Cardiac left ventricular remodeling is responsible for over 250,000 heart failure deaths each year in the United States. Myocardial infarct expansion, progressive thinning and deposition of non-contractile fibrotic tissue can result in compensatory dilatation of the nonischemic borderzone segments. Ultimately, cardiac output cannot be sustained under such progressive heart enlargement. The long term project goals are to noninvasively detect myocardial infarction edema, fibrosis and infarct stability using magnetic resonance imaging techniques based on endogenous $^1$H contrast generated by nuclear magnetic relaxation. The hypothesis is that the primary biological changes occurring within the first 8 weeks following myocardial infarction, particularly myocyte apoptosis, recruitment of cytokines and deposition of fibrotic scar tissue, have a significant effect on the magnetic environment of $^1$H nuclei in water molecules. The $^1$H magnetic environment changes can be detected using novel T1p magnetic resonance imaging (MRI) techniques developed by Dr. Witschey during graduate school. $^1$H T1p relaxation is sensitive to low frequency fluctuations of water molecules including the overall apparent exchange rate between free water $^1$Hs and proteins and macromolecules at low exchange frequencies and changes in total water mobility. In preliminary investigations performed on swine, T1p was shown to detect changes to the overall apparent exchange rate and rate of relaxation caused by rotational modulation of the magnetic dipole-dipole interaction at 8 weeks following ligation of the second and third branches of the circumflex artery. These findings present an opportunity to overcome limitations of current methods of endogenous contrast MRI based on T2 relaxation measurements. In this proposal, research will be performed to develop methods to overcome magnetic field heterogeneity (static and RF fields) and motion artifacts associated specifically with T1p cardiac imaging. During the independent phase, experiments will be carried out to (1) serially examine the effect of apparent $^1$H exchange rates and rotational modulation of magnetic dipole-dipole coupling on $^1$H T1p relaxation times from the moment of initial ischemia throughout the period of cardiac remodeling to 8 weeks in swine and (2) determine whether $^1$H T1p methods provide different information regarding biochemistry from perfusion based MRI techniques. Extensive additional training is proposed during the K99 phase to include didactic training, participation at conferences, seminars and workshops, biannual meetings of a career development committee, and hands-on training in the creation of animals models of cardiac dysfunction, state-of-the-art imaging and technique development, analysis, and development of research career skills. This training will prepare Dr. Witschey to achieve his career goals to advance our understanding and treatment of cardiac disease through novel and interdisciplinary techniques combined with basic science research.
Project Narrative

Left ventricular remodeling is a deadly condition during which the heart undergoes structural, functional and biochemical changes following myocardial infarction (MI). The initial and long-term macroscopic changes, edema, cell apoptosis, and deposition of collagen, are hypothesized to influence the nuclear magnetic relaxation of \(^1\)Hs in water. The implications are that the remodeling process can be noninvasively followed without expensive and potentially hazardous exogenous contrast based methods using magnetic resonance imaging (MRI).
Introduction to the Amended Application

I thank the reviewers for providing constructive criticism and enthusiasm. The most significant criticisms were the narrow focus of the career goals and objectives and career development plan, and the ambitiousness and novelty of the proposed research. We will address criticisms and elaborate on the most significant changes.

Career Development Plan and Career Goals

I significantly broadened the career goals and training program to prepare myself for research beyond the R00, to compete for extramural funding (e.g. R01) and to gain training beyond MR physics.

- Additional career training in cardiovascular disease-oriented and translational research are as follows:
  - Translational research training through the Patient Oriented Research Certificate program at Penn, which consists of biostatistics, patient research, ethics, and disease measurement.
  - Individualized training to provide training in clinical cardiology practice and to discuss current topics in heart disease research, problems and needs (with Victor Ferrari, MD).
  - Didactic training in cardiology through the School of Medicine (physiology, pathophysiology, anatomy and interventions/therapies).
  - Training in hands-on research to investigate modeling of heart disease and connect to diagnostic measurements of physiology (with James J. Pilla, PhD).
  - Participation at conferences (e.g. AHA), workshops and courses on career development.

- My career goals beyond the R00 phase are as follows:
  - Collaboration with several groups of MR physicists (Univ. Minnesota, Univ. Hospital Freiburg) to develop or apply other MR techniques to cardiovascular disease.
  - Implementation of T1ρ methods for clinical human imaging of heart disease.

Research Proposal

There were several concerns related to the ambitiousness, novelty of T1ρ of MI, and some ambiguity with respect to the goals in Aim 3, among a few other issues.

- We regret the inadvertent omission of some of the references pertinent to the work proposed in the previous proposal. Several important publications investigating T1ρ of acute myocardial infarction in humans (Huber, Radiology, 2006, but also Muthupillai, Radiology, 2004, Dixon, MRM, 1996) are now extensively referenced in the research proposal. The novelty of the current research is that we quantitatively investigate the time-varying relationship between water 1H nuclear relaxation and scar maturation via biochemistry (SA2), which has never been performed. Furthermore, the existing cine-T1ρ sequences are inappropriate for quantification because there is no mathematical expression for the change in T1ρ on the measured signal. The relationship between infarct size (an important indicator of clinical prognosis) as measured by T1ρ and LV performance has never been performed (SA3).

- Limitations of T1ρ to detect biochemical changes are now discussed. Concurrent biochemical events (edema, apoptosis) cannot be resolved at the spatial resolution of the MRI techniques.

- We introduce a novel approach in SA1 to numerically determine RF pulse phases and durations to reduce the sensitivity to field variations. Furthermore, a dark blood sequence for T1ρ imaging (SA1c) has never been implemented, but is needed to create contrast between the infarct and LV blood pool.

- SA3 has been substantially revised. We now seek to correlate measurements of total infarct size as determined by T1ρ and DE MRI with LV performance. There are differences in the underlying contrast mechanisms between T1ρ and DE, which may be responsible for differences in the infarct size measured using the two techniques. Therefore it is very important to determine whether the T1ρ infarct size is LV performance. The advantage of T1ρ over DE is that T1ρ is an endogenous contrast agent, therefore it has no added cost to the MRI scan, can be used in patients with renal failure and is not subject to protocol variations (timing of the contrast bolus), which improves quantification.

- Aims 2 and 3 will also occur during the K99 phase to reduce the ambitiousness of the proposal.

New Developments of 2010-2011. Notably, I have returned to Penn to continue cardiovascular research.

I have continued to be a productive scientist as demonstrated by my work in 2010-2011:

- I received the ‘Scientist of the Year’ award from the University Hospital Freiburg.
- I published 6 new manuscripts, 2 first author and 4 coauthor manuscripts.
- I will give 1 invited talk, Experimental Nuclear Magnetic Resonance Conference, Asilomar, CA
- 3 first author proceedings were accepted in the Intl. Soc. for Magnetic Resonance in Medicine.
2. Candidate’s Background

2.1 Overview: I am a trained biophysicist who specializes in magnetic resonance imaging (MRI) techniques. I have a special interest finding novel approaches to investigate cardiovascular disease. Although I have a two years of postdoctoral experience, I recognize that I need additional didactic training in cardiac anatomy, physiology, pathophysiology, and additional hands-on-training to become a competitive applicant for extramural funding. This training will enable me to complete the proposed research, which has the potential to broaden our understanding of the LV remodeling process and establish a basis for how fundamental MR properties change over the course of the disease progression. An essential aspect of the current proposal is for me to extensively broaden my knowledge under the mentorship of Dr. Robert Gorman.

2.2 Publication Record and Honors: I am a highly productive person and, since 2006, I have authored 6 published manuscripts and coauthored 9 others in the fields of novel MRI technology, cardiac disease and cartilage degeneration. In 2010, I was awarded the ‘Scientist of the Year’ together with my colleague Daniel Gallichan (2 of 85 people), for our collaborative effort on the INUMAC project at the University Hospital in Freiburg, Germany (see below). Four of my first-author manuscripts are published in two of the top journals in the field Journal of Magnetic Resonance and Magnetic Resonance in Medicine. In May 2009, this work culminated in my dissertation for which I received the award for ‘Outstanding Dissertation of the Year’ from the Biochemistry and Molecular Biophysics Graduate Group. In addition, as a postdoctoral researcher, I authored one manuscript ‘Rotating Frame, Spin Lattice Relaxation in a Swine Model of Chronic Myocardial Infarction’, which presents the preliminary results of this proposal and was recently published in Magnetic Resonance in Medicine.

2.3 Patented Technology: I am the co-inventor of six novel technologies, which were or are in the process of being patented as a result of my graduate and postdoctoral work. I personally maintain the software for T1p MRI techniques and these have been distributed to over 20 research sites to date through the MRI regional resource center (Center for Magnetic Resonance and Optical Imaging) at Penn. I believe that my expertise in T1p MRI makes me uniquely equipped to undertake the research proposal.

2.4 Postdoctoral Experience: My postdoctoral experience so far has been a combination of basic cardiac research pursued in the Gorman Lab at the University of Pennsylvania and technical developments at the University Hospital in Freiburg, Germany

Postdoctoral Research (Gorman Laboratory, University of Pennsylvania, June 2009-September 2009; February 2011-Current): I am currently a jointly appointed postdoctoral fellow with the Departments of Radiology and Surgery. Since returning to Penn in 2011, I have facilitated a diverse set of collaborations between the University of Pennsylvania and University Hospital in Freiburg, Germany, including: (1) (with collaborator PD Michael Markl) the introduction of 4D (spatial and temporally-resolved) blood flow measurements of the left ventricle to observe modulated blood hemodynamics in response to therapeutic interventions in the Gorman lab (polymer injections, gene therapy) and (2) (with collaborator Maxim Zaitsev) developing novel techniques for dynamic shimming and localization using PatLoc technology developed in Freiburg.

Postdoctoral Research (Medical Physics, University Hospital Freiburg, Germany, January 2010-January 2011): I was invited to be a postdoctoral fellow on the multinational project Imaging Neurodisease Using High Field MR and Contrastophores (NUMAC), sponsored as a 25 million euro, academic-industrial collaboration between the Deutsche Forschungsgemeinschaft (DFG), European Union (EU), Siemens AG and Bruker AG. The project has a nonrenewable funding period of 5 years (2006-2011) and was a once-in-a-lifetime opportunity to pursue imaging technology that will push the frontiers of MRI in parallel transmission, ultra high field MR, and parallel acquisition techniques with local gradient coils (PatLoc). The director of the Freiburg component of the project, Dr. Maxim Zaitsev, and the director of the MR center Dr. Jürgen Hennig are members of the proposed Career Development Committee, acting as both external advisors and contributing MR physics expertise. Although my work in Freiburg is not directly related to the current proposal, it demonstrates my broad range of interests. Furthermore, any advances in basic MR methodology have the potential to be useful to researchers studying any organ system. I continue to collaborate with the investigators on the use of local nonlinear gradient coils for multidimensional gradient encoding, localization and shimming. I have applied for two patents from this technology, one manuscript is in revision with Magnetic Resonance in Medicine and two manuscripts are in preparation.
3. Career Goals and Objectives

In the long-term I would like to confidently approach challenges in cardiovascular disease and contribute to the development of new therapeutic and diagnostic techniques. This is a significant undertaking: I need to have a diverse background to recognize and understand outstanding issues in cardiovascular disease, but yet have the skill to develop solutions. I recognize, however, that I need new skills to succeed as an independent investigator. To achieve my short- and long-term goals, I have revised the career development plan to fill gaps in my knowledge.

My long-term career goals are:

1. to be an independent researcher. I want the ability to successfully compete for independent funding to investigate problems in cardiovascular disease using quantitative methods and solve clinically important problems.
2. to be an educator. I want to teach others about the beauty of applied physics, especially MRI, which in my mind is one of the most fundamental applications of a nuclear spin and quantum mechanics. To achieve this goal, I need a broad interdisciplinary background such that I can address complex issues at the interface of health, biology, physics and mathematics.

My short-term career goals and interests are listed below:

Translation of Quantitative T1_\rho Methods to the Clinic

My short-term objectives are to translate T1_\rho relaxation methods to the clinic for investigation of cardiovascular disease. To achieve this goal, I have proposed additional training in Patient Oriented Research and individualized training in clinical cardiology (with Victor Ferrari, MD). One future hypothesis is that myocardial MR relaxation times change due to physiologic fibrosis in athletes. MRI would provide a noninvasive and ethical means to investigate the question of how physiologic cardiac remodeling in athletes is connected to pathophysiologic remodeling following myocardial infarction. Another future hypothesis is that MR-relaxation times will be a predictor of future outcome of LV remodeling intervention. This hypothesis is highly appropriate because I will gain training in interventional and therapeutic techniques to prevent adverse remodeling following MI.

Measurement of LV Hemodynamic Response to Infarct Intervention

Another future objective is to investigate 4D (3-spatial coordinates and time) flow analysis to measure changes in LV blood flow with a novel device that alters infarct material properties. The interventional device is placed on the epicardial surface and can be adjusted to stiffen the infarct region. In vivo testing has demonstrated that the device alters global function and redistributes the cavity volume from the infarct region to the normally contracting remote region. Using 4D blood flow imaging techniques, we can quantify the effect of infarct stiffening on the flow distribution. This project provides a perfect opportunity develop new skills with cardiac MRI image acquisition using state-of-the-art flow, strain, delayed enhanced, and real-time MR image acquisitions.

Development of MR Methodology for Dynamic Shimming and Steady-State MRI

I will continue to have an interest in basic MR methodology and techniques and to apply these techniques to cardiovascular disease.

I have a strong interest in the MR physics of nonlinear magnetic field gradients. Two potentially valuable techniques using nonlinear magnetic field gradients are dynamic shim updating of the magnetic field during MR acquisitions and gradient localization. I investigated both techniques while a postdoctoral fellow at the University Hospital in Freiburg, Germany. These techniques were accepted for presentation the 2011 meeting of the International Society of Magnetic Resonance in Medicine in Montreal, Canada. A manuscript related to target localization using nonlinear magnetic fields was submitted to Magnetic Resonance in Medicine and another manuscript related to dynamic shimming is in preparation.

I have been exploring rotating frame MR techniques with three collaborators at the University of Minnesota (Dr.’s Michael Garwood, Silvia Mangia, and Shalom Michaeli). Last September 2010, I visited the University of Minnesota to investigate several techniques related to adiabatic T1_\rho and steady-states. This work is summarized in a recent educational abstract accepted for presentation at the 2011 meeting of the International Society for Magnetic Resonance in Medicine.
4. Career Development Plan / Training Activities

The purpose of the proposed career development plan is to extensively broaden Dr. Witschey's cardiovascular and biomedical research expertise and diversify his knowledge so that he can compete at the highest level for extramural funding as an interdisciplinary scientist.

4.1 Career Development Committee: To guide the transition between the mentored phase and independent research phase, a career development committee will meet biannually (via teleconference if needed) in which Dr. Witschey will describe research results and obtain career development advice to help identify appropriate faculty positions and negotiate resources, personnel management techniques, define and operate a budget.

4.2 Didactic Coursework Training and Conferences: To achieve a better understanding of cardiac physiology and biomedical research, Dr. Witschey will take didactic coursework. Dr. Witschey proposes to take the standard cardiology block for 1st year medical students at Penn to gain a fundamental understanding of cardiac anatomy, physiology, pathophysiology, interventional and therapeutic techniques. Since the long-term career goals of the applicant involve teaching, knowledge dissemination and future course development, the applicant proposes to take the semester-long Teaching Seminar, individualized to biomedical research offered at Penn through a program offered by the NIH. Additional essential skills and support will be acquired through the Office of Biomedical Postdoctoral Programs, which has workshops for manuscript writing, seminar presentations, grant preparation, manuscript reviewing, grant reviewing, and laboratory management. Dr. Witschey will enroll in hemodynamics (BE 450) and continuum biomechanics (BE 455) at Penn.

4.3 Patient-Oriented Research Training: To gain additional skills to help the PI move beyond the R00 phase of the grant, training will be performed to assist in clinical implementation of the techniques. Dr. Witschey proposes to enroll in the Certificate Program in Translation Research (CTR) program, offered by the Institute for Translational Medicine and Therapeutics (ITMAT) at Penn, whose objective is to provide in-depth instruction in the fundamental skills, methodology and principles necessary for a translational investigator. The program is designed to meet these objectives through the provision of didactic coursework, a formal mentorship, formal laboratory training, and specific ongoing guidance with hands-on exposure to protocol and grant development. The core curriculum consists of Introductory Biostatistics, Fundamentals of Patient Oriented Research, Scientific and Ethical Conduct and Disease Measurement. Upon successful completion of the CTR program, Dr. Witschey is expected to have developed a strong foundation in the fundamental techniques of translational research through this program.

4.4 Individualized Training: Clinical Cardiology: To learn additional clinical aspects of cardiovascular disease and current topics in cardiovascular research to broaden his knowledge beyond techniques in MR physics, Dr. Witschey proposes one-on-one mentoring with Dr. Victor Ferrari twice a month for the duration of the K99 phase to discuss current research, problems and needs in the field heart disease. This individualized mentoring will involve discussions of the advantages and disadvantages of different radiologic approaches, interventional and therapeutic techniques, clinical image analysis, and other common diagnostic approaches in the field including echocardiograms, PET/SPECT and computed tomography. Post MI Ventricular Remodeling of Structural Heart Disease, Operative and Device-Based Therapeutic Approaches: Dr. Witschey will plan to meet at least twice monthly with Dr. Gorman to increase his knowledge of the pathophysiology of post infarct ventricular remodeling as well as other aspects of structural heart disease including valvular disease, coronary artery disease, ventricular aneurysm, pulmonary hypertension and aortic disease. Dr. Witschey will also gain a detailed understanding of current operative and device based therapeutic approaches for structural heart disease. He will also be kept abreast of the most cutting edge technologies that are under development in Dr. Gorman’s laboratory as well as other leading laboratories around the world.

4.5 Hands-On Training: Hands-on training in techniques used for assessment and quantification of heart disease is fundamental to completing the proposed research and future independent research. To achieve this goal, Dr. Witschey will become a fully integrated member of Dr. Gorman's research team. He will participate in all ongoing projects and will primarily focus on those that address LV remodeling and valvular heart disease. He will gain experience in finite element modeling of cardiovascular structures (LV, mitral valve, aorta) based on imaging data developed from 3D echocardiography and MRI. He will be particularly interested with ongoing research projects that employ cardiac MRI to assess regional myocardial stress-strain relationships in the
remodeling heart after MI (James J Pilla, PhD). Dr. Witschey will get extensive hands on experience with large animal models of post MI LV remodeling (pig, sheep and calf) while working with Dr. Gorman and his group of surgeons and surgical trainees. He will learn how the animal models are created and become familiar with all the tools and imaging techniques that are required to study large animals as their hearts remodel after MI. Specifically, he will become an expert in imaging large animals (MRI, echocardiography) as well as assessing the hemodynamic performance with catheter-based technologies.

4.6 Research Career Development Skills Training: The School of Medicine organizes workshops for grant writing, catered toward the development of the NIH’s early stage investigator and R01 awards. Dr. Witschey will take advantage of these opportunities, which should improve his grant writing skills and techniques needed to fund independent research. The University periodically organizes abstract and manuscript writing workshops and Dr. Witschey will take advantage of these to improve his writing skills. Ongoing opportunities for oral presentation will be present since Dr. Witschey will participate in conferences including the Society for Cardiovascular Magnetic Resonance (SCMR), the American Heart Association Scientific Sessions, the International Society for Magnetic Resonance in Medicine (ISMRM) and the Gordon Research Conference for In Vivo Magnetic Resonance, among others.

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<th>Timeline for Career Development/Training Activities</th>
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<td>Didactic Coursework</td>
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<td>Bioengineering</td>
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<td>BE 450: Hemodynamics</td>
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<td>BE 455: Continuum Biomechanics</td>
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<td>Certificate in Translational Research</td>
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<td>MTR 600: Introductory Biostatistics</td>
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<td>MTR 601: Fundamentals of POR</td>
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<td>MTR 602: Scientific and Ethical Conduct</td>
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<td>MTR 603: Disease Measurement</td>
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<td>Conference Participation (oral/poster presentation)</td>
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<td>American Heart Association Scientific Sessions</td>
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<td>Gordon Conference In Vivo Magnetic Resonance</td>
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<td>Research Career Skills Training</td>
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<td>Grant writing workshops</td>
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<td>Training in responsible conduct of research</td>
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<td>Mentoring undergraduate/graduate students</td>
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<td>Teaching Seminar for Researchers - 1 c.u.</td>
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<td>Individualized and Hands-On Training</td>
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<td>Topics and challenges in Cardiology (V. Ferrari)</td>
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<td>Topics and challenges in Cardiothor. Surg. (R. Gorman)</td>
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Dr. Witschey will participate in the Gorman Lab research meetings, Wehrli lab research meetings and Penn CVI journal club. The lab meetings are all structured as to encourage discussions and analytical thinking and presentation skills. Dr. Witschey understands the importance of building collaborations with other investigators, especially because his research will be conducted in an interdisciplinary fashion with researchers from physics, engineering, biology and medicine. Dr. Witschey has already initiated a number of collaborations and training opportunities with cardiologists (V. Ferrari), MR physicists (J. Hennig, M. Zaitsev M. Garwood), experts in flow dynamics (Michael Markl) and cardiac bioengineering scientists (James J. Pilla).
March 14, 2011

Scientific Review Committee
National Institutes of Health
Re: Walter R.T. Witschey, PhD

Statement of Support

Overview: It is my great pleasure to act as a mentor Dr. Walter Witschey during the K99 phase of the Path to Independence Award. The K99/R00 award is ideal for Walter because it was designed expressly to provide young researchers an opportunity to learn valuable new skills to begin their career as an independent researcher. His experience during the K99 as well as his significant past accomplishments will make him a very competitive applicant for independent faculty positions.

Proposed Research: Walter’s proposal is timely, innovative and highly significant. During the fall of 2010, Walter expressed an interest in applying his T1p MRI techniques to study post-myocardial infarction (MI) cardiac remodeling. I think that this is an exciting topic of research, which will be the first time that T1p is measured serially over the course of the post-MI period. It has the potential to provide many new insights about myocardial infarction because of the sensitivity of T1p to biochemical changes.

Career Development Plan: I am very enthusiastic about the proposed career development plan in the amended application and I am confident that it will provide Walter with an extensive background for an independent research career in heart disease. After a great deal of reflection, Walter has become highly motivated for cardiac research and together we have fleshed out a diverse career development plan. The plan involves didactic training in cardiovascular anatomy, physiology and pathophysiology in the University of Pennsylvania School of Medicine. Walter will also be introduced to state-of-the art therapeutic and diagnostic modalities for patients with cardiovascular disease through direct interaction with clinicians at the Hospital of the University of Pennsylvania. He will also take courses in biostatistics and patient-oriented research offered through the University’s Institute for Translation Medicine (ITMAT). Walter will receive hands-on training and experience in my laboratory with the creation and manipulation of large animal models of infarction induced left ventricular remodeling. He will also gain extensive experience with experimental imaging in these animal models by using several modalities including 2D and 3D echocardiography, sonomicrometry, MRI, and PET/SPECT. Finally Walter will gain experience with numerical and analytical modeling of heart dysfunction that
are ongoing in our laboratory. This training will be supplemented by selected chapter readings from the following volumes:

- Interventional Cardiology: Percutaneous Noncoronary Intervention. ed. Hermann H.
- Cardiac Remodeling and Failure. Singal, Dixon, Kirshenbaum, and Dhalla.
- Cardiac Contraction and the Pressure-Volume Relationship. Sagawa, Maughan, Suga, Sunagawa.
- Cardiac Surgery in the Adult. Cohn.

**Deliverables:** Walter and I have formulated deliverables, in terms of measuring the effectiveness of the mentorship that I provide (to be presented at the meetings of the career development committee):

- Submission of two new full-length manuscripts as lead author and at least one manuscript as contributing author in peer-reviewed journals per year with the expectation of 4 articles published or in press at the end of the award period.
- Submission of at least two meeting abstracts for presentation at AHA, SCMR or ISMRM.
- Active participation in scientific events at the School of Medicine, including presentations at the Penn Cardiovascular Institute.
- Participation in mentorship of graduate students and other trainees in my lab.
- Obtaining a faculty position in Year 2 of the postdoctoral fellowship K99 award.

**Mentors Qualifications:** I am a Professor of Surgery at the University of Pennsylvania and Director of the Cardiac Surgical Research Laboratory at Penn. I am cardiac surgeon who commits 85% of his time to laboratory and clinical research. My research has been focused primarily in the field of post-infarction left ventricular remodeling, valvular heart disease and cardiovascular imaging. My team has contributed significantly toward elucidating the mechanism of infarction induced heart failure with the use of sonomicrometry array localization, 3D echocardiography and MRI. I have a strong interest in developing algorithms to assess the risk for adverse long-term outcomes after myocardial infarction. We are currently developing novel treatment paradigms intended to be employed in the early post MI period with the intent of preventing the development of heart failure after MI. I have also involved in developing new therapeutic approaches to established heart failure. I believe my background and interests align well with those of Dr. Witchey and, therefore, make me uniquely qualified to facilitate his transition to an independent scientist during the period of funding that is requested in this application.

I have significant experience in advising, mentoring and supporting trainees at many educational levels. In the last decade I have mentored 23 postdoctoral fellows, 14 of which currently hold full-time tenure-track faculty positions in the
United States, Japan and Europe. The others have established productive private practice medical careers or careers in industry. I have mentor 16 pre-doctoral students all of whom have either been awarded PhD’s or have gained entry into medical or veterinary school. I have an established record of sustained mentorship. In 3 cases I have mentor trainees from predoctoral students until they obtained tenure track faculty positions. I must say that of all the bright young men and women that I have trained, Walter Witschey is among the most talented, innovative and motivated.

**Transition to Independent Phase:** Walter’s transition to independent research and securing a faculty position is a priority. I will ensure he has sufficient time to seek out and obtain a faculty position during the K99 phase. The animal models used in Specific Aims 2 and 3 require sophisticated resources that could not reasonably be put together by Walter during the R00 phase. Instead, we propose to find faculty positions that have access to sufficient shared resources. At the very least, I am highly interested and willing to collaborate on this project.

Sincerely,

Robert C. Gorman, M.D.
June 11, 2010

RE: Walter Witschey, Ph.D.

Dear Scientific Review Committee,

The University of Pennsylvania and Department of Surgery is highly committed to the development of Dr. Witschey into a productive, independent investigator. We will ensure that the University of Pennsylvania can fulfill all of Dr. Witschey's training and support needs to meet the requirements of the award. Our commitment to Dr. Witschey is not contingent upon the receipt of the K99/R00 award.

Young scientists need dedicated research time to pursue their research and develop into strong independent investigators. To meet this goal, Dr. Witschey will have protected time of at least 9 person-months to fully commit to the development of his research under the proposed K99 phase of the award. In addition, the remaining effort is protected so that he can seek out faculty positions and develop into an independent scientist.

Dr. Witschey has full access to the resources of the medical school. The training resources of the medical school courses, animal training, grant workshops, among others, are available. There are many faculty engaged in multitudes of diverse research fields in myocyte biology, heart failure, biotechnology and medical imaging, among other fields. Many of these collaborations can be found through the Penn Cardiovascular Institute (CVI). The interdepartmental structure of the CVI makes it possible for trainees to find many opportunities to collaborate on a wide variety of research topics. We encourage our trainees to seek out and collaborate on these projects.

It is very important that Dr. Gorman and the other members of the career development committee have adequate time to support Dr. Witschey in his research. We will provide the necessary time and support for the mentor, Dr. Gorman, and the members of the career development committee to meet biannually and informally to discuss the ongoing research projects and path to independence.

Best Regards,

Jeffrey A. Drebin, M.D., Ph.D.
Description of Institutional Environment

Overview: The University of Pennsylvania is an exceptional research institution and is the ideal location to carry out the K99 phase of Dr. Walter Witschey’s proposal. Penn has superb faculty, laboratory resources, and the Penn Cardiovascular Insititute (CVI) provides many opportunities to foster collaborative opportunities.

Collaboration: Translation of basic science and innovative technology into mechanisms to prevent, diagnose, treat and cure heart disease is one of the missions of the Penn CVI. The research proposal planned by Dr. Witschey is a perfect example of an innovative new technology that has the potential to yield new biochemical insights about myocardial infarction. To encourage collaboration and find new possibilities for this innovative new technology, Penn CVI has organized seven research programs designed to cultivate multidisciplinary cardiac research. This proposal is the interface between the two research programs: (1) biotechnology and imaging and (2) heart failure. There will be many opportunities, through planned seminar series and research fairs and presentations to find opportunities to collaborate.

Facilities and Resources: My laboratory offers the perfect opportunity to train Walter in mechanisms of post-MI cardiac remodeling. Over the past decade we have assembled a team of surgeons, veterinarians, molecular biologists, technicians, radiologists and engineers who are highly skilled in the manipulation of large animal models of post MI remodeling. Over the last decade we have performed experimental myocardial infarctions in over 2200 animals most of which were studied with some form of serial imaging. Our laboratory is housed in a highly unique facility that is dedicated to large animal cardiac research. The facility has 4 dedicated animal operating rooms, animal care facilities for 140 large animals, computer software programming, computer image analysis stations and a molecular biology laboratory. A Siemens 3T MRI is adjacent to the animal operating rooms and is dedicated to animal research. There are also 2 Phillips iE33 3D echocardiography machines and a state of the art catheterization/angiography suit. Portable fluoroscopy is available in all operating rooms. In addition, we have all the necessary resources for statistical analysis of data at the Penn Center for Epidemiology and Biostatistics. Penn has many faculty available to collaborate on projects through the Penn Cardiovascular Institute (CVI).
Specific Aims

Cardiac output cannot be sustained under the circumstances of progressive heart enlargement and remodeling following myocardial infarction (MI). The consequences of LV remodeling ultimately results in heart failure and are responsible for over 250,000 heart failure deaths each year in the US (1,2). There is a strong need to noninvasively monitor biochemical changes in the myocardial wall post-MI and in response to interventions or therapies. Existing T2-weighted MRI techniques have low sensitivity to MI (3,4), making them less than satisfactory for infarct tracking. Furthermore, gadolinium based contrast agents, while sensitive to the infarct region, can be costly (5,6) and are inappropriate for use in patients with renal failure (7-9). Recently, we used quantitative methods to measure rotating frame relaxation times (T1ρ “T-one-rho”) in vivo and demonstrated the sensitivity to collagen deposition in chronic MI. In this proposal, we will evaluate the following hypothesis:

1. Time-varying biochemical changes following MI have a substantial effect on the local magnetic environment of water molecules, such that they can be detected in vivo by T1ρ MRI.
2. Infarct size measured by T1ρ MRI is a strong determinant of LV performance.

Specific Aim 1: To develop and optimize T1ρ MRI techniques to overcome magnetic field heterogeneity and eliminate the LV blood signal to study MI in vivo.

Rationale: Quantitative T1ρ MRI techniques are sensitive to field variations and have poor infarct-blood pool contrast. Furthermore, existing cine-T1ρ MRI techniques to study MI are not quantitative and used very low power, reducing sensitivity (10). Therefore, there is a strong need to develop a new approach to field-insensitive quantitative T1ρ MRI and eliminate signals from the blood.

Specific Aim 1a: Magnetic field insensitive spin lock sequences will be found by numerical optimization of pulse sequence parameters over the range of $B_0$ and $B_1$ field variations encountered in cardiac applications.

Specific Aim 1b: Candidate sequences identified in SA1a will be programmed and tested under cardiac imaging conditions to evaluate their performance.

Specific Aim 1c: A method for dark blood T1ρ imaging will be generated to suppress LV blood pool water.

Specific Aim 2: To correlate apparent exchange rate ($k_{ex}$) and water rotational correlation times ($r_c$) measured by T1ρ MRI with cardiac biochemistry post-MI in swine.

Rationale: Water $^1$H chemical exchange and water rotational motion undergo changes 8 weeks post-MI and these changes can be detected by T1ρ, but not with conventional MRI techniques (11). Experiments performed on swine with chronic ventricular MI demonstrate changes in water $^1$H low frequency exchange rates ($k_{ex} = 0 – 2.5$ kHz) and tumbling, however, the timecourse of T1ρ relaxation time changes in response to biochemical changes is unknown, but is needed to assess interventional or therapeutic efficacy.

Specific Aim 2a: Five groups of eight animals will undergo cardiac MRI to determine baseline relaxation times and left ventricular performance.

Specific Aim 2b: MI will be induced in each of five groups of eight swine by a standardized posterobasal operation and a terminal in vivo and ex vivo MRI study will be performed on each group of swine at one of five time points post-MI (during occlusion, 5 days, 2, 4 or 8 weeks).

Specific Aim 2c: Nuclear relaxation times will be correlated with myocyte apoptosis, total collagen, collagen type and collagen crosslink density.

Specific Aim 3: To correlate T1ρ and delayed enhanced MRI infarct size with LV performance.

Rationale: Delayed enhanced (DE) MRI can quantitatively characterize the infarct region and the infarct size is directly related to LV performance and patient prognosis. T1ρ may provide a noninvasive method to obtain biochemical information from water tumbling instead of from contrast wash-in rates. Therefore, because the underlying mechanisms of DE and T1ρ contrast are different, it is important to determine if the infarct size measured by T1ρ correlates with LV performance.

Specific Aim 3a: Evaluate total infarct size on T1ρ MRI, DE MRI and from ex vivo optical measurements of scar volume.

Specific Aim 3b: Infarct size measurements will be correlated with LV performance using linear regression.
11. Research Proposal

A. Significance

1. Post-Myocardial Infarction (MI) Left Ventricular Remodeling

Immediately following MI, important structural and biochemical changes begin to occur. Alterations in the size, thickness and geometry of the left ventricle affect patient prognosis via augmentation of wall stress, impairment of systolic contractile function and the increased likelihood of aneurysm formation. Chronically, infarct expansion, progressive thinning, and deposition of non-contracile fibrotic tissue results in a decreased myocyte density together with compensatory dilatation of the noninfarcted remote and border zone segments. Ultimately, cardiac output cannot be sustained under the circumstances of such progressive heart enlargement, resulting in heart failure. The initial and long-term changes associated with left ventricular remodeling are responsible for over 250,000 heart failure deaths each year in the United States (1,2).

Primary Hypothesis

We hypothesize that the time-varying biochemical changes following MI, edema and myocyte death, and later deposition of collagen, have a substantial effect on the local magnetic environment of water molecules. We further hypothesize that we can detect these time-varying changes using \(^{1}H\) T1\(_p\) magnetic resonance imaging (MRI).

2. Limitations of Current T2 MRI Techniques to Detect Molecular Changes

T2 relaxation and T2-weighted MRI has been studied in several different fields of heart disease including acute and chronic MI (3-7). It was shown that T2 might discriminate early and late MI by differences in relaxation times, and that perhaps only small differences made chronic infarct and remote myocardium indistinguishable (8). In these previous studies, T2 relaxation in cardiac muscle was felt to be due to changes in the \(^{1}H\) rotational correlation time caused by tissue alteration and edema. Strong contributions to T2 relaxation other than changes in the rotational correlation time may include chemical exchange effects and diffusion through magnetic field gradients, among other mechanisms. These other contributions to T2 relaxation obscure endogenous contrast between scar, border zone, and healthy tissue, but may be overcome through the use of a moderate spin locking pulse used in a T1\(_p\) imaging experiment (9); that is, the spin locking pulse will prevent some relaxation mechanisms from having effect at low frequencies, prolong relaxation times and provide sensitivity to a greater range of molecular changes (10,11). Clinical studies using T1\(_p\) have shown sensitivity

3. The Advantage of Endogenous Contrast Agents

US sales of MR contrast agents were between $350 and 500 million during 2002 and 2006, and sales are expected to rise even further. Approximately 45% of all MR studies were contrast enhanced (12,13). On the other hand, T1\(_p\) is an endogenous contrast agent that originates from changes to the local magnetic environment of the water molecule. Although there will always be a role for contrast agents in diagnosis, it is critical to investigate endogenous contrast agents for patient safety, cost-effectiveness and patient comfort. It is vital to reduce the risk of rare allergic reactions and nephrogenic systemic fibrosis (NSF)(14-16), a deadly disease suspected to occur following administration of gadolinium-based MR contrast agents in patients with reduced renal function. Furthermore, because of these risks, institutional review boards are increasingly less likely to approve contrast agents for research studies on healthy subjects. Clearly, efforts to exploit noninvasive, endogenous based contrast mechanisms are desirable.

B. Innovation

1. First use of quantitative T1\(_p\) to detect remote, borderzone and infarct segments post-MI. Although signal intensity measurements of low power T1\(_p\) MRI using short spin lock times has been performed (17,18), this proposal would be the first time to investigate the use of T1\(_p\) relaxation times (via relaxation mapping) to quantify remote, borderzone and infarct zones in a serial post-MI model in any species. T1\(_p\) would provide useful information regarding infarct biochemistry indirectly through changes in water mobility and exchange. These features would then be used to distinguish acute from chronic MI due to replacement of necrotic myocytes and edematous tissue with collagen during wound-healing.

2. First correlation of T1\(_p\) with biochemical and DE MR measurements post-MI. This proposal also represents the first time use of T1\(_p\) relaxation times for correlation with volumetric measurements of infarct size serially post-MI in any species. This MRI technique may provide unique information compared to delayed enhancement (DE) MRI, which is commonly used to detect viable and nonviable myocardium due to dense
collections of fibrosis, but is insensitive to smaller areas of collagen developing between scar and viable myocardium during cardiac remodeling. This is also the first time that T1ρ relaxation times were correlated with biochemical characteristics of MI, collagen and cell death.

3. **T1ρ MRI enhances MI image contrast without exogenous contrast agents.** Using a swine model of chronic myocardial infarction, we have found that there are nuclear magnetic relaxation mechanisms that suppress endogenous contrast between myocardial tissues, healthy myocytes and mature scar, 8 weeks post-MI. By delivering sufficient RF power during a T1ρ MRI experiment, we found it is possible to overcome these relaxation mechanisms and reveal much greater differences in relaxation times than could be achieved using conventional, endogenous MRI techniques.

4. **Uniqueness and Expertise: Patented MRI Techniques.** Our group is uniquely positioned to undertake this research because of our expertise in T1ρ imaging and previous record of developing novel T1ρ techniques. This proposal is the first time that these techniques have been employed to measure T1ρ relaxation times post-MI in any species. While previous studies have examined MRI contrast under low power (ν1 < 200 Hz) and short spin lock (TSL < 10 ms) conditions (17,18), this study aims to quantify nuclear relaxation times using a range of molecular motion regimes (ν1 varies, TSL varies) to detect molecular changes.

**C. Approach**

1. **T1ρ Imaging of Mature MI and Comparison to Histology**

Water 1H T1ρ relaxation times could detect deposition of extracellular matrix material collagen as determined by biochemical staining of collagen and cells at 8 weeks post-MI in tissue collected from six swine (19). A T1ρ-weighted image was used to distinguish collagenous infarct regions from neighboring myocytes at a resolution of 100 μm² (Figure 1A). Water 1H in the infarct region have longer magnetic relaxation times and retain much of their signal following perturbation from their equilibrium magnetization. Quantification of nuclear relaxation through a T1ρ relaxation map (ν1 = 2500 Hz) was used to demonstrate that 1H relaxation times in the infarct region (T1ρ = 189 ± 26.2 ms) and remote myocardium (T1ρ = 60 ± 2.8 ms) were significantly different (Figure 1B). The T1ρ images (Figures 1A and 1B) provide the same information as the trichrome stain (Figure 1C) of an adjacent section of tissue, including wall thickness, collagen, wall narrowing and regional infarct transmurality.

2. **Model for T1ρ Relaxation in Cardiac Tissue**

Molecular mechanisms for water 1H nuclear relaxation in the myocardium were investigated using T1ρ relaxation dispersion. The change of the spin-lattice (T1) relaxation time with magnetic field strength may be used to examine molecular dynamics in tissues and protein solutions (20-23), however, applications are limited because of the inherently low polarizability of spins at the fields required to generate contrast. T1ρ has been used as an alternative method in vivo (9). T1ρ is necessary when the desired contrast generating mechanisms are at very low frequency (Hz-KHz). There are several possible mechanisms for in vivo T1ρ relaxation in cardiac tissue, which were considered in the present model and this treatment is similar to that in (24-32).

2.1 **Rotational Correlation Time and Dipole-Dipole Relaxation**

Fluctuations of the 1H local magnetic field because of other nearby 1Hs are responsible for homonuclear dipole-dipole relaxation. During a spin locking pulse (Figure 2), which is applied to induce T1ρ relaxation, the contribution to the nuclear relaxation rate is

\[
R_{1ρ dd} = \frac{1}{10k_{dd}} \left[ \frac{3}{1 + 4ω_1^2τ_c^2} + \frac{5}{1 + ω_0^2τ_c^2} + \frac{2}{1 + 4ω_0^2τ_c^2} \right]
\]

where
\[
\frac{1}{k_{dd}} = 2l(l+1)\hbar^2 \gamma^4 r^{-6} \tau_c
\]

Here the spin angular momentum quantum number \( l = \frac{1}{2} \), \( \hbar \) is Planck's constant and \( (1.58 \text{ Å}) \) is the internuclear distance. For a system in which \(^1\)Hs are in two-site chemical exchange (2SX), each site (A and B) has a contribution to the relaxation time \((R1\rho_A \text{ and } R1\rho_B)\). From this equation, it can be seen that changes in the relaxation rate will occur if either the Larmor frequency \(\omega_0\) or the RF field amplitude \(\omega_1\) coincides with the rotational correlation time \(\tau_c\).

### 2.2 Chemical Exchange Rate

Chemical exchange between \(^1\)H sites separated by a chemical shift difference may also cause relaxation dispersion if the exchange rate is on the order of the RF field amplitude. In particular, for two-site chemical exchange in the fast exchange regime, the relaxation rate is (33,34)

\[
R1\rho_{ex} = \frac{P_A P_B \delta \omega^2 \tau_{ex}}{1 + \omega_1^2 \tau_{ex}^2}
\]

where \(P_A\) and \(P_B\) are the fractional populations of the spin pools and the exchange time \(\tau_{ex} = \frac{1}{k_{ex}}\). This model should be only considered an approximation of true events, for which there are multitudes of exchanging pools of \(^1\)Hs within the frequency range of the experiment. However, it can be shown that combinations of exchanging pools can give rise to a similar overall shape and characterized by an apparent exchange rate rather than a definite single exchange rate.

#### 2.3 A Model for In Vivo T1\(p\) Relaxation

In several in vivo studies, the relaxation rate was modeled as sum of contributions from the dipole-dipole and chemical exchange relaxation rates.

\[
R1\rho = P_A R1\rho_{dd,A} + P_B R1\rho_{dd,B} + R1\rho_{ex}
\]

For two site chemical exchange, site A represents the free water fraction, which resonates with the RF field and has a rotational correlation time on the order of picoseconds. Site B represents a bound water fraction of unknown size with a single chemical shift \((\delta \omega \ll 2 \text{ kHz})\). The fractional size of the pools \(P_A + P_B = 1\).

On account of the limited range over which dispersion was measured \((\nu_1 = 500 – 2500 \text{ Hz})\), the contribution to the dipole-dipole relaxation rate was treated as approximately constant. Certainly, for the case of the free water fraction \(\omega_1 \tau_{c,A} \ll 1\), this is appropriate. Although, the rotational correlation time for site B is unknown, here, it is assumed also that \(\omega_1 \tau_{c,B} \ll 1\), so that

\[
R1\rho = \frac{A \tau_{ex}}{1 + \omega_1^2 \tau_{ex}^2} + B
\]

where \(A = P_A P_B \delta \omega^2\).

**Measurement of Water Apparent Exchange Rate \((k_{ex})\) and Rotational Rate \((k_{dd})\) in a Swine Model of Chronic Myocardial Infarction (Witschey, et al. Magnetic Resonance in Medicine, 2010).**

Recently, we used T1\(p\) dispersion MRI to quantify biochemical properties of the myocardium derived from the magnetic environment of \(^1\)H nuclei 8 weeks following MI in swine. We performed a series of experiments to measure the variation of \(^1\)H nuclear relaxation with the applied radiofrequency (RF) field to measure low frequency \((\nu_1 = 500 – 2500 \text{ Hz})\) \(^1\)H chemical exchange (via the apparent exchange rate) *Ex vivo* myocardial water \(^1\)H relaxation times varied with both the applied spin lock amplitude \((p < 0.05)\) and between infarct, borderzone and remote myocardium \((p < 0.05)\) (Figure 4). Moreover, the difference in T1\(p\) between infarct, borderzone and remote myocardium increased upon increasing the amplitude of the spin lock field \((p<0.05)\). The increase in relaxation time differences meant that for the same spin lock duration, it was possible to improve the contrast between each of the three regions (Figure 3 – e.g. compare \(\nu_1 = 2500\) and \(\nu_1 = 500\) or T2).
To explore possible mechanisms for T1 relaxation, the apparent exchange correlation time was quantified using the single Lorentzian model derived in (2). The relaxation rate was expressed as a sum over contributions from individual relaxation mechanisms and the fitted model is shown in Figure 3. The apparent exchange rate increased in the remote and borderzone regions compared to the infarct region.

Specific Aim 1: To develop and optimize T1p MRI techniques to overcome magnetic field heterogeneity and cardiac motion to study MI in vivo.

**Rationale of Approach:** T1p imaging is particularly sensitive to heterogeneity of the main magnetic B₀ and radiofrequency (RF) (B₁) fields (35,36), which is problematic in cardiac imaging (37-42). The sensitivity is on account of ineffective locking of the magnetization along the desired RF field. There are two types of artifacts that most significantly affect quantification: (1) variations or heterogeneity in the external magnetic field (B₀) and in the radiofrequency field (B₁) and (2) motion artifacts, which are associated with respiratory and cardiac motion, but also flowing blood in the heart. Existing T1p techniques were developed for musculoskeletal and brain imaging and are unsuitable for cardiac imaging for several reasons. The combined in vivo lower power requirements and increased B₀ heterogeneity, owing to differences in magnetic susceptibility between cardiac tissue and the alveolar spaces of the lung, acts as a vise, such that there may be no spin lock amplitude over which T1p experiments can be performed. Although it provides access to contractile information, cardiac cine T1p imaging of the heart (43) is unsuitable for quantification because of the short spin lock pulses and low RF power needed to limit SAR, and because the mathematical framework for T1p quantification has not been determined for this steady-state sequence. Recently a series of methods have been devised for numerical optimization of MRI pulse sequence timing to eliminate artifacts (44,45), however, these approaches has not been adapted for T1p imaging. Furthermore, dark blood imaging sequences are necessary to maximize contrast between the infarct region and blood pool to evaluate transmurality and infarct size (SA3) and these have not been implemented.

**Specific Aim 1a:** Magnetic field insensitive spin lock sequences will be found by numerical optimization of pulse sequence parameters over the range of B₀ and B₁ field variations encountered in cardiac applications.

**Numerical Estimation of Magnetization Response:** We will derive a relationship between nutation artifacts and the field variations using several types of spin locking sequences in which we vary the delay time or pulse phase between locking pulses, excitation or refocusing pulses. A series of T1p pulse phases and durations will be simulated over a range of magnetic field variations (RF field ν₁, main field Δν) and the deviation in magnetization from the ideal will be estimated \( \frac{M_{T1p}}{M₀} \) by providing the parameters as an input to the Bloch equations and solved using the Runge-Kutta (nonlinear optimization) method.

**Specific Aim 1b:** Candidate sequences identified in SA1a will be programmed and tested under cardiac imaging conditions to evaluate their performance.

Candidate sequences will be programmed in a pulse sequence environment for the Siemens MRI scanner and tested in vivo. The presence and appearance of artifacts will be qualitatively assessed by evaluating the R² goodness-of-fit of the T1p relaxation maps to the expected signal decay equation \( S = S₀e^{-\frac{T_{SL}}{T1p}} \).

**Specific Aim 1c:** A method for dark blood T1p imaging will be generated to suppress LV blood pool water. We will address motion artifacts by developing a black blood T1p imaging pulse sequence. In this sequence, slice-selective/non-selective inversion will be performed prior to the T1p pulse cluster to eliminate signal from...
inflowing blood. We will analyze the ranges of delays (inversion times) between inversion and inversion flip angles to determine what combination of parameters best suppress signal from the blood. The inversion efficiency will be estimated by measurements of the signal-to-noise ratio in the LV blood pool.

**Anticipated Results:** It is expected that we can greatly reduce the sensitivity of the pulse sequence to variations in the magnetic field homogeneity using a numerical approach. Although this has not yet been performed for a spin lock sequence, it shares similarities with the steady-state free precession sequence (45,46) that we may be able to exploit. We aim to reduce this sensitivity by 50%. Dark blood imaging should significantly improve delineation of contrast between the infarct and blood pools in the spin lock sequence by suppressing blood pool water. Implementing this technique should substantially improve planimetry of the infarct region in SA3.

**Specific Aim 2:** To correlate apparent exchange rate ($k_{ex}$) and water rotational correlation times ($\tau_c$) measured by T1p MRI with cardiac biochemistry post-MI in swine.

**Rationale of Approach:** Recently, we showed ex vivo that T1p was a more sensitive indicator of chronic MI than T2 relaxation methods because the spin locking pulse overcomes low frequency relaxation mechanisms of water $^1$H nuclei in myocardial tissue (47). These early results indicate that fibrotic myocardial collagen differentially effects nuclear relaxation from healthy tissue in the contractile region. Furthermore, relaxation times appear to be enhanced in edematous myocardium following acute infarction (17,18), yet relaxation times were not quantified and the time-dependent effect of scar maturation on nuclear relaxation is not known. It is hypothesized that T1p relaxation times may be an indicator of these time-dependent changes. Therefore we seek to correlate the time-dependent MR relaxation times with myocyte apoptosis, total collagen and collagen crosslink density in the remote, borderzone and infarct regions. Collagen type may also affect nuclear relaxation either through changes in $^1$H chemical exchange or indirectly through total collagen and crosslink density. Two parameters expected to change with the $^1$H magnetic environment are (1) the apparent exchange rate ($k_{ex}$) and the water rotational correlation time ($\tau_c$). The significance of this aim is that T1p MRI may be used to serially quantify biochemical changes (e.g. apoptosis or necrosis and collagen deposition) serially in patients or animal studies, such that the efficacy of intervention or therapy can be determined.

**Specific Aim 2a:** Five groups of eight animals will undergo cardiac MRI to determine baseline relaxation times and left ventricular performance.

**Baseline MRI Measurements:** For MRI, a pressure transducer (Millar Instruments, Houston TX) will be guided into the left ventricle for cardiac gating. The animal will be transported to a MRI scanner and undergo cardiac imaging.

**T1p MRI:** Animals will be transported to a 3 T clinical imaging system (Tim Trio Model, Siemens Medical Solutions, Erlangen, Germany) equipped with 40 mT/m nominal gradients, 6 channel spine array receive coil and birdcage body coil. A gradient echo localizer will be used to obtain short axis cardiac views. A T1p-prepared, centrically segmented, turboflash sequence is used to acquire T1p-weighted images during systole (48). The spin lock pulse cluster is delivered approximately 190 ms after the QRS complex.

**Specific Aim 2b:** Myocardial infarction will be induced in each of 5 groups of eight swine by a posterobasal operation.

**Rationale for the Swine Infarct Model:** The swine model of MI is ideal for use in this study. Swine hearts are nearly human size and have very consistent coronary arterial anatomy and limited collaterals. Dr. Gorman has determined the location and amount of infarcted LV mass following ligation of specific coronary arteries and combinations of coronary arteries. These studies were performed in live animals, and led to the development of models based on original ovine post-infarction heart failure models (49-51). For the proposed studies, we will utilize a posterobasal infarct model. Ligation of the second and third branches of the circumflex coronary artery infarcts 24.0%±4.5% of the LV mass at the posterior base of the heart. The current mortality with these infarct models is 18%. It is necessary to assess the changes at several time points (initial, 5 days, 2, 4 and 8 weeks) following MI to assess the efficacy of intervention or therapy.

**Creation of the Swine Infarct Model:** Yorkshire swine weighing between 20-25 kg will be used in this study. The animals are sedated with IM ketamine (25mg/kg). The animal is under complete cardiovascular monitoring. A left thoracotomy is done and the pericardial sac was opened. The left circumflex artery and mid posterior descending artery are ligated to create a 20-25% area of infarction. The borderzone is determined and mapped via color and contractility changes. During MRI the approximate location of the infarct can be identified and unconditionally confirmed at sacrifice and dissection.
**Ex Vivo MRI:** For each of the animals, the left ventricle will be separated from the heart. The tissue will be suspended in a custom-built, tissue imaging device containing saline. Imaging is performed on a 7 T system (Siemens). An additional T2-prepared (90°-180°-90°,) fast spin echo will be performed and the measured relaxation times reported as T1ρ (ν_1 = 0). Infarct size and T1ρ relaxation times will be determined from in vivo MRI data. Apparent exchange rates (k_ex) and rotational correlation times (τ_0) will additionally be measured from the T1ρ dispersion. The measured MR parameters, T1ρ, exchange and molecular rotation, will be correlated with biochemistry, collagen and cell apoptosis.

**Specific Aim 2c:** Nuclear relaxation times will be correlated with myocyte apoptosis, total collagen, collagen type and collagen crosslink density.

**Quantification of Myocyte Apoptosis:** ApopTag® Peroxidase In Situ Apoptosis Detection Kit (Chemicon International, S7100) stains for apoptotic DNA fragmentation. Entire tissue sections are scanned with an Aperio ScanScope, creating digital images of 20X magnification for data analysis. Positive and total nuclei are tallied for each image. An apoptotic index is then calculated. **Immunohistochemistry:** Paraffin embedded tissue sections are immunostained for active caspase-3, BCL-2, and Bax. Caspase 3 sections are pre-treated in boiling citrate buffer and the BCL-2 and Bax tissue sections are treated in boiling target retrieval solution. A histochemical score that incorporates intensity of staining and percentage of staining is used to assess tissue sections. **Immunoblotting:** Frozen tissue sections are pulverized under liquid nitrogen. Tissue powder is divided into two samples and lysed in RIPA buffer or CHAPS buffer, each containing protease and phosphatase inhibitors. After homogenization, samples are mixed at 4°C for 20 minutes, centrifuged at 1000g for 10 minutes at 4°C, and the supernatant collected and protein concentration determined. Eighty micrograms of total protein are separated on a 12% SDS-polyacrylamide gel, transferred to PVDF membrane, and probed with the following antibodies: BAX, BCL-2, caspase 3, caspase 8 and caspase. Bands are detected using an ECL Western blotting detection kit. Optical density values are obtained using Image Pro Plus. Tissue sections will be fixed in 10% neutral buffered formalin. Five micron sections were obtained for each paraffin embedded block and stained using Masson Trichrome staining. Collagen stained blue, while muscle, cytoplasm, and keratin stained red.

**Quantification of hydroxyproline, types I and III collagen content, collagen cross-linking** is performed on tissue samples obtained from infarct, borderzone myocardium and remote myocardium after the terminal study in each euthanized animal at every time point. **Hydroxyproline** is measured in weighed full thickness myocardial samples, hydrolyzed and dessicated. After treatment with chloramine-T, samples are reacted with aldehyde-perchloric acid and read at 550 nm in a spectrophotometer. **Quantitation of Phenotype I and III Collagen:** Myocardial collagen is extracted and digested with cyanogen bromide (CNBr) according to the procedure described by Mukerjee and Sen (52). An aliquot of the CNBr digested collagen is prepared for polyacrilamide gel electrophoresis that is performed on gels by stacking and separating gel concentrations of 4% and 12% respectively. Quantification of the relative amounts of the collagen phenotype I to III is determined by gel scanning as previously described (53-55). Phenotype I collagen in the myocardium is assessed as the product of the area scanned on the gel (corresponding to phenotype I collagen/area for type I and type III) and myocardial hydroxyproline. The myocardial concentration of type III collagen is determined similarly. **Collagen Cross-Linking.** A further aliquot of the CNBr-collagen digest is subjected to acid (HCl) hydrolysis. The respective amounts of non-cross-linked (soluble) and cross-linked (insoluble) collagen are determined from the product of the percentage of collagen soluble to CNBr digestion and the total myocardial collagen concentration, and the difference between the total collagen concentration and soluble collagen concentration respectively. The relationship between insoluble and soluble collagen is used as an index of the degree of collagen cross-linking as described previously.

**Anticipated Results and Limitations:** Acutely, we expect that T1ρ will detect edematous MI as hyperenhancement on T1ρ images and that this correlates with early loss of myocytes and edema concurrently. Increases in T1ρ will be strongly correlated with collagen deposition and possibly collagen type through indirect effect of collagen fibers and expansion of the extracellular space on the local magnetic environment of 1Hs. There are several limitations with T1ρ MRI. The spatial resolution in vivo is not high enough to resolve individual cells or collagen fiber bundles, so average changes are observed. Furthermore, because of the lack of spatial resolution, T1ρ MRI is unable to differentiate concurrent events below the resolution of the image, e.g. T1ρ cannot detect cell apoptosis or edema as distinct events when they occur concurrently.

**Specific Aim 3:** To correlate T1ρ and delayed enhanced MRI infarct size with LV performance.
Rationale of Approach: Delayed enhancement (DE) MRI has been reported to distinguish viable and nonviable myocardium (56,57), but it is uncertain whether it can detect cardiac remodeling in the injured, but viable regions. Furthermore, cine T1ρ MRI has been shown to highlight the area of acute infarction in clinical studies (17,18). Yet it is not known whether there exist differences in infarct size when compared to DE MRI on account of differences in the underlying mechanism – water 1H nuclear relaxation (T1ρ) or contrast wash-in kinetics and extracellular volume fraction. Importantly, infarct size is related to LV performance. It is necessary therefore to correlate T1ρ and DE MRI infarct size with other measures of LV performance, regional strain, ejection fraction and stroke volume. This is significant because infarct size and performance are important measures of clinical prognosis.

Specific Aim 3a: Evaluate total infarct size on T1ρ MRI, DE MRI and from ex vivo optical measurements of scar volume.

Delayed Enhanced Methods DE MRI will be performed at baseline and follow up MRI using a segmented inversion recovery (IR) turboflash sequence using the following imaging parameters during diastole: TE/TR/TI = 2.96/500/300 ms, segments = 26, flip angle = 25°. The segmented IR turboflash sequence is ideal for volumetric quantification of nonviable myocardium because of the high contrast-to-noise (CNR) between the suppressed signal from healthy myocardium and the hyperenhanced infarct region (58). DE perfusion MRI will be performed 15 minutes after intravenous injection of gadolinium-DTPA (Magnevist, Schering).

Estimation of Total Infarct Size Infarct size will be quantified from 3D T1ρ and DE images by planimetry. Determination of infarct boundaries will be determined quantitatively by signal intensity (on T1ρ-weighted images and DE images) or relaxation times (T1ρ relaxation maps). Infarct size will be reported in units of grams of infarcted myocardium. Fractional infarction will be calculated as percent infarcted to healthy myocardium and transmurality will be semiquantitatively computed as percent wall thickness (0, 25, 50, 75 and 100%) across the infarct boundary.

Specific Aim 3b: Infarct size measurements will be correlated with LV performance using linear regression.

Quantification of LV Performance (systolic and diastolic volume): LV performance will be estimated at baseline and follow-up MRI. LV volumes throughout the cardiac cycle will be measured by cine MR imaging. Cardiac and respiratory gated images will be acquired using a true fast imaging with steady-state (trueFISP) pulse sequence, which produces high blood/myocardium contrast. A total of 9 equally spaced radial long-axis images will be acquired at a temporal resolution of 25 ms. Epicardial and endocardial contours will be semi-automatically drawn on all images in the acquisition stack from which LV volume and mass will be calculated.

Regression: Multiple linear regression will be performed to correlate infarct size measurements with T1ρ and DE with measurements of LV performance, ejection fraction and stroke volume. The association of the T1ρ with these parameters will examined and a model will be performed to form a model between T1ρ infarct size, ejection fraction and stroke volume.

Statistical Analysis
Differences in each of the outcomes at each time point will first be assessed by treatment group using bivariate tests. For measured continuous outcomes, two-group comparisons will employ t-tests or Wilcoxon rank-sum tests. Analysis of variance (ANOVA) or Kruskal-Wallis tests will be used for k-group comparisons, with appropriate adjustments made to any pairwise comparisons to maintain the Type I error rate. 1-Way ANOVA will be used to measure relaxation time differences between infarct and remote compartments. Bonferroni multiple comparisons may be used for borderzone and remote compartments, for which differences among relaxation times are expected to be comparably smaller than in the infarct region. Dispersion curves will be modeled using the nonlinear estimation via the Runge-Kutta algorithm. Preliminary data was collected from ex vivo sections of tissue from 5 swine, which were part of an ongoing experiment. Sample sizes for Aims 2 and 3 were determined from differences in relaxation times between borderzone and remote myocardium measured for T2 (νc = 0 Hz). This is the most conservative estimate of the measured differences in vivo. To detect a significant difference between T2 in the remote and borderzone myocardium at a power level (power = 0.8) and significance (p = 0.05) requires 10 animals in each group (initial infarct, 5 days, 2 weeks, 4 weeks and 8 weeks). Preliminary data demonstrates that measurements of T1ρ should have relatively higher power than the most conservative T2 measurement.

12. Timeline All aims will be performed concurrently because development of the pulse sequence (SA1) is ongoing as further refinements occur. Furthermore, to ensure that there is sufficient time to complete the animal studies, these studies will begin during the K99 phase (Years 1 and 2) and continue through the R00 phase (Years 3, 4 and 5).