory gene expression. In conclusion, these studies suggest that the diabetic milieu, specifically increased levels of the SFA palmitate, induces expression of the epigenetic enzyme, JMJD3, in Mφs and this regulates inflammatory gene expression and cell function.

Oral Nitrite Supplementation Improves Rates of Wound Healing in Diabetic Mice

Karim M Salem, Nandn Nath, Ankur Aggarwal, Edith Tzeng, VA Medical Ctr and Univ of Pittsburgh, Pittsburgh, PA

Introduction: Nitric oxide (NO) is required for cutaneous wound healing. Impaired diabetic wound healing has been linked to a deficiency in local NO production and can be enhanced with NO delivery. NO is a short-lived, highly reactive molecule and local delivery is complicated by these properties. An alternate source of NO can be achieved through the ability of nitrite reductases to convert the stable NO end-product, nitrite, back to NO. We have previously demonstrated that skin and wound edge express high levels of xanthine oxidoreductase (XOR). XOR is a strong nitrite reductase. We hypothesize that dietary nitrite supplementation will improve wound healing in diabetic mice. Methods: Db/db mice (N>8/group) were pretreated for 1 week with sodium nitrite supplemented drinking water (50 mg/L), nitrite-free chow, or standard chow. Additionally, nitrite supplemented mice were gavaged with nitrite supplemented water (0.2 ml) every other day for the duration of the experiment. A 1 cm² excisional wound was created on the back of each mouse. Wounds were photographed every other day until closure and wound areas calculated with ImageJ and compared with ANOVA and Kaplan Meier. Results: Time to complete healing was significantly different between nitrite supplemented (NS), nitrite depleted (ND), and control mice (18.9±0.7, 21.6±3.3, and 23±3.5 days, respectively, P = 0.035). NS mice reached 75% and 100% healed faster than ND or control mice (P = .009 and P = .042, respectively) [Figure]. Initial wound expansion on day 2 was decreased in NS mice compared to ND mice. XOR activity was increased in wounds compared to skin but similar between all treatment groups. Conclusion: Nitric oxide is a critical component of wound healing. Oral systemic nitrite supplementation improves diabetic wound healing in mice. Dietary nitrite may be an inexpensive and safe method of augmenting NO production through wound XOR expression to improve wound repair.

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Fatty Acid Binding Protein 4, FABP4, Causes Impaired Wound Healing in Diabetes

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Wound healing in diabetes is impaired due to failed resolution of inflammation. Macrophages play a significant role in the establishment of a regulated inflammatory response during wounding. Macrophage function is dictated by metabolism, which alters gene expression. Recent studies suggest that a fatty acid binding protein, FABP4, may control macrophage function in diabetes by altering metabolism. Thus, we examined whether FABP4 controls macrophage function and hence inflammation in diabetic wound healing. To investigate this, C57BL/6 mice were fed either a normal (12% saturated fat) diet or a high-fat (60% saturated fat) diet (HFD) for 12 weeks to induce physiologic “pre-diabetes.” Wounds were created and CD3-CD19-NK1.1-CD11b+ cells (macrophages) were isolated each day following injury and FABP4 expression was quantified by qPCR and Western blot. We found that HFD wound macrophages demonstrated a significant increase in FABP4 gene expression and protein production on day 3 post-injury compared with controls.

To determine if FABP4 alters inflammatory gene expression in wound macrophages, we isolated wound macrophages with an FABP4 inhibitor, treated them, and analyzed for IL-1β and TNFα expression. IL1β and TNFα gene expression were significantly reduced (P<0.01) in diabetic wound macrophages treated with the FABP4 inhibitor, suggesting that inflammatory gene expression can be controlled in diabetic wound macrophages through FABP4 modulation. As we have previously identified, epigenetic mechanisms often dictate gene expression during wound healing, thus we examined whether FABP4 expression in diabetic macrophages was regulated by histone modifications. Chromatin immunoprecipitation (ChIP) analysis of the FABP4 promoter in wound macrophages revealed a significant increase in H3K4 trimethylation, an activating mark, on the FABP4 promoter in diabetic wound macrophages suggesting that epigenetic regulation may play an important role in the differential expression of FABP4 in diabetic wounds.

In conclusion, FABP4 appears to be upregulated in diabetic wound macrophages and contributes to increased macrophage inflammation. Modulation of FABP4 or its expression may help resolve inflammation in diabetic wounds and promote healing.

Defining the Mechanisms of Autologous Bone Marrow Cell Therapy in Critical Limb Ischemia

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Introduction. Here we present a composite of proteomic, cellular, and radiological analyses that define the mechanisms by which autologous concentrated bone marrow mononuclear cells (cBMNC) promote limb preservation in patients with critical limb ischemia. Methods. CD45+, CD34+, CD105+, and VEGFR-2+ cells were enumerated using fluorescent activated cell sorting (FACS) from aliquots of the cBMNC from each patient enrolled in the Phase III MOBILE TRIAL. Direct limb perfusion was measured with Positron Emission Tomography/Computed Tomography (PET/CT) with radiolabeled water (15H2O). Anterior tibialis muscle (ATM) into which cBMNC was injected prior to below knee amputation in the Phase I CHAMP trial were collected for capillary density and proteomic analyses. Results. There were no differences in the number of CD45+ (636 ± 388 vs. 868 ± 699 x 10⁶, p= 0.279), VEGFR-2+ (0.4 ± 0.8 vs. 0.3 ± 0.6 x10⁶, p=0.757) and CD34+ (21 ± 13 vs. 35 ± 30 x 10⁶, p= 0.156) cells in the cBMNC product injected in those patients undergoing amputation and those with a preserved limb (n=90). There was a significant association between CD105+ (7 ± 4 vs. 16 ± 13 x 10⁶, p= 0.05) cells in patients and freedom from amputation. A Blood Perfusion Index (BPI) was calculated by comparing the ratio of H2O15 peak tracer uptake level of the untreated: treated leg with an increase from 0.38 at baseline to 0.54 (42%) at 12 weeks (n=4, p< 0.05). There was an increase in CD31+ capillaries in the ATM after injection of cBMNC. ATM specimen also showed increases in VEGF-A, angiopoietin-2, and MMP-9 compared to the untreated specimen. Conclusion. This first in man analyses provides conclusive evidence that cBMNC improves limb perfusion via capillary formation. This study suggests that bone marrow cell mediated angiogenesis may be dependent on CD105+ mesenchymal progenitor cells.

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Muscle Ischemia Induces Nlrp3 Inflammasome Activation in Platelets via Tlr4, Promoting Platelet Aggregation and Interfering With Perfusion Recovery

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Introduction: Angiogenesis is an adaptive response to chronic ischemia, but is deficient in peripheral arterial disease (PAD). We have recently shown that the pattern recognition receptors toll-like receptor 4 (TLR4) and nucleotide-binding domain leucine rich repeat containing protein 3 (NLRP3) expressed by platelets control aggregation and thrombosis. The role of platelet TLR4 and NLRP3 in PAD is unexplored.

Methods: Unilateral femoral artery ligation (FAL) was performed in transgenic mice with platelet-specific ablation of TLR4 (TLR4 PF4) and in global NLRP3 knockout (NLRP3 KO) mice. Platelet NLRP3 inflammasome activation was monitored by caspase-1 activation (fluorescent labeled inhibitor of caspase-1, FLICA) and cleavage of IL1β (Western blot). Platelet aggregation was evaluated with impedance aggregometry. Laser Doppler perfusion imaging (LDPI) verified perfusion in the ischemic (right) and non-ischemic (left) limb over time. Angiogenesis and myoblast regeneration were measured histologically.

Results: Platelet NLRP3 inflammasome activity was significantly upregulated following FAL (p<0.001), and reversed with TAK242, a TLR4 inhibitor. FAL significantly increased aggregation of circulating platelets, which was significantly suppressed in TLR4 PF4 (p<0.01) and NLRP3 KO mice (p<0.001). Down-regulation of platelet aggregation and caspase-1 activity in TLR4 PF4 mice was nearly completely reversed by nigericin, a NLRP3 activator. Ischemic limb perfusion (Fig 1A) was significantly higher in TLR4 PF4 (Fig1B, p<0.05) and NLRP3 KO mice (Fig1C, p<0.001) than in controls 14d after FAL. Angiogenesis and regeneration were significantly improved in TLR4 PF4 mice (p<0.05).

Conclusion: We show that the platelet NLRP3 inflammasome is activated in muscle ischemia via platelet TLR4, which upregulates platelet aggregation and deters recovery from FAL. Thus, platelet TRL4/NLRP3 activation may be a therapeutic target to improve limb salvage in PAD.

S. Vogel: None. P. Murthy: None. X. Cui: None. B. Xie: None. U. Sachdev: None.
Hyperglycemia Enhances Pro-inflammatory Properties of Macrophage-derived Exosomes to Drive Hematopoiesis in Apolipoprotein E-deficient Mouse

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Background and Purpose:
Diabetes is recognized to enhance the frequency and severity of atherosclerosis and cardiovascular disease. Recent studies have shown that hyperglycemia is associated with enhanced hematopoiesis and macrophage accumulation in atherosclerotic lesions. We explored whether high glucose concentrations can enhance intercellular communication between mature macrophages and hematopoietic progenitors via exosomes to promote inflammation and diabetic atherosclerosis.

Methods: Bone marrow derived macrophages (BMDM) from C57BL/6 mice were cultured with normal (5mM) or high glucose concentrations (25mM). Exosomes were isolated with our cushioned-density gradient ultracentrifugation method followed by nanoparticle tracking and western blot analysis. Pro-inflammatory properties of high glucose exosomes (HGexo) were tested in vitro by exposing them to BMDM cultured in normal low glucose. The capacity for BMDM-derived exosomes to alter systemic and vascular inflammation were next tested by infusing 25-30 weeks-old ApoE/− mice fed a chow diet with 3 x 10¹⁰ exosomes three times a week, for four weeks.

Results: Our data show that HGexo can stimulate the expression of inflammatory cytokines (IL-6, IL-1β) as well as NADPH oxidases (Nox-1 and Nox-4) in cultured BMDM. Furthermore, our findings show that intraperitoneally injected exosomes distribute to numerous organs and tissues including the bone marrow and the spleen. Lastly, HGexo enhance the expansion of multipotent and lineage committed hematopoietic progenitors.

Conclusions: We identify that exosomes derived from cultured BMDM exposed to high glucose have the capacity to exert intercellular communication in vitro, and in vivo. Our findings suggest that exosomes produced by macrophages exposed to hyperglycemia could represent an unsuspected source of inflammation to accelerate atherosclerosis in diabetes.

ABSTRACT SESSION II:
PERIPHERAL ARTERIAL DISEASE
Hydrogen Sulfide Limits the Development of Intimal Hyperplasia in a Mouse Model of Femoral Wire Injury and in Human Veins

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Objectives: Mainstays of contemporary therapies for this arterial occlusive disease include angioplasties, stents, endarterectomies and bypass surgery. However, these treatments suffer from high failure rates due to re-occlusive vascular wall adaptations, namely intimal hyperplasia (IH). IH develops in response to endothelium injury, leading to inflammation, vascular smooth muscle cells (VSMC) dedifferentiation, migration and proliferation at the site of injury. Hydrogen sulfide (H₂S) is a ubiquitous signaling gazotransmitter, which exhibits antioxidant, anti-inflammatory, and vaso-relaxant properties. Thus, we hypothesized that H₂S could reduce IH formation.

Methods: WT male C57BL6/J mice submitted to femoral wire injury surgery to induce IH were treated with an H₂S donor (NaHS) in the drinking water. IH was measured 28 days post-surgery by histology. In addition, segments of great saphenous vein obtained from patients undergoing bypass surgery were maintained in culture ex-vivo for 7 days in presence of various H₂S donors (NaHS, GYY4137, diacyltrisulfide). Finally, primary human umbilical vein endothelial cells (HUVEC) and primary human VSMC were treated in-vitro with the same H₂S donors to study cellular proliferation and migration.

Results: NaHS treatment significantly reduced IH development in the mouse model of femoral wire injury (Figure 1). Similarly, the various H₂S donors prevented the development of IH in vein segments ex-vivo. In vitro, the same H₂S donors stimulated human endothelial cells (HUVEC) migration and proliferation, while inhibiting migration and proliferation of primary VSMC (Figure 2).

Conclusions: Exogenous H₂S prevents IH formation in mice in-vivo and in human veins ex-vivo. Importantly, H₂S reduces VSMCs but stimulates ECs proliferation and migration. These data suggest that exogenous H₂S therapy could be used in human to minimize IH, thus limiting vascular reconstruction failure.
Short-term Oral Supplementation with a Novel Marine Oil Fraction Alters Resolution Phenotype in Healthy Subjects and Patients with Peripheral Arterial Disease

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Objectives:
Peripheral arterial disease (PAD) is a chronic disease characterized by systemic inflammation. Recent work suggests that the resolution of inflammation is orchestrated by specialized pro-resolving lipid mediators (SPM), largely derived from n-3 PUFA. We hypothesize that PAD is associated with defective resolution, and is modifiable via oral intake of marine lipid fractions enriched for SPM.

Methods:
In an oral dose finding study, 10 PAD subjects and 10 healthy subjects received three escalating doses (1.25, 2.5, and 5 g/d) of a novel marine lipid supplement for 5-day periods over 1 month. The RBC content of n-3 PUFA, the omega-3 index (O3I), was measured. We profiled plasma lipid mediators, phagocytic activity of PMN and monocytes (Mo) to E.coli, Mo surface markers, and Mo-derived macrophage (MDM) gene expression.

Results:
Compared to baseline, all subjects had an increase in the ratios of n-3 PUFA:arachidonic acid (P<0.00005) and SPM:prostaglandins (P=0.08) in plasma, an increase in the DPA-derived maresins (P=0.01), and an increase in the O3I (24%, P<0.0001). In the PAD cohort, there was an increase in the DHA-derived resolvins (P=0.09). Mo phagocytosis increased (P=0.02) after treatment and correlated with increase in O3I (r=0.45, P=0.055). PMN phagocytosis also increased (P=0.003) post-supplementation. We observed decreased expression of the Mo adhesion molecule CD18 (P<0.00005), and the scavenger receptors CD163 (P=0.0006) and CD36 (P=0.0001). Within the PAD cohort, Mo expression of ICAM-1 (P=0.003) and CCR2 (P=0.006) were decreased. A decrease in MDM gene expression of iNOS and MCP-1, both associated with M1 phenotype, and an increased expression of MRC1, a M2 marker, was observed.

Conclusion:
Short-term, oral supplementation with a novel marine oil fraction increased plasma SPM levels, increased the phagocytic activity of Mo and PMN, decreased the expression of Mo surface markers associated with systemic inflammation and atherosclerosis, and promoted a resolution phenotype in MDM. Collectively these data demonstrate a basis for further studies of oral SPM supplementation on inflammation and resolution pathways in patients with PAD.

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Role of PDE10A in Arterial Calcification

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Vascular calcification is highly prevalent in patients with diabetes mellitus and chronic kidney disease. When located in the media, arterial calcification is strongly associated with increased cardiovascular morbidity and mortality. The second messenger cyclic nucleotides cAMP and cGMP, controlled by distinct cyclic nucleotide phosphodiesterase (PDE) isozymes, play important regulatory roles in a variety of human diseases. Using a qPCR PDE array, we found that PDE10A was the most highly induced among all PDE genes in a rat model of medial artery calcification. PDE10A expression was markedly increased in calcified arteries from rats with chronic kidney disease and in tibial arteries from patients with peripheral artery disease. Interestingly, it co-localized with osteogenic markers in these specimens. In vitro, PDE10A knockdown using siRNA, and inhibition with a synthetic inhibitor markedly reduced osteogenic transformation and calcification of vascular SMC exposed to high phosphate levels. Aortic rings from PDE10A knockout mice showed significantly less Pi-induced medial calcification than those from wild-type controls. Deficiency of PDE10A also reduced medial calcification in a mouse medial calcification model in vivo. Mechanistic studies to elucidate the signaling alterations invoked by PDE10A are ongoing. These findings suggest that PDE10A plays a crucial role in the development of medial artery calcification, and that targeting it may provide a novel therapeutic strategy for reducing medial calcification and improving outcomes in patients with PAD.

Y. Cai: None. X. Wang: None. T. Lin: None. R.J. Guzman: None.
Genome Wide Association Study in the Million Veteran Program Identifies a Novel Role for Thrombosis in the Pathogenesis of Peripheral Artery Disease


Introduction: PAD is a leading cause of cardiovascular morbidity and mortality. Previously published GWAS have been limited by small sample sizes and have only identified 3 genome-wide significant (P<5x10^-8) risk loci to date. Hypothesis: DNA sequence variants affecting multiple biological pathways are associated with PAD risk. Methods: Using electronic health record data, we identified individuals with and without clinical PAD from the 353,323 Million Veteran Program (MVP) participants genotyped on a customized Affymetrix Biobank array. We tested 32 million genotyped and imputed DNA variants for association with clinical PAD separately in participants of European (EUR), African (AFR), and Hispanic (HIS) ancestry using logistic regression models controlling for age, sex and population structure, and then performed trans-ethnic meta-analysis. The results were replicated with data from the UK Biobank. Results: We identified 31,307 individuals (24,009 EUR, 5,373 AFR, 1,925 HIS) with, and 211,753 individuals without, PAD. Following meta-analysis and replication, there were 19 genome-wide significant risk loci associated with PAD. We replicated a known association at 9p21 (P=4.3 x10^-39), and identified several novel loci associated with PAD that were previously known to be associated with atherosclerosis (LPA, HDAC9), diabetes (TCF7L2), lipid levels (LPL, CELSR2), and tobacco use (CHRNA3). We also identified a novel association with PAD for the Factor V Leiden (FVL) mutation (OR 1.20, P=1.6x10^-12), which remained significant after controlling for venous thromboembolism (OR 1.10, P=8.7x10^-4). Sensitivity analysis demonstrated FVL is associated with increasing risk estimates for PAD severity (claudication OR 1.19, P=0.0012; rest pain OR 1.41, P=0.004; tissue loss OR 1.57, P=7x10^-9). Conclusions: Using the MVP, we assembled the largest reported cohort of individuals with clinical PAD and genetic data worldwide. Our data replicate known causal risk factors and identify a novel association for FVL and its putative role for thrombosis in the development of clinical PAD.

Characterization of Peripheral Artery Disease Severity by Near-Infrared Spectroscopy

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Background: Peripheral artery disease (PAD) frequently is undertreated. ABI is the standard for diagnosis of PAD but is limited in its ability to predict functional status and risk of disease progression. Previous work with Near-Infrared Spectroscopy (NIRS) has demonstrated impaired oxygen utilization in PAD muscle and may allow for improved diagnosis and stratification of disease severity. We hypothesize that exercise produces characteristic changes in lower-extremity muscle oxygenated heme percent (StO2) which differentiates PAD from control and predicts PAD severity better than ABI.

Methods: We recruited 31 PAD subjects with intermittent claudication (IC) (ABI < 0.9) and 9 controls (ABI ≥ 0.9 and no IC). All subjects completed a Gardner maximal treadmill test. PAD subjects walked until IC was prohibitive (peak walking time, PWT) and controls walked for 540 seconds. StO2 measurements were taken from the lateral gastrocnemius with the wireless MOXY NIRS monitor. StO2 was documented at baseline, 60s, claudication onset time (COT), and PWT. For each subject, StO2 values were expressed as percent of baseline to allow for comparison across subjects. Data were analyzed by student’s t-test and linear regression.

Results: Mean baseline StO2 values were 46±11% and 58±17%, respectively, for PAD and control subjects. Among controls, StO2 dropped below baseline at 140±101s whereas PAD subjects dropped below baseline at 6±10s (P<0.001). PAD StO2s at 60s, COT, and PWT were compared to control StO2s at the corresponding time points. PAD patients’ StO2 at 60s, COT, and PWT were 65.2±37.9% (n=31), 66.4±35.5% (n=31), and 73.2±26.0% (n=25) lower than controls, respectively (P < 0.001). PAD subjects were separated into tertiles of PWT. Relative to baseline, StO2 decreased 84.2±18.4% (n=11) in the lowest tertile compared to 29.8±30.6% (n=10) in the highest tertile (P<0.05). Percent decrease in StO2 at 60s correlated to PWT (R² =0.56) in contrast to ABI (R²=0.018).

Conclusion: During exercise, PAD patients exhibited a characteristic change in StO2. In contrast to ABI, StO2 predicted PAD-related exercise limitation. These data suggest NIRS in conjunction with traditional methods could be used to guide diagnostic and treatment decisions.

Circulating Exosomes from PAD Patients Modulate Vascular Repair and Inflammation

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Objectives: Peripheral arterial disease (PAD) is a chronic disease characterized by inflammation. Recent work suggests that circulating exosomes may contribute to vascular injury and remodeling. We hypothesize that exosomes from PAD subjects negatively modulate vascular repair via miRNA and bioactive lipid mediators (LM).

Methods: Exosomes (particle size 30-100nm) were isolated from plasma of healthy (n=6) and PAD (n=6) subjects. Exosome miRNA was isolated and assessed by qPCR. Targeted metabolo-lipidomics was performed by liquid-chromatography-tandem mass spectrometry. VSMC and EC migration were assessed via scratch assay. Monocyte-derived macrophage gene expression after exposure to exosomes was assessed via RT-qPCR.

Results: Compared to healthy subjects, exosomes from PAD subjects contained lower levels of pro-angiogenic miR-126 and miR-210 (25.2±6.4 vs 8.3±1.7, p<0.05 and 0.29±0.07 vs 0.08±0.02, p<0.05, respectively). Exosomes contained arachidonic acid, eicosapentaenoic acid and docosapentanoic acid, as well as both pro-inflammatory and pro-resolving bioactive LMs and their pathway markers, including prostaglandins, leukotrienes, lipoxins, resolvins (D- and E-series) and maresins. By principle component analysis, exosome LM profiles differed significantly between healthy and PAD subjects. Exosomes from PAD subjects increased VSMC migration (1.5±.09-fold vs 1.0±.09-fold wound closure, p<0.005) and decreased EC migration (1.5±.04-fold vs. 1.8±.06-fold wound closure, p<0.005) compared to healthy controls. Both PAD and healthy exosomes increased MDM expression of pro-inflammatory genes TNF-α and MCP-1.

Conclusion: Plasma-derived exosomes from PAD patients contain an altered profile of vascular-active miRNA and LMs and confer effects on VSMCs and ECs that may impair vessel remodeling. We describe the first known evidence that plasma exosomes contain pro-resolving LMs. Collectively these data suggest that circulating exosome-based signaling may modulate vascular inflammation and repair in PAD patients.

Abstract Session III: Vascular Endothelium and Thrombosis
PARP-1 Silencing Upregulates FOSL1 Transcription, Enhances Angiogenesis and Accelerates Ischemic-Diabetic Wound Healing


Objective: People with combined ischemic and diabetic wounds of the lower extremities have the highest risk for limb loss, especially for those without surgical revascularization options. We have demonstrated that Poly-ADP-Ribose polymerase (PARP-1) is hyperactivated in hyperglycemic/hypoxic cells and in ischemic/diabetic murine wounds. This study elucidates the molecular mechanisms of PARP-1 mediated impairment of angiogenesis in diabetic/ischemic wounds.

Methods: A model of dorsal bipedicle flap-ischemic wounds on diabetic mice was used. The wounds were treated topically with nanoparticle-encapsulated siPARP-1 or vehicle. Wound closure rate and perfusion was analyzed using digital photography and Laser Doppler scanning, respectively. Angiogenetic markers in the tissues were measured by immunohistochemistry. In-vitro endothelial tube formation assay was performed using HUVECs cultured under hyperglycemic and hypoxic conditions.

Results: Wounds treated with topical siPARP-1 significantly accelerated wound healing compared to vehicle (from 25% ± 5% to 40%± 8% (n=7, p < .05) by day 6 and from 50% ± 15% to 75%± 3% (n=7, p < .05) by day 12, and also exhibited improved tissue perfusion (50%± 5% increase in perfusion units over control on day 6, n=47 p <0.05). Improved capillary density was also observed in the siPARP-1 treated wounds detected by immunohistochemistry for SMA (250%±35% increase in mean fluorescence intensity over control on day 12, n=4, p<0.05) and CD31 (125% ± 15% increase in mean fluorescence intensity over control on day 12, n=4, p<0.05). In-vitro angiogenesis assay showed that PARP-1-silencing significantly enhanced endothelial tube formation of hyperglycemic/hypoxic HUVECs (15± 4 complete polygons as compared to 0 in untreated, n=4, p<0.05). Human angiogenesis PCR-array analysis of pro-angiogenic factors revealed that PARP-1 silencing upregulated FOSL1 transcription by 5-fold (n=4, p<0.05). Interestingly, co-silencing of FOSL1 in PARP-1 silenced HUVECs resulted in loss of endothelial tube formation.

Conclusions: PARP-1 silencing is an effective strategy to promote ischemic-diabetic wound healing. Our data suggest that PARP-1-FOSL1 is a potential novel axis in angiogenesis and PARP-1 could be a promising therapeutic target for improving angiogenesis in these wounds.

Clinical and Genetic Determinants of Varicose Veins: a Prospective, Community-Based Study of ~500,000 Individuals

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Background:
Varicose veins are a common problem with no approved medical therapies. While it is believed that varicose vein pathogenesis is multifactorial, there is a limited understanding of the genetic and environmental factors that contribute to their formation. Large-scale studies of risk factors for varicose veins may highlight important aspects of pathophysiology and identify groups at increased risk for disease.

Methods:
We applied machine learning to agnostically search for risk factors of varicose veins in 493,519 individuals in the UK Biobank. Predictors were further studied using univariable and multivariable Cox regression analysis. A genome-wide association study (GWAS) of varicose veins was also performed among 337,536 individuals (9,577 cases) of white British descent, followed by eQTL and pathway analyses. Because height emerged as a new candidate predictor, we used LD score regression to estimate the genetic correlation between height and varicose veins. Finally, we performed Mendelian randomization analyses to assess for a causal role for height in varicose vein disease.

Results:
Machine learning confirmed several known (age, gender, obesity, pregnancy, history of deep vein thrombosis) and identified several new risk factors for varicose vein disease. The most important novel predictors were leg bioimpedance (HR: 0.44, 95% CI: 0.39-0.50, P < 0.0001) and height (HR: 1.74; 95% CI: 1.51-2.01, P < 0.0001), which both remained independently associated with varicose veins after adjusting for traditional risk factors in Cox regression. A GWAS identified 30 new genome-wide significant loci, identifying pathways involved in vascular development and skeletal/limb biology. Mendelian randomization analysis provided evidence that increased height is causally related to varicose veins (IVW: beta = 0.266, P = 1.28 x 10^-16).

Conclusions:
Using data from nearly half a million individuals, we identified novel clinical and genetic risk factors which provide pathophysiological insights and could help future improvements of treatment of varicose vein disease.

Fenofibrate Induces Endothelial Cell Tubule Formation Independent of Phospholipogenesis

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Fenofibrate, a proliferator-activated receptor (PPAR)α agonist, is the only oral medication demonstrated to prevent lower extremity amputations in diabetic patients. Phosphatidylcholines, generated by choline-ethanolamine phosphotransferase 1 (CEPT1) via the Kennedy Pathway, also induce PPARα activation, but their metabolism is altered in the setting of diabetes. It is unknown whether CEPT1 is essential for fenofibrate-mediated endothelial cell (EC) function. To evaluate this, we generated a murine model for conditional knockdown of Cept1 in the endothelium (Cept1ECKO). Heart ECs (MHECs) were harvested from 6wk old Cept1ECKO and wildtype (WT) littermates, and cultured in vitro on growth factor-reduced Matrigel. Cultures were then supplemented with VEGF (50ng/mL), bFGF (50ng/mL), and fenofibrate (25uM), then assessed longitudinally at 0, 4, and 6 hours. We observed that compared to WT, Cept1ECKO MHECs had significantly less tubule formation (p < 0.0001). VEGF and bFGF failed to rescue Cept1ECKO MHECs, but demonstrated a robust agonist response in WT MHECs (bFGF: p=0.003; VEGF: p=0.0002). Interestingly, fenofibrate demonstrated complete rescue of Cept1ECKO MHECs at 4 and 6 hours of culture. This finding demonstrates that fenofibrate restores EC function even in the setting of impaired phospholipid biosynthesis. This observation may partially explain how fenofibrate confers added benefits in subjects with diabetic and peripheral arterial disease. Future work will further elucidate this mechanism of action in diabetic subjects.

Background: The role of antiplatelet agents in the modulation of arterial disease is well described, but a paucity of data exists regarding their role in chronic venous insufficiency (CVI). We hypothesize that platelet responses to various antiplatelet agents are altered when comparing platelet function within refluxing and non-refluxing vein segments. Additionally, changes in platelet phenotype may alter vein wall biology.

Methods: Isolated platelets were obtained simultaneously from the patient antecubital vein (ACV) and a refluxing greater saphenous vein (GSV) during surgical phlebectomy and compared to platelets from healthy individuals. Non-refluxing GSV was harvested for coronary bypass. Platelet surface receptor activation was assessed through P2Y12 (clopidogrel), PAR1 (vorapaxar), and thromboxane (aspirin) pathways by flow cytometry for p-selectin. Immunoblotting assessed CD41 (platelet) and CD45 (WBC) within the wall of vein samples.

Results: Platelets from refluxing GSV showed a significant increase in reactivity via all platelet signaling pathways, especially P2Y12 and thromboxane when compared to platelets from the ACV in the same patient. Conversely, platelets collected from the ACV in CVI patients showed a significant decrease in reactivity to all agonists compared to ACV in healthy individuals without CVI. Most notably, GSV from a patient with CVI had a reduction in CD41 content, but a seven-fold increase in the CD45:CD41 ratio, compared to GSV from healthy people (Figure).

Conclusions: Platelet activation by these clinically relevant pathways is enhanced locally in the refluxing GSV, yet systemic, circulating platelets isolated from CVI patients are 2-3-fold less active than systemic platelets from healthy people. Our data suggest that reflux may locally alter the circulating platelet phenotype and in turn also have a role in remodeling the vein wall.
Vascular Injury-Induced ATP release leads to IL-1β Production and Endothelial Dysfunction

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Introduction
Injury leads to inflammation and modulates vein graft responses that result in vein graft failure. Previous work has shown that vascular injury (mechanical and chemical) leads to membrane injury, ATP release, and endothelial dysfunction. These studies were performed to determine the role of inflammation after vascular injury on endothelial dysfunction.

Methods
Endothelial-dependent responses (EDR) of isolated rat aorta (RA) were determined in a muscle bath. IL-1β production in response to exogenous ATP treatment was determined in TNFα and IFNγ-primed human saphenous vein endothelial cells (HSVEC) in the presence and absence of P2X7R inhibitors A438079 (A43) and oxidized ATP (oATP), the ATP hydrolyzing enzyme apyrase, the p38MAPK inhibitor SB203580 (SB), and the MAPKAP kinase (MK2) inhibitor MMI-0100. p38MAPK and MK2 phosphorylation, and VCAM protein levels were determined by immunoblotting.

Results
ATP treatment led to impaired EDR that was partially restored by oATP and apyrase (A). ATP treatment of HSVEC led to p38MAPK and MK2 activation (data not shown), delayed (2 hrs) IL-1β production (B) and increased VCAM expression (24hrs after treatment, C). P2X7R antagonism and p38MAPK/MK2 inhibition inhibited IL-1β production (D). VCAM expression was reduced by P2X7R antagonism. IL-1β treatment of RA (3hrs) led to impaired EDR (D).

Conclusions
These data suggest that injury, leading to release of ATP and activation of the P2X7R/p38MAPK/MK2 signaling axis, leads to increases in the inflammatory cytokine IL-1β and expression of the endothelial inflammatory marker VCAM. Phosphorylated MK2 is known to stabilize cytokines, hence these data provide a direct link between vascular injury and endothelial inflammatory responses. Finally, IL-1β directly impaired EDR, suggesting that these data also provide a plausible mechanism for injury induced endothelial dysfunction (E).
Application of a Cryogel-Coated Prosthetic Vascular Graft Material for Delivery of Targeted Gene Therapies in a Rabbit Model

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Objectives: Long-term success of prosthetic grafts (PG) in peripheral arterial disease is limited by development of anastomotic neointimal hyperplasia. We have constructed a bioactive prosthetic graft material (BPGM) capable of delivering biologic agents in vitro, and evaluate our BPGM in vivo with a rabbit carotid interposition bypass model.

Methods: We synthesized our BPGM by cryopolymerization of RGD with methacrylated alginate and heparin, coating 1.5cm by 2mm electrospun PET (ePET) grafts, and dipcoating in fluorescent siRNA for 3 hours. Three rabbits received bare ePET and 3 received cryogel-coated ePET for a carotid interposition bypass (Figure 1). After 24 hours, bypass patency was assessed, and cell toxicity of the proximal anastomosis, mid-graft, and distal anastomosis examined with H&E staining. Confocal microscopy was used to visualize fluorescence, correlating with ability to deliver siRNA in vivo.

Results: Graft patency was equal between groups, with no increased cell toxicity in rabbits receiving cryogel-coated ePET. Confocal microscopy demonstrated no difference in retained fluorescence between rabbits receiving cryogel-coated or bare ePET, and no increased transfection of cells at 24 hours (Figure 2).

Conclusion: Creation of the optimal PG demands a material that is biocompatible, responsive, and nonthrombogenic. We have constructed a modified PG capable of in vitro delivery of targeted gene therapies, with comparable patency and biocompatibility in our large animal model. Additional optimization to achieve predictable and sustained release is needed to validate this as an effective and practical method to deliver biologic agents in vivo.
Figure 1. Cryogel-coated ePET graft with fluorescent siRNA implanted in a rabbit carotid interposition bypass model. Intraoperative images of a rabbit undergoing carotid artery interposition bypass using our bioactive prosthetic graft material at initial surgery (left) and after 24 hours (right).

Figure 2. Cross-sections of the distal anastomosis in rabbits with bare ePET graft material compared to cryogel-coated ePET. Confocal microscopy demonstrates no difference in retained fluorescence and no increased transfection of cells at 24 hrs in cross-sections of the distal anastomosis from rabbits receiving bare ePET graft material (left), and cryogel-coated ePET (right), with arterial elastic lamina in green, fluorescent siRNA in red, and cell nuclei in blue.

Abstract Session IV: Vascular Inflammation and Injury
Efficacy and Mechanisms of Metformin Therapy in Established Experimental Abdominal Aortic Aneurysms

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Objective: Diabetes reduces the risk for abdominal aortic aneurysm (AAA) disease. In prior work in rodent models, pre-treatment with metformin was protective against experimental AAA initiation and progression. This study examined the influence of metformin therapy on progression of existing experimental AAAs.

Methods: AAAs were created in 10-12 week old male C57BL/6J mice via transient intra-aortic infusion of porcine pancreatic elastase (PPE). Mice were treated with metformin (250 mg/kg via oral gavage), metformin plus AMPK inhibitor Compound C (the later via 10 mg/kg intraperitoneal injection) or vehicle alone for 10 days starting on day 4 following AAA creation. Outcomes were assessed by serial transabdominal ultrasonographic assessment of aortic diameter during treatment, as well as histological examination at sacrifice.

Results: The principal findings are summarized in the Figure. Vehicle-treated mice experienced progressive, time-dependent aortic enlargement from day 3 to 14 following PPE infusion. Metformin treatment substantially attenuated further enlargement of existing AAAs. Treatment with Compound C partially rescued the AAA phenotype in metformin-treated mice. Histologically, characteristic aneurysmal pathologic changes, including medial elastin degradation, smooth muscle cell (SMC) depletion, mural leukocyte accumulation and neoangiogenesis (assessed by CD31 staining), present in PPE-infused, vehicle-treated mice, were substantially attenuated with metformin treatment. Co-treatment with Compound C abrogated the effects of metformin on media elastin degradation, SMC depletion and mural macrophage infiltration, with less influence on mural angiogenesis.

Conclusion: Metformin therapy suppresses further expansion of existing experimental AAAs. This effect is abrogated by co-treatment with Compound C. These findings further justify performance of a clinical trial of metformin for medical management of AAA disease.
Figure. Metformin treatment limits the progression of established experimental AAAs. Male C57BL/6 mice were daily treated with vehicle (n=7), metformin (250 mg/kg via oral gavage) (n=6), or metformin plus AMPK inhibitor compound C (10 mg/kg ip) (n=6) starting day 4 following PPE infusion. (A): Mean and SD of aortic diameters. Two-way ANOVA followed by two sample comparison, *P<0.05 and **P<0.01 between two group. (B-G): Quantification of medial elastin degradation, SMC depletion, leukocyte infiltration (macrophages and T cell subsets) and angiogenesis (CD31). Nonparametric Mann-Whitney test, *P<0.05 and **P<0.01 between two groups.

Retrograde Hemorrhage and Ischemic Injury after REBOA in a Porcine Model of Uncontrolled Aortic Injury

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Introduction Resuscitative Balloon Occlusion of the Aorta (REBOA) has gained popularity as a less invasive approach to temporize traumatic noncompressible hemorrhage, yet mortality remains over 70%. Although associated injuries may account for some deaths, contributions from ischemia and ongoing retrograde bleeding are also likely, with REBOA occlusion frequently in Zone 1 (descending thoracic aorta) and often greater than 20 minutes. This study examined retrograde blood loss and ischemic injury after REBOA in a porcine model of aortic injury.

Methods Six anesthetized swine with invasive hemodynamic and neurophysiologic monitoring (Muscle Evoked Potentials and Somatosensory Evoked Potentials) underwent 8 Fr femoral access and Zone 1 positioning of a REBOA balloon prior to aortic injury. The thoracic aorta was injured with a 22 Fr dilator, followed by aortography and immediate REBOA inflation proximal to the injury. Profound deterioration of the first three animals with one hour of REBOA prompted the next three animals to undergo only 30 minutes of REBOA. Blood loss was recovered with a cellsaver. Animals underwent permanent stent repair of the aortic injury and resuscitation with the intent to recover.

Results: Despite proximal hemorrhage control documented angiographically, blood loss from retrograde bleeding was substantial averaging 3.7 L and 3.5 L for the 30- and 60-minute groups, respectively. After balloon inflation, mean pressure fell an average of 62 mmHg within 20 minutes (p < 0.001), while cardiac output decreased 20-40%. In the lower extremities, Neuromonitoring revealed ischemic loss of motor signals at a mean of 27 minutes. Even after resuscitation with blood, bicarbonate, saline and pressors, all six animals arrested shortly after balloon deflation, amidst falling bicarbonate (p less than 0.001), and rising lactate (p less than 0.01) relative to baseline. Conclusions: Retrograde hemorrhage is an underappreciated event during REBOA control of aortic injuries, that may contribute to spinal cord ischemia, tissue ischemia and death. This study suggests that improved outcomes for noncompressible hemorrhage will require balance of competing goals of hemorrhage control and distal perfusion.

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Cell Mimetic Liposomal Nanocarriers Tailored for Vascular Smooth Muscle Cell Molecular Therapeutics

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Introduction: Our laboratory aims to develop biocompatible nanocarriers for molecular therapeutics aimed at vascular pathology. We have previously established a liposome platform that is an effective delivery system for RNAi in vascular smooth muscle cells (VSMC). Tailoring liposome membranes to mimic vascular cell membrane lipid constituents may be a promising strategy for increased delivery to target cells. Here we test our previously established liposome platform with the incorporation of naturally occurring signaling lipids known to influence vascular cell function as a method to increase VSMC association.

Methods: Established cell-penetrating neutral liposomes (R8-PLPs) were assembled and fluorescently tagged as previously described. The propensity of diacylglycerol (DAG) and/or phosphatidylinerine (PS) to increase the association of R8-PLP to VSMCs was tested by the incorporation of gradient percentages DAG/PS alone and in combination at 5-20% membrane occupancy. Liposome stability and siRNA encapsulate retention was analyzed via dynamic light scattering and Ribo-green, respectively.

Results: DAG and PS incorporation increased VSMC association of R8-PLP, with 10% PS increased over all other groups (P10; Fig1A). Combinatorial formulations were screened for optimal DAG content with PS fixed at 10%. DAG20%+PS10% (D20P10) performed best, with increased VSMC association over all other combinatorial groups or independent P10 modification (Fig1B). Stability profiles were consistent (~50nm size and ~80% drug retention) and not significantly different among groups.

Conclusion: Signaling lipid incorporation into the nanocarrier architecture potentiates VSMC association of established R8-PLP liposomes, without sacrificing stability or drug retention. These results suggest cell mimetic tuning of liposomes to generate specificity and increase delivery efficacy is a viable strategy for advancing targeted liposomal drug delivery.
Increased Plasma Sulfide in Vascular Surgery Patients Correlates with Reduced Post-Operative Mortality

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Objective: Hydrogen sulfide (H2S) is an endogenously produced gaseous signaling molecule with the potential to modulate vascular functions. Free plasma sulfide can be measured by various techniques, but no consistent relationship with cardiovascular disease has yet emerged. For example, sulfide levels are decreased in CHF patients, but elevated in PAD. We therefore sought to compare plasma sulfide levels from PAD patients to matched controls, and explore links between mortality rates and sulfide levels using two assays. Approach & Results: Patients undergoing carotid endarterectomy (n=49), open lower extremity revascularization (n=44) or leg amputation (n=22) were enrolled (mean age 68.9±9.6, 67% male). Blood was collected from 20 matched control patients, without PAD or CAD (mean age 67.9±1.3, male 65%). Plasma sulfide was measured using two methods, first detection using lead acetate, and second using mass spectrometry. Controls had increased plasma sulfide levels measured by both methods (lead acetate, Fig. A; mass spec, Fig. B) compared to PAD patients (p<0.001, p=0.013). Also, PAD patients were divided into high (n=57) and low (n=58) sulfide (lead acetate) groups by median split. Low sulfide PAD patients had increased probability of post-op mortality (p=0.0337, Fig. C). To determine the source of plasma H2S detected by lead acetate, we tested the effects of detergent and proteolytic denaturation of plasma as well as of reducing agents on H2S release. We found denaturation increased plasma sulfide release, and that dithiothreitol was most effective at liberating H2S, suggesting bound sulfane sulfur as source of H2S detected using the lead acetate assay. Conclusions: Plasma free and bound sulfide were reduced in PAD patients compared to controls, and correlated with mortality. These findings provide evidence linking circulating sulfide to clinically meaningful events, and support directed H2S investigations toward diagnostic and therapeutic purposes.
Parthenolide Inhibits Inflammatory Dysfunction of Human Aortic Endothelial Cells and Proliferation of Smooth Muscle Cells in vitro and Restenosis in a Rat Model

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Background: Cardiovascular diseases remain the leading cause of death in developed countries. Endovascular surgical interventions trigger uncontrolled proliferation of smooth muscle cells (SMCs) in the intima (intimal hyperplasia) leading to lumen re-narrowing (restenosis). Despite extensive efforts devoted to therapeutic methods with a focus on inhibiting SMC proliferation, restenosis persists at a significant rate. In recent years, a growing body of knowledge has underscored a crucial role for endothelium damage in the pathophysiology of restenosis, suggesting that preservation of the endothelium would provide an effective approach for effectively curbing restenosis.

Methods and Results: We utilized high-throughput screening methods to search for small molecules that could inhibit the proliferation of human aortic SMCs without damaging human aortic endothelial cells (ECs). We identified such a lead compound in Prestwick Library; i.e. Parthenolide, a sesquiterpene lactone extracted from feverfew. Treatment with a low dose (1 µM) of Parthenolide for 96h inhibited SMC but not EC proliferation. When the cells were stimulated with inflammatory cytokines (TNF-α or IL-1β), Parthenolide mitigated cytokine-induced proliferation and MCP-1 production of SMCs, but rescued cytokine-induced endothelial dysfunction, including apoptosis, proliferation, decrease of eNOS, and increase of inflammatory markers. Mechanistic studies showed that while Parthenolide treatment in both SMCs and ECs induced NRF-2 activation (nuclear translocation), NRF-2 knockdown with siRNA diminished the aforementioned beneficial effects of Parthenolide in both cell types. In contrast, NFkB activation was not significantly affected by Parthenolide. In a rat balloon angioplasty model, perivascular delivery of Parthenolide in Pluronic gel effectively inhibited intimal hyperplasia and restenosis 14 days after surgery.

Conclusion: These results indicate that the natural compound Parthenolide differentially attenuates pathological behaviors of both human vascular SMCs and ECs, whereas known agents as such are scarce. Thus Parthenolide may serve as a promising lead compound for future development of next-generation anti-restenotic therapeutics.

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Targeted Nanotherapy for the Treatment of Atherosclerosis

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Objective: Atherosclerosis is the leading cause of death and disability in the United States. We hypothesize that systemic administration of a novel nanofiber will target areas of atherosclerosis and regress atherosclerotic lesions.

Methods: Self assembling peptide amphiphile (PA) nanofibers were synthesized. An 18 amino acid sequence which retains the cholesterol efflux actions of apolipoprotein-A1 (apoA1) along with a Liver X Receptor (LXR) agonist, known to enhance cholesterol efflux, were incorporated into the nanofiber to both target and treat atherosclerosis. To assess the ability of the nanofiber to target and treat atherosclerosis in vivo, LDL receptor knockout mouse (LDLR KO) mice were fed a high fat diet (HFD) for 14 weeks after which they received bi-weekly injections of the therapeutic or control for 8 weeks. Optimum dose, concentration, binding duration, and biodistribution was determined using fluorescent microscopy and pixel quantification. Treatment groups included: PBS control, PA nanofiber with a scrambled targeting sequence, LXR agonist alone, targeted PA nanofiber (ApoA PA), and targeted PA nanofiber incorporating LXR agonist (ApoA-LXR PA). n=10/treatment group.

Results: ApoA PA and ApoA-LXR PA nanofibers effectively targeted atherosclerotic plaque in the aortic root. Optimum concentration of nanofiber was 2mg/mL, and optimum dose was 6mg/kg. There was no difference in optimum dosing or concentration between males and female mice. ApoA PA nanofiber localized to the aortic root for approximately 2-3 days, and was cleared from the aortic root by 7-10 days. Concentrations of ApoA PA in the aortic root was 5-fold higher than in the lung, liver, and kidney one day post injection. After only 8 weeks of treatment, male and female mice treated with ApoA-LXR PA had 11 and 9% plaque area reduction compared to PBS treated controls, respectively. Differences in treatment conditions vs. controls showed sex dependence, with only male mice demonstrating significantly higher plaque reduction from ApoA1-LXR PA treatment in comparison to scrambled PA treatment.

Conclusions: Our results demonstrate that a novel targeted nanofiber binds specifically to atherosclerotic lesions and reduce plaque burden after a short treatment duration.

Poster Session
**Notch1 Activation in Endothelial Cells Promotes Progression of Atherosclerosis**

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**Introduction.** Notch signaling plays pivotal roles in vascular development and cardiovascular disease. We previously showed that the Notch pathway is activated in luminal endothelial cells (EC) at atherosclerotic plaques, implicating a potential involvement of Notch signaling in atherosclerosis. We further demonstrated that loss of Notch1 signaling in EC suppresses progression of atherosclerosis. Here, we test the effect of activation of the Notch1 pathway in EC on atherosclerosis using mouse model with inducible EC-specific Notch1 activation on an ApoE⁻/⁻ background.

**Methods.** We created a Tamoxifen-inducible EC-specific N1IC (Notch1 intracellular domain, an active Notch1 mutant) expression mouse line on atherosclerosis-prone ApoE⁻/⁻ background: ROSAᵀᴹSL-N1IC/VE-cadherinCreERT²/ApoE⁻/⁻. Mice were fed with high-fat-diet (HFD) along with or without Tamoxifen treatment (i.p. 2 mg/day for 5 consecutive days) starting from 4-wk old and terminated on 16-wk old. Aortas were harvested to study the effects of Notch1 activation in EC on the progression of aortic atherosclerosis. Expression of N1IC in EC was validated by immunostaining. Aortic plaque burden was evaluated by quantification of proportion of total aorta containing atherosclerosis detected by Oil-Red-O and hematoxylin-eosin staining using computer-aided image analysis. Blood was tested to measure lipid profile.

**Results.** Enforced Notch1 activation in EC is induced in Tamoxifen-treated mice. EC gain-of-function Notch1 signaling significantly increased aortic plaque burden \(n=10\) in both (Tamoxifen -) and (Tamoxifen +) group, \(P<0.05\). The serum levels of total cholesterol, HDL, LDL, triglycerides and glucose were comparable between Tamoxifen-treated and -untreated mice.

**Conclusions.** Activation of the Notch1 signaling pathway in EC facilitates atherosclerotic plaque formation. The increase in atherosclerosis was not due to changes in the serum levels of lipid, glucose, triglycerides, HDL, LDL and total cholesterol. Our findings demonstrate that activation of the Notch1 signaling pathway in EC promotes progression of atherosclerosis. It highlights Notch1 signaling as an emerging therapeutic target for the treatment of atherosclerosis.

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Evaluation of Peripheral Calcium Score as a Measure of Peripheral Artery Disease Severity

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The current gold standard for diagnosing PAD is Ankle Brachial Index (ABI). However, vascular calcification can falsely elevate ABI. No studies have compared the diagnostic value of peripheral calcium score (PCS) in lower extremity arteries with ABI.

Primary aim of this study was to describe the association of PCS with continuous ABI values and categories of ABI in a retrospective cohort design. We identified 50 patients who underwent CTA and ABI measurements [ABI categories for PAD severity: severe (<0.5), moderate (0.5-0.9), normal (0.9-1.4), noncompressible (>1.4)]. We evaluated CTAs imaged from abdominal aorta through lower extremities and determined total calcium volume of plaques with density >130 HU and area >1mm² from infrarenal abdominal aorta to the foot using TeraRecon by two independent readers (Intra class correlation 99%). We explored the association between ABI and PCS in SAS using multiple linear regression and analysis of covariance adjusting for age, race, smoking status, hypertension, hyperlipidemia, type II diabetes, and chronic kidney disease.

We found that ABI was inversely associated with PCS in linear regression (p<0.01, Figure 1A). Differences in mean PCS were statistically significant across ABI categories [F(3, 29)= 5.03, p=0.01, Figure 1B]. Across subgroups, the mean PCS was significantly different for ABIs <0.5 and 0.5-0.9 (p=0.02), <0.5 and >1.4 (p<0.001), 0.5-0.9 and >1.4 (p=0.04), 0.9-1.4 and >1.4 (p=0.05). Proportion of tibial calcium to overall PCS was much lower in ABI<0.5: 0.0003 vs ABI>1.4: 0.357 (p=0.02). Mean PCS may be a valid measure of PAD severity and percentage of tibial calcium may help quantify PAD burden in non-compressible vessels. Our study serves as proof of concept for a comprehensive PCS system to diagnose and evaluate PAD severity, particularly in high-risk subpopulations where non-invasive studies may be unreliable.

Collateral Development in Swine after Ligation of Native Leg Arteries

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Introduction:
The development of collateral vasculature is a key mechanism compensating for arterial occlusions in patients with Peripheral Artery Disease (PAD). We aimed to examine the development of collateral pathways after ligation of native vessels in a porcine model of PAD.

Methods:
Right hindlimb ischemia was induced in domestic swine (N=11, male, 26-57 kg) using two different versions of arterial ligation. Version 1 (N=6) consisted of ligation/division of the right external iliac, profund femoral (RPFA) and superficial femoral arteries (RSFA). Version 2 (N=5) consisted of the ligation of Version 1 with additional ligation/division of the right internal iliac artery (RIIA). Development of collateral pathways was evaluated with standard angiography at baseline (prior to arterial ligation) and at termination (4-8 weeks later). Relative luminal diameter of the arteries supplying the ischemic right hindlimb were determined by 2D angiography, as percent of the size of the distal aortic diameter.

Results:
The pathway connecting the RIIA to the RPFA and RSFA/popliteal artery of the ischemic limb was the dominant collateral pathway in version 1. Mean luminal diameter (± standard error) of the RIIA at termination increased by 39.4± 5.5% (P<0.01) compared to baseline. There were two co-dominant collateral pathways in version 2. The first pathway connected the common internal iliac trunk and left internal iliac artery to the reconstituted RIIA which then supplied the RPFA and RSFA/popliteal arteries. The second pathway connected the left profunda artery to the reconstituted RPFA. Mean diameter (± standard error) of the common internal iliac trunk and left profunda artery increased at termination by 23.7± 7.6% and 24.8± 7.4%, respectively (p < 0.05).

Conclusion:
Two versions of hindlimb ischemia induction (right ilio-femoral artery ligation with and without right internal iliac artery ligation in swine produced differing collateral pathways along with changes to the diameter of the inflow vessels. Radiographic and anatomical data of the collateral formation in this porcine model has value in investigation of the pathophysiology of hindlimb ischemia, and assessment of angiogenic therapies as potential treatments for PAD.

Differential Matricellular Protein (CCN) Expression in Atherosclerotic and Aneurysmal Smooth Muscle Cells

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Introduction: Recent evidence implicates reduced activity of CCN3 as a mediator of MMP production and aortic wall degeneration in the process of model aortic aneurysm. We have previously shown that AAA-derived VSMC have increased elastolytic activity. We hypothesized that there would be differential CCN expression and production by VSMC derived from human AAA or derived from human atherosclerotic plaque.

Results: Early passage cell lines of VSMC lineage as confirmed by flow cytometry were analyzed by Illumina expression array. The VSMC were derived from infrarenal aneurysm (AAA, n=22) or atherosclerotic plaque (Pl, n=29). There were no differences in expression of CCN1, 2, 5 or 6 (Fig A) between the two cell types. Expression of CCN3 was significantly reduced in AAA compared to Pl, CCN4 was significantly greater in AAA than in Pl-derived VSMC. Western blot of cellular extracts from these cells (n=3 each) confirms qualitatively greater CCN3 in Pl vs AAA VSMC, while no appreciable difference was seen in the production of CCN4 (Fig B). Production of CCN1, 2 and 6 are qualitatively similar and there may be slightly lower CCN5 production in Pl vs AAA-derived VSMC (Data not shown). Matricellular protein expression show distinct patterns between Plaque-derived and AAA-derived VSMC and may be related to the enhanced elastolytic activity of cells in AAA.

The Aortic Luminal Area is a Potential Marker of Increased Rupture Risk in Abdominal Aortic Aneurysms


Introduction: Diameter is currently the only factor used to estimate rupture risk of abdominal aortic aneurysms (AAAs). Many large AAAs, however, do not rupture, and a significant portion of small AAAs do. Our aim was to investigate if simple two-dimensional geometric measurements can improve rupture risk prediction in AAAs, and relate these measurements to biomechanical determinants of AAAs.

Methods: Thirty patients with ruptured AAAs (mean age was 77 ± 5 years and 23 were male) and 60 patients (mean age 60 ± 8 years, and 46 were male) with asymptomatic AAAs were included. At the location of the maximal diameter, the diameter, the luminal area and the vessel area were measured. Finite element analysis was used to compute 3D-geometric and biomechanical parameters of the asymptomatic AAAs, using A4 Clinics Software (VASCOPS, Austria). An automatic matching function was used to construct diameter-matched groups.

Results: Analysis of all stable AAAs (n=60) and ruptured AAAs (n=30) showed that ruptured AAAs had a significantly larger diameter, 77 ± 15 mm vs. 62 ± 13 mm (p<0.01) and significantly larger luminal area 2281 ± 1964 mm² vs. 1059 ± 674 mm² (p<0.01). In order to control for diameter as a confounder, two diameter-matched groups, one with ruptured AAAs (n=28) and one with stable AAAs (n=15) were formed (74 ± 12 mm vs 73 ± 11, p = .67). Diameter-matched ruptured AAAs had a larger luminal area (1954 ± 1254 mm² vs. 1120 ± 623 mm², p = .02) and a lower relative ILT area (55 ± 24 % vs 68 ± 24%, p= .03). In multivariate regression of 60 asymptomatic AAAs, including the maximal diameter, the luminal area explained the largest amount of variance in the biomechanical rupture risk parameters, followed by the ILT-area.

Conclusions: We demonstrate that the luminal area is increased in ruptured AAAs compared to stable AAAs. Further, we show that this finding may in part be explained by a correlation between luminal area and biomechanical rupture risk parameters.

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Fatty Acid Synthase Expression in Peripheral Arterial Plaque

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Introduction: Diabetic patients exhibit distinct patterns of diffuse and recalcitrant peripheral artery disease, which subsequently predisposes them to poor wound-healing, infection, and critical limb ischemia. Fatty Acid Synthase (FAS), an enzyme responsible for de novo fatty acid synthesis, exhibits elevated content in the liver tissue in diabetic mouse models and elevated serum levels in diabetic subjects with carotid artery stenosis. It is currently unknown whether tissue FAS and serum circulating FAS (cFAS) correlate with diabetic status in patients with femoral arterial-occlusive disease.

Materials and Methods: We enrolled diabetic and non-diabetic subjects undergoing femoral endarterectomy (FEA) in an IRB-approved vascular biobank. Serum and femoral endarterectomy plaque samples were collected from participants, and tissue FAS and serum cFAS content was evaluated using ELISA. Differences in cFAS content between cohorts were summarized as mean ± SEM, and statistical significance was determined via T-test and Pearson correlation analysis.

Results: 33 patients (16 DM, 17 non-DM) who underwent femoral endarterectomy for high grade occlusive disease were evaluated. No significant difference in key demographics were observed. Tissue plaque FAS content was 69.8% (13.3 vs 7.81 ELISA: Total protein) higher in DM compared to non-DM subjects (p-value = 0.011). cFAS was also elevated by 41.7% (1.39 vs 0.98 ELISA: Total protein) in diabetic compared to non-DM subjects (p-value = 0.048). Correlation analysis of 23 patients’ paired samples revealed a significant correlation between cFAS and plaque FAS content (Pearson r = 0.47, p-value = 0.023).

Conclusion: Our study is the first to evaluate cFAS levels in patients with high grade, symptomatic, lower extremity PAD, and demonstrates evidence that cFAS and tissue FAS levels correlate in subjects with diabetes. Future studies will help determine whether cFAS is a relevant biomarker for disease severity and progression in diabetic subjects.

Serum Resistin is Associated with Impaired Endothelial Function and Poor Outcomes in Patients with Peripheral Artery Disease


Objective: Resistin is a hormone secreted by adipocytes that has been associated with metabolic syndrome and cardiovascular disease. However, less is known on the role of resistin in patients with PAD. This study seeks to understand the relationship of serum resistin with endothelial function and major adverse cardiac events (MACE) in patients with PAD.

Methods: One-hundred and six patients with intermittent claudication and an ankle-brachial index <0.9 or history of revascularization for symptomatic PAD were recruited between 2011-2016. Resistin was assayed using commercially available ELISA kits. Endothelial function was measured via brachial artery flow-mediated vasodilation (FMD) at baseline. Incident MACE were identified by subsequent chart review and defined as a composite endpoint of MI, coronary revascularization, stroke, or death from a cardiac cause. Cox proportional hazards models were used to calculate hazard ratios for MACE.

Results: At baseline, despite similar Rutherford scores, medical comorbidities, and medication use, FMD was significantly lower with increasing resistin quartile (I: 9.1±3.3%, II: 7.1±3.5%, III: 5.8±4.0%, IV: 5.6±3.5%, p=.002). A univariate linear regression demonstrated that increasing resistin quartiles predicted lower FMD (II: -1.99, 95% CI -3.96 to -0.02, p=.05. III: -3.34 95% CI -5.29 to -1.39, p=.001. IV: -3.52, 95% CI -5.48 to -1.55, p=.001). In multivariate linear regression, resistin quartiles III and IV predicted lower FMD relative to the first quartile after adjusting for several patient characteristics and comorbidities (III: -2.26, 95% CI -4.51 to -0.01, p=.05, IV: -2.53, 95% CI -4.87 to -0.20, p=.03). During a median follow-up period of 36 months (IQR: 29-45), 21 patients experienced a MACE. In a Cox proportional hazards model adjusted for smoking status and CAD, resistin was independently associated with an increased rate of MACE (HR: 1.11, 95% CI 1.02-1.21, p=.02).

Conclusion: Resistin is associated with impaired endothelial function and an increased rate of MACE in patients with PAD. Further research is needed to determine the mechanism by which resistin may increase MACE, and prospective studies should determine whether decreasing resistin has therapeutic benefit in patients with PAD.

Microvascular Pathology in Peripheral Artery Disease

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Background: Peripheral Artery Disease (PAD) is caused by atherosclerotic narrowing of arteries supplying the legs. PAD-induced myopathy is characterized by myofiber degeneration and progressive fibrosis. Qualitative histological review suggests pathological changes in the microvasculature of PAD muscle, in association with advancing fibrosis. We tested the hypothesis that microvessel architecture, pericyte coverage, and collagen profiles systematically change with advancing disease and are consistent with advancing microvascular pathology.

Methods: Biopsies of PAD patients at Fontaine Stage II (n=15) and IV (n=16), and controls (n=15) were labeled with antibodies specific for Col I, Col IV, αSMA, or CD31 and analyzed by quantitative wide-field, fluorescence microscopy. Thickness of the basement membrane (BM), peri-microvascular Col I density, and BM lumen diameter were measured. Pericytes were identified by abluminal location within the microvascular BM and αSMA* labeling. Group differences were tested by ANOVA and a post hoc pairwise T Test with Bonferroni correction. Correlations were determined by linear regression analysis.

Results: Thickness of the BM was greater in Stage II patients (1.58 µm) compared to controls (1.42 µm) (p<0.043) and in Stage IV (1.75 µm) compared to Stage II patients (p<0.021). Microvascular BM lumen diameter was increased (p<0.001) in Stage IV patients (3.97 µm) compared to control (3.25 µm) and Stage II patients (3.29 µm). Thickened PAD microvessels had greater pericyte coverage than control microvessels. BM thickness correlated positively with microvascular BM lumen diameter (R²= 0.513, p<0.001). Peri-microvascular Col I deposition correlated positively with microvascular lumen diameter (R²= 0.162, p=0.006), and was greater in Stage IV compared to Stage II patients (p=0.040).

Conclusions: Increased perivascular Col I deposition, BM thickening, and BM lumen diameter represent advancing microvascular disease in PAD patients. Pericytes, which deposit BM collagen, are more abundant in thickened microvessels. Pericyte replication and secretion of Col IV may be determining factors in the microvascular pathology of PAD muscle.

Are There Sex-specific Differences in Arteriovenous Fistula Maturation?

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The arteriovenous fistula (AVF) is the preferred method of dialysis access due to its proven superior long term outcomes. However, women have lower rates of AVF maturation than men (38% vs. 60%), preventing optimal AVF use. Using a novel mouse AVF model that recapitulates human AVF maturation, we tested the hypothesis that there is a difference in male and female AVF maturation. Aortocaval fistulae were created in male and female C57BL/6 mice (9-10wk). At days 0, 3, 7, 14 and 21, aortic and IVC diameters and flow velocity were monitored by Doppler ultrasound. We then calculated shear stress. Using qPCR, we measured messenger RNA (mRNA). AVF were examined at day 21 and AVF wall thickness was measured by computer morphometry.

Female mice weighed less preoperatively and at day 21 (p<0.05). They also had larger suprarenal aortic diameter (p=0.002) but smaller infrarenal IVC diameter (p<0.05) at baseline. After AVF creation, there was similar dilation of the infrarenal aorta and IVC in both male and female mice (p>0.05). Infrarenal IVC mean velocity was decreased in female mice at baseline and at day 3, 14 and 21 (p<0.05). Similarly, mean laminar shear stress magnitude in the infrarenal IVC was decreased in female mice at day 7 (p=0.03), 14 (p=0.04) and 21 (p=0.01). There was no difference in the infrarenal aorta shear stress magnitude (p=0.80). mRNA of KLF2, a marker of laminar shear stress, was decreased in the venous limb of female AVF on day 21 (p=0.048). Preoperatively, female mice had thinner venous walls (p=0.035). However, at day 21, AVF wall thickness was similar (p=0.18).

AVF in female mice have lower magnitudes of laminar shear stress, lower expression of KLF2 mRNA and a higher percent increase in wall thickness. These findings suggest a mechanism underlying the diminished rates of AVF maturation in women.