Swine Models for NAD$^+$ Supplementation in Heart Failure

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Synopsis

We are exploring the hypothesis that nicotinamide adenine dinucleotide (NAD+) metabolism can be targeted to improve functional capacity in failing human hearts. NAD+ is a ubiquitous molecule that is required as a redox cofactor or substrate for hundreds of enzymes within the cell. NAD+ concentration falls in failing human hearts and in some rodent models of heart failure. High doses of precursors including nicotinamide riboside (NR) have therapeutic effects in rodent models. However, the doses used exceed what is tolerable in humans and the potential for benefits at human-relevant doses remains uncertain. Our preliminary and published results suggest that high doses of NR may be required in rodent models because it is extensively metabolized in the intestines and liver when delivered orally, with only a tiny fraction reaching the circulation intact. In contrast, intravenous delivery allows a much higher proportion of the dose to reach organs such as the heart. In addition to questions about dosing, the mechanism of protection has remained unclear. It is presumed to involve cardiac NAD+ levels, but whole-body supplementation studies leave open the possibility that other tissues mediate protection, for example through lowering blood pressure. Others have proposed that intermediates in NAD+ synthesis might influence cardiac function through binding to ion channels, rather than via NAD+, but this possibility has not been tested due to challenges in measuring conductance in the mouse heart. Here, we propose that swine provide an ideal system in which to test these questions. Swine have a more human-like cardiovascular system than do rodents and faithfully recapitulate many aspects of human heart failure. Unlike humans, swine can be subjected to invasive analyses and tested using higher “mouse-like” doses and alternative delivery routes such as i.v. that are not yet proven safe for human study. We will take advantage of state-of-the-art facilities available at Penn to assess cardiac function in swine, including echocardiography, magnetic resonance imaging, and invasive hemodynamics. These studies will allow us to obtain much more information than prior studies in rodents, and to obtain a more uniform heart failure phenotype as well as perform higher dosing and invasive tissue-level analyses that cannot be tested in human trials. This is precisely the information that is needed by the field to determine whether the promising data in rodents are likely to translate to viable human therapeutics. Our proposal illustrates the potential for synergistic interactions between Dr. Baur, an expert in NAD+ metabolism in the Dept. of Physiology, and Dr. Tschabrunn, an expert in large animal models of heart failure in the Dept. of Medicine. The major weakness cited for an R01 on this project that was submitted but is not yet funded was the lack of preliminary data using the test compound (NR) in the proposed swine model. Our proposal to the Synergy program is intended to obtain these preliminary data in support of a resubmission.

Specific Aims

(These aims are designed to fit the budget and time frame of the Synergy program - our planned R01 will include more variables: additional compounds and doses, i.v. delivery, reversibility of established heart failure.)

Aim 1) Demonstrate that high-dose oral NR is tolerable and raises blood and tissue NAD+ levels in swine
Aim 2) Provide a preliminary assessment of the effect of high-dose oral NR on cardiac remodeling, function, and electrical activity in swine

Significance

Heart failure - the syndrome in which the heart fails to adequately pump blood throughout the body, leading to insufficient tissue perfusion and nutrient delivery - is a leading cause of morbidity and mortality in the US and worldwide. It is estimated to affect more than six million Americans and to contribute to about 1 in 8 of deaths (1). Supplemental precursors for NAD+ have recently been shown to protect against heart failure (HF) across multiple preclinical models in rodents (2-6). Combined with the observation that NAD+ concentration is lower in failed human hearts (7), this work has inspired great interest in the translational potential of supplementing NAD+ levels in human HF patients. However, humans have variable presentation and environments necessitating large studies, cannot be dosed at the levels used in rodents and cannot be subjected to invasive studies to determine mechanisms. Moreover, safety data are lacking for alternative delivery methods such as i.v. that might allow lower doses to be effective. Studying the role of NAD+ in swine will allow us to determine whether
supplementation holds promise in a more human-like model of heart failure, to characterize the underlying mechanisms, and to address key questions in the field, such as: 1) Are higher “mouse-like” doses required for similar efficacy of oral NAD\(^+\) precursors in larger animals? 2) Does the route of delivery determine the dose required? and 3) Does NR work better than “conventional” vitamin B3 precursors? Such studies are urgently needed to assess the translatability of rodent findings and guide early-stage human trials.

Approach

Animal Study Timeline: Swine will be randomized into 2 groups: Placebo (n=4) or High Dose Oral NR (500mg/kg, n=4); Prior to initiation of daily oral therapy, all animals will undergo a baseline study that will include echo imaging, hemodynamic data collection, and laboratory assessment. One week later, myocardial infarction will be initiated, and repeat echo imaging and hemodynamic/laboratory data collection will be performed 2 weeks and 4 weeks post-infarction. The terminal study will be performed 8 weeks post-infarction and will include repeat echo, hemodynamic, and laboratory assessment in addition to electrophysiology study, and high-resolution intracardiac mapping.

We will employ a well-characterized swine model of post-infarction heart failure that has been developed and used extensively in the Tschabrunn lab (8). Briefly, utilizing fluoroscopy, vascular access is obtained via the common femoral artery, percutaneous coronary angioplasty wire and balloon are advanced into the LAD and the balloon is inflated just distal to the first diagonal coronary artery. Following 180 minutes of occlusion, the balloon is deflated and vascular perfusion is restored. The resulting infarct size typically covers about 15% of the left ventricle and results in an ejection fraction of ~30%.

Animals will receive placebo or high dose oral NR (comparable to the dosing in rodent studies) beginning one week prior to surgery in order to establish whether the beneficial effects of this NAD\(^+\) precursor are conserved in a large animal model. In addition, we will take advantage of the advanced capabilities in the Tschabrunn lab to study changes in hemodynamics and electrophysiology as well as perform high-resolution intracardiac mapping (Figure 1). None of these methods are available in rodent models, which have generally relied on fractional shortening (a surrogate of ejection fraction) as the only functional readout.

Post-sacrifice, NAD\(^+\) levels will be measured in both infarcted and non-infarcted cardiac tissue, whole blood, and liver. Cardiac tissue will be examined histologically for evidence of fibrosis, necrosis, and inflammatory pathology.

Impact and Future Work

The proposed experiments will provide critical preliminary data to support a resubmission of our R01 to explore the impact of NAD\(^+\) metabolism in heart failure comprehensively in swine. These studies will fill a key gap in the field, which is currently struggling to translate successful findings in rodents (high-dose, short term, uniform but poorly measure cardiac phenotypes, invasive analyses) to viable therapies for humans (lower doses tolerated, longer term disease course, variable but well measured phenotypes, no invasive analysis). Swine allow a range of doses to be characterized in a model with a more uniform presentation, detailed phenotyping, and invasive analyses, making it possible to rationally guide future human trials.
References


NAME: Joseph A. Baur

eRA COMMONS USER NAME (credential, e.g., agency login): joebaur

POSITION TITLE: Professor of Physiology

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

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<tr>
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<td>Harvard Medical School, Boston, MA</td>
<td>Postdoctoral Fellow</td>
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<td>Molecular Biology of Aging</td>
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A. Personal Statement

My career has been dedicated to understanding the molecular mechanisms that lead to aging, and how they can be influenced to promote health and longevity. I am a Professor of Physiology and my lab is located within the Institute for Diabetes, Obesity, and Metabolism at the Perelman School of Medicine of the University of Pennsylvania. I also direct the Rodent Metabolic Phenotyping Core of Penn’s Institute for Diabetes, Obesity, and Metabolism. Current projects in the lab are focused on elucidating the molecular mechanisms by which dietary factors exert beneficial effects on longevity and metabolism in mammals. A major area of interest is the role of changes in nicotinamide adenine dinucleotide (NAD⁺)-metabolism in mediating aging and susceptibility to disease. NAD⁺ falls over the course of natural aging, and can be increased by dietary restriction, exercise, or supplemental precursors. To better understand the roles of NAD⁺ in normal physiology, we have characterized strains of mice with tissue-specific overexpression or deletion of the key NAD⁺ biosynthetic enzyme nicotinamide phosphoribosyltransferase (Nampt). These genetic changes drive corresponding changes in NAD⁺ levels in target cell types, allowing the design of more precise experiments than those conducted with precursor supplements or disease models. We have also developed extensive methodology for measuring NAD⁺-related metabolites, including via tracer studies to determine flux. A major challenge for the field is to understand the compartmentalization of NAD⁺ within mitochondria and other organelles. We have recently identified SLC25A51 as the long-sought transporter that allows NAD⁺ to enter the mitochondrial matrix, which will enable experiments to manipulate this pool specifically. In unpublished studies, we have demonstrated that decreased cardiomyocyte NAD⁺ content is sufficient to recapitulate features of heart failure. The rich environment at Penn, including my co-Investigator Cory Tschabrunn and collaborators such as Dan Kelly has made this extension of our work into cardiac biology possible. Cory Tschabrunn has dedicated the early part of his career to developing and standardizing the swine model that we propose to use, and has assembled a state-of-the-art suite of equipment for this purpose. We have worked together closely throughout the preparation of this proposal. Dan Kelly, who provides extensive expertise on rodent and human heart failure, has served as a co-mentor for a postdoc trained in my lab, Tim Luongo, and we are coauthors on a submitted manuscript from Tim’s work, as well as a separate manuscript in Nature Cardiovascular Research, in addition to frequently consulting on each other’s projects. Over the course of our studies on NAD⁺, my lab has also developed a number of synergistic collaborations outside of Penn, including with Marie Migaud, who is a co-Investigator via subcontract on the present proposal. In particular, we have made substantial use of isotopically labeled NAD⁺ precursors and synthetic intermediates created by the Migaud lab. We have also dramatically extended out analytic capabilities by working with Josh Rabinowitz, a world-renowned expert in mass spectrometry and metabolomics who directs a joint Penn/Princeton core. Our broad goal is to identify
molecular mechanisms that are amenable to nutritional or pharmacological manipulation and can be translated into therapies to prevent or treat age-related diseases in humans. Thus, together with Cory Tschabrunn, I am well suited to lead this team exploring the role of cardiac NAD+ in heart failure in swine.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022-Present  Professor, Institute for Diabetes, Obesity and Metabolism and Department of Physiology, Perelman School of Medicine at the University of Pennsylvania
2016-Present  Director, Rodent Metabolic Phenotyping Core, Institute for Diabetes, Obesity and Metabolism, Perelman School of Medicine at the University of Pennsylvania
2015-Present  Associate Editor, Journals of Gerontology, Series A: Biological Sciences
2015-Present  Editorial Board Member, AGE – The Journal of the American Aging Association
2013-Present  Editorial Board Member, ScienceOpen
2011-Present  Associate Editor, Frontiers in Genetics of Aging
2017-2022  Associate Professor, Institute for Diabetes, Obesity and Metabolism and Department of Physiology, Perelman School of Medicine at the University of Pennsylvania
2009-2017  Assistant Professor, Institute for Diabetes, Obesity and Metabolism and Department of Physiology, Perelman School of Medicine at the University of Pennsylvania
2008-2009  Instructor, Institute for Diabetes, Obesity and Metabolism and Department of Physiology, University of Pennsylvania School of Medicine
2003-2008  Post-Doctoral Fellow, lab of David Sinclair, Department of Pathology, Harvard Medical School
1998-2003  Ph.D. (Integrative Biology), Shay/Wright lab, Department of Cell Biology, UT Southwestern Medical Center, Dallas, Texas (UTSW)

Honors and Awards

2020  Collaborative Scientist Grant, American Society of Transplant Surgeons
2018-2020  Keith Michael Andrus Cardiac Research Award, Friedreich's Ataxia Research Alliance
2013  Joseph A. Pignolo, Sr. Award in Aging Research
2010-2014  New Scholar Award, Ellison Medical Foundation
2008-2012  K99/R00 Pathway to Independence Award
2004-2006  Post-doctoral Fellowship 0425834T, American Heart Association
2003  Finalist for the Nominata Award (top UTSW Graduate Student)
2001  Sigma Xi and GSO Poster Awards
2001  New Opportunities Award NO-0005-00, Ellison Medical Foundation
1999  Honorary Mention for the Howard Hughes Fellowship, Howard Hughes Research Institute
1998  NSERC Post-Graduate Fellowship, National Science and Engineering Research Council (declined)
1997  Colville Award/Huggins Scholarship, Acadia University
1997  Malcolm W. Orchard Memorial Scholarship, Acadia University
1996-1997  Clarke K. McLeod Scholarship, Acadia University
1996  Chester W. Small Scholarship, Acadia University
1995  Dr. Leverett Chipman Dev. Scholarship, Acadia University
1995  NSPI Scholarship, Nova Scotia Power Inc.
1995  Manning Scholarship, Acadia University
1994  Gold level Duke of Edinburgh’s Award (Citizenship)

C. Contributions to Science

1. As a graduate student, I demonstrated the existence of telomere position effect in human cells. Telomeres are complex DNA/protein structures that cap the ends of human chromosomes, and that shrink with each cell division due to incomplete replication of the underlying DNA. My work established
that this shortening of telomeres can relieve transcription silencing of adjacent genes, which may have implications for aging as well disorders involving chromosome rearrangements. Ongoing work has identified several genes that are regulated by this mechanism and could thereby contribute to age-related phenotypes.


2. During my postdoctoral studies, I was among the first to demonstrate that resveratrol, a small polyphenol that has many biological activities, including activation of sirtuin enzymes, has potent anti-diabetic effects in mice. Although high doses can lead to weight loss, we found that in obese mice moderate doses of resveratrol restored insulin sensitivity and longevity independently from body weight and had transcriptional effects comparable to those of consuming fewer calories. These studies have contributed to the rationale for many ongoing clinical trials, with an emerging consensus that resveratrol has mild, but beneficial effects in type II diabetics. I have continued to pursue mechanistic aspects of these findings collaboratively, with the hope of improving our ability to translate the success in rodents.


3. A major focus of my independent career has been testing how physiological fluctuations the availability of nicotinamide adenine dinucleotide (NAD) influence the activities or various enzymes that employ it as a redox cofactor or cosubstrate. NAD levels vary in a circadian fashion, and as a function of age and diet, and multiple studies have shown improvements in metabolism and disease phenotypes in animals given supplements of precursor vitamins. I have developed strains of mice with tissue-specific overexpression or loss of NAMPT, the rate-limiting enzyme for NAD biosynthesis from nicotinamide, which have led to important insights in the field. We have shown that NAD depletion in skeletal muscle causes progressive myopathy and mitochondrial dysfunction that is rapidly reversible with nicotinamide riboside, an NAD precursor. We have also shown that NAD levels become limiting during liver regeneration, and that increasing NAD levels genetically or through supplementation with precursors accelerates the process, which may be a model for tissue regeneration more broadly. We have worked with the Migaud lab at the University of Southern Alabama and the Rabinowitz lab at Princeton to develop isotopic tracer methods to understand NAD turnover and the metabolism of supplemental precursors. Most recently, we have identified SLC25A51/MCART1 as the mitochondrial NAD transporter in mammalian cells, allowing compartment-specific manipulation of mitochondrial NAD for the first time.
4. I have made significant contributions to our understanding of how rapamycin affects mammalian physiology, particularly with respect to its undesirable metabolic consequences. Rapamycin’s effects are of significant interest, because it robustly extends mouse lifespan and attenuates age-related diseases, yet has side effects, including metabolic dysfunction, that limit its potential utility in humans. Together with Dudley Lamming, my lab published the first direct evidence that off-target inhibition of mTORC2 is the cause of rapamycin-induced insulin resistance. We further revealed that rapamycin impairs the generation of beige adipocytes, which are metabolically active and have potential roles in glucose and lipid metabolism. Most recently, we have shown that loss of mTORC1 in adipocytes is sufficient to cause hyperlipidemia in mice due to a failure to suppress lipolysis upon feeding. We continue to work actively in this area with the hope of developing alternative approaches to capture the benefits of rapamycin without the side effects.


Complete List of Published Work in MyBibliography:

h-index (Google scholar): 64
NAME: Cory Michael Tschabrunn

eRA COMMONS USER NAME (credential, e.g., agency login): CORYTSCHABRUNN

POSITION TITLE: Research Assistant Professor of Medicine

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

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<td>Ph.D.</td>
<td>08/2016</td>
<td>Health Sciences – Cardiac Electrophysiology</td>
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A. Personal Statement

I am an Assistant Professor in the Division of Cardiovascular Medicine and Director of Translational Cardiac Electrophysiology at Penn. I devote a majority of my effort to clinical and translational research, with a primary focus in cardiovascular medicine, cardiac electrophysiology, and large animal comparative disease modeling. Our multidisciplinary research program integrates clinical, translational, and basic science investigators with engineers, physicists, geneticists, and molecular/cell biologist to further elucidate the mechanisms of complex cardiac arrhythmias and develop novel therapeutic treatment strategies for such conditions. In addition, my laboratory focuses on the development of clinically relevant translational research models for mechanistic investigations and new technology development for various cardiovascular diseases. My team has developed and refined the use of minimally invasive percutaneous large animal models, including a well-characterized human-like model of chronic myocardial infarction that is the foundation of this proposal. I am fully confident with my background, expertise in large animal experimentation, and existing relationships with the study team, this proposal will be successful.

Ongoing grant support that I would like to highlight includes:

R42-HL-139309
Tschabrunn (PI)
Catheter Based Cardiovascular Device Retrieval System
9/24/2018-6/30/2022

R44-HL-158375
Tschabrunn (PI)
Utility of Esophageal Cooling Therapy for the Prevention of Thermal Injury During Atrial Fibrillation
9/15/2021-7/31/2024

R01-HL135090
Atluri (PI), Role: Co-Investigator
A Novel Shear Thinning Hydrogel System for Advanced Cellular Therapy in Ischemic Heart Disease
8/1/2017-5/31/2022

R44-HL-140645
R. Gorman (PI), Role: Co-Investigator
Myocardial Delivery of MMP Inhibiting Hydrogels
07/15/2020-06/30/2022
B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020-Present  Assistant Professor of Medicine (Research Track), Division of Cardiovascular Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
2017-Present  Director of Translational Cardiac Electrophysiology Laboratory, Division of Cardiovascular Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
2017-2020  Instructor of Medicine, Division of Cardiovascular Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
2014-2017  Technical Director, Experimental Electrophysiology Laboratory, Beth Israel Deaconess Medical Center, Boston, MA
2012-2017  Research Associate, Division of Cardiovascular Medicine, Cardiac Electrophysiology Section, Beth Israel Deaconess Medical Center, Boston, MA
2009-2012  Research Assistant, Division of Cardiovascular Medicine, Cardiac Electrophysiology Section, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
2006-2009  Senior Research Analyst, Division of Cardiovascular Medicine, Cardiac Electrophysiology Section, Stony Brook University School of Medicine, Stony Brook, NY
2006-Present  Member, Heart Rhythm Society

Honors

2017  Conscientious Researcher IACUC Award, Beth Israel Deaconess Medical Center
2022  Conscientious Investigator Award, University of Pennsylvania IACUC

C. Contributions to Science

I have published over 70 peer-reviewed original research manuscripts and over 70 book chapters, editorials, reviews, and abstracts. My h-index according to google scholar is 28. A complete listing of my PubMed-referenced papers can be found at: https://www.ncbi.nlm.nih.gov/pubmed/?term=tschabrunn.

1. I developed a human-like model of post-infarction reentrant ventricular tachycardia. This model is utilized to better understand the physiology underlying these arrhythmia circuits and develop novel imaging, histopathologic, and mapping/ablation system algorithms to identify and target critical regions.


2. The model systems developed have been used to foster collaborations with industry, engineers, and cardiovascular magnetic resonance imaging experts to develop and validate new mapping/ablation system and imaging based technologies for the treatment of cardiac arrhythmias.


3. Long-term outcome following cardiac ablation procedures have improved, but remain sub-optimal. In the clinical electrophysiology laboratory, I have participated in several investigations using electroanatomical mapping technologies to better understand the mechanisms and substrate underlying complex cardiac arrhythmias.


4. Additional clinical investigations have been performed focused on ways to improve catheter-based mapping and ablation therapies in challenging patient populations. These studies, 2 of which had
evolved from initial translational laboratory-based investigations, introduced new techniques and technologies to improve clinical outcomes.


Budget Justification ($100,000 total, 1 year)

Principal Investigators

Joseph Baur, PhD (co-PI, no salary support requested): Dr. Baur’s research focuses on understanding the basic mechanisms that lead to aging, and how interventions such as restricting nutrient intake are able to slow the process. A central focus of the lab since its inception has been understanding the role of nicotinamide adenine dinucleotide (NAD⁺) in controlling the metabolic response to changes in nutrient status. Dr. Baur has published several important studies in this area and has developed genetic models that are currently used by many in the field, most recently creating a model of cardiomyocyte-specific NAD⁺ depletion. Dr. Baur is also the Director of Penn’s Rodent Metabolic Phenotyping Core and has considerable experience in assessing mouse physiology.

Cory Tschabrunn, PhD (co-PI, no salary support requested): Dr. Tschabrunn’s research focuses on structural heart disease, post-infarction remodeling, and mechanisms of cardiac arrhythmias. His laboratory develops and utilizes clinically relevant large animal models for mechanistic investigation and new technology/therapeutics development for various cardiovascular diseases. Dr. Tschabrunn and his team have significant experience in chronic infarct model creation and post-infarct phenotyping with various imaging and electroanatomic mapping techniques. Dr. Tschabrunn is also Director of Translational Cardiac Electrophysiology at Penn.

Other Personnel

Tara Oster, BS (Research Specialist, 2.4 CM, $13,260): Ms. Oster has several years of large animal experience and has been an integral member of the Tschabrunn lab for the last year. She will provide anesthesia support, post-operative care for all surgical and imaging procedures outlined in the proposal.

James Davis, PhD (Research Specialist, 1 CM, $6,265): Dr. Davis has over a decade of experience studying NAD⁺ metabolism in human and mouse tissues. He helped develop the NAD⁺ assays used in the lab and routinely analyzes the NAD⁺ contents of cells and tissues. Dr. Davis will assist with tissue collections and sample preparation and will take primary responsibility for assaying the NAD⁺ content of blood and tissues derived from these studies.

Materials and Supplies

Animal purchase/delivery/intake ($9,600): Eight pigs will be purchased at a cost of $1200 each.

Animal per diem ($10,675): Each animal will be housed for 9 weeks at a cost of $21.18/day. Eight animals x 63 days x $21.18 = $10,675.

Infarct procedure ($20,240): The infarct procedure costs $2,530/animal. This includes use of the translational cardiovascular laboratory facility, anesthetic agents, medications, all surgical supplies, and coronary angioplasty equipment.

Two-week assessments ($9,360): All animals will be assessed by transthoracic echocardiogram, hemodynamics, and basic labs at a total cost of $1,170/animal two weeks after the infarct procedure. Costs include anesthetic agents, medications, supplies for hemodynamic data collection, and laboratory assessment.

Four-week assessments ($9,360): All animals will be assessed by transthoracic echocardiogram, hemodynamics, and basic labs at a cost of $1,170/animal four weeks after the infarct procedure. Costs include anesthetic agents, medications, and facility costs. The Tschabrunn laboratory will provide intracardiac mapping disposables at no cost to allow completion of this pilot study.

Terminal Study ($18,240): All animals will be assessed by intracardiac electroanatomic mapping, repeat transthoracic echocardiogram, hemodynamics, and basic labs at a cost of $2,280/animal eight weeks after the infarct procedure. Costs include anesthetic agents, medications, and facility costs. The Tschabrunn laboratory will provide intracardiac mapping disposables at no cost to allow completion of this pilot study.

General laboratory supplies ($3000): General laboratory supplies including reagents for determination of NAD⁺ concentrations and preparation of histology sections, disposable gloves, plastics, glassware, etc.