autophagy and eNOS activation in response to 60-min of treadmill-running (i.e., a physiological approach to increase (5-month) mice that display compromised vascular autophagy, we show suppressed upregulation / initiation of vascular nitric oxide synthase (eNOS), the enzyme responsible for nitric oxide generation. Importantly, in older (24 month) vs. adult revision describes a novel mechanism that is responsible for this observation. Specifically, endothelial cells with oxide generation is prevented when vascular autophagy is genetically compromised. A second manuscript currently in recapitulated in primary endothelial cells from old vs adult mice, and primary endothelial cells from mice with inducible vascular shear stress). Of note, nodes of dysfunction that exist in cells with genetic repression of autophagy, are compromised autophagy display impaired glycolysis, ATP production, and subsequent purinergic signaling to endothelial in the process of healthy aging. This is relevant because the population worldwide is aging, aging-related cardiovascular complications are the primary cause of death in individuals ≥ 65 y old, and patient-management costs to the healthcare system are enormous and unsustainable. Current support for this work is provided by grants from the NIH NIA (RO3) and AHA (Grant-In-Aid). The process of autophagy is repressed in many cell types in the context of aging, and might play a role in neurodegenerative diseases such as Alzheimers and Parkinos. One study reports that autophagy is repressed in primary endothelial cells from aged vs. adult humans, and might play a role in compromised arterial function that is associated with the process of aging. In 2014 we sought to determine whether genetic repression of autophagy per se influences the ability of endothelial cells to generate nitric oxide. We were the first to report that shear-stress induced nitric oxide generation is prevented when vascular autophagy is genetically compromised. A second manuscript currently in revision describes a novel mechanism that is responsible for this observation. Specifically, endothelial cells with compromised autophagy display impaired glycolysis, ATP production, and subsequent purinergic signaling to endothelial nitric oxide synthase (eNOS), the enzyme responsible for nitric oxide generation. Importantly, in older (24 month) vs. adult (5-month) mice that display compromised vascular autophagy, we show suppressed upregulation / initiation of vascular autophagy and eNOS activation in response to 60-min of treadmill-running (i.e., a physiological approach to increase vascular shear stress). Of note, nodes of dysfunction that exist in cells with genetic repression of autophagy, are recapitulated in primary endothelial cells from old vs adult mice, and primary endothelial cells from mice with inducible deletion of autophagy specifically in endothelial cells. It now is time to test the hypothesis that compromised endothelial cell autophagy in the context of aging, impairs physiological initiation of autophagy in primary arterial endothelial cells of older vs. adult humans, to an extent that impairs eNOS activation and subsequent arterial vasodilation. Thus, my new direction is a shift from basic research using cells, vessels, and animals, to clinical research using primary arterial endothelial cells from adult and older males and females. My new collaboration is with Dr. Joel Trinity from the Utah Vascular Research Laboratory (UVRL), who has developed a rhythmic handgrip model wherein brachial artery and radial artery shear-rate can be elevated similarly in adult and old subjects. Drs Ash Nelson and David Morgan, both Physician-Scientists associated with the UVRL and consultants on this project, will obtain primary endothelial cells from the radial artery before and after the exercise intervention, in adult and old patients. Results from this study will determine whether our findings from cells, vessels, and mice with genetic repression of autophagy can be translated to primary endothelial cells obtained from, and arterial function observed in, humans. Data obtained from these studies will lay important groundwork for Aims embedded in upcoming grant applications to address a “human component,” together with basic science-related Aims. Demonstrated experience with using these procedures will increase our competitiveness for extramural funding.

### B. Positions and Honors

#### Research and/or Professional Experience

- **1985-1987**
  Natural Sciences and Engineering Research Council of Canada Visiting Fellowship; Defense and Civil Institute of Environmental Medicine, Environmental Physiology Section, Downsview, ON, Canada

- **1987-1990**
  Postdoctoral Fellowship; University of California-Davis, Department of Internal Medicine, Division of Cardiovascular Medicine, Davis, CA, USA

- **1990-1994**
  Assistant Research Physiologist; University of California-Davis, Department of Internal Medicine, Division of Cardiovascular Medicine.

- **1994-1995**
  Visiting Senior Research Scientist, Cardiovascular Pharmacology, Alliance Pharmaceutical Corporation, San Diego, CA.

- **1995-2001**
  Assistant Adjunct Professor (non-tenure track); University of California-Davis, Department of Internal Medicine.
C. Contributions to Science

1. Postdoctoral Fellowship. I developed/refined a minipig model to study mechanisms responsible for coronary collateral growth and development. Swine were instrumented to evaluate regional and global myocardial function and to quantify regional blood flow at rest and during dynamic exercise. We reported that: (i) myocardial ischemia is not requisite to precipitate collateral development; (ii) flow-induced shear stress was an important requirement for collateral development; (iii) collateral vessels that do grow and develop are adequate to maintain function at rest but are inadequate to do so during the stress of dynamic exercise.

- Symons JD, Pitsillides KF, Longhurst JC. Chronic reduction of myocardial ischemia does not attenuate coronary collateral development in miniswine. Circulation. 1992 86 (2) : 660-71. PMID: 1638730

2. Very early independence. Myocardial ischemia and reperfusion impair coronary vascular function. Exercise training improves coronary vascular function. I developed a rodent model to determine whether prior exercise training is protective concerning subsequent myocardial ischemia. We reported that: (i) while exercise-training is protective in this regard; (ii) high-intensity interval training is even more efficacious; and that (iii) the Na-H exchanger contributes importantly to ischemia-induced coronary vascular dysfunction.

• Symons JD, Rendig SV, Stebbins CL, Longhurst JC. Microvascular and myocardial contractile responses to ischemia: influence of exercise training. J Appl Physiol. 2000 88 (2) : 433-42. PMID:10658008
• Symons JD, Hayashi Y, Ensunsu JL. Improved coronary vascular function evoked by high-intensity treadmill training is maintained in arteries exposed to ischemia and reperfusion. J Appl Physiol. 2003 95 (4) : 1638-47. PMID:12819213
• Symons JD, Schaefer S. Na(+)/H(+) exchange subtype 1 inhibition reduces endothelial dysfunction in vessels from stunned myocardium. Am J Physiol Heart Circ Physiol. 2001 281 (4) : H1575-82. PMID:11557546

3. Early independence. Hyperhomocysteinemia was hypothesized to be a cardiovascular disease risk factor, but its impact on vascular function was unknown. I developed several rodent models to show that endogenously produced pathophysiological concentrations of homocysteine are sufficient to: (i) impair endothelial function in resistance and conductance sized arteries, (ii) increase vascular permeability, and (iii) evoke arterial stiffening.

• Symons JD, Rutledge JC, Simonsen U, Pattathu RA. Vascular dysfunction produced by hyperhomocysteinemia is more severe in the presence of low folate. Am J Physiol Heart Circ Physiol. 2006 290 (1) : H181-91. PMID:16143648

4. Independence (lipotoxicity focus). Obesity and T2DM precipitate endothelial dysfunction but precise mechanisms are unclear. I have used cell systems, isolated vessels, and intact animals with / without genetic manipulation to show that endothelial dysfunction and hypertension that exist in the context of diet-induced obesity is secondary to: (i) elevated free fatty acids (FFAs) in general; and (ii) the FFA metabolite ceramide in particular. Further, we have shown that (iii) ceramide disrupts interactions among Akt-Hsp90-eNOS by activating protein phosphatase 2A (PP2A) in a manner that can be prevented in vivo by using a novel small molecule PP2A inhibitor.

• Symons JD and Abel ED. Lipotoxicity contributes to endothelial dysfunction: a focus on the contribution from ceramide. Reviews in Endocrine and Metabolic Disorders. 2013 14 (1) : 59-68. doi: 10.1007/s11154-012-9235-3 PMID: 23292334

5. Independence (autophagy focus). The mechanism whereby vascular autophagy contributes cardiovascular function is unclear. In 2014 we showed that autophagy could be upregulated in: (i) endothelial cells by nutrient deprivation; (ii) blood vessels by 14 h fasting in mice; and (iii) blood vessels from mice in response to acute exercise. Further, after showing that a protein required for autophagosome formation (Atg3) was lower in arteries from aged vs. adult mice, we silenced Atg3 in endothelial cells and reported that shear-induced NO generation was prevented. A manuscript in R-1 reveals a novel mechanism responsible for this observation in vitro. Ongoing studies are determining whether this (or other) mechanism(s) can be translated aged humans.

Bharath LP, Ruan T, Li YY, Mueller R, Bean T, Reese V, Richardson RS, Sargsyan A, Pires, K, Babu PVA, Boudina S, Graham TE, Symons JD. Endothelial cell autophagy maintains shear-stress-induced nitric oxide generation via glycolysis-dependent purinergic signaling to eNOS. R-1 2017


A complete list of ~70 publications is at: http://www.ncbi.nlm.nih.gov/sites/myncbi/1hc1qC8WC8iAe/bibliography/46643892/public/?sort=date&direction=descending

D. Current Research Support

• 16GRNT31050004 American Heart Association Western States Affiliate Grant-In-Aid
  07/01/2016 – 06-30-2018
  Aging limits autophagic flux in endothelial cells
  154K over 2 years Role: PI
  The purpose of this grant is to determine the time course of autophagy repression in endothelial cells during aging.

• 1RO3 AG052848-01A1 National Institutes of Health
  01/01/2017 – 12-31-2019
  Characterizing the phenotype of young and old mice with disrupted vascular autophagy
  149K over 2 years Role: PI
  The purpose of this grant is to elucidate mechanisms whereby compromised autophagy limits endothelial cell nitric oxide generation.

• 16UFEL31810001 American Heart Association Western States Affiliate Undergraduate Fellowship
  07/01/2016 – 06/30/2017
  Vascular adaptations require intact endothelial cell autophagy
  $6,500 Student (G Hestwood) Mentor (JD Symons)
  The purpose of this grant is to determine whether EC autophagy must be intact for training-induced vascular adaptations to be observed.

• Undergraduate student support through University of Utah Undergraduate Research Opportunities Program (UROP), 3 students at present x 2400.00 per award.

Completed Research Support relevant to this application

• WU13237P02917459W Washington University (WU) Diabetes Research Center (DRC) Pilot / Feasibility Grant
  Pathophysiological and genetic disruption of EC autophagy lowers endothelial cell NO production
  40K / 1 year Role: PI
  This is an NIH NIDDK award to Washington University, that solicits applications externally for Pilot/Feasibility Grants

• University of Utah, Diabetes and Metabolism Center : Pilot and Feasibility Grant
  Characterizing a mouse model to study vascular autophagy in obesity and type 2 diabetes
  01-01-2015 – 12-12-2016
  25K / 2 year Role: PI
  This grant is designed to provide funds to generate / characterize iecAtg3KO mice.

• University of Utah, Center on Aging : Pilot and Feasibility Grant
  Links among autophagy, mitophagy, and nitric oxide bioavailability in aging vasculature.
  01-01-2015 – 12-12-2015
  20K / 1 year Role: PI
  This grant is designed to provide funds to collect preliminary data for extramural applications.

• Undergraduate student support through University of Utah Undergraduate Research Opportunities Program (UROP), 4 students x 2400.00 per award; American Heart Association, 2 students x 6000.00 per award; American Physiological Society, 1 student x 7500.00 per award; American Diabetes Association Minority Undergraduate Internship Award, 1 student x 3000.00; Native American Research Internship Program, 1 student x 6000.00 per award; 2 students x Science Without Borders program; Brazil).

Completed Research Support – last 4 years only

• 2R15HL091493-02 National Institutes of Health
  The role of ceramide in contributing to vascular dysfunction in diet-induced obesity.
  08/2012 – 07-2016
  100K/year 3 years Role: PI
This grant provides undergraduate students with research training experience.

• 1-12-BS-208 American Diabetes Association Research Grant 01/2012 – 12/2014
  Mechanisms whereby endogenous ceramide impairs eNOS signaling and arterial function
  This grant is a renewal that uses intact animals, isolated vessels and cell systems assays to determine mechanisms responsible for ceramide-induced vascular dysfunction.
  100K per year / 3 years Role: PI

• UU Research Committee Faculty and Research and Creative Grant 07/2008 – 06/2010
  Determining the contribution from ceramide to cardiovascular complications associated with obesity in Spltcb2+ mice.
  The goal of this research is to determine whether ceramide evokes endothelial dysfunction in a tissue autonomous manner using a genetic approach. Role: PI

• UU Interdisciplinary Research Grant 01/2010 – 12/2010
  Determination of reactive oxygen / nitrogen species in cell culture models of oxidant stress using electron paramagnetic spectroscopy (EPR). Optimize the use of EPR to test the hypothesis that ceramide accumulation generates superoxide anion which combines with nitric oxide to form peroxynitrite and disrupt agonist mediated eNOS dimerization. Role: PI

• 1R15HL091493-01 01/2008 – 11/2011
  National Institutes of Health R15 A.R.E.A. Grant; The role of ceramide in contributing to vascular dysfunction in diet-induced obesity. To provide undergraduate students with research training experience. Role: PI

• 7-08-RA-164 07/2008 – 11/2011
  American Diabetes Association Research Grant
  Role of ceramide in obesity-related vascular dysfunction.
  To test the hypotheses that ceramide contributes to lowering nitric oxide bioavailability using cell culture, isolated vessel, and whole animal systems. Role: PI

• University of Utah Funding Incentive Seed Grant 07/2011 – 06/2012
  Mechanisms for ceramide-mediated vascular dysfunction; This grant provides funds for investigators to obtain preliminary data to be used for extramural grant applications. These funds will be used to optimize cell imaging studies for mapping intracellular protein movement. 28 K per year; 1 year; Role: PI

• University of Utah College of Health Research and Creative Grant Fund 06/2011 – 05/2012
  Ceramide binds I2PP2A and activates PP2A. This grant funds preliminary experiments to determine how ceramide activates PP2A. 6K per year; 1 year, Role: PI

Pending Research Support

• 1RO1 HL135592-A1 National Institutes of Health 07/01/2017 – 06-30-2022
  Autophagy maintains vascular function through a novel glycolysis-linked pathway regulating eNOS
  2,097,000 over 5 y Role: PI
  The purpose of this grant is to elucidate mechanisms whereby compromised autophagy limits endothelial cell nitric oxide generation.

• UU Office of Vice President – Research Instrumentation Grant
  55K to purchase an additional isobaric pressure perfused myograph apparatus
  Submitted 01-15-2017