1. **Title:** Is Heart Failure with Reduced Ejection Fraction (HFrEF) an Outcome of Coronary Microvascular Disease.

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**Location:** NEOMED

2. **Abstract.** The goal of this proposal is to test the hypothesis that impairments in the connection between flow and metabolism are linked to heart failure. In the heart, the connection of flow to aerobic metabolism, metabolotransduction, is essential for our livelihood, because anaerobic metabolism is inadequate to maintain cardiac pump function. Despite the critical dependency of cardiac work on continual delivery of oxygen and nutrients, a complete understanding of mechanisms involved in the coupling of flow to metabolism is evasive. Previously, we found that mice null for Kv1.5 channels rapidly develop heart failure (HFrEF) when subjected to a hemodynamic challenge. These mice show evidence of coronary insufficiency, i.e., inadequate perfusion of the myocardium and impairments in ventricular contraction, minute areas of hypoxic tissue and ST segment changes in the ECG consistent with ischemia. To determine if this heart failure phenotype is a nuance of specific deletion of Kv1.5 channels (or not), we propose to study if mice null for Kv1.2 channels show the same alterations in myocardial blood flow and also show a propensity to develop heart failure. This is done by using a genetically modified mouse, which has smooth muscle specific deletion of Kv1.2 channels. If our results show that Kv1.2 mice show a similar heart failure phenotype to Kv1.5 null mice, this will be a paradigm shift in the understanding of heart failure as a potential form of ischemic heart disease. If reconnecting metabolism to flow halts or reverses the disease, such a result may provide new insights into the treatment of patients with heart failure.

3. **Background and Rationale.** A Medline analysis of heart failure and coronary metabolic dilation/hyperemia yielded 46 "hits," but none addressed heart failure as caused by inadequate metabolic dilation. What we find interesting is that there is anecdotal literature and clinical practice supporting the role of impaired metabolic dilation in heart failure. Specifically, beta-adrenergic antagonists are now one of the drugs of choice for all but the most severe cases of failure, and a priori one would think that a negative inotrope, which reduces pump function, would be contraindicated to treat the myocardium with impaired contractility. Our explanation is that with a reduction in oxygen demands the imbalance between metabolism and flow is lessened, thereby reducing microareas of ischemia and apoptosis, and the progression to failure is slowed. Thus our goal of elucidating the specific ion channels involved in metabolotransduction in the heart may help our understanding of the basis of certain types of heart failure. Within this context, below is a summary of some known concepts that we have elucidated that provide some background into our interrogation of Kv1.2 channels and the potential role they may play in the disassociation of flow from metabolism in heart failure.

- Metabolic coronary dilation is in part mediated by hydrogen peroxide
- Hydrogen peroxide mediated activation of redox sensitive Kv1.5 channels plays a key role in coronary metabolic dilation.
• Heart failure is a progressive condition characterized by microvascular ischemic foci leading to diffuse fibrosis and impaired pump function.
• Kv1.5 null mice show a much higher propensity to develop HFrEF (compared to wild type [WT] mice) when subjected to a hemodynamic stress.

Our hypothesis is supported by our preliminary data showing that Kv1.2+/- mice show impaired metabolic dilation; accordingly, we believe they will progress rapidly into HFrEF when subjected to a hemodynamic challenge (high blood pressure).

4. Goals and objectives. The goal of this summer research is to test the hypothesis that expression of the Kv1.5 family channels after the onset of cardiac dysfunction and heart failure during imposition of hypertension will stop the progression of dysfunction or restore normal function. This will be accomplished in a transgenic mouse we have created which has tet-on smooth muscle specific expression of Kv1.5 channels in a Kv1.5 null background. The null mouse shows impaired metabolic dilation and rapidly develops heart failure (within 2 weeks) after imposition of hypertension produced by transaortic constriction. We will express these channels to reconnect myocardial blood flow with metabolism in one group of transgenic mice and compare the results to a group without channel expression. Given that we currently have all the resources necessary for this project, the goal is attainable over the course of the summer. We are not studying wild type animals in this design because we have a plethora of data showing the responses of wild types to the hypertension; importantly these animals do not develop heart failure in response to the heightened cardiac metabolism.

5. Investigative Methods.

| METHODS (all studies and surgeries will be performed in anesthetized (isoflurane) mice) |
|----------------------------------|------------------|-----------------|-----------------|
| Model                           | Measurement      | Method           | Groups and Protocol                        |
| Kv1.2<sup>WT</sup> and Kv1.2<sup>/-</sup> subjected to transaortic constriction (TAC) | Myocardial Blood Flow | Contrast Echocardiography | Protocol: 1) Baseline (Hexamethonium), 2) Varying doses of norepinephrine, i.v., to increase cardiac work up to 4 fold. |
|                                 | Arterial Pressure, Heart Rate | Solid state catheter in aorta via femoral | Groups: 1) Kv1.2<sup>/-</sup> treated with tamoxifen and 2) Kv1.2<sup>WT</sup>, without tamoxifen |
|                                 | Stroke Volume    | Echocardiography, M-mode |                              |
|                                 | Electrocardiogram | Standard limb leads |                              |
|                                 | Pulmonary edema  | Lung dry/wet wet |                              |

The deletion of the channel is a tamoxifen activated Cre transgenic. If the mice do not receive tamoxifen, they have a WT phenotype, and the channel is expressed. If treated with tamoxifen, the channel is deleted. After the measurements and the end of the protocol, mice will be euthanized by a high dose of isoflurane followed by either i.v. KCl or Fatal Plus.
6. **Proposed method of data analysis.** One-way ANOVA and t-tests where appropriate. P<0.05 will be accepted for statistical significance.

7. **Significance of anticipated findings.** If our hypothesis is correct (we reject the null hypothesis), we will show that re-establishment of the connections between flow and metabolism in the heart may stop, or reverse, the progression of heart failure. To date in clinical medicine, the therapies for heart failure just slow the progression of the disease. Our results may open a new vista in the interrogation of the problem and could lead to a new strategy to control this debilitating condition.
8. Appendix: Summer Research Fellow Training/Mentoring Plan. The plan we have for the student (an M1, Jacqueline Graham, has contacted us about her desire to work on this project) is arranged in a hierarchical manner.

a. First, Jennifer will interact in a 1:1 manner with Dr. Ohanyan, who will teach her the surgical and experimental procedures, including echocardiographic measurements and data analysis.

Second, Jennifer will interact with Dr. Chilian in a 1:1 manner reviewing the data, the protocols, the rationale and the interpretations. Dr. Chilian and Dr. Ohanyan will serve as co-mentors.

Third, Jennifer will attend the lab meetings of the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Bratz, Raman, Thodeti, Meszaros, Yin, Ohanyan, Jones, and Yun) and will present her results in this weekly meeting.

Fourth, Jennifer will participate in a summer journal club that will involve all the summer research students and faculty. Each summer student will be expected to participate.

Fifth, Jennifer will be expected to present a poster at the research day when all summer fellows present a synopsis of their work.

b. All the necessary resources (echocardiographs, Millar system to measure arterial pressure, anesthesia machines, computer for measuring evaluating echo images, transgenic mice, surgical instruments, surgical supplies, ultrasonic contrast) and financial resources for completing the research are available.

c. The research will be completed at NEOMED.