Patients in heart failure are sometimes implanted with a left ventricular assist device (LVAD) to bridge them to transplant. Clinical data shows that ~15% of these patients recover pump function and can subsequently have their LVAD removed. The goal of our study was to determine whether cellular level maximal power output of the left ventricle improves after patients are fitted with LVADs. Myocardial samples were obtained (1) from the left ventricle apex of patients when they were implanted with a LVAD and (2) from the left ventricle free wall when they subsequently received a heart transplant. Non failing cardiac samples were also obtained from organ donors for comparison. All samples were snap frozen in liquid nitrogen and stored in the vapor phase of liquid nitrogen. Multicellular preparations were then obtained by chemically permeabilizing the samples in 1% Triton solution after mechanical homogenization. These preparations were connected between a force transducer and a motor and maximally activated in a saturating Ca²⁺ solution. Once force had reached steady state, the preparations were allowed to shorten against pre-set loads imposed using SLControl software. Maximum power output was determined from the force-velocity curves. Preliminary results suggest that LVAD treatment improved cellular level maximum power output and isometric force. There was also a trend towards a lower maximum shortening velocity post-LVAD. Further studies, will determine whether intrinsic ventricular function is improved by LVAD implantation as well as the molecular mechanisms that may mediate any observed responses.

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Superinhibitory Phospholemman Mutants as Potential Therapeutics for Heart Failure
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Heart failure is characterized by a decrease in cardiac contractility and is a leading cause of morbidity and mortality. Treatment with cardiac glycosides such as digoxin increases cardiac contractility by elevating intracellular calcium through inhibition of the sodium-potassium ATPase (NKA). However, the therapeutic window for this class of drugs is narrow as high calcium levels lead to increased SR load, calcium leak, and cardiac arrhythmias. Alternatively, the endogenous inhibitor of NKA, phospholemman (PLM), is dynamically regulated. We hypothesize that a superinhibitory mutant of PLM will increase the contractility of cardiac myocytes. This effect will be inherently self-limiting since inhibition of NKA is relieved by phosphorylation or elevated intracellular sodium. Here we used a high-throughput screen of PLM mutants utilizing fluorescence microscopy to simultaneously measure PLM-NKA and PLM-PLM binding. By tagging NKA with CFP and PLM with YFP, we can measure NKA-PLM binding with heterotransfer FRET (CFP to YFP) and oligomerization of PLM with homotransfer FRET (YFP to YFP). We observed that several mutants have a decreased affinity for oligomerization (PLM-PLM binding) leading to an increased apparent affinity for NKA. Future plans include creating and screening many more mutants and measuring the functional effect of PLM-based superinhibitors in vitro and in vivo.

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