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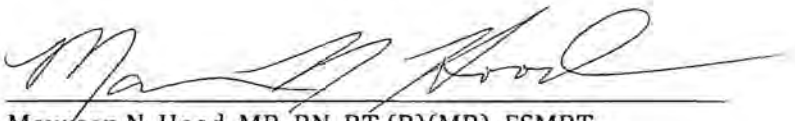

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DISSERTATION DEFENSE

MAGNETIC RESONANCE IMAGING OF HEART FAILURE USING A SWINE MODEL

BY

MAUREEN N. HOOD, MR, RN, RT (R)(MR), FSMRT

21 March 2011

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LINKING PAPER/EXECUTIVE SUMMARY

MAGNETIC RESONANCE IMAGING OF HEART FAILURE USING A SWINE MODEL

MAUREEN N. HOOD, MR, RN, RT (R)(MR), FSMRT

Background

Unlike every other form of heart disease, the prevalence and incidence of heart failure is still rising, and heart failure has become one of the leading causes of morbidity and mortality in the United States (US)(1). Furthermore, heart failure is commonly associated with sudden death at any stage of the condition (2-22). Heart failure is a burden on the health care system with an incidence of 10 per 1,000 persons over the age of 65 in the US (23, 24). The US is also in a nursing shortage. It is estimated that by the year 2025, there will be a short fall of nearly 260,000 nurses (25). Nurses are the primary care givers in the US. The nursing shortage poses a threat to the quality of life for the heart failure patient. More research is needed to better understand heart failure so that care plans, mediations and/or devices can be refined to help streamline patient care.

There is great variability in symptomatic expression among heart failure patients and during the course of the condition for a single patient. For instance, some patients may feel exercise intolerance, but may not ever experience peripheral edema; whereas, another person may experience pulmonary congestion, peripheral edema, and exercise intolerance. It was once thought that heart failure was always a progressive condition, but studies using pacing-induced tachycardia in animals to produce heart failure have demonstrated reversible myocardial remodeling when the tachycardic pacing stimulus is removed (26, 27), unless significant fibrosis has already infiltrated the myocardium (28). More recently, coronary bypass surgery has been found (in unusual cases) to reverse left ventricular cardiomyopathic remodeling in human patients (29, 30). Due to the many underlying causes of heart failure and the many associated co-morbidities such as diabetes mellitus, chronic obstructive pulmonary disease and ischemic heart disease, heart failure continues to be a complex and multifaceted challenge for care providers and researchers to understand and manage.

Therefore, the primary aim of my dissertation studies and research focused on heart failure, the associated methods and mechanisms of heart failure and muscle damage, and magnetic resonance imaging (MRI). Leading up to the research, methods and theories of conducting research and of muscle damage were addressed. The first paper published was on the method of cohort

studies (31). It is important to understand the different designs and statistics that apply to the study of a specific group. Cohort studies have direct applicability to the study of cardiovascular disease. It is important to be able to properly set up and define groups to be studied as well as how to define the numbers needed in order to be able to glean meaningful information from the data. Cohort studies can be applied to epidemiological studies, clinical research and even applied bench science.

The paper of excellence is on the theory of muscle damage (32). Although this paper focused on theories of skeletal muscle damage related to exercise, it still has considerable overlap into this dissertation research. Skeletal muscle can be damaged from complex mechanical and biochemical changes as a direct result from overuse. Similarly, tachycardia is an assault on the heart that also has complex mechanical and biochemical changes that result in pathological remodeling of the myocardium. When a person exercises, it is not just the skeletal muscles that are involved in the specific activity that get affected, the whole body does. Consequently, when the heart is not functioning properly, it affects the whole body as well. Understanding the complex array of physiologic responses in muscle is critically important for being able to maximize wellness.

As the first two papers were completed, the dissertation research protocol was waiting approval. Two more papers have been generated from the research study, one on the MRI T_1 mapping technique with histology to support the new pulse sequence (33), and the last paper that looked at heart dysfunction and morphologic changes found on MRI as compared to collagen and protein pathway protein changes (34).

The research was based as follows: The *central hypothesis* of this research is that MRI will be able to quantify the functional changes induced by myocardial remodeling from tachycardic pacing in a swine model.

Aim 1: MRI can quantify myocardial T_1 changes in a tachycardia-induced heart failure model using a new free-breathing T_1 Mapping pulse sequence.

- T_1 mapping values without the use of a contrast agent
- T_1 mapping values after administration of a gadolinium (Gd)-chelate

Aim 2: Correlate the pattern of fibrosis in post-mortem heart failure histological specimens with the tissue characterizing MRI techniques of T1 mapping values and myocardial delayed enhancement (MDE).

- Masson's Trichrome Blue Histology
- Immunohistochemistry of Col I, III, VI

Aim 3: Measure the quantity of protein changes in the TGF-Beta 1 pathway of fibrosis in the myocardium of swine in heart failure as compared to controls using quantitative Western blotting from post-mortem tissue specimens

Methods

This Institutional Animal Care and Use Committee (IACUC) approved protocol consisted of two groups of swine: a heart failure group and a control group. MRI was only performed in the heart failure group. The control group consisted of Yorkshire swine (N=10) who had been euthanized after been involved in this protocol as a control animal only, or from non-cardiovascular IACUC protocols that were available for tissue harvesting. Additional heart failure animals were also included that were not scanned with MRI for the bench science parts of this study.

Magnetic Resonance Imaging

After obtaining IACUC approval, Yorkshire swine (N=9) were implanted with pacemakers (St Jude Medical Integrity SR, Sylmar, CA) and paced for 3 to 5 weeks at 200 beats per minute. Each animal was scanned in a 1.5 Tesla MRI scanner (GE Signa, Waukesha, WI) at baseline (non-paced pacemaker in situ) and then at heart failure [confirmed by echocardiography as half the fractional shortening as compared to baseline (from approximately 32 percent at baseline to 16 percent at failure), with pacer turned off]. The animals were sedated and intubated with spontaneous breathing supplemented by oxygen, for the MRI examinations. The animals received a ketamine/propofol drip to maintain anesthesia as approved by the IACUC. The animals were continuously monitored by a porcine experienced anesthesia crew and then recovered after each MRI examination.

The animals received a comprehensive cardiac electrographically-gated MRI examination that included a modified Look-Locker with saturation recovery SSFP sequence for T_1 mapping (35). For the contrast administration, a standard 0.2 mmol/kg dose of Gd-chelate contrast agent (Gadoteridol, Bracco, Princeton, NJ) was administered intravenously.

Histology

Immediately post-euthanasia, myocardial tissue was collected from septum and left ventricular free wall and then was immediately fixed in 10% buffered formalin. The tissue was later embedded in paraffin and cut into 5 μm sections using standard methods. Slides were stained using Accustain Masson Trichrome Blue and microscopic images captured with a Nikon Eclipse 80i microscope with digital DXM 12000c camera (Nikon Instruments Inc., Melville, New York).

Western Blots

Myocardial tissue was acquired from the swine immediately post-euthanasia. Tissue was cut from the left ventricular free wall and immediately immersed in liquid nitrogen. Protein extraction was performed on the heart failure (N = 10) and control (N = 10) samples. The proteins were separated using electrophoresis and then transferred to membranes. Primary antibodies were used for Anti-TGF Beta Receptor Type 1, Smad 2, Smad 3, MADH7 and MMP14. Conjugated secondary antibodies were used for detect with an Odyssey-Infrared Imaging System (Li-cor Biosciences, Lincoln, NE) and then quantified.

Immunohistochemistry

The tissue from the histology was used to create slides for immunohistochemistry. The tissue sections were then incubated with primary antibodies for Collagen I, Collagen III, and Collagen VI. Then the tissue slides were incubated with secondary antibodies, and stained with DAPI. The slides were captured with specific wavelength fluorescence and photographed using a

Nikon Eclipse 80i microscope with digital DXM 12000c camera (Nikon Instruments Inc., Melville, New York).

Findings

Magnetic resonance imaging can demonstrate morphological and functional changes in a tachycardic swine model of heart failure. As tachycardia stress the heart, the myocardium remodels itself in an attempt to retain function. MRI using a T_1 mapping sequence can quantify the changes in fibrosis in the heart by measuring the T_1 value of the myocardium allowing for detection of diffuse or global heart disease in a non-invasive manner. The T_1 mapping sequence values in heart failure were consistent with the increase in collagen found on histological analysis. There is a trend toward correlation between T_1 values and collagen percentage, but more animals need to be evaluated. This free-breathing version of the T_1 mapping technique offers the potential to be able to quantitatively evaluate a wide range of patients, including patients with high acuity levels and children, with diffuse fibrosis of the myocardium.

In this study we found increases in collagen that were not depicted on traditional T_2-weighted or myocardial delayed enhancement images during the MRI. The functional and morphologic changes demonstrated on MRI may be related to the increase in collagen Types I, II and VI. As collagen type I linkages break, and the more elastic type III collagen increase, the heart is allowed to expand and the muscle also, thus creating DCM and a decrease in cardiac function as seen on MRI. In addition, an up regulation of TGFBR1, the receptor Smads 2 and 3, and the inhibitory Smad 7 is related to increased fibrosis and were slightly up regulated in this study, but still needs further investigation.

Impact

The biggest impact for patient care is that MRI can be used as a non-invasive tool to help manage heart failure patients more frequently and accurately. MRI gives us a better tool to identify those patients at greater risk for sudden cardiac death through improved detection of fibrosis in the myocardium. MRI with T_1 mapping may be able to enable physicians to improve the selection of patients who may benefit from a specific mode of therapy or which patients may need the very expensive implanted device support. The improvement in diagnosis should lead to improvements in treatment and prevention approaches for heart failure. Since heart failure is such as huge chronic condition, nurses need evidence based knowledge that will help make the delivery of care to this population as efficient and effectual as possible. Research such as this should help improve case specific needs for optimal functioning of the heart failure patient and hopefully improve their overall quality of life for as long as possible.

Future

More research needs to be done with the MRI of heart failure as well as with the changes in collagen, especially in the specific types of collagen and their developmental pathways. The next phase of research will be focused on more of the TFGF B1 pathway proteins and the collagens of the myocardium.

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Skeletal Muscle Physiology and Damage Models from Exercise

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Running head: SKELETAL MUSCLE PHYSIOLOGY

Skeletal Muscle Physiology and Damage Models from Exercise

Abstract

The mechanisms of skeletal muscle fatigue leading to muscle damage during exercise are complex. When a person exercises, it is not just the muscles actively involved in the specific activity that get affected; the whole body is involved. The classic theory of muscle damage and fatigue is attributable to AJ Hill and colleagues dating back to 1924, which focuses on muscle failure as a catastrophic event based on changes in ion exchange from ATP and oxygen depletion and waste product build up. However, this theory cannot account for the variability found in human athletics and endurance activities. The Central Governor hypothesis and the mechanism of mechanical stress are two other models of muscle damage that can also account for many aspects of muscle fatigue and damage during exercise, but they are also unable to account for all aspects of exertional muscle injury. There are still gaps in our understanding of muscle physiology and damage mechanisms that need to be explored. Since exercise is complex, trying to discern the actual damage mechanisms in a controlled, objective research study is difficult. Advanced diagnostic tools such as magnetic resonance spectroscopy (MRS) can investigate muscle metabolism in humans *in vivo*. However, MRS is difficult to perform in skeletal muscle, technically challenging and expensive, thus, limiting its use. None the less, more research is essential to help more thoroughly explain the differences in athletes and within individuals when they exercise in order to maximize performance and prevent injury.

Key Words: muscle fatigue, muscle injury, exercise, magnetic resonance, biological/physiological models

Introduction and Background

Strenuous exercise produces a complex array of physiologic responses that consume energy and oxygen, and produce byproducts. Prolonged exercise or the addition of eccentric exercise can lead to muscle damage through increased metabolic heat production, a high demand for adenosine triphosphate (ATP), accelerated oxidative, chemical and mechanical stress, and metabolic acidosis (Krustrup, Ferguson, Kjaer, Bangsbo, 2003; Harkema, & Meyer, 1997; Clarkson & Hubal, 2002; Blei, Conley, & Kushmerick, 1993) When exercise causes an elevated or sustained level of stress to the muscle tissues, cellular level changes begin to occur, followed by muscular changes and sometime systemic reactions. As sustained elevation in cellular respiration and transient tissue hypoxia continue, activated muscles have been shown to generate more reactive oxygen species (ROS) than the antioxidant systems can scavenge (Murrant, Andrade & Reid, 1999). Peroxide may play a role in the activation of andenosine monophosphate-activated protein kinase which plays a role in glucose transport in skeletal muscles (Coderre, Kandror, Vallega, & Pilch, 1995; Lund, Holman, Schmitz, & Pedersen, 1995). Sandstrom, Zhang, Burton, Silva, Reid, Westerbald, and Katz, (2006), found that excessive levels of ROS disrupt glucose transport; although low to moderate levels stimulate glucose transport to skeletal muscles.

It is also thought that auto-oxidation of oxymyoglobin in the cells leads to changes that enhance superoxide and peroxide formation, causing release of ferric heme proteins, which cause further disturbances in muscle membrane integrity. When consumption of nutrients outpaces the transport of nutrients to the muscles and cells, disruption of the sarcolemma membrane, sarcotubular system, and myofibril contractile components may occur as is found in sustained or eccentric exercise (McNeil & Khakee, 1992; Lieber & Friden, 2002). These changes in the

components of the musculature can lead to swelling, weakened contractility of the muscle, myofiber matrix derangement, intracellular and extracellular electrolyte imbalances and pain as muscle stress progresses (Kasper & Talbot, 2002). The damages to the muscle cells lead to decreases in pH, accelerated ATP depletion, the release of intracellular muscle constituents into the circulation (CK, lactate, heme, and potassium), and a massive unregulated release of Ca^{++} from the sarcoplasmic reticulum through the Ryanodine receptor (RYR1), which can overwhelm the normal regulatory mechanisms leading to severe muscle injury (Isaeva, Shkryl, & Shirokova, 2005).

The biochemical and structural pathways leading to muscle damage from exertion or eccentric exercise, although studied extensively, are still not clearly understood. Furthermore, it is also not known why some individuals sustain muscle damage in response to strenuous physical activity when others do not (Clarkson, Hoffman, Zambraski, Gordish-Dressman, Kearns, Hubal, Harmon, & Devaney, 2005). This is significant in that there are rare and severe complications such as renal failure, disseminated intravascular coagulation and compartment syndrome and rhabdomyolysis that can occur from exertion to the muscles (Kring, 2005; Warren, Blumbergs, & Thompson, 2002). Since severe muscle damage can be life threatening in rare cases such as rhabdomyolysis and malignant hyperthermia, it is essential that the mechanisms surrounding the pathways to muscle damage from exercise be better understood. This paper will serve as a general review of the basic myofibril types in skeletal muscles, the energy sources used during different types of exercise, and a discussion of the complex physiological processes surrounding muscle fatigue and damage with the theories behind why muscle injuries occur during exercise.

Main Myofibril Types

There are three main type of muscle fibers found in human skeletal muscle, Type I (slow twitch, oxidative), Type IIA (fast twitch, glycolytic, oxidative), and Type IIB (fast twitch, glycolytic), based on histochemical staining techniques. In addition to these main types, scientists have also discovered many other fiber types, but there is disagreement as to the classification of these types and subtypes. There are intermediate staining types of fibers referred to as type IB and IIC, and subtypes IIAB and IIAC (Schantz & Dhoot, 1987; Bye, Gronnerod & Vogt, 1989; Hather, Mason, & Dudley, 1991). In addition, studies have found that the fiber types are not static. They have the ability to alter their characteristics based on environmental demands and innervation change (Guth, 1968; Guth & Yellin, 1971; Fluck, 2006). Furthermore, the characteristics of muscle fibers between muscles as well as within a muscle can vary somewhat making the distinction of exact fiber types even more difficult (Spurway, 1981). In essence, the make up of the fibers within a muscle are complex, adding to the challenge of discerning how our muscles metabolize energy during exercise and how they may become damaged from exercise or exertion.

Energy Use

Immediate energy

When muscles need to work, they call on a form of immediate energy to fuel their contraction, called the ATP-PCr System (McArdle, Katch, Katch, 2007; Conley, Kemper, & Crowther, 2001). The ATP-PCr System uses intramuscular high-energy phosphates, ATP, and PCr to generate the energy necessary for muscular contraction. Each Kilogram of skeletal muscle generates 3 to 8 mmol of ATP and 4 to 5 times more PCr, enough to fuel a person for one minute at a brisk walk pace, fuel a marathon runner for about 20-30 seconds, or supply 5-8

seconds of intense exercise such as in a sprint (McArdle, Katch, & Katch, 2007). This immediate energy source runs out quickly, but essentially buys the body time to allow for another energy source to kick in.

Short-term energy

Adenosine triphosphate (ATP) must be made to further provide muscles with energy needed for contraction. There are two main pathways for muscle to obtain energy, glycolysis and oxidative phosphylation. The short-term ATP synthesis system is known as glycolysis or the lactic acid system, which is considered an anaerobic pathway for ATP synthesis. Fast twitch muscle fibers are considered to be mitochondria poor, and thus have a low capacity for making ATP through oxidative phosphorylation. Therefore, glycolysis is the main pathway for energy production in white, fast twitch muscle fibers (Type IIa), which need to create energy quickly without the aid of oxygen from the blood supply. Type IIb fast twitch muscle fibers also use glycolysis, but are also capable of a moderate amount of oxidative ATP production, making them a mixed energy muscle fiber type.

Glycolysis provides a quick mechanism for the cytosol of cells to provide ATP from glucose, although it only coverts about 5 percent of the energy from a glucose molecule (Kimura 2006, Krustrup, Ferguson, Kjaer, Bangsbo, 2003; Conley, Kemper, & Crowther, 2001). Glycolysis supplies the energy to phosphorylate ADP at the substrate level during intense exercise (about 45% of high-energy phosphates) and results in lactate formation, a blood marker of anaerobic exercise (McArdle, Katch, & Katch, 2007). Under aerobic conditions, lactate formation is balanced by lactate removal. As muscle exertion levels increase, and muscle cells can no longer meet energy needs through aerobic oxidative phosphorylation alone, lactate starts to accumulate as the level of lactate formation starts to out pace lactate removal. This is known

as the blood lactate threshold or "anaerobic threshold," which is an important indicator of fitness (Mader & Heck, 1986; McArdle, Katch, & Katch, 2007). At continuous, high intensity exercise, anaerobic metabolism increases (Conley, Kemper, & Crowther, 2001). The buffering capacity eventually becomes outpaced by the production of lactate and free hydrogen ions in the muscle, causing a decrease in pH, which in turn, reduces muscle's ability to contract. In addition, exercising near maximal performance not only consumes glucose rapidly and produces reactive oxygen species such as lactic acid, the combination of changes that are occurring are expressed by the individual in the form of discomfort and breathlessness (Killian & Campbell, 1983).

Long-term energy

Steady-state oxygen consumption, referred to as the aerobic system of energy transfer, can occur during steady exercise, usually after 3-4 minutes. The pathway used by the aerobic energy system is called oxidative phosphorylation and works mainly to sustain the slower, red (Type I fibers) skeletal muscles. This system, as suggested by its name, requires oxygen for the production of ATP. The oxymyoglobin in muscles is used in the production of ATP within the mitochondria, which, although it's a longer pathway than glycolysis, produces substantially more ATP per glucose molecule (Kimura, Hamaoka, Kurosawa, & Katsumura, 2006). Glycolysis generates a net of 2 ATP, but does so very quickly (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2002). The citric acid cycle, electron transport chain and the oxidative phosphorylation process within the mitochondria produce a net gain of 30 ATP for each molecule of glucose catabolized; a much more energetic but slower process (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2002). Figure 1 shows a simplified schematic of theses energy processes to help understand their close relationship. Oxidative metabolism (the constant resynthesis of ATP from phosphphocreatine (PCr)) predominates in long term exercise as it takes longer for the waste

products produced to overwhelm the system. Unlike anaerobic exercise, aerobic exercise is able to remove lactic acid and other waste products at approximately the same level as it is being produced. However, even the aerobic system has its limits to being able to sustain homeostasis.

A number of factors influence how long a person can sustain aerobic exercise. For instance, glycogen availability plays a dramatic role in exercise capacity. The availability of cellular ADP is the main regulator of phosphylation of ATP. ADP concentration controls the enzymes that regulate the oxidative pathways and the metabolism of macronutrients (McArdle, Katch, & Katch 2007). The fine balance of nutrients and waste products is crucial. Since oxidative phosphorylation is adversely affected by acidosis, the accumulation of hydrogen ions (waste) contributes to the reduction of ATP synthesis (Conley, Kemper, & Crowther, 2001).

Along with the availability of glucose, oxygen must also be available to the muscles performing the work. The cardiovascular system carries oxygen from the lungs to the muscles. Exercise physiologists use the term maximum rate of oxygen uptake (VO_2-max) to refer to the fastest rate at which an individual can utilize oxygen (Mitchell & Blomquist, 1971). Breathing rate and the exchange of oxygen (O_2) and carbon dioxide (CO_2) are critical measures of exercise performance and fitness. Impaired transport of oxygen and nutrients to the muscles can reduce the rate at which the mitochondria can produce ATP through oxidative phosphorylation as well as the rate at which waste products can be carried away or neutralized by superoxide dismutase (SOD) and other free radical scavengers (Isaeve, Shkryl, & Shirokova, 2005; Murrant, Andrade, & Reid, 1999).

The supply of oxygen starts to affect the muscle after a while during aerobic exercise. The muscles produce heat in addition to ATP during energy conversion. This process can cause the muscles to swell. When the muscles swell, the blood vessels carrying oxygenated blood to

the muscles and waste products away from the muscles become constricted (mechanical compression). Muscles have an intramuscular oxygen reserve that allow the mitochondria to continue to produce ATP via oxidative phosphorylation for a short while after arterial oxygen supply is depleted, but even lowered levels of oxygen create an imbalance of waste products in the muscles cells (Sadamoto, Bonde-Petersen, & Suzuki, 1983; Jarvholm, Styf, Suurkula, & Herberts 1988; Aratow, Ballard, Crenshaw, Styf, Watenpaugh, Kahan, & Hargens, 1993). Eventually, fluid loss and electrolyte depletion add to the problems encountered for muscle contraction to continue. The pH of the muscles decreases similarly to anaerobic exercise conditions, reducing muscle contractility.

Discussion of Muscle Fatigue and Damage from Exercise

Models

Muscle damage from exercise is a complex phenomenon. The work of the Nobel Laureate, A.V. Hill and colleagues, still dominates the central theory of fatigue and skeletal muscle failure as a catastrophic event. The classical model of exercise physiology, which has help up well to much scrutiny over the years, essentially states that skeletal muscles will have a catastrophic failure, or fatigue, when the muscles reach an excessive build up of waste products (lactic acid) from the depletion of the ATP (Hill, 1924; Hill, Long, & Lupton, 1924). A build up of lactic acid lowers the pH leading to an acidic environment in which the muscles lose their ability to contract with force (Fabiato & Fabiato, 1978). For instance, Lieber and Friden, (2002) found that intracellular glycogen stores in skeletal muscle fibers become depleted under repetitive activation. What this implies is that when muscles are activated to contract, they signal the myocytes to convert glycogen to ATP. Under anaerobic conditions, glycolysis is the process

called upon that rapidly makes ATP from glycogen in the cytosol of the cell, but it is an inefficient process, depleting the glycogen stores quickly. Lactate and protons are also quickly generated during gylcolysis - too quickly for the blood to carry away the waste products during intense exercise, resulting in an ionic imbalance that causes a decline in performance.

The muscles can produce much more ATP from glycogen under the longer process of oxidative phosphorylation, which is found predominately under aerobic exercise conditions. This slower system can call upon the blood supply bringing additional glycogen, oxygen and other nutrients, while sweeping away waste products so that muscle cells can sustain exercise for a much longer period of time. However, even under aerobic conditions, without enough oxygen getting to the skeletal muscles, ATP production can be hampered. If the muscle tissue swells too much, the blood flow to the activated muscles is decreased. When O2 decreases to muscles, ATP production through oxidative phosphorylation also decreases (Sadamoto, Bonde-Petersen, & Suzuki, 1983; Jarvholm, Styf, Suurkula, & Herberts 1988; Aratow, Ballard, Crenshaw, Styf, Watenpaugh, Kahan, & Hargens, 1993). This is thought to be what happens in compartment syndrome injuries (Clayton, Hayes & Barnes, 1977; Birtles, Rayson, Jones, Padhiar, Casey, & Newham, 2003).

However, the classical model does not take into account such phenomenon as a second wind or a runner's kick at the end of a race. Furthermore, the ATP level in myocytes does not get deleted completely during exercise, even during maximal exercise (Noakes & St. Clair Gibson, 2004; Noakes 2007). Myocytes retain nearly 50% of their capacity in order to protect the muscle from damage. Myocytes have feedback mechanisms and a molecular plasticity that control the conversion of ADP to ATP and work to clear waste products from the muscles to help maintain a homeostatic environment (Wu, Jensen, Beard, 2006; Noakes, St Clair Gibson,

Lambert, 2007; Fluck, 2006). However, even with the human body's defense mechanisms that fight to maintain homeostasis, there are exercise situations that force or allow the body to move beyond the homeostatic yearnings of the myocytes and allow damage and myocyte death to occur. The central governor model of muscle fatigue focuses on central nervous system (CNS) control of homeostasis and fatigue (Noakes, Peltonen & Rusko, 2001; Noakes 2007). This model postulates that the CNS controls how many myofibrils will be called upon during a given situation so that the body can strive to keep itself in homeostasis. The information the CNS receives from the body, such as hypoxia, during exercise guides how the central governor will respond. The central governor is also responsible for telling the body how intense and which muscles need to be called upon. The subconscious brain works to maintain homeostasis and the conscious brain can tell the body to override the subconscious brain and push the muscles to go beyond their normal safety levels and sometimes allow muscle damage. The central governor model helps to describe why our muscles can be pushed beyond homeostatic levels that can produce muscle damage such as in marathon running, (Noakes, 2007), but even this model has its critics as it still does not account for fatigue and muscle damage at submaximal exercise levels or short intense bouts of exercise (Weir, Beck, Cramer, & Housh, 2006).

Muscle fatigue and damage during aerobic exercise cannot be explained well by the part of Hill's classical model that relies on oxygen and ATP depletion in the muscles. As muscles are exercised, the body increases its cardiac output to send an increase of blood to the muscles being activated. Gonzalez-Alonso and Calbert (2007) found that metabolic changes are occurring, especially under heat stress conditions that cause muscle fatigue even when ATP, PCr and oxygen levels are still high. Instead, it is thought that the body is simply producing excessive waste products (Pi, ADP, CO_2 and H^+) during heavy exercise that can inhibit muscle contraction

when skeletal muscle temperatures rise above 40 degrees Celsius. This phenomenon is referred to as heat stress. The build up of waste products is consistent with Hill and colleagues part of the model that focuses on waste product build up, which lower the pH to such an acidic level that the structural integrity of the muscle fiber proteins can become disrupted from the breakage of amino acid bonds (Hill, 1924; Hill, Long, Lupton, 1924).

It was once thought that protons [H+] were the main correlative between acidosis and contractile force reduction (fatigue). However, muscle fiber experiments have demonstrated a reduction in contractile force without a drop in pH (Westerbald & Allen, 1992). In addition, several studies have found that pH does not change significantly at temperatures near normal body temperature (Pate, 1995; Westerbald, 1997; Chin & Allen, 1998). The Ca2+ release needed for contraction is somehow reduced in acidic conditions. It is not clear what is causing this reduction in Ca2+, but it is postulated by Chin and Allen, (1998) that the contractile proteins have a lower Ca2+ sensitively due to a reduction in pH.

An additional consideration surrounding the pH debate deals with other waste products produced during exercise. It has been found that when reactive oxidative species (ROS) are being formed at a rate too great to be chelated and removed at a balanced rate, they may play a role in muscle fatigue and damage. Generally, ROS in Type I and IIa fibers are held in check by ROS Scavengers (SOD and catalase), but when exercise is too intense, they increase (Isaeva, Shkryl & Shirokova, 2005; Murrant, Andrade, & Reid, 1999). It is more probable that the fatigue is caused by a combination of factors such as a drop in pH and an increase in ROS.

Another possible mechanism for exercise induced muscle damage is mechanical stress. Mechanical stress seems especially likely in unaccustomed and eccentric exercise (Lieber & Friden, 2002; McNeil & Khakee, 1992; Morgan & Talbot, 2002). Morgan's (1990) popping

sarcomere hypothesis states that when sarcomeres are over stretched, they will become weak and can predispose the myofibrils to shearing and deformations in the membranes and t-tubules. Eccentric exercise is well documented with muscle damage from being stretched beyond optimal sarcomere length, but unaccustomed length of exercise and unaccustomed force of exercise is also though to provoke mechanical damage to the myofibrils (Ebbeling & Clarkson, 1989; Morgan & Proske 2004). In a study by Kasper (1995) which looked at exercising rats after allowing their muscles to atrophy for 28 days, demonstrated that sarcolemmal disruption occurs with muscle reloading. When muscles are not able to withstand the exercise demands put on them, they fatigue and may become injured. However, the cascade of events that follows mechanical stress such as Ca2+ overflow and the release of proteases, doesn't always lead to the death of the cells. Muscle fibers have the ability to seal the tears in the sarcolemmal membranes, so not all myocytes that sustain damage continue on to necrosis (Kasper, 1995). It was also demonstrated that smaller muscle fibers were more likely to become damaged, which is consistent with the findings of Morgan and Proske (2004) of the effects of unaccustomed exercise on skeletal muscles.

To help skeletal muscles repair themselves in response to injury, muscle fibers contain satellite cells. Satellite cells are the progenitors of myocytes and some investigators refer to them as stem cell like since they are essential to postnatal muscle growth and repair in skeletal muscle (Schultz 1985; Bischoff 1990; Schultz and McCormick 1993). Satellite cells are essentially quiescent until called upon in response to injury, such as with exercise induced damage (Bischoff 1975; Bischoff 1986; Grounds, White et al. 2002). An interesting related observation in a study on rats recovering from hindlimb suspension, type IIc myofibers were reported during the muscle rehabilitation phase (Kasper, white & Maxwell, 1990). Type IIc

fibers are associated with developing muscles and muscles that are transitioning from one type to another (Kugelberg, 1976; Whalen, Sell, Butler-Brown, Schwarta, Bouveret, & Pinset-Harstrom, 1987). Although it is known that satellite cells can give rise to any of the myofibril types, any link between satellite cells, type IIc fibers and muscle cell transition or muscle regeneration is still unclear. None the less, skeletal muscles have various responses to injury from exercise induced injuries.

However, is mechanical stress truly a separate phenomenon, or is it a combination of mechanical breakdown and a chemically mediated reaction from Ca2+ changes. Studies on rats found that mechanical events from eccentric contractions can lead to disrutpion in desmin (a cytoskeletal structural protein) integrity through calpain-mediated digestion (increased calcium ion) and sarcolemma damage (Lieber & Friden, 2002; Barash, Peters, Friden, Lutz & Lieber, 2002). Desmin disruption thus represents a very early structural manifestation of muscle injury during eccentric contraction (Lieber, Thornell, & Friden, 1996; Lieber & Friden, 1999; Lieber & Friden, 2001). In addition, skeletal muscle contraction is a Ca2+ dependent mechanism. Release of Ca2+ from the SR – initiates muscle contraction (depolarizes the transverse tubular membrane and the SR Ca2+ release channels (ryanodine receptors) (Isaeva, Shkryl & Shirokova, 2005; Gissel, 2005). It may be that this combination of mechanical and chemical damage at the cytoskeletal level predisposes the contractile apparatus to the types of muscle soreness and injury reported with eccentric exercise injuries (Lieber, Thornell, & Friden, 1996; Lieber & Friden, 2001; Overgaard, Lindstrom, Ingemann-Hansen, Clausen, 2002). A hypothesis on damage caused by eccentric exercise put forth by Yeung, Balnave, Ballard, Bourreau and Allen (2002) postulates that the sarcomeres in muscle fibers are of varying length. During eccentric exercise, the shorter sarcomeres are overstretched by shearing forces, causing an increase in Ca2+, Na+

and partially disrupting the t-tubule system. These consequences of overstretching eventually lead to inflammation, swelling and a disruption in homeostasis of metabolic products.

Magnetic Resonance Imaging and Spectroscopy

The advent of nuclear magnetic resonance imaging (MRI) and nuclear magnetic resonance spectroscopy (MRS) finally gave scientists the ability to start to evaluate the metabolic changes that occur during exercise *in vivo* (Hoult, Busby, Gadian, Richards, & Seely, 1974; Dawson, Gadian, & Wilke, 1977). ^{31}P MRS can measure metabolites such as ATP, PCr and P_i. In addition, perfusion (Toussaint, Kwong, M'K paru, Weisskoff, LaRaia, & Kantor, 1996b) and diffusion tensor (Basser, Mattiello & LeBahn, 1994) during exercise can be calculated to help investigators gain a better understanding of the dynamic movement of fluids and the ultrastructure that influences these metabolic pathways in skeletal muscle.

The main advantage of using MRS is to evaluate metabolic processes of skeletal muscle in their natural environment instead of cutting and stripping individual myofibrils to be investigated in the laboratory. This will also allow for more investigation with human subjects. Much of our knowledge of muscle damage and fatigue has been gleaned from animal studies. Unfortunately, sometimes translating findings from animal studies to the human condition does not work well. MRS allows for the metabolites to be studied in ways that single myofibrils cannot such as monitoring glycogen conversion with the natural feedback and control mechanisms in place (Hoult, Busby, Gadian, Radda, Ricahrds, & Seeley, 1974; Newcomer, Sirikul, Hunter, Larson-Meyer, & Bamman, 2004). Furthermore, ^{31}P MRS can evaluate and monitor both aerobic and anaerobic types of exercise conditions. In a case study of an athlete being subjected to unaccustomed exercise, ^{31}P MRS was able to demonstrate a reduction in

muscular oxidative capacity and increased anaerobic ATP production that were correlated well with increased fatigue (Newcomer, Sirikul, Hunter, Larson-Meyer, & Bamman, 2004).

However, even ^{31}P MRS studies are somewhat constrained in that there are limited types of exercise that can be performed inside an MR scanner. Cycling ergometers are available to attach to certain MR scanners, but they are very expensive. Other types of simple exercise can be performed in the MR scanners, such as having the participant push their feet against a block, but these can be difficult to validate and measure consistently. Lastly, ^{31}P MRS examinations are difficult and expensive to perform, limiting their broad application (Carlier, Brillault-Salvat, Giacomini, Wary, & Bloch, 2005; Toussaint, Kwong, M'K paru, Weisskoff, LaRaia, & Kantor, 1996). None the less, more research with *in vivo* types of studies need to be performed and then correlated with more traditional exercise physiology measures such as breathing rates, pain and exertion scales, and VO_2 max, demographics and genetic variability. Increases in technology will aid in the future understanding of muscle physiology and damage so that injuries from exercise can be better prevented.

Limitations

What Hill's classic theory, the Central Governor hypothesis and mechanical stress model demonstrate is that muscle fatigue and damage from exercise it is complex phenomenon. Exercise can be continuous or intermittent, last a short or long duration of time, be concentric, eccentric, or isometric in nature, and have variable intensity levels. To further complicate matters, muscle fiber type and intensity of exercise also play a role in the amount of fatigue and damage presented (Metzger & Moss, 1987; Barclay, 1995). In addition, the fitness level of the individual, relative familiarity of the exercise, genetic make up, hydration, nutrition, and

environmental factors such as temperature and humidity play roles in muscle damage from exertion. The hardest aspect of modeling muscle damage from exercise is that these factors are intertwined and vary between and within individuals. The concept of dynamics of complex systems suggests that muscle damage from exercise is a system of interwoven parts and processes (Bar-Yam, 1997). When a person exercises, it is not just the muscles actively involved in the specific activity that get affected. The whole body is involved, but this is difficult to objectively quantify. In addition, it is difficult to see what is happening in a live body at the cellular and intracellular level. Singling out a single factor to study in the live human is a most difficult challenge for investigators, so it is not surprising that investigators disagree on the details of the mechanisms that cause muscle damage and fatigue.

Significance

Understanding the biochemical and mechanical processes that can induce fatigue and damage during exercise can help improve not only athletic performances, but also rehabilitative measures. New knowledge is critical for designing exercise programs that maximize performance while minimizing adverse effects. For nursing, an improvement in understanding muscle fatigue and injury from exercise will help improve the design of care plans for patients. Nurses will be able to better educate their patients in how to avoid fatigue and injury during exercise, which should help patients be more compliant in their rehabilitation programs.

Summary

It is clear from this review that the study of muscle fatigue and damage is complex. Myofibrils can vary within and between muscles as well as between individuals. The classical

theory of muscle physiology as postulated by AV Hill and colleagues has served the exercise physiology community well for a long time, but it is clear that there are gaps that cannot be explained such as what allows people to run marathons. The central governor model tries to fill these gaps through a hypothesis that the CNS controls how muscles are recruited and perform. The mechanical stress model also demonstrates many aspects of muscle damage. No single model explains everything, thus the need to continue to study fatigue and damage from exercise.

The advent of nuclear magnetic resonance, particularly 31P NMR, has enabled scientists to study muscle physiology *in vivo*. As technology improves, studying muscles in vivo during various types of exercise using 31P NMR should add to the body of knowledge in exercise physiology. Muscle damage is a complex problem that will require much more research in the future. Through better understanding of the complex processes, it is hoped that prevention of exertional injuries will improve and the rehabilitation of skeletal muscle injuries will be maximized.

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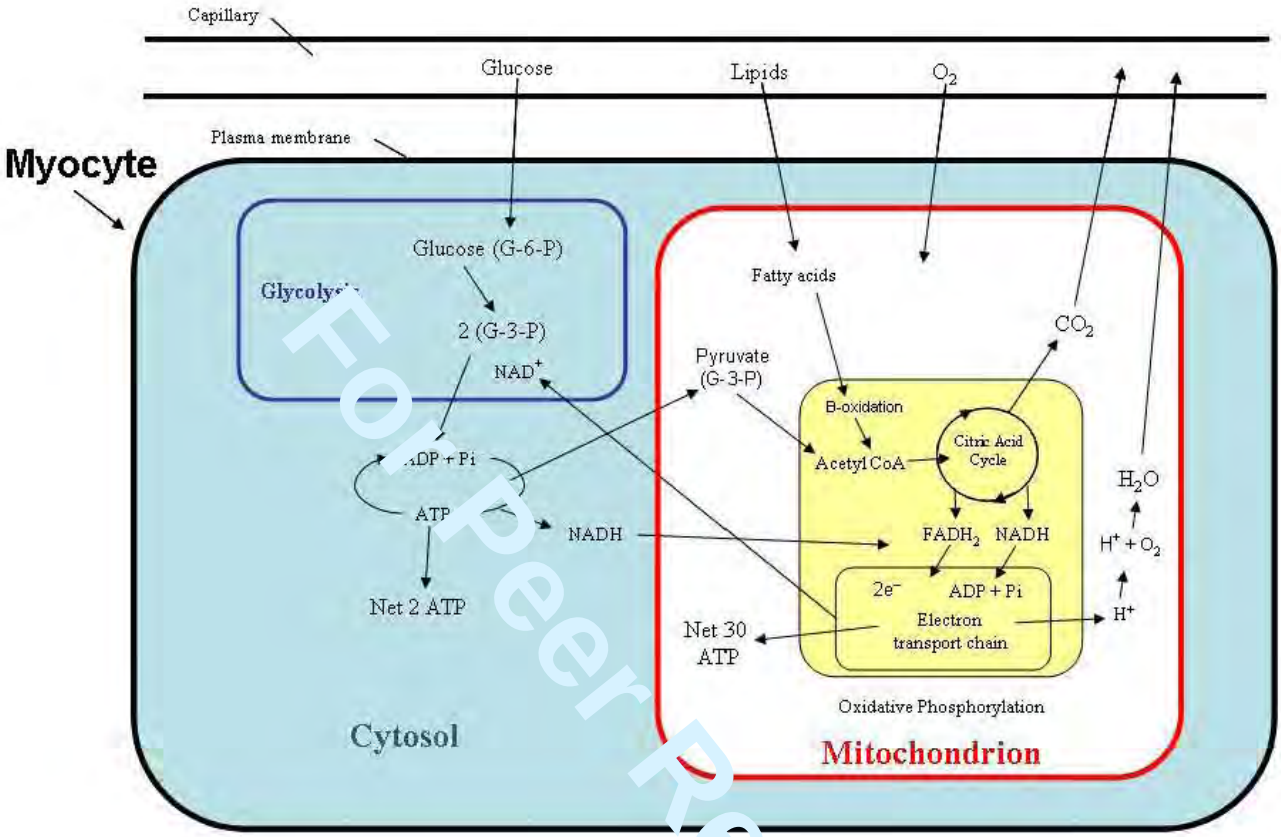
Figure 1. Simplified metabolic processes for the production of energy in muscle fibers. Myocytes use both anaerobic glycolysis and aerobic oxidative phosphorylation to produce the energy needed for cellular metabolism. Gylcolysis is a quick process for fast twitch fibers to produce a short burst of energy. Oxidative phosphorylation takes a bit longer and requires oxygen to be present in the myocyte, but it produces a far great number of ATP that can be utilized for longer energy needs. Adapted from Fluck, (2006), and Alberts, Johnson, Lewis, Raff, Roberts, and Walter (2002).

Figure 1.



Magnetic Resonance Imaging of Heart Failure Using a Swine Model

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A. Specific Aims

Unlike other forms of heart disease, the prevalence and incidence of heart failure is still rising, making heart failure one of the leading causes of morbidity and mortality in the United States. Furthermore, heart failure is a chronic, progressive condition that is associated with sudden death in 50% of afflicted individuals and gradual pump failure in the remainder. The cause of sudden death in heart failure is predominately (but not exclusively) due to ventricular tachyarrhythmias. ***At present there are few reliable predictors of risk for sudden death in heart failure. Implantable defibrillators can significantly reduce the risk of sudden death in heart failure, but the reduction is modest, resulting in increased morbidity and expense without benefit in the majority of recipients.*** An improved understanding of the causes of sudden death and mechanisms of progressive heart failure should lead to optimized patient care strategies.

Dilated cardiomyopathy (DCM), the most common type of nonischemic heart failure, is associated with increased ventricular chamber size and decreased systolic function. Persons with DCM are at risk for sudden death and persistent tachycardia is a common cause of DCM. The degree of myocardial fibrosis, especially central fibrosis, is thought to play a critical role in determining the risk of sudden death in DCM. This proposal will focus on the development of fibrosis in tachycardia-induced DCM in Yorkshire swine, a large animal model of human heart failure.

Magnetic resonance imaging (MRI) provides excellent, noninvasive images of cardiac structure and function, and is the only modality that can quantify fibrosis. Our ***long-term goal*** is to develop MRI techniques that can identify persons at high risk for sudden death from heart failure and to improve patient care through more specific condition identification. To meet this goal, the ***overall objective*** is to define the functional and morphologic characteristics of DCM on MR imaging techniques, including quantification and phenotypic identification of pathologic fibrosis. To validate MRI findings, extracellular matrix analysis of the myocardium will be performed using histology and quantitative Western blot analysis. The ***central hypothesis*** is that MRI will be able to quantify the functional changes induced by myocardial remodeling from tachycardic pacing in a swine model.

The **specific aims** of this research are:

1. Measure the difference in cardiac function between baseline and tachycardic pacing-induced heart failure in swine using MRI. Rationale: MRI can noninvasively measure wall thickness, ventricular dimensions, calculate ejection fraction and assess wall strain. The swine will be imaged before and after heart failure induction. The heart failure time-point will be determined from serial echocardiograms (when fractional shortening is decreased by two standard deviations (from 32 to 16%)). Several experimental MR techniques will be piloted that may improve MRI's ability to characterize heart failure.

2. Measure the quantity of Type I and Type III collagen in the myocardium of swine in heart failure as compared to controls using quantitative Western blotting from post-mortem tissue specimens. Rationale: It is well documented that pacing-induced tachycardia increases the percentage of fibrotic proteins in the extracellular matrix. Quantitative Western blots will be performed on tissue from the swine in Aim 1 and from control pigs to assess for a difference in collagen amount and type. Additional tissue samples will have histologic assessment for fibrotic changes.

3. Correlate the pattern of fibrosis in post-mortem heart failure histologic specimens with the tissue characterizing MRI techniques of T1 values, T2 values, and delayed myocardial enhancement (MDE). Rationale: By correlating the pattern of fibrosis in the heart failure tissue to the MRI results, providers can more confidently use MRI as a tool to help discern the degree and pattern of dilated cardiomyopathy (heart failure) an individual is experiencing. T1 and T2 values can quantify the tissue characteristics and mathematically find differences in tissue composition related to changes in myocardial remodeling. MDE can show areas of focal fibrotic replacement/scarring in the myocardium.

Upon completion of this study, we expect that MRI should be able to detect differences between baseline and heart failure induced myocardium in swine, quantifying the degree of loss of function and degree of fibrosis as a result of DCM. Histological evaluation and quantitative Western blot analysis of collagen types I and III should demonstrate an increase in fibrosis and change in collagen ratios in the myocardium from baseline to heart failure. This study will provide improved insight to the degree of cardiac remodeling that is induced from sustained tachycardia, allowing providers to more accurately assess the degree of DCM and potentially help reduce the risk of sudden death through appropriate therapies based on the individual's degree of heart failure. It is hoped that these improvements in the diagnosis and understanding of heart failure will optimize care plans for patients with DCM so that nurses can help maximize quality of life.

B. Background and Significance

Introduction

Unlike every other form of heart disease, the prevalence and incidence of heart failure is still rising, and heart failure has become one of the leading causes of morbidity and mortality in the United States (US) (1). Heart failure is a chronic, progressive condition that is commonly associated with sudden death at any stage of the condition (2-22). Heart failure is also associated with a wide range of co-morbidities making heart failure difficult and costly to manage. Heart failure is a burden on the health care system with an incidence of 10 per 1,000 persons over the age of 65 in the US (23,24). In addition, heart failure has one of the highest re-hospitalization rates, with 20-50% of all persons hospitalized with heart failure requiring readmission to the hospital within 3-6 months (25,26). The mortality rate of heart failure within the first year alone approaches 10% and the five year mortality rate is approximately 50%. Nearly half of these patients die suddenly, and the rest die of progressive pump failure (27). In 2007 the American Heart Association estimated that the direct and indirect burden of heart failure on the US economy has now risen to roughly $33.2 billion (1). This staggering amount of money is projected to worsen as the baby boomer generation reaches retirement age.

This growth in heart failure will impact nursing, especially in light of the nursing shortage in the United States. It is estimated that by 2020, there will be a shortage of approximately 340,000 nurses (28). Since heart failure is a chronic condition, often associated with other co-morbidities, it is feared that the quality of care and, consequently, quality of life of heart failure patients will be adversely impacted with the nursing shortage (29). To help overcome these potential problems, nurses are becoming more involved in translational health care research. The National Institute of Nursing Research supports the board NIH goal of encouraging more translational research (30). Through improved knowledge from healthcare research, registered nurses, nurse practitioners and nurse scientists will have better tools to aid in decision making, care plan assessments and areas for continued improvement. Heart failure is one area of research where more research is needed in order to improve patient care.

Heart failure, previously termed congestive heart failure, is defined by the ACC/AHA (1) as the impaired ability of the ventricle to fill or eject blood that may be induced through any structural or functional cause. It is classified as a clinical syndrome, defined by clinical symptoms from a history and physical, not from a quantitative laboratory value. Heart failure has many underlying manifestations which complicate its management. Heart failure may be acute or chronic, and be acquired or inherited. The general clinical manifestations of heart failure are fatigue, dyspnea, and fluid retention. These clinical symptoms may also be associated with exercise intolerance, pulmonary congestion and peripheral edema, but not all persons with heart failure present the same (1). There is great variability in symptomatic expression among heart failure patients and during the course of the condition for a single patient. For instance, some patients may feel exercise intolerance, but may not ever experience peripheral edema; whereas, another person may experience pulmonary congestion, peripheral edema, and exercise intolerance.

Heart failure can occur as a result of a variety of structural and/or functional conditions such as dilated cardiomyopathy, hypertension, valvular disease and left ventricular dysfunction. These specific entities can be evaluated and treated to help slow the progression of or reverse the symptoms of heart failure. It was once thought that heart failure was always a progressive condition, but studies using pacing-induced tachycardia in animals to produce heart failure have demonstrated reversible myocardial remodeling when the tachycardic pacing stimulus is removed (31,32), unless significant fibrosis has already infiltrated the myocardium (33). More recently, coronary bypass surgery has been found (in unusual cases) to reverse left ventricular cardiomyopathic remodeling in human patients (34,35). Due to the many underlying causes of heart failure and the many associated co-morbidities such as diabetes mellitus, chronic obstructive pulmonary disease and ischemic heart disease, heart failure continues to be a complex and multifaceted challenge for care providers and researchers to understand and manage.

The American College of Cardiology and the American Heart Association (ACC/AHA) publishes practice guidelines based on the current state of diagnostic and management practices. The ACC/AHA bases its recommendations on three classification levels and three evidence levels (1). The levels of evidence range from multiple randomized control trials and meta-analysis to simply current state of practice. There are many gaps in the understanding of heart failure since many of the ACC/AHA recommendations are not supported by evidenced based research findings.

However, the cardiology community is actively working to search for better ways to diagnose and manage heart failure, updating their practice guidelines approximately every five years. Clinicians and scientists are conducting clinical trials, basic research and retrospective reviews to investigate the mechanisms of heart failure, diagnostic options and treatment options for heart failure patients. One area of current research regards loop diuretics. Furosemide, a non-potassium sparing loop-diuretic, is the ACC/AHA current practice recommendation for the treatment of fluid overload in patients with heart failure (1,36). However, the Studies Of Left Ventricular Dysfunction (SOLVD), found that the use of diuretics was associated with an increased risk of progression of congestive heart failure and increased mortality from heart failure (37). The Randomized Aldactone Evaluation Study (RALES) has contributed to the debate surrounding furosemide (38). Loop-diuretic use is associated with prolonged activation of the rennin-angiotensin-aldosterone system (RAAS) and the RALES trial tested the hypothesis that spironolactone, a blocker of aldosterone, would reduce mortality. The RALES study found that aldosterone antagonists reduced mortality and hospitalization rates when it was added to the patient's standard care (which included a loop diuretic), demonstrating the adverse effects of prolonged RAAS activation by furosemide. Other researchers have suggested that chronically elevated levels of aldosterone due to the activation of the RAAS is detrimental but no previous randomized, blinded, controlled trials have been preformed to confirm the risk of chronic, elevated aldosterone (39,40). A recent animal study by McCurley et al, (2004)(41), found that furosemide-treated animals developed left ventricular heart failure faster than placebo treated animals, as well as significantly increased sodium-calcium exchanger (NCX) current and a significant depression in the in beta-adrenergic responsiveness of the NCX. The mechanism causing the increase in heart failure progression by furosemide, however, is still unknown, and so these changes must be studied further.

Determining the actual mechanisms influencing the development of heart failure is critical to the proper management of the patient. The current guidelines for the care and management of heart failure are outlined by the ACC/AHA (1). The initial clinical workup and diagnostic examinations are based on general standard of care practices. The goal of the diagnostic work ups is to classify the patient for management. The current guidelines recommend that myocardial structure and function be evaluated, differentiation of ischemic versus non-ischemic heart failure be made, and risk stratification for potential therapeutic options be made (1). All patients need to have a full history and physical, to include drug and alcohol consumption history. In addition, routine laboratory analyses should be performed that include complete blood count, urinalysis, serum electrolytes, blood urea nitrogen, creatinine, glycohemoglobin, lipid panel, liver function and thyroid stimulating hormone levels. The initial work up also generally includes a twelve-lead electrocardiogram (ECG) and chest x-ray. Echocardiography is a recommended diagnostic tool to help assess left ventricular (LV) size, wall thickness, and valvular function in patients suspected of heart failure. Radionuclide ventriculography can also be performed to assess LV ejection fraction (EF) and volumes. Coronary arteriography is reserved for those patients who are having significant angina or ischemia that would benefit from intervention.
Patients presenting with more advanced heart failure or with additional symptoms or co-morbities require further considerations so that the cause of heart failure can be more definitely classified and potentially corrected or at least managed optimally (42-46). For instance, hypertension is a common cause of heart failure that can usually be controlled with proper medication and lifestyle management. Valvular disease is another cause of heart failure that can usually be corrected surgically. The ACC/AHA guidelines for 2005 (1) suggest clinical, diagnostic and treatment protocols for each successive stage of heart failure. Non-invasive modalities such as treadmill stress tests, Holter monitoring, nuclear scintigraphy, electron beam computed tomography (EBCT), computerized tomography (CT), and magnetic resonance imaging (MRI) are useful diagnostic tools. Coronary arteriography is a common diagnostic tool for patients with unstable angina or non-ST-elevation myocardial infarction, but is an invasive test (47). Another invasive test is the cardiac biopsy (48-51). Cardiac biopsies are used to acquire tissue from the heart to monitor patients for cardiac allograft rejection, and to help diagnose inflammatory illnesses such as myocarditis, or infiltrative disorders such as cardiac amyloid and cardiac sarcoid (1,52,53). With the advent of more non-invasive tools such as CT and MRI, cardiac biopsies are being performed less frequently (54,55).

There are also other selected tests to assess for diseases such as amylodosis, sarcoid, rheumatic disease and infections such as human immunodeficiency virus. However, once a patient is diagnosed with heart failure, serial assessments need to be planned to manage the patient over time. But, again, the majority of these diagnostic recommendations are based on consensus opinions, case studies or just current standard of care, not from evidenced based clinical trials. Little of the underlying cellular changes have been

investigated in conjunction with advanced imaging tools such as MRI. More investigations that compare histologic findings to advanced imaging techniques will help clinicians better understand heart failure, its causes, mechanisms of progression, diagnostic tools, and treatment options so that each heart failure patient can be optimally managed.

Cardiovascular Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has been used for more than 25 years as a non-invasive tool in diagnostic radiology. Unlike plain film, x-ray fluoroscopy, invasive catheter angiography and computed tomography (CT), MRI is capable of obtaining images without the use of ionizing radiation (x-rays). MRI images are generated by placing the subject into a magnetic field and applying radio-frequency pulses. The response to the applied radio-frequency pulses (i.e. the radio-frequency signal emitted from the subject in response to the radio-frequency pulse) is processed by the MRI scanner into images or quantitative information that can be used to measure phenomenon such as blood flow. The manner and type of radio-frequency pulse which the MRI scanner applies is called a pulse sequence. A pulse sequence contains the computer instructions given to the MRI scanner for the application of the radio-frequency pulses. The resultant image contrast is highly dependent on the pulse sequence. In MRI, there is a large variety of pulse sequences which can be combined for an examination. The specific combination of pulse sequences depends on the pathology suspected and the organ of interest (56). For example, imaging the heart requires pulse sequences that use electrocardiogram triggered acquisitions to compensate for the motion from the heart beating, whereas imaging the brain does not since the brain is a stationary organ. Additionally, pathology such as ischemic disease in the brain require sequences such as diffusion weighted imaging (57), whereas the heart requires contrast enhanced perfusion and delayed enhancement imaging (58-60).

Although MRI has long been used for cardiac imaging, significant improvements in MRI scanners for cardiac specific pulse sequences have only been commercially available since the late 1990's. The gradient sets and computer processors that run the pulse sequences must be fast and powerful in order to acquire the image data fast enough to minimize motion from the beating heart (61,62) Over the past several years, many new techniques have evolved for assessing the heart. In 2005, the American College of Radiology set equipment and training standards for cardiac MRI, which included minimum field strength requirements, minimum gradient performance, minimum software requirements and minimum post-processing capabilities (63). Cardiac MRI is a complex and difficult examination that needs not only state-of-the-art MRI scanners, but also staff that is experienced with performing cardiac MRI studies. Although the clinical use of cardiac MRI is growing, reimbursement is still slow, so centers offering comprehensive cardiac MRI remain limited to academic centers (64). Further research is needed to help make cardiac MRI a generally available tool for routine clinical practice.

Cardiac MRI is a relatively new diagnostic method for the assessment of heart failure, but its value is starting to be appreciated (65). The main advantages of cardiac MRI are that it can provide information on myocardial morphology, function, blood flow, myocardial perfusion, and myocardial injury or infiltrative/fibrotic changes in a non-invasive examination during a single session (66). Cardiac MRI is the single modality that can offer a comprehensive set of tools to evaluate and monitor the heart failure patient.

There are a variety of cardiac MRI techniques. These techniques can be categorized by the signal appearance of blood ("bright blood" or "dark blood"). Cine steady state free precession (SSFP) "bright blood" images, provide structural and function images in any plane needed. Long axis, short axis, two-chamber, 3-chamber and 4-chambers sets of images can be acquired with high image quality using the cine SSFP technique. These techniques can assess for wall motion abnormalities, thickness of myocardium, volume size, ejection fraction, valvular disorders, and global function (67-75). SSFP sequences have become the work horse sequence for cardiac MRI, but these sequences are still usually acquired during a breath hold, one image location at a time.

"Black blood" images, such as double inversion recovery, are useful with and without the use of fat saturation techniques for assessment of anatomy and pathology (76-81). Black blood imaging serves as a way to assess the vessels walls such as the ascending aorta and also the right ventricle for pathologies such as arrythmogenic right ventricular dysplasia. These black blood techniques have long breath holds, making them difficult for some patients. Since dilated cardiomyopathy is a condition that tends to see

increases in fibrosis instead of fat, the black blood sequences have little to add that cannot be already obtained by the SSFP sequences.

Phase contrast (PC) images are used to assess flow velocities within vessels. MRI pulse sequences collect both amplitude and phase information. The sequence can apply a bi-polar velocity encoding gradient that will collect directional and flow information and moving spins (blood flow). The phase information collected from the use of the bi-polar gradient allows for the flow of moving spins to be calculated because of the relationship between flow velocity and signal intensity. PC can be used to calculate pulmonary to systemic (Qp/Qs) flow ratios in the case of left to right shunts (52,82-84). PC images are also used to assess for areas of hemodynamically significant stenoses, aortic coarctations, venous anomalies, atrial septal defects, the presence of anomalous pulmonary veins and other vascular abnormalities. PC images can help assess for ventricular velocities and valvular abnormalities (83,85-87), which may sometimes occur in dilated cardiomayopathy as the left ventricle dilates, but this is often a late feature of the condition or may be related to other cardiac conditions. Although PC can be used to help calculate cardiac volume and regurgitant volumes, PC is not suitable for calculating ejection fraction or ventricular wall motion problems that are associated with dilated cardiomyopathy.

T2 weighted fast spin echo (FSE) with fat saturation sequences are able to help assess for areas of infection and lesions that are high in water content. T2 FSE is a technique that is not widely used, although it is sometimes used for infarct imaging and a few other cardiac disease entities such as myocarditis, tumors and sarcoid (88-91). T2 FSE with fat saturation may be able to discern a difference in the left ventricular myocardium when compared before and after heart failure induction. If the dilated myocardium develops enough fibrosis in focal areas, this T2 FSE fat saturated technique may be able to show the acutely fibrotic areas or areas of inflammation. Lastly, the T2 FSE fat saturated sequence is an excellent set of images to compare to post contrast images to look for possible congruence by comparing the bight areas on the T2 images to any bright areas on the delayed enhancement images.

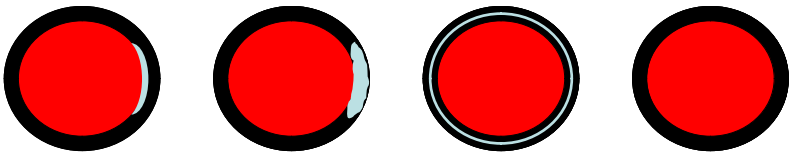
Measurement of wall thickness and movement during contraction and relaxation help to determine the functional aspects of the heart. Although cine SSFP can demonstrate a qualitative measure of the thickness and movement of the myocardium, a more quantitative technique, is needed. Tagging and wall strain sequences are two such techniques that can help quantify myocardium movement. Tagging is a cine technique that uses thin saturation planes to create a grid pattern across cine MR images that can be followed throughout the cardiac cycle (92-99). The grid lines appear hypointense and will displace with the contraction and relaxation of the myocardium (92,100). The current techniques for tagging use a spatial modulation of magnetization (SPAMM) tagging grid that is performed from end-diastole to end-systole (98). Strain analysis programs can be run on the tagging sequences to quantify the degree of strain in different zones. Unfortunately, the quantitative strain analysis for tagging is time consuming and difficult to perform, so it is rarely done.

Wall strain can be performed using another technique called mixed echo train acquisition displacement encoding with stimulated echoes (DENSE), which is still considered to be an investigational technique (101). DENSE produces color coded strain maps that can quantitatively map local strain through the use of eigenvalues. Thus, DENSE can show large or small displacement in the contracting and relaxing myocardium, with relatively fast post-processing time. This technique has not been validated yet, and would benefit greatly from being used in this animal experiment to help validate the technique. This sequence will be tested in this study to assess wall strain.

Assessing the myocardium for viability is an area on intense interest and one of the areas where cardiac MRI excels. Viability assessment requires the use of a contrast agent. There are currently five gadolinium (Gd)-chelate based agents commercially available. These paramagnetic contrast agents have a relaxivity that predominately causes a shortened T1, or longitudinal relation effect (102). Gd-chelate contrast agents shorten T1 relaxation of blood resulting in bright vascular signal on T1-weighted pulse sequences. Bright or positive enhancing agents have unpaired electrons that can be up to 18,000 times stronger than the hydrogen nucleus (103). At low concentrations, paramagnetic agents are positive enhancers, however, at high concentrations, paramagnetic agents can cause significant T2* shortening effects which can overpower the T1 contributions such that an actual decrease in vascular signal may be seen. Clinically, this T2* effect is most commonly seen as a result of a fast contrast injection rate (e.g. > 3 mL/sec), urinary concentration of Gd in the renal collecting system and bladder, or in the great vessels during the arterial phase imaging of a left antecubital venous injection (104).

The five Gd-chelate contrast agents currently approved for commercial use in the US are broadly classed as extracellular contrast agents (Gadopentate dimeglumine, Gd-DTPA (Magnevist); Gadoteridol (ProHance); Gadodiamide (Omniscan); Gadoversetamide (OptiMARK); and Gadobenate Dimeglumine, Gd-BOPTA (MultiHance)) (105). Since none of these agents are currently approved for cardiac use, the use of Gd-chelates in cardiac MRI is considered an off-label use. However, the use of Gd-chelates is considered to be standard practice in cardiac MRI. These gadolinium based agents are also called "first pass" or "non-specific" agents and are relatively small in size. They have relatively short intravascular duration secondary to their quick extravascular distribution into adjacent background tissues and urinary excretion within minutes of their intravenous administration. Of these five agents, Gadobenate Dimeglumine, Gd-BOPTA has a weak interaction with serum proteins in vivo (106). Clinical evaluations comparing Gd-BOPTA to other traditional Gd-chelate contrast agents (e.g. Gd-DTPA) have demonstrated Gd-BOPTA to have increased T1 relaxivity (signal enhancement) and a prolonged duration for vascular enhancement—qualities ideal for CE MRA (106-108).

The properties of these Gd-chelates can be exploited for cardiac MRI. The Gd-chelates can be used for first-pass perfusion of the myocardium, and they can also be used for delayed enhancement evaluation. Starting with the former, Gd-chelate perfusion MRI can be performed at rest, or with stress. Pharmacological stress using adenosine is the most commonly performed cardiac MRI perfusion analysis procedure (109). The patient is stressed with a two-minute adenosine infusion at 140 ug/kg/min and then a short axis fast steady state gradient echo cine sequence is acquired in a multi-phase fashion for over one minute during a dynamic injection of 1 mmol/kg Gd-chelate, infused at 3 to 5 mL/sec. Perfusion defects will appear as dark areas within the normally enhancing myocardium because areas where the myocardium is damaged will take up the contrast agent more slowly. At approximately 8 to 10 minutes after the Gd-chelate injection, a higher resolution, using a segmented inversion recovery technique is then acquired in two planes, usually the short axis and the long axis of the heart. This technique of waiting a few minutes after the Gd-chelate injection is generally referred to as a delayed myocardial enhancement (MDE) technique. Hyperintense areas will appear in the myocardium where necrosis, scar, fibrosis, or infections occur because they retain the contrast agent longer than healthy myocardium (91,108,110-130). The combination of perfusion imaging and MDE can help to evaluate which areas of the myocardium are still viable. Hyperenhancement patterns can be found with different types of pathology. Ischemic heart disease typically manifests as a subendocardial region of hyperenhancement, which if extensive, may become transmural, but it is specific to vascular distribution (Figure 1) (113).



A. Subendocardial B. Transmural C. Midwall D. No hyper-enhancement

athology. Ischemic heart disease is commonly associated with subendocardial (A) and transmural (B) types of enhancement patterns. Mid-wall or central enhancement (C) is often seen in late stages of heart failure or dilated cardiomyopathy. No hyper-enhancement (D) is seen in both healthy tissue and diffuse fibrosis.

Dilated cardiomyopathy tends to have either a normal enhancement pattern (e.g. no delayed hyper-enhancement), or a circumferential mid-wall enhancement pattern (Figure 1)(99,111,113) These findings with MDE seem to be consistent with ischemic versus non-ischemic patterns of heart failure in that the ischemic heart failure is associated with coronary artery disease territories, where as dilated cardiomyopathy is thought of as a more diffuse or global pathology.

What is still unclear is why some patients with non-ischemic dilated cardiomyopathy have a negative MDE whereas others have the mid-wall enhancement. Having a negative MDE finding for the dilated cardiomyopathy cases in which fibrosis is diffuse rather than centralized along the mid-wall suggests that the technique is most likely the problem with the detection of the fibrosis. The MDE technique is based on using an inversion pulse that causes the enhancing myocardium to become dark or nulled. The inversion pulse

allows areas of focal scar to become hyperintense (bright) as compared to the rest of the myocardium. Pervious studies using MDE to assess patients with dilated cardiomyopathy have all found that the diffuse fibrosis is not detected with MDE (99,113,116).

The finding of hyper-enhancement within the wall of the myocardium for patients with dilated cardiomyopathy can be significant and have an impact on the course of their care. A study by Nazarian, Bleumke, and Lardo, et al (2005)(114), found that those patients with 26% to 75% thickness of mid-wall enhancement (scar) were at significantly greater risk of inducible ventricular tachycardia (VT). Although this is only one study, it is consistent with the coronary artery disease study by Bello, Fieno, and Kim , et al (2005)(131), that found infarct size based on MDE cardiac MRI measurements to be a stronger predictor of inducibility of VT than left ventricular ejection fraction. Non-sustained VT is associated with an increased risk of mortality from sudden death (15). In addition, it is generally accepted that persons with dilated cardiomyopathy are at risk for sudden death (21).

There are no clear predictors for risk of sudden death in heart failure and few management protocols and devices that can significantly reduce the risk of sudden cardiac death from dilated cardiomyopathy. Most patients are still managed individually. Modest improvements in risk reduction have been made by the use of implanted cardiac defibrillators (ICD) in Trials such as the Marburg Cardiomyopathy Study (MACAS) and the Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT) (6,31). The Defibrillators in Nonischemic Cardiomyopathy Treatment Evaluation (DEFINITE) found a 5.7% decrease in mortality (a 35% risk reduction) though ICD use which was not statistically significant in this trial, but was similar to results in the MACAS and SCD-HeFT trials (6,31,132). Approximately 30% of persons with dilated cardiomyopathy are expected to die from sudden cardiac death. The improvement in risk reduction though these ICD trials has prompted the ACC, AHA, and European Society of Cardiology (ESC) 2006 guidelines to help prevent sudden cardiac death recommend that an ICD be implanted in persons with dilated cardiomyopathy who have sustained VT and an expected survival of at least one quality year (133).

The Animal Model

Heart failure is a chronic condition that has used animals to increase our understanding of the pathophysiology of the condition and the success or failure of treatment regiments. Although humans are common research subject for heart failure, sometimes research can be justified to be done on animals, particularly when pathologic specimens are needed. For instance, biopsies are difficult and dangerous to perform making histochemical comparisons rare in human heart research. Most human histochemical comparisons are done on post-mortem subjects. Studying early to mid ranges of heart failure can benefit from the use of animals as the progression of the disease can be more closely followed and systematically studied so that improvements in the understanding of these pathologic conditions can be translated into improved treatment and quality of life. The gaps in our knowledge of heart failure are too great to be able to use computer simulations to study heart failure, so use of animal models is required at this time.

Heart failure can be induced through animal modeling through a number of different mechanisms, depending upon the type of heart failure the researcher wishes to try to simulate. No model can perfectly reflect human physiology and response to disease, but there are certain questions in research that cannot be studied ethically using human models. The pig model fairly closely simulates the human heart which is why the pig is such a popular model for studying heart disease. The pig is a large animal model that more closely matches the heart and circulatory system of humans than do rats, mice or dogs (134-137). Chronic tachycardia is well known to produce heart failure in humans in the form of dilated cardiomyopathy. Rapid pacing is a well known technique for inducing dilated cardiomyopathy in animal models (Power and Tonkin, 1999). Heart failure can also be induced in an animal model by causing ischemic areas in the heart through a number of techniques such as coronary ligation, timed balloon occlusion, coronary embolization, thrombus generation and chronic hypoxia (136,138-144). Unfortunately, ischemic models produce uneven pathology in the myocardium; some areas will be necrotic, with varying degrees of fibrosis and scarring, and some areas will be normal. This may make systematic study of molecular changes in the myocardium problematic to study, or at the very least, more complicated to study. The pacing model produces a fairly uniform disease model. The myocardium will have a more uniform pattern of fibrosis occurring (136,137,145-152). Since this study wishes to assess the changes in the cells and fibrosis that are occurring with heart failure, the pacing model is the more logical choice for consistency in the model.

Laboratory Analysis

Until very recently, the predominant opinion in cardiology was that the heart was terminally differentiated, and has little, if any, ability to regenerate cardiac myocytes when they become damaged. If cardiac myocytes do not have the ability to replace themselves, then cardiac myocyte loss must be extremely slow as these cells must last the organism's lifetime (153). It is now believed that the myocytes in the heart are not static; that they actually do have some ability to renew themselves to help maintain heart homeostasis. The human body is constantly trying to maintain homeostasis so that it can survive. In recent years, cardiac remodeling has become an area of increased research. Pacing the heart in animals will cause dilated cardiomyopathy to develop through the remodeling process (154-164). Several studies have demonstrated that the myocardium can remodel itself to account for the increased assault on the cardiac myocytes during tachycardia (32,152,162,165).

The remodeling process is complex and not entirely understood. The composition of the myocardium is approximately 85% myocytes (166). The matrix surrounding the cardiomyocytes is a complex, highly ordered array of supportive material called the extracellular matrix (ECM). The ECM consists of a complex network of proteins that provide a fibrous network with biologically active molecules that work in conjunction with the myocytes to provide the overall structure and function of the heart (167). The main fibrous materials supporting the functioning of the left ventricle are fibrillar collagens type I and type III (167). Collagen is a triple helical protein structure that can have very strong tensile strength through its characteristic cross-striations with neighboring fibrils every 67 nm (168) Type 1 collagen (molecular formula $[\alpha1(I)_2\alpha2\ (I)\ )$ has exceptionally high tensile strength, thus it has been associated with myocardial stiffness (169,170). Type III collagen (molecular formula $[\alpha1(III)_3\ )$ is much thinner than type I collagen and has less tensile strength (171). Collagen has been found to increase in patients with ischemic heart failure, with type III collagen being the primary collagen that increases (172), lowering the type I to type III ratio in the myocardium. Mukherjee and Sen (1991) suggest that the increase in type III collagen and the lowering of the type I:III may play a role in myocardial dysfunction, but the features of the relationship between type I and type III collagen and how this effects myocardial function are not completely understood.

Both mechanical and chemical stimuli can change the compositional and structural components in the myocardium depending on the type of insult (173-177). This remodeling process with overloading types of insults cause the myocytes to increase in size and the extracellular matrix to expand in order to compensate with the increased demand (178,179). It is also well known that the assault on the heart from rapid pacing causes remodeling to occur, changing the molecular and cellular composition of the myocardium, but the exact changes will most likely be somewhat different from the overloading model. More research is still needed to identify the quality and quantity of the ECM changes from a rapid heartbeat (180). For example, in a study of collagen content in transgenic rats and angiotension-converting enzyme induction, the ECM of the myocardium was found to have an increase of collagen content in heart failure rats (181). Similarly in paced canine, collagen volume increased significantly after several weeks of rapid pacing as compared to sham dogs with the midwall and epicardium exhibiting the greatest accumulation of collagen fibers (Weber 1990). However, the ECM is a complex network of proteins and proteoglycans. The mechanisms and features of the remodeling in the myocardium from rapid pacing are still uncertain (182,183).

In this study, pig hearts will be paced at 200 bpm to induce heart failure through myocardial remodeling. Specifically, the magnitude of ECM is expected to increase. It is estimated from previous work done by McCurley and colleagues (2004)(184), using a paced-pig model, that the pacing in this studying is expected to induce the ECM to increase the overall quantity of fibrotic material within the myocardium by approximately 6 percent. This change in ECM composition in the myocardium will be assessed in two methods. In order to quantify some of the specific fibrotic proteins in the myocardium, Western Blot analysis will be performed. This will be compared to histochemical analysis of tissue samples to look for correlation of the laboratory analyses. This combination approach has been used by many other researchers on heart failure (181,185-187). For instance, in a previous study by Pokharel, et al, 2004,(181) that studied the global insult of increased angiotension-converting enzyme on transgenic rats, the Western analyses were able to confirm the increases in type I and type III collagen as compared to left ventricle collagen volume change in the transgenic rats as compared to control rats. In a study on rabbits that looked at an ischemic model of heart failure, Western Blot analyses and histochemical comparisons were performed that supported the change in matrix metalloproteinases (MMP) that occur in the ECM after an ischemic assault (186).

In order to maximize the potential information from the tissue samples in this study, additional tissue samples will be collected and frozen for analysis later of possible progenitor or satellite cells in the myocardium. In the last ten years, stem cell and progenitor cell markers have been discovered in the myocardium (188-195). Recent discoveries of various cell markers in the myocardium suggest that satellite cells or some other form of myocyte progenitors do exist within the myocardium (196). Satellite cells are the progenitors of myocytes and some investigators refer to them as stem cell like since they are essential to postnatal muscle growth and repair in skeletal muscle (197-199). Satellite cells are essentially quiescent until called upon in response to injury (200-202). In this pacing model, the myocytes are not killed quickly like they are in an ischemic model. Instead, the assault is steadier and may in theory be stimulating the cardiac myofirils to signal to the matrix that new cells are needed to offset the assault the cells are experiencing. It is definitely known that the ECM is stimulated to produce fibroblasts. The ECM may play also a part in the potential of the cardiac myocytes to regenerate since the matrix contains a vast array of structural and molecular substrates for the cardiomyocytes. This is unknown. The presence of progenitor markers suggests that satellite cells or a similar type of cell exists within the myocardium, but the relevance of these findings to the treatment of heart failure is hotly debated. The evidence based on progenitor markers and the knowledge we have of skeletal myocytes strongly suggest that more research will eventually lead to the finding of cells that replenish cardiac myocytes. For these reasons, it is important to continue to look for the cells that may be responsible for stimulating myocyte growth in the myocardium.

Innovation.

Although MRI has been used to assess heart failure patients clinically, few studies exist that have studied dilated cardiomyopathy using non-invasive and tissue comparisons in a pacing animal model. Scientists have studied pacing-induced heart failure in swine using echocardiography and western blot analyses to measure connective tissue growth factors that occur with myocardial remodeling (150,161). However, studying paced-swine in the MRI environment is uncommon since pacemakers have been found to be risky in MRI scanners (203,204) A couple of groups have investigated spin-tagging techniques in paced dogs to assess left ventricular wall strain (205,206). Another group has studied contractile dysfunction and MDE in minipigs using a pacing technique, but they compared their MRI findings to Positron Emission Tomography, not to histology (207). Ischemic studies assessing MRI and Western Blot comparisons have been done (208) , but the ischemic type of heart failure is heterogeneous, with large areas of focal scar. The paced model of heart failure is more uniform in nature and thus, may yield different data than can be acquired from ischemic models. Additionally, looking specifically at collagen types I and III of the ECM and then correlating the heart failure collagen finding with the MRI imaging techniques has not been done. If MRI techniques can accurately measure the degree of fibrosis in dilated cardiomyopathy, then MRI can be used as a non-invasive tool to help manage heart failure patients and may discern those patients at greater risk for sudden cardiac death as well as mode of therapy or device support. This ultimately should allow nurses to improve upon the care plans for patients with dilated cardiomyopathy. Being able to assess a patient's progress with a non-invasive study allows for more frequent and accurate assessment of the physiologic state of the patient so that plans for assisting patients with maintaining optimal functioning and quality of life can be designed for them by their nursing case managers.

In summary, heart failure is a chronic condition that costs the US healthcare system billions of dollars a year (1), in addition to degrading patient quality of life. This study will explore the ability of MRI with existing and experimental techniques to define the dilated cardiomyopathy phenotype. In addition, histological evaluation and quantitative Western blot analysis of collagen types I and III should demonstrate an increase in fibrosis and change in collagen ratios in the myocardium between baseline and heart failure.

This study should be able to measure the functional loss of myocardium detected by MRI that can be correlated with histological and western blot analysis of the myocardium. MRI has the potential to provide improved insight to the degree of heart failure with a non-invasive procedure, allowing providers to more accurately assess the degree of DCM and potentially help reduce the risk of sudden death through appropriate therapies based on the individual's degree of heart failure. This study will allow for treatment and prevention approaches specific DCM rather than global treatments, thus improving case management of the heart failure patient.

C. Preliminary data

For the MRI imaging, phantom studies were performed using a smoked ham to simulate a pig. The proposed MRI sequences were performed using the ham to establish an imaging protocol for the study. In addition, a single lead pacemaker was inserted into the ham to simulate the conditions the pigs will be scanned under while in heart failure. This is important from both a safety standpoint for the pigs and for imaging quality reasons since metal can deflect MRI signals causing degradation of the image data. The pacemaker and lead were not attracted to the magnetic field of the scanner. It was noted that the pacemaker lead produced only a small (5 mm) localized deflection on the images. However, the pacemaker caused considerable deflection when it was within 10 cm of the imaging area of interest. Moving the pacemaker to a distance of at least 15 cm from the region of interest eliminated the deflection artifacts from the images. This test did not measure inducible current in the pacemaker lead, which is another potential safety concern. Recent research on pacemakers and implanted defibrillators has demonstrated that pacemakers manufactured within the past 5 years have been safely scanned in humans (209-212) . The pacemakers in this study will be turned off prior to the MRI scanning, so the safety and well fare of the pigs with the implanted pacemakers during MRI scanning should be relatively safe.

D. Research Design and Methods

Overview

The proposed methods for this study are based on an existing heart failure protocol being conducted by Dr. Mark Haigney at the Uniformed Services University of the Health Sciences (USUHS). This study is using Yorkshire pigs to study the effects of a diuretic, furosemide, on heart failure progression using a pig model. Heart failure will be induced using tachycardic pacing, which induces dilated cardiomyopathy in four to six weeks. After a baseline physical exam, blood work and imaging (echocardiogram and MRI), the animals will have single-lead pacemakers inserted into the right ventricle. The pigs will have weekly blood draws and echocardiograms to monitor their heart failure progression. The second MRI will be performed shortly before they are euthanized.

After the completion of the imaging the Yorkshire pigs will be euthanized according to a standard IACUC approved protocol with pentobarbital sodium followed by KCl. The hearts will be surgically extracted for pathologic investigation. Ventricular dimensions will be acquired from base to apex and in anterior to posterior dimensions. Care will be taken not to include the papillary muscles in the calculations. The thickness of the ventricular wall will be measured.

Tissue samples will be taken from the left ventricular free wall and septum for tissue analysis for each of the heart failure pigs. The samples will be frozen in liquid nitrogen for the Western blot analyses. Another set of samples will be fixed for histological sectioning. In addition, an equal number of samples will be taken from control pigs from non-heart failure Yorkshire pigs of similar age from other training protocols to serve as controls for the histopathology comparisons. The tissue will be analyzed for percent fibrosis (histology) and for collagen Type I and Type III content (western Blot).

Conceptual Model

The model for this study is based on scientific methodology that has its roots in empiricism. Many of the current recommendations for care of the heart failure patient from the American College of Cardiology and the American Heart Association (ACC/AHA) are simply based on current practice standards, not systematic trials. Heart failure needs to have more systematic research performed in order to better understand the mechanisms of heart failure and the tools used to diagnose heart failure.

This proposed study will be a quasi-experimental mixed-method study with two time points for each animal for the MRI. This study will not meet the strict demands of being a randomized, double blinded, trial. Assessing pigs through the use of MRI for DCM with pacemakers implanted has not been done before, thus it is unknown how successful the imaging will be. The other rationale for a quasi-experimental study is for the welfare of the pigs. It is prudent to start with a small trial to gain preliminary data before moving to a larger study with more animals. None the less, the methods in this study will be consistently applied to all of the animals in the study to ensure as controlled a study as possible. In addition to imaging the pigs with MRI,

histologic specimens will be collected and compared to the findings on the MR images. Statistical analyses will be performed to power the study and to look for correlations between the imaging results and the laboratory findings. This study is not trying to investigate any treatment; it is trying to assess the ability of MRI to accurately depict the extent of fibrosis, myocyte loss, and remodeling in dilated cardiomyopathy. The knowledge gained from this study could lead to a larger, randomized, controlled study that tests techniques or treatments in a similar model.

Experimental Design and Methods

Participant Selection

Working with animals in research is considered a privilege and the utmost care of the animals must be rigorously maintained. The Uniformed Services University of the Health Sciences (USUHS) has an Institutional Animal Care and Use Committee (IACUC) that oversees the animal research at USUHS. All Federal laws, regulations and policies pertaining to animal research must be followed in any animal research study at USUHS. The rationale and justification for animal research in the proposed heart failure study will be based on the USUHS IACUC policies and guidance (213). All members of the proposed animal research study will be required to pass the IACUC research training course and the swine specific research course. A veterinarian will be consulted for to help with the care of the animals used in a heart failure protocol. In addition, the Anesthesia Department at USUHS will be consulting and assisting in the conduction of the research in order to safely comply with the animal care requirements needed for the research.

For this study, Yorkshire pigs will be the study participants. Pacemakers will be implanted into Yorkshire pigs and then paced for 3 to 6 weeks of pacing at 200 beat per minute, in which time heart failure in the form of dilated cardiomyopathy will be produced (41). Weekly cardiac ultrasound recordings will be made until fractional shortening is decreased by two standard deviations (from 32 to 16%). Rapid pacing causes several responses similar to human heart failure such as elevated neurohumoral activation, blunted calcium transients, and action potential prolongation. By using the rapid pacing model, homogeneous, global dysfunction can be achieved. This phenotype is typical of dilated rather than ischemic cardiomyopathy where there are local and region variances depending upon the coronary artery that was occluded. The pigs will be assessed with MRI at baseline, and then again just before sacrifice when the pigs will be in heart failure. A potential pitfall will be that the pigs will have their heart failure scans at different time end points due to inter-individual variability in the speed of heart failure progression. However, the proposal requires that all pigs be in an equivalent state of severe systolic failure at sacrifice. An alternative would be to sacrifice the pigs at the same end-point, but then some of the animals may either not be in heart failure or not survive to the time point (Dr. Mark Haigney, personal communication, 2007).

There are many animals that have been used to study heart failure such as mice, rats, rabbits, goats, dogs and pigs. The IACUC stresses that the species selected should be the least sentient that can provide the information needed. Rabbits, rodents and non-mammalians are more difficult and problematic to translate findings to humans in heart research. In addition, rabbits, rodents and non-mammalian animals used in research are smaller than animals such as pigs and goats, which would necessitate the use of a higher field MRI scanner than is currently available on the National Naval Medical Center campus. Pigs and goats fall into the next possible level of animals for ethical consideration. Goats have been used in heart research, but they are not often used due to their large size and expense. Pigs have been used extensively in heart research (214-216) and the pig heart is remarkably similar to the human heart (134). Pigs and goats have actually been considered to be a better heart model than dogs since dogs have an extensive collateral circulation (136). For this proposed heart research using MRI, pigs make the best justified model since they make a relatively good comparator to humans, they are lower than dogs on the IACUC sentient list, the USUHS Laboratory of Animal Medicine is accustomed to caring for pigs, and there is an existing heart failure study using pigs that wishes to collaborate on this MRI research.

Inclusion/Exclusion Criteria

The following are the inclusion and exclusion criteria:

Inclusion Criteria into the study groups are: initially healthy, male Yorkshire pigs at baseline assessment.

Exclusion Criteria from the study group are: Yorkshire pigs that become too ill after induction of heart failure to be safely transported to MRI for scanning.

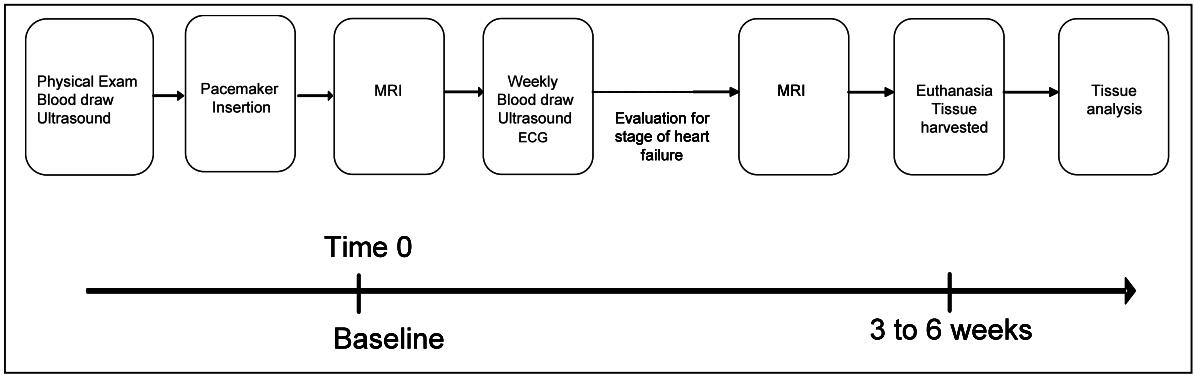


Figure 2. Flow design and time line of for the animals as they progress to heart failure. Animals are expected to vary in time it takes to reach a decrease of two standard deviations in fractional shortening of the functioning of the myocardium.

Setting

The subjects will be housed at the USUHS Laboratory of Animal Medicine (LAM). All testing will be performed in the USUHS LAM facility except for the MRIs. The MRIs will be performed at the National Naval Medical Center (NNMC) MRI Clinic on the research MRI Scanner. NNMC is on the same campus as USUHS, so the transportation of the animals will be minimal. NNMC recognizes the USUHS IACUC.

Participant Burden and Attrition

After a baseline physical exam, blood work and imaging echocardiogram, the animals will have single-lead pacemakers inserted into the right ventricle. After a recovery period, the animals will have a baseline MRI performed. Then the swine will be paced for approximately 3 to 6 weeks of pacing at 200 beat per minute, in which time heart failure in the form of dilated cardiomyopathy will be induced. The pigs will have weekly blood draws, echocardiograms (Echo's) and electrocardiograms (ECG's) to monitor their heart failure progression. The second MRI will be performed shortly before they are euthanized. There is a chance that a pig may die or become too ill before the second MRI is performed. For this reason a 10% attrition rate is planned into the sample size determination. After the completion of the imaging the Yorkshire pigs will be euthanized according to a standard IACUC approved protocol with pentobarbital sodium followed by KCl. The hearts will be surgically extracted for pathologic investigation.

Determination of Sample Size

Sample size for the MRI study was determined based on three criteria: a) alpha level of 0.05 (two-tailed tests); b) power of 0.80 (ß = 0.2) according to standard statistical procedures; and c) review of the literature. A power analysis was performed using nQuery Advisor software with the assistance of Cara Olsen in the USUHS Biostatistics Consulting Center. The specific power analyses for each aim are listed below under each aim. The overall power analysis yielded a sample size of 8. To account for attrition (20%), we have increased our total number of pigs to 10.

Experimental Procedures

Aim 1. Measure the difference in cardiac function between baseline and tachycardic pacing-induced heart failure in swine using MRI.

MRI

MRI was selected as the method to evaluate the myocardium because of MRI's ability to differentiate between tissues that have different relaxation times, and thus can detect the inherent tissue differences in various components such as water content. Magnetic resonance imaging uses a powerful magnetic field to align protons in a patient's body along the direction of the main magnetic field. A radiofrequency pulse that is tuned to specifically match the resonant frequency of the protons in a person's body is then sent to tip the spinning protons into the xy plane where a sensitive radio receiver can detect the signal produced. A sophisticated computer with mathematical calculations can convert the signals into images, a spectrum of the metabolite species in the tissue or other quantifiable physiologic information. The main factors that are observed in MRI experiments are T1 (longitudinal) and T2 (transverse) relaxation rates. Different tissues have different relaxation rates which are why MRI can provide contrast between structures, help define composition of tissues and characterize pathology.

The pigs will be sedated with ketamine (intramuscular) and have a catheter placed in an ear vein. Anesthesia with be induced via intravenous (IV) propofol, the pigs will then be intubated, and anesthesia maintained with an IV infusion of propofol for the duration of the MRI procedure. The pigs will be allowed to spontaneously breathe via a Mapleson Anesthesia Circuit attached to an oxygen tank. The pigs will be carefully monitored and kept warm during transportation to and from the laboratory and while in the MRI suite.

MRI Scanning

MRI scanning will be performed on a 1.5 Tesla superconducting scanner (General Electric Health Care, Waukasha, WI) with a phased-array send and receive coil. A total of 9 Yorkshire pigs will be positioned supine and feet first, with padding and blankets placed to help keep the animal comfortable. The MRI scanning protocol will be the same for all subjects. The pigs will be sedated prior to transportation to the MRI clinic. The animals will be continuously monitored while under sedation with the assistance of anesthesia support from USUHS Anesthesia Department and the Anesthesia support staff from the USUHS Veterinary Surgical Division of the Laboratory of Animal Medicine. MRI monitoring equipment and medication infusion pumps will be available and used to ensure the welfare of the pig while in the MRI scanner.

Sequences

The MRIs will be performed in the NNMC MRI Clinic, 1.5 T General Electric MR Scanner, v 14x. Localizers will be performed first in all subjects. The sequences that will be performed are outlined in Appendix A.

Post Processing of MRI Data

Post processing will be performed on MR workstations with advanced software for post-processing. Ejection fraction, left ventricular wall thickness, left ventricular volumes and strain maps will be calculated.

Outcome Measures

Dependent variables include:

Ejection fraction
Left ventricular wall thickness
Left Ventricular wall strain

Data Analysis

This aim is designed with the pigs being scanned twice, once before pacing and once after they are in severe heart failure. Since the pigs will be used as their own controls, dependent t-tests will be performed for each outcome variable. If deemed necessary because of the small sample size, Bonferroni corrections may need to be performed on these t-tests as well. The computer package SPSS will be used for most statistical analyses.

For wall thickness, a sample size of eight (8) pigs per group will have an 80% power to detect a difference in means of 0.405, assuming a standard deviation of 0.350 based on a paired t-test with a 5%

two-sided significance level, as well as with a conservative Bonferroni correction at a significance level of 0.017) (Table 1). For ejection Fraction, a sample size of eight (8) pigs per group will have an 80% power to detect a difference in means of 14.50, assuming a standard deviation of 12.5 based on a paired t-test with a 5% two-sided significance level, and 80% power to detect a difference in means of 18.129 assuming a standard deviation of 12.500 at a conservative Bonferroni correction at a significance level of 0.017). For wall strain, a sample size of eight (8) pigs per group will have an 80% power to detect a difference in means of 0.162, assuming a standard deviation of .140 based on a paired t-test with a 5% two-sided significance level, and 80% power to detect a difference in means of .203 assuming a standard deviation of .140 at a conservative Bonferroni correction at a significance level of 0.017). (nQuery Advisor Software).

	Wall Thickness (mm)		Ejection Fraction (%)		Wall Strain (λ^1)	
Sig level	.05	.017	.5	.017	.05	.017
Mean diff	.405	.35	14.450	18.129	0.162	0.203
Effect size	1.156	1.450	1.156	1.450	1.156	1.450
n	8	8	8	8	8	8

Table 1. Power analysis table based on a study by Ahmed, von Lueder, Kjekshus, & Attramadal, 2005(150) and Marcus, Gotte, Van Rossum, et al, 1997 (98). Based on this table, 8 pigs is sufficient to detect sizes of clinical significance.

Aim 2. Measure the change in quantity of Type I and Type III collagen in the myocardium between swine in heart failure as compared to controls using quantitative Western blotting from tissue specimens.

Tissue from the left ventricle in at least two places will be obtained from the excised heart from the pigs at sacrifice from Aim 1. Tissue samples will be taken from the left ventricular free wall and septum for microscopic analysis for each of the heart failure pigs. In addition, an equal number of samples will be taken from control pigs from non-heart failure Yorkshire pigs of similar age to serve as controls for the histopathology comparisons. Two sets of samples from each site will be collected. Each site will have tissue that is flash frozen in liquid nitrogen for the Western Blot experiments and tissue that will fixed in a 10% neutral buffered formalin solution for histologic preparation.

Western blot methods:

Sections from each layer of the heart will be immediately frozen in liquid nitrogen at the time of sacrifice and stored at -80 C. The frozen tissue will be used for Western electroblotting. The frozen tissue samples will be pulverized with a mortar and pestle and then homogenized in immunoprecipitation buffer. The tissue will be prepared for protein size separation using a sodium dodecyl sulfate (SDS) – polyacrylamide gel electrophoresis (PAGE) technique. This technique can resolve different sizes of the extracellular matrix proteins through their charge, molecular size, and abundance. The separated proteins will be blotted to an inert membrane for visualization after staining. The blots will be quantified for the amount of collagen types I and III present through density detection with digitization and software (NIH-image).

Detailed protocols are in Appendix B.

Histological methods:

For histological analysis, transmural sections will be obtained from two areas of the left ventricle, one in the mid septal region and one in the lateral left ventricular wall. The tissue fragments will be fixed in 10% neutral buffered formalin, embedded in paraffin, and then sectioned into 5µm-thick sections. Sections will be mounted onto glass slides and stained with hematoxylin and eosin stain (H & E), and also with a collagen specific stain Masson's Trichrome. The Masson's Trichrome should help further differentiate the collagen from the myocytes as this stain depicts the collagen as blue and the muscle cells as pink (217,218) . Volume fraction of fibrosis will be determined by quantitative morphometry, using a camera hooked to a light microscope to capture the images. The technique involves dividing the magnified area into quadrants and then randomly selecting four fields from each quadrant from each of the three layers of the myocardium (epicardiaum, midmyocardium and endocardium). The connective tissue will be selected

and traced for each field, which will then be analyzed with computer software to calculate the area fraction of collagen and muscle. Blood vessels and perivascular interstitial cells will be excluded from the connective tissue.

Outcome Measures

Dependent variables include:

Percent extracellular matrix fibrosis
Quantity of Collagen Type I
Quantity of Collagen Type III

Data Analysis

The tissue samples will be compared between control pigs and the heart failure pigs. Two samples will be taken from each pig, one in the septal region of the LV and one from the lateral wall of the LV. The tissue samples will be further separated by layer of the myocardium, epicardium, mid myocardium and endocardium. This data will be compared both between groups and within groups. T-tests will be performed to look for changes between the two different areas of the heart for each animal. If there is no significant difference, the data from the two areas of each pig will be pooled. Pooling should essentially double the number of samples per pig, increasing the statistical power.

T-tests will be done to compare the control animal tissue to the heart failure tissue for each of the three tissue layers. The histology samples measuring percent fibrosis will be analyzed in one set of analyses. The quantitative Western Blot samples will be analyzed statistically as two separate independent variables, collagen I and collagen III. All of the tissue samples will have the same sort of statistical comparisons performed. The samples will be tested for homogeneity of variances. If met, Multivariate Analysis of Variance (MANOVA) will be performed to look for changes between the three tissue layers with a Hotellings T-squared test since there are two groups. If the homogeneity of variances is not met Box M tests will be used instead. The computer package S PSS will be used for most statistical analyses.

For percent fibrosis, a sample size of eight (8) pigs per group will have an 80% power to detect an effect size of 1.507 using a two group t-test with a 0.05 two-sided significance level. Based on fibrosis studies by Ohtani, Yutani, Nagata, et al, (1995)(219) and Batlle, Perez-Villa, Garcia-Pras, et al, (2007)(220), the effect size for this study is expected to be at least 1.5.

For Type I collagen, a sample size of 7 in each group will have 80% power to detect a difference in means of 95.5 (the difference between a group 1 mean of 195.5 and a group 2 mean of 100.0) assuming the group 1 standard deviation is 64.0 and group 2 standard deviation is 35.5, using a two group Satterthwaite t-test with a 0.050 two-sided significance level. For Type III collagen, a sample size of 10 in each group will have 80% power to detect a difference in means of 76.9 (the difference between a group 1 mean of 176.9 and a group 2 mean of 100.0) assuming the group 1 standard deviation is 57.0 and group 2 standard deviation is 53.0, using a two group Satterthwaite t-test with a 0.050 two-sided significance level. The collagen data is from Seeland and colleagues, (2002)(221), observed effect sizes for Type I collagen as 0.80 and effect size for Type III collagen as 0.84.

Aim 3. Correlate the pattern of fibrosis in post-mortem heart failure histologic specimens with the tissue characterizing MRI techniques of delayed myocardial enhancement (MDE), T1 values and T2 values.

The relationship between histology and MRI findings is critical if non-invasive studies such as MRI are to be used for the diagnosis and follow-up surveillance of persons with heart conditions. In ischemic models of heart disease, MRI has had a strong correlation between the histologic sections demonstrating myocyte loss and with hyperenhancement patterns on MRI (222). The cellular mechanism for MDE is not fully agreed upon (223). One hypothesis is that acute myocardial infarctions result in myocyte rupture, which allows the extracellular gadolinium agents to passively diffuse into the intracellular space where they collect to produce a hyper-enhancement effect in damaged areas (224). However, there is an alternate hypothesis that the sarcolemmal membrane integrity becomes compromised, causing myocyte loss and an increase in collagen fibers to fill the interstitial space may yield a reason for the gadolinium compound to concentrate in these

damaged areas resulting in hyperenhancement (224,225). Areas that have hyperenhancement may display several different appearances. The region, pattern, depth and degree of enhancement will be evaluated.

Outcome measures:

MRI:

- T1 value
- T2 value
- Wall strain
- Delayed enhancement
  - Region
  - Pattern
  - Depth
  - Degree

Myocardium

- Percent fibrosis

Data Analysis

Pearson's product moment correlation (Pearson's r) will be used to assess for correlation between the MRI and percent tissue fibrosis for T1 value, T2 value, Wall strain and simple MDE. For the MRI data that has hyperenhencement present (positive MDE), additional zero order analyses will be performed to look for correlations at lower levels of the dependent variable. These additional analyses will most likely not be powered significantly, but may serve as pilot data to look for trends to further explore on further studies. The computer package S PSS will be used for most statistical analyses. A 0.05 two-sided Fisher's z test of the null hypothesis that the Pearson correlation coefficient $\rho = 0.000$, will have 80% power to detect a ρ of 0.790 when the sample size is 10.

Data Management

MRI Data will be stored on magneto optical disks that will be secured in a locked office. In addition, data will be stored on a desktop computer and on a laptop computer, both of which will be kept secured in locked offices. Although this is an animal study, the data will be protected as it is with any clinical study.

Limitations

A potential limitation will be that the pigs will have their heart failure scans at different time end points due to inter-individual variability in the speed of heart failure progression. However, the proposal requires that all pigs be in an equivalent state of severe systolic failure at sacrifice (fractional shortening is decreased by two standard deviations (from 32 to 16%). An alternative would be to sacrifice the pigs at the same end-point, but then some of the animals may either not be in heart failure or not survive to the time point.

Another limitation is that the Western blot and histologic controls are not the same pigs as the controls scanned for MRI since the pigs will not be sacrificed until after they have heart failure. A separate group of Yorkshire pigs will be used as the controls for the Western blot and histologic analyses and they will be weight and age matched as closely as possible. We do not expect the difference in control population to have a significant impact on the results of this study.

Project Time Table

PROJECTED TIMETABLE	MONTHS											
YEAR ONE	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Notification of Funding	**X**											

Begin Organizational Aspects of Study	**X**	**X**										
Finalize IACUC information and approval	**X**	**X**	**X**									
Buy supplies			**X**									
Begin testing subjects				**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Start biochemical analyses						**X**	**X**	**X**	**X**	**X**	**X**	**X**
Data Entry				**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Annual Report												**X**
YEAR TWO	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Continue testing subjects	**X**											
Continue biochemical analyses	**X**											
Data Entry	**X**											
Complete testing		**X**										
Data analyses	**X**	**X**	**X**									
Final Report			**X**									

Participant Information

This is a research study on animals and the results are not intended for any clinical diagnosis.

Storage of Participant Samples

Tissue samples will be stored in a freezer in Dr. Mark Haigney's laboratory or Dr. Christine Kasper laboratory.

Privacy and Confidentiality

All information in this study is derived from animals.

Use of Sample by Investigators

The protocol participates with the USUHS tissue sharing agreement.

Withdrawal from Research

Subjects will be removed from the study at the discretion of the PI or veterinarian if the animals are deemed to ill to continue. The protocol will follow all Federal laws, regulations and policies pertaining to animal research.

Commercial Interest

Tissue obtained from this research will not be used for any commercial purposes.

E. Vertebrate Animals

All experimental protocols will be reviewed and approved by the USUHS Institutional Animal Care and Use Committee. The investigation will conform to the *Guide for the Care and Use of Laboratory Animals,* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Risks MRIs

The risks of MRI are considered minimal by the FDA but include those related to the physiological effects of the radio-frequency pulses and those related to being in a static magnetic field. Concerning the risks related to physiological effects of radio-frequency pulses, these risks are regulated in terms of specific absorption rate (SAR) which the FDA limits to less than 0.4 W/kg whole body. Fortunately, the MRI scanner calculates these limits for each pulse sequence prior to their performance and will NOT allow the pulse sequence to begin if the SAR limits are exceeded. The pigs will be closely monitored via an MRI monitoring system, plus anesthesiologists will be present to help care for the animal while it is away from the USUHS

Laboratory of Animal Medicine. Preliminary safety studies were performed and well as a recent review of MRI safety literature to assess the pacemaker risk to the pigs. It is expected to be minimal.

F. Select Agent Research
n/a

G. Multiple PI Leadership
n/a

H. Consortium/Contractual Arrangements
n/a

I. Resource Sharing
n/a

J. Literature Cited

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Magnetic Resonance Imaging of Heart Failure Using a Swine Model

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1

Introduction

- The prevalence and incidence of heart failure is rising
  - Heart failure has become one of the leading causes of morbidity and mortality in the US
- American Heart Association - 2007
  - Direct and indirect burden of heart failure on the US economy has now risen to ~ $33.2 billion

Hunt, et al, 2005

2

Introduction - Nursing Impact

- The nursing shortage
  - Estimated shortage by 2020 = 340,000 nurses
- Nursing shortage poses a threat to quality patient care
- People living longer with chronic conditions, many of which need a lot of care
- HF is complicated and associated with other chronic conditions

Auerbach, et al, 2007; Kane, et al, 2007

3

Introduction - Nursing Impact

- RNs and NPs will be impacted with the growth of HF patients
- Nurses need more information to manage the HF patient more effectively
  - Cardiac rehab
  - Device management
  - Home care
  - Assisted care facilities
  - Nursing homes

4

Background

- Heart failure (aka CHF), is defined as:
  - The impaired ability of the ventricle to fill or eject blood that may be induced through any structural or functional cause
- Gaps in the understanding of HF
  - Many of the ACC/AHA recommendations are NOT supported by evidenced based research findings
- Determining the actual mechanisms influencing the development of heart failure is critical to the proper management of the patient

Hunt, et al, 2007

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Background

- HF is a chronic, progressive condition*
  - Associated with sudden death in 50% of individuals
    - Most due to ventricular tachyarrhythmias
  - Gradual pump failure in the remainder
- ***At present there are few reliable predictors of risk for sudden death in heart failure*****
- ***Implantable defibrillators can significantly reduce the risk of sudden death in heart failure, but the reduction is modest***

*Kannel and Schatzkin, 1985; Nerheim, et al, 2005

**Kadish, et al, 2000, 2004; Grimm, et al, 2004, Zipes, et al, 2006

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Background

- HF can occur as a result of a variety of structural and/or functional conditions
  - myocardial infarctions,
  - hypertension,
  - dilated cardiomyopathy,
  - valvular disease and
  - left ventricular dysfunction
- Dilated cardiomyopathy (DCM) is the most common type of non-ischemic heart failure

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Background

- Persons with DCM are at risk for sudden death as well as chronic HF
- Degree of myocardial fibrosis – especially central fibrosis, is thought to play a critical role in determining the risk of sudden death in DCM
- Persistent tachycardia is a common cause of DCM
  - Rapid pacing is a well know method to produce DCM in animal models

Grimm, et al, 2004; Peters and Kienzle, 1988; Burkett, et al, 1994

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Background

- Magnetic resonance imaging (MRI) is a non-invasive diagnostic tool that can provide images of:
  - Cardiac structure and function
  - The only modality that can quantify fibrosis – detect scarring in the myocardium
- This proposal will focus on the development of fibrosis in tachycardia-induced DCM in Yorkshire swine, a large animal model of human heart failure

Stork, et al, 2007; Knopp, et al, 2002; Assomull, et al, 2007; Assomull , et al, 2006; Dewey , et al, 2004; McCrohon, et al, 2003; Nazarian, 2005; Teraoka et, al, 2004; Vohringer, et al, 2007; Brodoefel, et al, 2007; Soriano, et al, 2007; Casolo, et al, 2006;White, et al, 2006; Soriano, et al, 2005; Mahrholdt, et al, 2005; Tandri, et al, 2005; De Cobelli, et al, 2006; Weinsaft, et al, 2007; Sechtem, et al, 2006; Moon, et al, 2004; Moon, et al, 2003; Jackson , et al, 2007; Gupta, et al, 2004.

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Central Hypothesis

- **MRI will be able to quantify the functional changes induced by myocardial remodeling from tachycardic pacing in a swine model**
- ***Overall objective*** is to define the functional and morphologic characteristics of DCM on MR imaging techniques, including quantification and phenotypic identification of pathologic fibrosis
  - To validate MRI findings, extracellular matrix analysis of the myocardium will be performed using histology and quantitative Western blot analysis

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Goals and Objectives

- Our ***long-term goal*** is to develop MRI techniques that can identify persons at high risk for sudden death from heart failure, and to enable more efficient patient care and nursing management of the HF patient

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Specific Aim #1

- **Measure the difference in cardiac function between baseline and tachycardic pacing-induced heart failure in swine using MRI**
  - MRI can measure wall thickness, ventricular dimensions, calculate ejection fraction and assess wall strain
  - The swine will be imaged before and after HF induction
  - The heart failure time-point will be determined from serial echocardiograms - when fractional shortening is decreased by two standard deviations (from 32 to 16%)
  - Several experimental MR techniques will be piloted to that may improve MR's ability to characterize HF

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Specific Aim #2

- **Measure the quantity of Type I and Type III collagen in the myocardium of swine in heart failure as compared to controls using quantitative Western blotting from post-mortem tissue specimens**
  - It is well documented that pacing-induced tachycardia increases the percentage of fibrotic proteins in the extracellular matrix
  - Quantitative Western blots will be performed on tissue from the swine in Aim 1 and from control pigs to assess for a difference in collagen amount and type
  - Additional tissue samples will have histologic assessment for overall fibrotic percent changes

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Specific Aim #3

- **Correlate the pattern of fibrosis in post-mortem heart failure histologic specimens with the tissue characterizing MRI techniques of T1 values, T2 values and myocardial delayed enhancement (MDE)**
  - By correlating the pattern of fibrosis in the heart failure tissue to the MRI results, providers can more confidently use MRI as a tool to help discern the degree and pattern of dilated cardiomyopathy (HF) an individual is experiencing
  - T1 and T2 values can quantify the tissue characteristics and mathematically find differences in tissue composition related to changes in myocardial remodeling
  - MDE can show areas of focal fibrotic replacement/scarring in the myocardium

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Theoretical Framework

- Scientific methodology – (empiricism)
- The basis for the scientific methods used in the US and Great Britain are based on the British empiricist philosophy of John Locke
  - Ideas must be supported by evidence, not dogma or superstition
- Much of the current recommendations for care of the heart failure patient from the ACC/AHA are simply based on current practice standards, not systematic trials

Yolton 2001

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Animal Model

- Although humans are common research subject for HF, sometimes research can be justified to be done on animals, particularly when pathologic specimens are needed
  - Humans are less uniform
  - Humans may have concomitant conditions that may complicate the study
- Most human histochemical comparisons in HF studies are done on post-mortem subjects
  - Heart biopsies are invasive
  - Heart biopsies have risk

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Animal Model

- The pig model fairly closely simulates the human heart which is why the pig is such a popular model for studying heart disease.
- The pig is a large animal model that more closely matches the heart and circulatory system of humans than do rats, mice or dogs
- This study will use Yorkshire pigs

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HF Models

- Ischemic models produce uneven pathology in the myocardium
  - some areas will be necrotic, with varying degrees of fibrosis and scarring
  - some areas will be normal
  - more complicated to study
- Ischemic models may make systematic study of molecular changes in the myocardium problematic to study

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Pacing Models

- The pacing model produces a fairly uniform disease pattern
  - The myocardium will have a more uniform pattern of fibrosis occurring as compared to an ischemic model
- **Since this study wishes to assess the changes in the myocardium, especially fibrosis that are occurring, the pacing model is the more logical choice for consistency in the model**

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Team/Resources

- **Dissertation Committee**
- NNMC MRI Clinic Support
  - MRI scanner use
  - Monitoring equip
- GE Healthcare Scientists
- USUHS Anesthesiology
- USUHS LAM
- USUHS Laboratory Support
- Consultants
  - Cara Olsen – Biostatistics Consulting Center
  - Frank Shellock, PhD – Physiology and MRI Safety
  - Roopa Biswas, PhD – Western Blots

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Study Design

- Quasi-experimental, mixed-method study with two time points for each animal for the MRI and control pigs for the histology comparisons
  - This study will not meet the strict demands of being a randomized, double blinded, trial
  - Assessing pigs for non-ischemic DCM with pacemakers implanted has not been done before using MRI
  - Standard as well as select investigational MRI techniques will be used

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Study Design

- Rationale for a quasi-experimental study is for the welfare of the animals since research on animals is a privilege
  - It is prudent to start with a small trial to gain preliminary data is this area before moving to a larger study with more animals

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Methods

- Study subject: Yorkshire pigs
- MRI performed at two time points
  - Prior to pacing
  - At heart failure shortly before sacrifice
- Laboratory analysis – quantitative Western Blots and histology
  - Tissue from pigs at sacrifice
  - Tissue from control pigs

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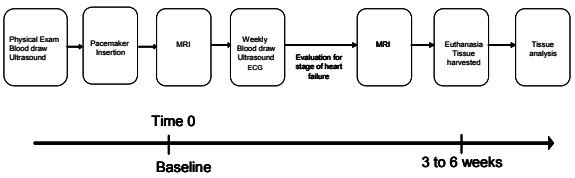
Methods - timeline



24

Specific Aim #1

- **Measure the difference in cardiac function between baseline and tachycardic pacing-induced heart failure in swine using MRI**

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Methods – Aim #1

- Aim #1 is designed with the pigs being scanned twice
  - once before pacing
  - once after they are in severe heart failure
- Ejection fraction, LV wall thickness, LV wall strain will be calculated on MRI
- Since the pigs will be used as their own controls, dependent t-tests will be performed for each outcome variable. If deemed necessary because of the small sample size, Bonferroni corrections may need to be performed on these t-tests as well

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Methods – Aim #1

- Power Analysis
- Wall thickness, a sample size of eight (8) pigs per group will have an 80% power to detect a difference in means of 0.405, assuming a standard deviation of 0.350 based on a paired t-test with a 5% two-sided significance level
- Ejection Fraction, a sample size of eight (8) pigs per group will have an 80% power to detect a difference in means of 14.50, assuming a standard deviation of 12.5 based on a paired t-test with a 5% two-sided significance level
- Wall strain, a sample size of eight (8) pigs per group will have an 80% power to detect a difference in means of 0.162, assuming a standard deviation of .140 based on a paired t-test with a 5% two-sided significance level (nQuery Advisor Software)

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Specific Aim #2

- **Measure the quantity of Type I and Type III collagen in the myocardium of swine in heart failure as compared to controls using quantitative Western blotting from post-mortem tissue specimens**

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Methods – Aim #2

- The tissue samples
  - Control pigs
  - HF pigs
- Two samples will be taken from each pig heart
  - Septal region of the LV
  - Lateral wall of the LV
- The tissue samples will be further separated by layer of the myocardium: epicardium, mid myocardium and endocardium
  - If possible compared between and within groups
- T-tests will be performed to look for changes between the two different areas of the heart for each animal.
  - If there is no significant difference, the data from the two areas of each pig will be pooled

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Methods – Aim #2

- T-tests will be done to compare the control animal tissue to the heart failure tissue for each of the three tissue layers
- The histology samples measuring percent fibrosis will be analyzed in one set of analyses
- The quantitative Western Blot samples will be analyzed statistically as two separate independent variables, collagen I and collagen III
- Multivariate Analysis of Variance (MANOVA) will be performed to look for changes between the three tissue layers with a Hotellings' T since there are two groups
  - If the homogeneity of variances is not met Box M tests will be used instead.

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Methods – Aim #2

- For percent fibrosis, a sample size of 8 pigs per group will have an 80% power to detect an effect size of 1.507 using a two group t-test with a 0.05 two-sided significance level.
- For Type I collagen, a sample size of 7 in each group will have 80% power to detect a difference in means of 95.5, assuming the group 1 standard deviation is 64.0 and group 2 standard deviation is 35.5, using a two group Satterthwaite t-test with a 0.050 two-sided significance level.
- For Type III collagen, a sample size of 10 in each group will have 80% power to detect a difference in means of 76.9, assuming the group 1 standard deviation is 57.0 and group 2 standard deviation is 53.0, using a two group Satterthwaite t-test with a 0.050 two-sided significance level.
  - Significance most likely will not be valid with the multiple layers of the dependent variables with the small sample size
  - It is noted that the sample size will only allow for descriptive analysis

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Specific Aim #3

- **Correlate the pattern of fibrosis in post-mortem heart failure histologic specimens with the tissue characterizing MRI techniques of T1 values, T2 values and delayed myocardial enhancement (MDE)**

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Methods Aim #3

- Pearson's product moment correlation (Pearson's r) will be used to assess for correlation between percent tissue fibrosis and MRI data for T1 value, T2 value, Wall strain and simple MDE
- For the MRI data that has MDE present, additional zero order analyses will be performed to look for correlations at lower levels of the dependent variable
  - These additional analyses will most likely not be powered significantly, but may serve as pilot data to look for trends to further explore on further studies
- A 0.05 two-sided Fisher's z test of the null hypothesis that the Pearson correlation coefficient ρ = 0.000, will have 80% power to detect a ρ of 0.790 when the sample size is 10

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Discussion

- Projected results
- It is expected that MRI will demonstrate significant differences in the pigs between baseline and heart failure for:
  - wall thickness
  - ventricular dimensions (volume)
  - ejection fraction
  - wall strain

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Discussion

- It is expected that the MDE images will most likely be negative
  - If positive, the enhancement is expected to be in the midwall area of the LV
  - Midwall enhancement is consistent with sudden death and poor prognostic outcomes
- The T1 and T2 values are expected to be different between baseline and HF time points
  - No comparative data on non-ischemic models of heart failure
  - Pilot data to help prepare for further studies if the descriptive statistics are promising

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Discussion

- It is also well known that the assault on the heart from rapid pacing causes remodeling to occur, changing the molecular and cellular composition of the myocardium
- It is estimated from previous work done by McCurley, Haigney and colleagues (2004), using a paced-pig model, the pacing in this studying is expected to increase the overall quantity of fibrotic material within the myocardium by approximately 6 to 7 percent

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Discussion

- Study by Pokharel (2004), on global insult of increased angiotension-converting enzyme on transgenic rats
  - Increases in type I and type III collagen
- Histological evaluation and quantitative Western blot analysis of collagen types I and III in our HF pacing model should demonstrate
  - Increase in fibrosis
  - Increase in collagen quantities
  - Possibly a change in the collagen I & III ratio

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Weaknesses and Limitations

- Pigs unable to be sacrificed at the same time period as pigs will take differing amounts of time to acquire the same level of heart failure
- MDE images are expected to be negative
- The T1 and T2 value sequences also have not been tested in this model and are most likely under-powered
- No direct comparison of before and after heart failure from the same animals for the tissue analysis
  - Control pigs need to be used for the tissue comparisons

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Special Thanks

- My Committee:
  - Christine Kasper, PhD, RN, FAAN, FACSM
    - Chair
  - Mark Haigney, MD, FAHA
  - Dorraine D. Watts, PhD, RN
  - Laura Talbot, APRN, BC, EdD, PhD
  - John Capacchione, MD, IACUC
  - Vincent B. Ho, MD, MBA, FAHA

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Summary

- HF is a chronic condition that costs the US healthcare system billions of dollars a year and degrades quality of life
- This study will explore the ability of MRI with existing and experimental techniques to define the dilated cardiomyopathy phenotype.
- The histological evaluation and quantitative Western blot analysis of collagen types I and III should demonstrate an increase in fibrosis and change in collagen ratios in the myocardium between baseline and HF
- This study should be able to measure the functional loss of myocardium detected by MRI that can be correlated with histological analysis

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Summary

- If successful, MRI can be used as a non-invasive tool to help manage heart failure patients more frequently and accurately
  - Identify those patients at greater risk for sudden cardiac death
  - Help select mode of therapy or device support
- Improved treatment and prevention approaches specific to DCM
  - Improve nursing case management of the HF patient
  - Improve case specific needs for optimal functioning
  - Improve quality of life

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Questions?

"The improvement of understanding is for two ends: first, our own increase of knowledge; secondly, to enable us to deliver that knowledge to others."

John Locke
(1632-1704)

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References

- Entire proposal text and references (225) available upon request.

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Uniformed Services University of the Health Sciences

Graduate School of Nursing

Request for Appointment of Dissertation Chairperson (Form C)

Name of Student: Maureen N. Hood

Semester: Fall 2007 Area of Concentration Heart Failure

Name of Selected Dissertation Chairperson: Christine Kasper, PhD, RN

Phone Number 295-1092

The above named student has selected the named faculty member to serve as Dissertation Chairperson.

The undersigned faculty member agrees to serve as the Dissertation Chairperson, understanding all responsibilities that are part of this critical role:

Christine Kasper — Christine Kasper
Printed Name — Signature

Maureen N. Hood — Maureen N. Hood
Printed Name of Student — Signature

Approval/Disapproval

Signature: Christine Kasper Date: 1/3/08
Director, Doctoral Program

Approval/Disapproval

Signature: Lynne Schwartzkopf Date: 4 Jan 08
Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing

Request for Appointment of Dissertation Advisory Committee (Form D)

Name of Student: Maureen N. Hood

Semester: F07 Area of Concentration Heart failure

Dissertation Chairperson: Christine Kasper, PhD, RN

Selected Faculty to Serve as Dissertation Advisory Committee:

1. Dorraine Watts Phone # 727-983-9424

2 Mark Haigney Phone # 295-3826

The above named student has selected the named faculty members to serve as the Dissertation Advisory Committee.

The undersigned faculty members agrees to serve as the Dissertation Advisory Committee, understanding all responsibilities that are part of this critical role:

Mark Haigney — Printed Name of Faculty Member — Signature

Dorraine Watts — Printed Name of Faculty Member — Signature

Maureen N. Hood — Printed Name of Student — Signature

Approval/Disapproval

Signature: _____ Date: 1/3/08
Director, Doctoral Program

Approval/Disapproval

Signature: _____ Date: 4 Jan 08
Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing

Request for Appointment of Dissertation Advisory Committee (Form D)

Name of Student: Maureen N. Hood

Semester: F '07 Area of Concentration Heart failure

Dissertation Chairperson: Christine Kasper, PhD, RN

Selected Faculty to Serve as Dissertation Advisory Committee:

3. Vincent Ho Phone # 295-1898

4. John Capacchione Phone # 295-3141

The above named student has selected the named faculty members to serve as the Dissertation Advisory Committee.

The undersigned faculty members agrees to serve as the Dissertation Advisory Committee, understanding all responsibilities that are part of this critical role:

John Capacchione — Printed Name of Faculty Member
Signature

Vincent B. Ho MD, MBA — Printed Name of Faculty Member
Signature

Maureen N. Hood — Printed Name of Student
Signature

Approval/Disapproval

Signature: Date: 1/3/08
Director, Doctoral Program

Approval/Disapproval

Signature: Date: 9 Jan 08
Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing

Request for Appointment of Dissertation Advisory Committee (Form D)

Name of Student: Maureen Hood

Semester: Summer 2010 Area of Concentration Heart Failure in MRI

Dissertation Chairperson: Christine Kasper, PhD

Selected Faculty to Serve as Dissertation Advisory Committee:

John Maye Phone # 301-295-1194

Patricia A. Kelley Phone # 301-587-8315 (H) 301-295-1123

The above named student has selected the named faculty members to serve as the Dissertation Advisory Committee (attach NIH biotech and rationale for the selection of each).

The undersigned faculty members agree to serve as the Dissertation Advisory Committee, understanding all responsibilities that are part of this critical role:

John Maye
Printed Name of Faculty Member — Signature

Patricia Kelley
Printed Name of Faculty Member — Signature

Maureen N. Hood
Printed Name of Student — Signature

Approval/Disapproval

Signature: _____ Date: 7-26-10
Director, Doctoral Program

Approval/Disapproval

Signature: _____ Date: 9-9-10
Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing

Report of Proposal Defense Examination for the Doctor of Philosophy Degree (Form E)

The proposal defense of Maureen Hood

entitled: Magnetic Resonance Imaging of ~~Heart~~ failure Using a Swine Model was held

on 3/20/08 from 1300 to 1430. The decision of the Examining Committee is:

PASS

A. Both the proposal and the oral explanation are satisfactory: ✓

B. Minor changes are recommended by the Dissertation Advisory Committee and are to be made to the satisfaction of the Dissertation Chairperson: _____

DEFER

A. Major change(s) in the proposal is/are required. Changes must be made to the satisfaction of the Dissertation Chairperson: _____

B. Major change(s) is/are required. Changes must be made to the satisfaction of the Dissertation Advisory Committee: _____

C. Remediation required prior to making major changes. Completion of remediation must meet the satisfaction of the Dissertation Advisory Committee: _____

FAIL

Neither the oral performance nor the proposal is adequate: _____

Signatures of the Committee

Chairperson: _____

Member: Vincent B. Ho

Member: _____

Approval/Disapproval

Signature: _____ Date: 3/23/08
Director, Doctoral Program

Approval/Disapproval

Signature: _____ Date: 4-4-11
Dean, Graduate School of Nursing, USUHS

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A Review of Cohort Study Design for Cardiovascular Nursing Research

Maureen N. Hood, MS, RN

Nursing research encompasses a wide array of study areas that oftentimes follow specific groups of patients or patient types. The cohort study design is a useful method to study any group, especially to track outcomes or to evaluate exposure or risk factors. Several different cohort study designs can be applied to the general population or to specific subpopulations or groups, such as those with cardiovascular disease. Cohort designs provide a temporal view of groups and exposures that can uncover outcomes and exposures that may be difficult to separate out in smaller, traditional experiments. There are several types of cohort designs, each with their unique advantages. Cohort designs may be prospective or retrospective. Although most cohort designs are longitudinal, there are also cross-sectional types of studies that are useful. As with any type of research design, selection of the study participants and control groups must be made carefully. It is important for the variables to be clearly defined and measurable. The investigator must also be aware of potential biases and weaknesses associated with different cohort study designs and account for these problems when they arise. Reports from cohort studies should be presented clearly, addressing the potential confounding problems. This article explores the many types of cohort designs, with examples from cardiovascular disease research to demonstrate how nurses can use this design in their research.

KEY WORDS: cardiovascular, cohort studies, experimental design, nursing, research

Outcomes research is an important focus for nursing research because of high demand for professional accountability and awareness of the need for evidence-based practice.[1,2] Traditionally, the randomized controlled trial (RCT) is considered the "gold standard" of healthcare research. However, RCTs cannot always be done easily or ethically in the clinical realm of healthcare research. Often, groups of people need to be studied in their natural settings, and sometimes, randomizing people into treatment versus nontreatment groups may be unethical in circumstances where nontreatment exposes patients to harm.[3] For instance, in a patient fall prevention study in a hospital, one can randomize patients into groups such as experimental group versus usual care (UC), but you cannot use a no-treatment control group because of the potential harm to the patient. For a study such as fall prevention, the new intervention is commonly piloted on a small scale and then compared with the existing prevention intervention strategy. Comparing a new intervention to an existing intervention yields important information while maintaining the current standard of care.

Cohort studies are one of the more powerful study designs for describing the natural course of a disease and for analyzing associations between variables and outcomes.[4] Cohort study designs have become important because their results have been found to be comparable with those of RCT studies.[5] Some types of cohort studies, such as the nested case-control, adopt features of the RTC, making them a strong type of study design. John Snow (1813–1858) was a pioneer in cohort design for the study of illness in a population. He studied cholera in England during a time when the airborne miasmata from zymotic materials were the main accepted cause for disease.[6] Snow's evaluation of different cohorts based on their water source found a difference in death rates that he was able to attribute to a water source. Snow was able to convince the local authorities to remove the Broad Street pump in Soho where a major outbreak of cholera was occurring although his theory was not generally accepted by scientists and physicians at the time.[7] Snow's cohort study established important information about the mode of communication of the disease without knowing the exact agent of the disease.

Cohort studies are for use not just in epidemiological research as they can also be useful in nursing

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research anytime a group or population needs to be studied. Because most nurses are interested in studying people and their health, nurse scientists should consider cohort study design. Cohort studies can help determine risk factors, causality, and the incidence of a disease or condition in a group or population. Nursing science studies humans and human needs, health promotion, illness management, and a wide variety of other nursing topics that are often difficult to precisely define.[1]

Many details need to be considered before deciding if cohort design is the most desirable methodology and which cohort design might be most suitable to answer a given research question. This article describes cohort study designs; explores the different types of cohort research designs; details the potential uses of cohort studies, including their strengths and weaknesses; and presents some strategies for presenting information found through cohort study designs. This article is intended to help nurse researchers explore the cohort research design to see how cohort study designs may fit into their plan of study.

Definitions

The term *cohort* comes from the Roman word for a fighting force of 300 to 600 men.[8] This "Roman Cohort" or fighting unit would move forward in hopes of finding favorable outcomes or a victory. The epidemiology community adopted the term *cohort* to mean "any designated group of individuals who are followed or traced over a period of time" for use in outcomes research on populations, a fitting analogy to the Roman meaning of the term.[9] It is generally accepted that a cohort is a group whose members fit into a described category. Cohort studies are a mainstay of epidemiological research that can be easily translated into nursing research. For example, to study pressure sore risk among the elderly in a skilled nursing facility, a cohort study design can help to determine risk factors that lead up to the development of pressure sores.[10] Through cohort studies in nursing, best predictors and risk factors can be identified so that nursing care strategies can be modified to minimize adverse outcomes.

Using cohort studies, investigators look at relationships between exposures and/or risk factors and outcomes. An outcome is defined as "something that follows as a result or consequence."[11] Exposures can be defined as conditions where one is submitted to or made accessible to a particular action or influence.[11] A risk factor as defined in statistics is a "clearly defined occurrence or characteristic that has been associated with the increased rate of a subsequently occurring disease."[12] These definitions help to guide the types of research questions that can be studied using the cohort design. It is critical for the researcher to define exposures, risk factors, and outcomes as well as the cohort groups themselves. Careful and thorough design supports the validity and efficacy of the cohort study.

The study of cardiovascular disease is especially suited for cohort-designed research studies. For example, women's heart disease has been of great interest in the past few years. One way to study the differences in cardiovascular disease symptoms in women is to use a cohort design so that the symptoms experienced by women can be elucidated. In a recent study by DeVon et al,[13] they compared the symptoms of men and women with acute coronary syndrome. By comparing the 2 cohorts (men and women), they were able to define the symptoms that each cohort with acute coronary syndrome experienced, which helped to demonstrate to clinicians several important differences in symptoms that women experience as compared with men. They found that women experienced more jaw and neck pain compared with men, felt overall higher intensity of symptoms, and had chest pain that was more like a feeling of fullness rather than crushing pain as men report. Cohort studies such as this help clinicians to better diagnose and care for specific patient groups.

Cohort Types

There are many ways to conduct cohort studies (Figure 1). Cohort study designs provide a strong, straightforward methodology with which to study populations or groups. The main strengths of cohort studies are their usefulness in studying relatively uncommon exposures and outcomes and their ability to calculate relative risk ratios (RRs).[3,4,8,14] Cohort studies also have a temporal scheme that allows them to help establish causal inferences in the relationships between exposures and outcomes.[15] Cohort studies can also be

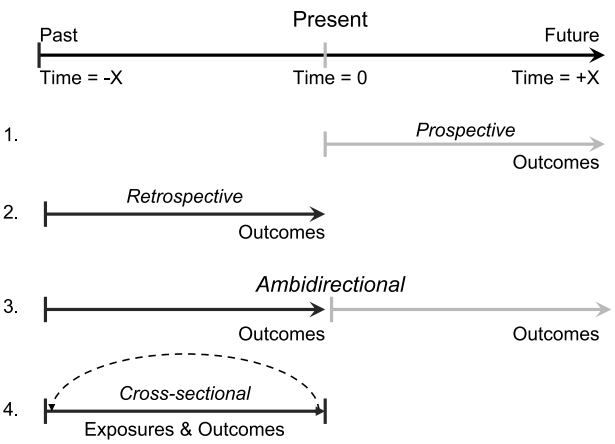

FIGURE 1. *General cohort design schemes. Cohort studies may be prospective, retrospective, cross-sectional, or a combination, depending on the question of interest.*

used to study more than 1 risk factor or exposure at a time and more than 1 outcome at a time, including varying degrees of outcome level and exposure levels.[4,8,14] The main weaknesses of cohort designs are the large sample sizes needed, the length of time often required to follow a cohort, the potentially high cost to conduct the study, confounding factors, selection bias, and existing bias of the participants.[3,4,8,14] For example, Wyatt et al[16] studied cardiovascular disease in more than 5,000 African Americans in the Jackson metropolitan area in a study referred to as the Jackson Heart Study (JHS). The JHS looked at hypertension prevalence, awareness, treatment, and control while also collecting a wide range of other factors that could potentially be important to understanding cardiovascular disease in this cohort, such as demographic, lifestyle, health status, and access to care. Although the JHS found that the public health interventions were demonstrating an improvement for this population, they were able to also document many risk factors in the population, such as obesity, smoking, heavy drinking, and lack of physical activity, as continuing problems. The JHS involved funding from multiple funding sources as well as investigators from multiple institutions, but it obtained a rich source of data from which to study.

Not all cohort study designs are large and expensive. There are many types of cohort studies, which lend to different types of research questions. The main types of cohort study designs and their chief strengths and weaknesses are outlined in Table 1. Nearly all cohort studies are longitudinal, where a group is followed over time, but there are cross-sectional types as well. The most common cohort designs are the prospective and retrospective studies, both of which are longitudinal in nature.

Prospective Design

In the prospective cohort design (Figure 2), the investigator defines a sample and predictor variables before any outcomes have occurred and then collects the data for the study in a prospective manner.[4] The advantages of prospective studies are their ability to define the incidence of a condition, describe the most probable causes of a condition, control or measure the exposure, measure confounding variables, and measure the predictor variable levels, all of which help lower bias.[4,14] The weaknesses of a prospective study are the typically long duration for completion, potentially large sample size, potentially high cost, and inefficiency. The study of heart disease is well suited for the prospective cohort study design. For example, atrial fibrillation (A-fib) is a chronic cardiac condition where nurses can play an integral role in the care of the patient. Inglis et al[17] conducted a study to assess nurse-led home-based care for the patient with chronic A-fib. The study looked at a chronic A-fib cohort. The patients were randomized into 2 groups, one received home-based intervention (HBI) care and the other group received UC, and followed over a 5-year period.

TABLE 1 The Main Cohort Designs With Strengths and Weaknesses Outlined

Cohort Type	Strengths	Weaknesses
Prospective	Able to control or measure the exposure Able to measure confounding variables Avoids recall bias Able to calculate relative risks	Can be costly Can be lengthy
Retrospective	Inexpensive Quick Able to study longitudinally with long latent periods	Risk of confounding variables Sometimes unable to determine exact exposure Selection bias Measurement error
Ambidirectional	Captures exposures that can cause both short-term and long-term outcomes	Can be costly Can be lengthy Risk of confounding variables Exposure determination problems
Cross-sectional	Prevalence of outcomes can be studied Collects a snapshot view of the study population	Risk of confounding variables Risk of ignoring exiting Selection bias Measurement error
Nested case-control cohort	Control group from the study population Helps provide risk factor prevalence Helps reduce costs by using control ''cases'' to represent all cases when testing is expensive or impractical	Number of cases can be low in rare conditions. Risk of confounding variables Control cases may not account for risk factors appropriately.
Multiple cohort	Good for rare exposures Multiple exposures can be considered Ranges of outcomes can be studied.	Risk of confounding variables Inefficient for rare outcomes Often expensive Existing bias Comparison groups may not be easily comparable.

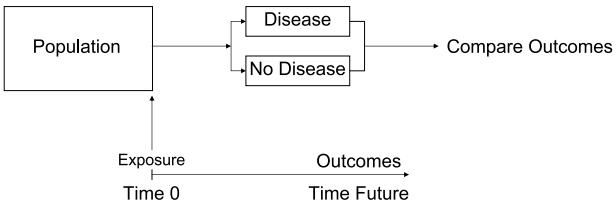

FIGURE 2. *Cohort design: prospective cohort. The prospective cohort design uses a forward look at a population.*

Although this particular study was underpowered (149 HBI patients and 148 UC patients), the results found were similar to those of other studies that have demonstrated a trend toward HBI reducing rates of rehospitalization and mortality. This study points out how the prospective cohort study can be used to evaluate nursing care in specific patient populations so that the foundations of nursing science can be expanded. Unfortunately, this A-fib study also demonstrates the cost of prospective cohort studies. A power analysis is crucial to help plan for prospective studies so that a statistically valid number of participants can be recruited into these studies so that the information gleaned from the research can be meaningful.

Retrospective Design

In the retrospective design, the investigator defines a sample and then collects the data about the predictor variables after outcomes have occurred.[4] In other words, the data already exist and the investigator uses the existing data to answer a question(s) of interest. Retrospective studies are relatively inexpensive and quick to perform and have many of the same strengths of prospective cohort designs. In addition, conditions that have long latent periods or are intermittent in nature can be studied more easily using retrospective cohort designs.[15] Retrospective studies are subject to higher risk from selection bias, confounding variables, and indeterminate exposure values.[4] Moreover, retrospective studies are often limited by the completeness of the existing data, which, if not obtained originally, will result in deficiencies in data, potentially rendering more definitive conclusions unachievable.

Ambidirectional Design

Two other cohort designs that are not used very often are the ambidirectional and the cross-sectional cohort studies. The ambidirectional study is a hybrid of both the prospective and retrospective studies. An investigator searches through existing data to answer a specific question but, in addition, collects similar data prospectively to collect more information to help answer the question of interest. The ambidirectional cohort study design is useful when exposures can cause both short-term and long-term outcomes[8] or when the information on a disease is available but the analysis of risk factors has not been done.[18] Infection outbreaks in hospitals can be studied using an ambidirectional cohort design to help determine the causal agent. For instance, in a study of multidrug-resistant *Pseudomonas aeruginosa* in an intensive care unit, the investigators looked back at the intensive care unit patients retrospectively and then followed them prospectively to collect information to analyze all the possible contributing factors to the infection outbreak.[19]

Cross-sectional Design

Cross-sectional cohort studies are unique in that they evaluate a single time point, unlike most cohort studies that are longitudinal and often take years to perform. Cross-sectional designs help to look at the prevalence of a problem in the population at a given point in time. The advantage of the cross-sectional design is that it takes less time to acquire the data and is therefore generally a less expensive type of study to perform. For instance, heart failure is a growing problem in the United States despite improvements in technology and medical treatment. Zambroski et al[20] looked at the heart failure patient cohort with a cross-sectional study design to assess not only the prevalence but also several factors that were associated with heart failure. This quick study found the heart failure patient to have an unacceptably high level of symptoms impacting quality of life. Cross-sectional studies like this one can be very useful to nurses to help target their care and interventions to decrease the frequency and severity of specific symptoms, which then may improve quality of life in the patient.

Nested Case-Control Cohort Design

Nested case-control cohort designs are cohort studies that use a control group randomly selected from within the study population (Figure 3). At first glance, nested case-control cohort studies seem very much like prospective or retrospective studies. These studies use selected cases and controls from within a given cohort or population. The "control group" population (subpopulation of the entire cohort) will have "cases" that develop or have the outcome of interest. In addition, a group of selected controls who are time matched to the cases are used to provide information on the prevalence of risk factors in this smaller group, which, in turn, can be interpolated to represent risk for the entire cohort.[21] The main advantages of nested case-control cohort design is the ability to obtain a control group from within the population being studied.[4,8] By studying the control cases, information on the prevalence of risk factors in the population can be

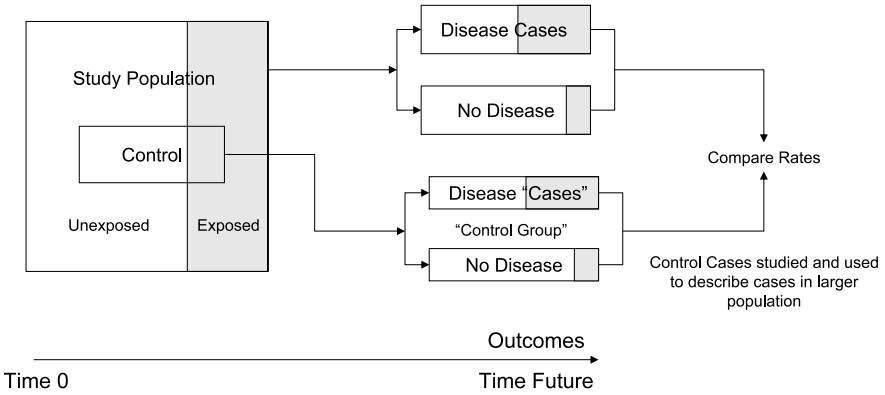

FIGURE 3. *Cohort design: nested case cohort. Nested case-cohort design takes advantage of the larger population and the control group that has already been selected to study a targeted question within the larger study.*

determined; also, when the cost of studying the cases is expensive or impractical, costs can be reduced by using the control cases to represent all the cases found in the study. Unfortunately, in studies where the outcome condition is rare, a low number of control cases will be available for study, which can statistically threaten the validity of the findings because they may not account for the risk factors appropriately.[4] In addition, as in most cohort studies, the risk of confounding variables is to be considered. A good example of how nested case-control cohort design can be used is demonstrated by a study that looked at coronary patients.[22] They extracted 2 groups from the main cohort of coronary patients, those who had repeated myocardial infarctions (MIs) and those who did not have a second MI. The 2 groups were matched for sex, age, and hospital. They were able to find that those patients who regularly visited the nurse center were 52% less likely to have a reinfarction. They also looked at smoking, adherence to medications, regular visits to the primary care physician, and lifestyle modifications. This was a surprising finding that is important and points to the need for further research into the use of primary care nursing clinics to help care for the post-MI patient.

Multiple-Cohort Design

Another cohort design type uses 2 or more separately defined cohorts that can be compared (Figure 4). This type of design is referred to as the multiple-cohort study design or the cohort study with external controls.[4] This type of cohort study is akin to a multicenter type of study but is focused on similar cohorts from different places. The cohorts may have similar exposures or different exposure levels or one group may not have any exposure to the predictor of interest. There are numerous ways to define the cohorts in multiple-cohort design studies, including secondary data analyses. Sometimes, the multiple-cohort design is the only feasible method of studying rare exposures to health hazards.[4] Unfortunately, confounding variables can be greatly accentuated in this design, making the actual predictors of outcomes difficult to determine. A study by Fower-Brown et al[23] on the utility of using exercise testing to screen for CAD is one example of how multiple-cohort study design can be used to utilize existing information to search for the sensitivity of a screening tool. The exercise tolerance test data came from multiple-cohort studies of asymptomatic individuals. The setup and analysis were done somewhat like a meta-analysis and investigated several outcomes such as prognostic values like ST-segment depression, heart rate recovery, and development of premature ventricular contractions. The cohorts yielded varying rates of findings for the exercise tolerance tests, with as little as 3% of young aviators having a minor ST-segment elevation finding as opposed to 29% of persons with diabetes in the Finland cohort having an ST-segment elevation during the exercise tolerance screening test. The multiple-cohort design allowed for the researchers to also look at combinations of risk factors separate from and in combination with the exercise tolerance testing to evaluate if this type of screening could be cost-effective in the asymptomatic population. This

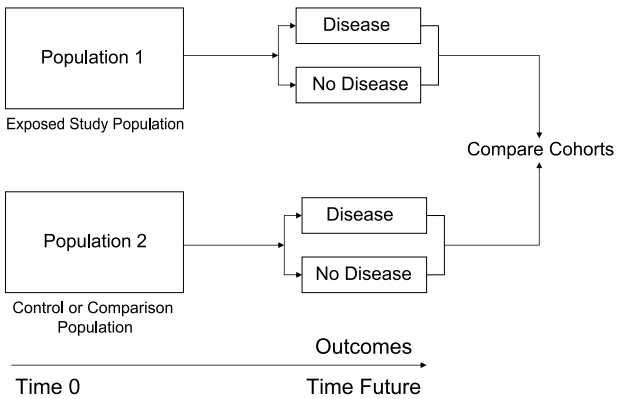

FIGURE 4. *Cohort design: multiple cohort. Multiple-cohort design uses 2 separate populations to study a question.*

paper on exercise tolerance screening found a potential for using exercise testing as a cost-effective tool only in those persons who are already at risk. This is valuable information, and because it was based on 55 similar research studies, the findings seem to be solid. However, because this was a secondary data analysis from multiple sources, there is potential for error. When investigating data from multiple studies, it is possible that the variable constructs were not always interpreted the same way by all of the investigators. Therefore, secondary data studies with data from multiple investigators have a chance for interference from confounding variable constructs and variable disagreement. The authors of this multiple-cohort analysis of exercise tolerance testing also recommended that a more rigorous prospective study be conducted to further evaluate exercise tolerance testing as a prognostic tool, but at least multiple-cohort studies can help find areas where more evidence-based studies are needed.

Sample Selection

Sample selection is the key to the cohort design. The definition of the group to be studied must be considered carefully. Participants must fit into the description of the population and be available for the entire study period (unless death is an outcome of interest), and the number of participants needs to be large enough so that the study has sufficient power for valid conclusions to be obtained.[4] Examples of cohort samples are the general population, easily definable populations such as cardiology nurses, and special populations such as those with rare exposures or rare outcomes from exposures (eg, small-pox vaccine–associated myocarditis[24]) or outcomes due to certain genetic variations (eg, Turner syndrome women have an increased risk of cardiovascular abnormalities[25]). Sample participants need to be free of the outcome of interest at the start of the study period. The measurement of exposure needs to be recorded as accurately as possible in cohort studies to calculate relative exposure rates or predisposition rates and help define the strength of the relationship between the exposure/predisposition and the outcome.[3,14] Measurements of exposure should consist of intensity, duration, regularity, and variability. Measurements in genetic associations need to look at differential gene dosages, allele variability, and, possibly, environmental triggers.[26,27] Feasibility also needs to be addressed for both the sample size and exposure. For some exposure measures, scales or "cutoff" values need to be assigned (eg, Modified Bruce Protocol is used to quantify the degree of exercise attained during an exercise treadmill test. The speed and gradient of the treadmill need to be defined for each stage of the test so that it can be compared in a standard fashion). Just as in any clinical study, participants in both the exposed and unexposed groups need to be measured as consistently as possible in a cohort study.

Potential Bias

Bias is a potential problem in all research studies; cohort studies are no exception. When reviewing a cohort study, several potential problem areas should be addressed (Table 2). The investigator must look for several key sources for bias—selection bias, information bias, existing loss, and confounding variables[3]—and address any possible bias in the results dissemination. The selection of study population(s) should address inclusion and exclusion criteria. It is extremely important that populations selected are free of the outcome of interest at the baseline or starting time of the study period, except for cross-sectional cohort studies. Biases in information can lead to systematic differences in measurement between the cohorts or even between the diseased versus nondiseased participants (diagnosis bias) in a study.[14] In a study of oral contraceptives and the risk of thromboembolism, for example, there was a concern that patients who were taking oral

TABLE 2 Questions to Ask When Assessing a Cohort Study

Problem Area	Questions
Selection bias	Was the study population clearly defined?
	Is the study population a representation of the general population or the clearly defined population?
	Is the study population size adequate?
Information bias	Were the information and measures to study the population and the control population conducted similarly?
	Did the investigators attempt to limit investigator bias?
	Were all participants treated in a similar fashion?
Existing bias	Did the investigators attempt to limit participant loss?
	What were the factors contributing to the losses?
	Was the study duration long enough?
Confounding variables	Did the study use a comparison cohort or a control?
	Were the exposed and unexposed groups different?
	Did the investigators account for confounding variables in their analysis?

TABLE 3 Example of a Summary of Cohort Risk Data

Outcome	Exposed	Unexposed	Total
Disease	A	B	A + B
No disease	C	D	C + D
Total	A + C	B + D	A + B + C + D

A represents exposed persons who get the outcome of interest; B, unexposed persons who get the outcome of interest; C, exposed persons who do not contract the outcome of interest; and D, unexposed persons who do not contract the outcome of interest.

contraceptives were being admitted to the hospital for thromboembolism more often than were women with similar symptoms who were not taking oral contraceptives because of the physician knowledge that oral contraceptives can cause thromboembolism.[28] The relationship between exposure and disease presents a mixed association of disease determinants and participation determinants.[29]

Subject loss, or exiting, is a real threat that can affect internal and external validity.[29] The best way to deal with losses is to design the experiment to not have any or very few. If participant dropout is significant in 1 population, then the factors contributing to the losses need to be evaluated. The loss of the participants can have a significant impact on the study results and should be addressed by the investigators.

Analysis and Reporting

Analyzing and reporting the information from a cohort study can be an arduous task because of the large sample sizes and large data synthesis normally associated with cohort design studies. Computer programs such as SAS (SAS Institute, Inc, Carey, North Carolina) and SPSS (SPSS, Inc, Chicago, Illinois) can be very helpful in the statistical analysis phase of the study. The study must have clearly defined independent and dependent variables, the definition of the exposure(s) or risk factor(s) must be clearly defined and measurable, and the outcome(s) of interest must be clearly stated and measurable. As with any research study, if the study variables are not clearly defined and the research design not clearly followed, the validity and reliability of the results can become questionable.

The investigator needs to remember that not all confounding variables or influences can be identified and corrected for. Each type of cohort study has weaknesses that the investigator needs to focus on (Table 1). For example, nested case-control studies are vulnerable to low validity if the number of control cases is low. Therefore, the investigator may need to adjust for possible type I error. Known confounding variables must be considered and dealt with appropriately for the information generated to be as valid as possible. Statistical analyses such as covariate analysis or standard Cox regression[30] are useful in cohort studies. Confounding can also be minimized by using a dual cohort technique where the comparison (control) population has been matched for comorbidities or other similar characteristics such as age and sex.

Cohort studies often study multiple variables within a population. In some cases, such as in nursing informatics where extensive lists of variables can be examined, the amount of information can be daunting. The research questions need to be defined clearly in the beginning of the study so that the information that is important to the study question can be extracted and presented. Tables help present data in a logical manner. An example of a very simple data display from a cohort study is demonstrated in Table 3. Tables give the reader a view of the sample size and the relative numbers of results before statistical

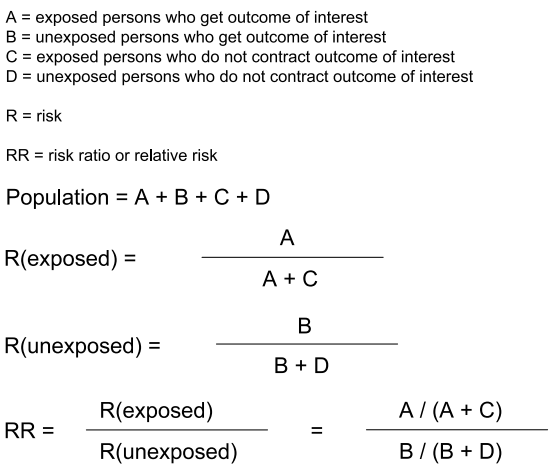

FIGURE 5. *Relative risk ratio. Calculating the relative risk ratio provides a measure of the probability of the risk due to certain factors or reduction of risk from an intervention. Adapted from Greenberg et al.*[3]

analyses have been applied. Tabulating data forms a starting point from which to base a more in-depth statistical analysis.

Relative Risks

Cohort studies are one of the research designs used to calculate relative RR because they are probabilities calculated through the incidence of the disease in the population of study (Figure 5). The RR is a simple calculation that provides a mathematical probability of the risk of a person exposed within a population contracting the disease compared with the general population of study. In addition, RRs are not to be confused with odds ratios (a ratio of the chance, or odds, of an event occurring, not the percentages). Odds ratios are often chosen because they can make the results look more impressive, but they are more complicated calculations that may not accurately reflect the actual risk to an individual.[31] Therefore, when reading the statistics from a cohort study, look at the statistics section to look to see which risk measurement was used by the authors. A great example of how RRs are used in research is demonstrated by a targeted assessment of women with diabetes from the Nurses' Health Study cohort. Hu et al[32] looked at the association between fish consumption and omega-3 fatty acid intake as they relate to the risk of cardiovascular disease. The nurses in this study who reported higher fish consumption and omega-3 fatty acids were found to have much lower RRs for cardiovascular disease and mortality than were the low-intake women (intake of fish or omega-3 fatty acids of <1 per month resulted in RR = 1.0; 1–3 per month, RR = 0.75; 1 per week, RR = 0.66; 2–4 per week, RR = 0.67; ≥5 per week, RR = 0.48; *P* for trend = .005). The researchers had to account for multiple possible confounding variables such as other dietary intake differences and the lack of direct measurements of variable such as diabetes confirmation and severity of disease. There are inherent problems with self-reporting questionnaires that need to be taken into consideration with the study design and statistics of a study such as this. However, because this study showed a significant trend that was nearly identical to that of other studies of women without diabetes and had a cohort size of more than 5,000 nurses, this type of cohort study still can demonstrate valuable information. Studying people in real-life situations is difficult with the confounding variable that must be accounted for, but these are studies that need to be done.

Summary

Cohort study design is a useful method used to study a population, especially when the interest is the prevalence of an outcome or the natural history of an exposure or risk factor. Several different cohort study designs can be applied to the general population or to specific subpopulations or groups, such as those with cardiovascular disease. Cohort designs provide a temporal view of groups and exposures that can help to find outcomes and exposures that can sometimes be difficult to separate out from smaller, traditional experiments. Investigators must be careful to select the participants and variables that can be clearly defined and are measurable. The investigator must also be aware of potential biases and weaknesses associated with different cohort study designs and account for these problems when they arise. Reports from cohort studies should be presented clearly, addressing the potential confounding problems. Cohort designs are easily adaptable for nurses to study cardiovascular disease and should be considered as a potential method when exploring a question.

Clinical Pearl

- Cohort study design is well suited for the cardiovascular nurse researcher who is studying a defined group or population.
- Cohort study design is a useful method to evaluate the prevalence of an outcome or follow the natural history of an exposure or risk factor.
- Cohort study design provides a temporal view of groups and exposures/risks, which may help to establish causal inferences in the relationships between exposures and outcomes that may otherwise be overlooked.

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Free-breathing T1 Mapping MRI for Quantification of Myocardial T1 in Swine with Heart Failure

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Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure

Type of Article: Original Research

Advances in knowledge:

1. The free breathing T_1 mapping technique is a promising method for quantifying diffuse myocardial fibrosis in heart failure.

2. The myocardium in heart failure patients has an increase in the percentage of fibrosis that not often detected by traditional T2-weighted and myocardial delayed enhancement imaging.

3. In this tachycardia swine model of heart failure, T_1 mapping MRI is shown to quantify changes in T_1 related to changes in fibrosis.

4. T_1 mapping values are measurable with or without the use of a gadolinium chelate contrast agent.

Implications for Patient Care:

1. This free-breathing version of the T_1 mapping technique offers the potential to be able to quantitatively evaluate myocardium in a wide range of patients, including patients with high acuity levels and children.

2. Since T_1 mapping works without the use of a contrast agent, this sequence offers a completely non-invasive way to evaluate the myocardium for changes in fibrosis.

Abstract

Purpose: To evaluate a new, free-breathing pulse sequence to quantify myocardial T_1 changes in a swine model of tachycardia-induced heart failure.

Methods: Procedures were approved by the Institutional Animal Care and Use Committee. Yorkshire pigs were implanted with pacemakers and paced at 200 bpm to induce heart failure. Animals were scanned twice using MRI, once at baseline and once at heart failure. A T_1 mapping sequence was performed prior to and after the administration of a gadolinium-chelate contrast agent. T_1 mapping values were compared between the baseline and heart failure scans. Percentage fibrosis of heart failure tissue was compared to similar left ventricular tissue from control animals using Trichrome Blue histology.

Results: Significant differences were found between the baseline and heart failure T_1 mapping values prior to the administration of contrast (960 ±96 ms, 726 ± 94 ms respectively, p = 0.02), and after contrast (546 ±180 ms, 300 ±171 ms, p = .005). There was also a significant difference in the percentage of collagen between the control and heart failure animals per histological analysis (5.4 = ±1%, 9.4 = ±1.6%repsectively, p <.001).

Conclusions: The free breathing T_1 mapping technique is a promising method for quantifying diffuse changes in fibrosis in the myocardium between normal and heart failure animals without the use of a contrast agent. The myocardium of heart failure has diffuse increases in the percentage of collagen that in this study were not detected by traditional delayed enhancement imaging, which was detected as a lower T_1 mapping value.

Key Words: Magnetic Resonance Imaging, Heart failure, Collagen, Histology

Introduction

The ability to accurately detect critical myocardial changes in heart failure provides an opportunity to properly select treatment and to monitor therapeutic outcome. Cardiac magnetic resonance imaging (MRI) is an emerging diagnostic tool capable of comprehensive assessment of heart failure (1). The main advantages of cardiac MRI are that it can non-invasively provide information on myocardial morphology, function, blood flow, myocardial perfusion, and myocardial injury or infiltrative/fibrotic changes during a single session (2).

In order to assess the myocardium using MRI, a variety of techniques can be used. Myocardial delayed enhancement (MDE) imaging, using a gadolinium (Gd)-chelate contrast agent, has been shown to be particularly useful (3-7). Gd-chelate contrast agents have relatively short intravascular duration during their “first pass” secondary to their quick extravascular distribution into adjacent background tissues and urinary excretion within minutes of their intravenous administration. MDE imaging is performed approximately 5-10 minutes following the intravenous administration of a Gd-chelate contrast agent injection. On MDE images, normal myocardium is generally dark and abnormal regions appear brighter (i.e. hyperenhancement) than adjacent myocardium. These hyperenhanced areas have been associated with myocardial infarction, scar or fibrosis, and inflammation. Hyperenhancement of abnormal myocardium is believed to occur secondary to the retention of Gd-chelate contrast agent in the abnormal regions relative to healthy myocardium (8-21).

The pattern of hyperenhancement on MDE imaging, moreover, can suggest the underlying pathology. Myocardial infarction in ischemic heart disease, for example, typically manifests as a subendocardial region of hyperenhancement, which if extensive, may become transmural, but bounded by a vascular distribution (10). Dilated cardiomyopathy may have a circumferential mid-wall enhancement pattern that corresponds to mid-myocardial fibrosis (9, 10, 22), or may be normal on MDE (i.e. no abnormal regions of hyperenhancement (10, 13, 22)).

The degree of myocardial fibrosis is thought to play a critical role in determining the risk of sudden cardiac death in patients with heart failure., As previous studies using MDE to assess patients with dilated cardiomyopathy have been unable to detect diffuse myocardial fibrosis reliably (10, 13, 22), better MRI methods are necessary for detection of fibrosis in heart failure patients. Cardiac T_1 mapping MRI is a new technique that can enable signal quantification on a standard scale (23).

The purpose of this study was to investigate a new free-breathing T_1 mapping MRI pulse sequence to quantify myocardial T_1 changes in a tachycardia-induced heart failure swine model, both without and with the use of a Gd-chelate contrast agent. This study hypothesizes that the T_1 mapping technique can differentiate between healthy and heart failure animals based on T_1 mapping values of the myocardium. The T1 mapping technique should be able to demonstrate T_1 value changes that relate to a change in the amount of fibrosis, specifically collagen, present in the myocardium between baseline and heart failure as supported by histological analysis.

Methods

This Institutional Animal Care and Use Committee (IACUC) approved protocol consisted of two groups of swine: a heart failure group and a control group. MRI was only performed in the heart failure group. The control group consisted of Yorkshire swine (N=10) who had been euthanized after been involved in this protocol as a control animal only, or from non-cardiovascular IACUC protocols that were available for tissue harvesting.

Magnetic Resonance Imaging

After IACUC approval, Yorkshire swine (N=6) were implanted with pacemakers (St Jude Medical Integrity SR, Sylmar, CA) and paced for 3 to 5 weeks at 200 beats per minute. Each animal was scanned in a 1.5 Tesla MRI scanner (GE Signa, Waukesha, WI) at baseline (non-paced pacemaker in situ) and then at heart failure [confirmed by echocardiography as half the fractional shortening as compared to baseline (from approximately 32 percent at baseline to 16 percent at failure) , with pacer turned off]. The animals were sedated and intubated with spontaneous breathing supplemented by oxygen, for the MRI examinations. The animals received a ketamine/propofol drip to maintain anesthesia as approved by the IACUC. The animals were continuously monitored by a porcine experienced anesthesia crew and then recovered after each MRI examination.

The animals received a comprehensive cardiac electrographically-gated MRI examination that included, sagittal steady state free precession (SSFP) scout, parallel imaging calibration, short axis cine SSFP, radial long axis cine SSFP, T2-weighted fast spin echo (T2W-FSE) with fat suppression, T_1 mapping , cine inversion recovery (cine IR), and inversion-

recovery (IR) MDE pulse sequences. For the contrast administration, a standard 0.2 mmol/kg dose of Gd-chelate contrast agent (Gadoteridol, Bracco, Princeton, NJ) was administered intravenously. The MDE pulse sequences were acquired between 6 and 12 minutes post-contrast with the optimal inversion time for each MDE determined from the cine IR sequence performed prior to the respective MDE sequence. MDE sequences consisted of a fast gradient echo pulse sequence with an IR prep pulse and the following parameters: 20^0 flip angle, 31 cm field of view, ± 31.25 receive band width, 1 R-R interval, 10 views per segment, 8 mm slice thickness and 256x160 image matrix.

For T_1 mapping, a modified Look-Locker with saturation recovery SSFP sequence (24) with three Look-Locker imaging blocks (2, 2, and 6 heartbeats respectively) was used. The sequences starts with an initial inversion time (TI) of 50 ms, then adds TI increments of 40 ms (e.g. if the heart rate is 100 bpm (RR interval is 600 ms), thus acquiring ten TIs of 50, 90, 130, 650, 690, 730, 1330, 1930, 2530, and 3130 ms (50, 50+40, 50+2x40, 50+RR, 50+40+RR, 50+40x2 +RR, 50+40x2 +2xRR, 50+40x2 +3xRR,50+40x2 +4xRR, 50+40x2 +5xRR)). SSFP imaging was performed at each of the TI times with the following parameters: 1.9 ms echo time (TE), 4.3 ms repetition time (TR), 45° flip angle, 256x160 matrix, 3 signal averages, 20 views per segment, 8mm slice thickness, with 1-2 min free breathing scan duration. T_1 mapping sequences were performed before contrast, as well as at 5 minutes after contrast administration.

MRI Image Analysis

Ejection fraction was calculated using the short axis cine SSFP images with IDL volume analysis software (Cine Tool, GE Healthcare, Niskayuna, NY). The MDE images were read and

graded by an experienced cardiac MRI Radiologist. T_1 mapping images were also analyzed using an in-house specially designed IDL-based analysis software program using two methods. The myocardium was analyzed selecting the entire myocardial region to assess for global T_1 values. The second set divided the myocardium into six segments starting at the mid septal wall so that the septal wall could be compared to the free wall of the left ventricle (Figure 1).

Tissue Handling and Histology

Immediately post-euthanasia, myocardial tissue was collected from septum and left ventricular free wall and then was immediately fixed in 10% buffered formalin. The tissue was later embedded in paraffin and cut into 5 µm sections using standard methods. Slides were stained using Accustain Masson Trichrome Blue. Ten images per tissue slide were captured with a Nikon Eclipse 80i microscope with digital DXM 12000c camera (Nikon Instruments Inc., Melville, New York), and analyzed using an IDL program written to separate image intensity levels to calculate area quantification. Histological images were analyzed three times with the image measurement averaged in order to reduce observer error. Areas of tissue with blood vessels were excluded from the image analysis.

Data Analysis

Descriptive statistics were performed using SPSS Statistical Software, v 16, (SPSS Inc., Chicago, IL). The MRI T_1 Mapping data was analyzed using dependent T-test, using a two-tailed significance level of 0.05. The histology data was analyzed using independent T-tests with a two-tailed significance level of 0.05.

Results

The MRI studies in the heart failure swine demonstrated a significant difference at heart failure in several structural and functional parameters (Table1). Short axis cine SSFP images clearly show a difference in ventricular dimension (Figure 2), with the animals in heart failure having a much wider end diastolic left ventricular dimension (52 mm at heart failure; 34.7 mm at baseline) and end systolic left ventricular dimension (46.6 mm at heart failure; 26.3 mm at baseline), larger left ventricular volumes (end-diastole: 127.4 cc at heart failure; 47.6 cc at baseline; end-systole: 108.8 cc at heart failure; 25.6 cc at baseline) and thinner end systolic wall thickness (5.75 mm at heart failure; 7.41 mm at baseline). Baseline average left ventricular ejection fraction was 46 ± 3% at baseline and 15 ±7% at heart failure. On both T2W FSE and MDE imaging, the left ventricular myocardium had a normal appearance on all baseline and all heart failure MRI studies.

An example of the T_1 color map and the T_1 mapping decay curve generated by the computer analysis software is shown in Figure 3. The mean T_1 value for the pre-contrast T_1 mapping sequence was 960 ±96 ms at baseline; and for the swine at heart failure, 726 ±94 ms, (Paired t-test, N= 6, p = .020)(Table 2). The 5 min post contrast T_1 value for the pigs at baseline was 546 ±180 ms; and 300 ±171 ms, at heart failure (p = .005). There were no differences found in T_1 mapping values between the left ventricular anteroseptal and inferolateral walls. Grouped mean differences are shown on a graph in Figure 4. The swine at heart failure had significant and equivalent declines in T_1 values prior to contrast administration as well as with both time points after the use of a Gd-chelate contrast agent. Individual animal results are

depicted in Figures 5 and 6. All heart failure animals exhibited at least some decline in T_1 value at the heart failure end point.

Histological analysis using Trichrome Blue staining was performed in left ventricular anteroseptal and inferolateral myocardial tissue from both the control group and heart failure group. Slides from the left ventricular anteroseptal and the inferolateral walls showed an average of 5.4 ± 1.0% of collagen calculated for the control animals (N = 6) and 9.4 ± 1.6% ($p < .001$) for the heart failure animals (Figure 7). A scatter graph showing the collagen percentages of heart failure and control animals is shown on Figure 8. All of the heart failure animals have a higher percentage of collagen than the control animals.

Discussion

This study supports previous observation (23, 25) that T_1 mapping can quantify myocardial changes in heart failure. The tachycardia-induced swine model produces a classic model of dilated cardiomyopathy hallmarked by a reduction in left ventricular ejection fraction and enlargement of the left ventricular chamber. In this study, the T_1 mapping MRI technique demonstrated a measurable and significant decrease in T_1 value between normal baseline myocardium and myocardium at heart failure. T_1 values measured by MRI were significant and similarly reduced with or without Gd-chelate contrast agent potentially obviating the need for the use of Gd-chelate contrast agents which has benefits in terms of cost, scan time and overall patient safety. Interestingly, the T_1 value changes in this model were acquired during tidal respiration (Controls Post: 546 ±180 ms, Failure Post T_1: 300 ±171 ms, Mean collagen: 9.6 ±1.5%), but are consistent with the values described for breath hold studies performed in a

patient population by Iles, et al (2008) (23) (Controls Post T_1 mean: 564 ±23ms, Failure Post- T_1 mean: 383 ±17ms, Mean collagen: 8.4 ±4.3%) , and by Messroghli, et al (2006)(25) in an healthy volunteers (Messroghli: normal human T_1 Pre contrast: 982 ±46ms vs. Pre contrast Control Pigs: 960 ±96 ms) (Table 3).

The changes in myocardial T_1 values measured on MRI appear to reflect an increase in collagen (fibrosis) in the heart failure myocardium as compared to control animals as supported by histological evidence. There was no overlap in percentage of collagen between control animals and heart failure animals (Figure 8). All of the heart failure animals had a higher percentage of collagen (9.4% vs. 5.4%, $p< .001$)) which is consistent with the lower baseline T_1 values found on MRI in the heart failure group. Unfortunately, the sample size is too small to properly correlate the percentage of collagen with T_1 mapping value. It is hoped that with continued research, T_1 values can be correlate with the percentage of collagen within the myocardium in order to better stratify the degree of heart failure and potentially improve clinical decision making, especially by providing improved risk assessment for risk of sudden cardiac death.

The free (spontaneously)-breathing T_1 mapping technique allows for a wider population to be imaged versus other proposed methods which require breath-holding. Many people with moderate to severe heart failure, or those with concomitant conditions cannot hold their breath reliably, making any type of breath hold MRI technique problematic or even unobtainable. Current breath hold MDE pulse sequences often have problems with artifacts when patients cannot hold their breath adequately or if they have arrhythmias – two conditions very ill patients often have. In addition, MDE sequences require the addition of a Gd-chelate

contrast agent to be used. As shown in this study, the T_1 mapping technique can provide meaningful T_1 data without the use of a Gd-chelate contrast agent. Moreover, the T_1 mapping values are valid using the T_1 mapping technique after the administration of Gd-chelate contrast media. T_1 mapping may provide an additional MRI tool for myocardial assessment if MDE pulse sequences are suboptimal or non-diagnostic.

One other important aspect of the T_1 mapping technique is that changes were noted at heart failure but other standard myocardial techniques of T2W FSE and MDE revealed normal myocardial signal (i.e. were negative). T_1 mapping may provide even earlier detection of myocardial abnormalities than current traditional MRI techniques, offering a means by which earlier and potential reversible myocardial changes are identified. The central hyperenhancement (i.e. scarring or fibrosis) that is visible with the MDE technique in patients with dilated cardiomyopathy is typically found in patients with long term or late stage disease (10, 13). This degree of fibrosis may be too advanced to be able to remodel back to a more normal state if the offending stimulus is corrected. T_1 mapping has the potential to identify patients with lowering T_1 mapping values before the condition has progressed to the stage of having so much central fibrosis that it is visible with MDE.

Motion from breathing causes artifacts on the images which can introduce error into the T_1 calculations. In addition, arrhythmias common in heart failure can also introduce artifacts and blurring of the images. This study has a small sample size (N=6), so there is not enough statistical power to assess a correlation between T_1 mapping values and collagen levels. However, based on the difference in percent collagen found between the control group and heart failure group, it can be inferred that that fibrosis contributes to our observed T_1 value

differences shown by T_1 mapping between baseline and heart failure. However, more research is required to fully establish this correlation between the MRI T_1 values and the histology. The Modified Look-Locker with Saturation Recovery SSFP sequence appears to be a promising technique to identify and monitor heart failure progression in a wide population.

T_1 mapping MRI can quantify the changes in fibrosis in heart failure by measuring the T_1 value of the myocardium. We describe a free-breathing T_1 mapping sequence that has broader patient applicability for the detection of diffuse heart disease without requiring the use of a contrast agent.

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Tables

Table 1. MRI measurements obtained for the 6 paired animals. Each animal was scanned twice, once at baseline and the second time at heart failure. Dependent T-test significance level was based on a two-tailed analysis of < .05.

Parameter	Baseline	Heart Failure	Significance
Ejection fraction	46.15 ± 3%	15.55 ± 7%	.000*
LV End Diastolic Diameter	34.67 mm	52 mm	.002*
LV End Systolic Diameter	26.33 mm	46.58 mm	.002*
LV End Diastolic Volume	47.64 cc	127.44 cc	.005*
LV End Systolic Volume	25.59 cc	108.80 cc	.005*
LV Stroke Volume	22.07 cc	18.63 cc	.292
LV End Diastolic Wall Thickness	4.75 mm	4.92 mm	.638
LV End Systolic Wall Thickness	7.41 mm	5.75 mm	.001*
Heart rate	103 bpm	94 bpm	.190

Table 2. Statistical results for T_1 mapping using dependent T-tests. T_1 values are consistently lower for the failure animals. Dependent T-test significance level was based on a two-tailed analysis of < .05.

Acquisition Period	Baseline T1 Mapping	Heart Failure T1 Mapping	Significance Values
Pre Contrast	960 ±96 ms	726 ±94 ms	p = .020
5 Min Post Contrast	546 ±180 ms	300 ±171 ms	p= .005

Table 3. Comparison of T_1 values between breath-hold human studies and the animals in this study. The T_1 values obtained in the free-breathing swine model are similar to the T_1 values obtained from studies by Iles, et al (2008) (23) and by Messroghli, et al (2006)(25).

Parameter	Human Breath hold T_1 Value	Swine Free-Breathing T_1Value
Healthy Control Pre Contrast T1(25)	982 ±46ms	960 ±96 ms
Healthy Control Post Contrast T1(23)	564 ±23ms	546 ±180 ms
Heart Failure Post T1 (23)	383 ±17ms	300 ±171 ms

Figure Legends

Figure 1. Short axis 2D Cine SSFP End Systolic Images at baseline (A), and heart failure (B). The heart failure animals have a much wider LV chamber than the baseline animals. Pacemaker leads can be seen as dark deflection artifacts in the RV (thin arrows). The tip of the lead can be seen at the edge of the LV (solid arrow).

Figure 2. An example of a T_1 decay curve and T_1 map acquired in short axis. This T_1 map shows a range of values from 50-1000 ms. The myocardium of the left ventricle can be seen as a circular green object near the center of the image (arrows).

Figure 3. The T_1 values were calculated two ways. The first method was a large region of interest (ROI) drawn to encompass the entire LV myocardium (A). The second method involved drawing a new ROI and then segmenting it into six separate regions. The regions were then compared to each other and against the global measurement. There was no difference found among any of the ROIs showing not only no change between septum and free LV wall, but also consistency of the T_1 mapping calculation.

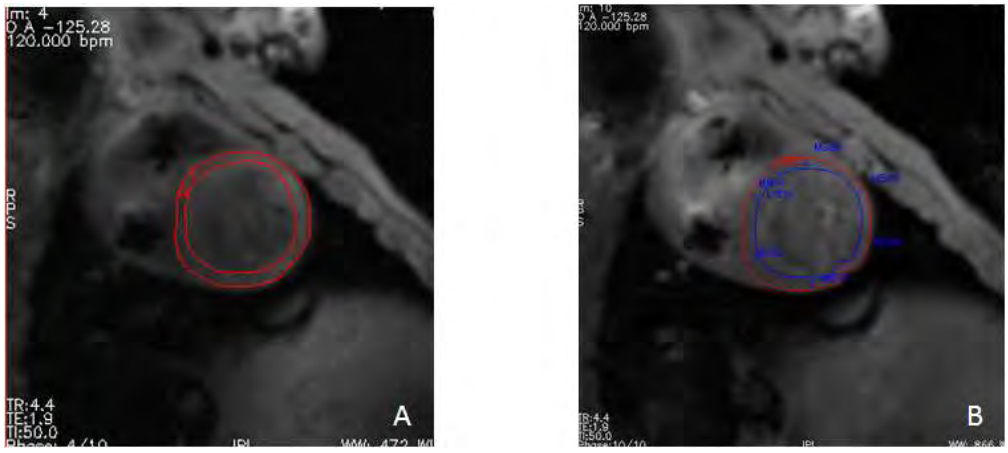
Figure 4. Aggregate T_1 mapping results. The mean baseline T_1 mapping values for the myocardium of all animals was higher at all three time points as compared to the heart failure animals. The slope of the line between pre-contrast, 5 minutes post contrast and 12 minutes post contrast as similar.

Figure 5. T_1 mapping results of individual animals without the use of a contrast agent. All of the heart failure animals exhibited a drop in T_1 value as compared to the baseline measurements (HF = 726 ± 94 ms, baseline = 960 ± 96 ms, p = .020).

Figure 6. T_1 mapping results of individual animals at 5 minutes post contrast administration. The animals received a 0.2 mmol/kg injection of a gadolinium chelate contrast agent. Similar to the pre-contrast results, all heart failure animals demonstrated a drop in T_1 value as compared to their baseline measurements. The 5 minute post values were heart failure = 300 ±171 ms, and baseline 546 ±180 ms (p = .005).

Figure 7. Myocardium stained with Masson's Trichrome Blue shown at 20x power, resolution 1.264^{e+007} px^2, 0.32 µm/px captured at 1372x1024. Myocytes are red, nuclei are black and collagen is blue. A small amount of structural collagen (thin blue lines) is seen in the control tissue (A), whereas, considerably more fibrotic (collagen) blue streaks are seen in heart failure tissue (B). Control animals (N=6) had a mean collagen percentage of 5.4%, where as the heart failure animals (N=6) had a mean collagen of 9.4%.

Figure 8. Graph showing the collagen percentage measured in each individual pig. Heart failure pigs 1-6 (red diamonds) had a range of 7.64-11.84%, and the control pigs 7-12 (green diamonds) had a collagen range of 3.56-6.23% (p < .001).



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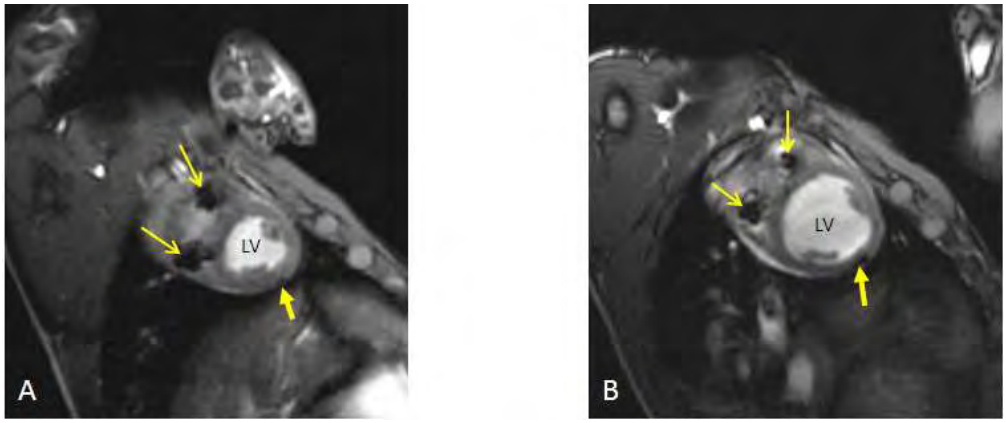

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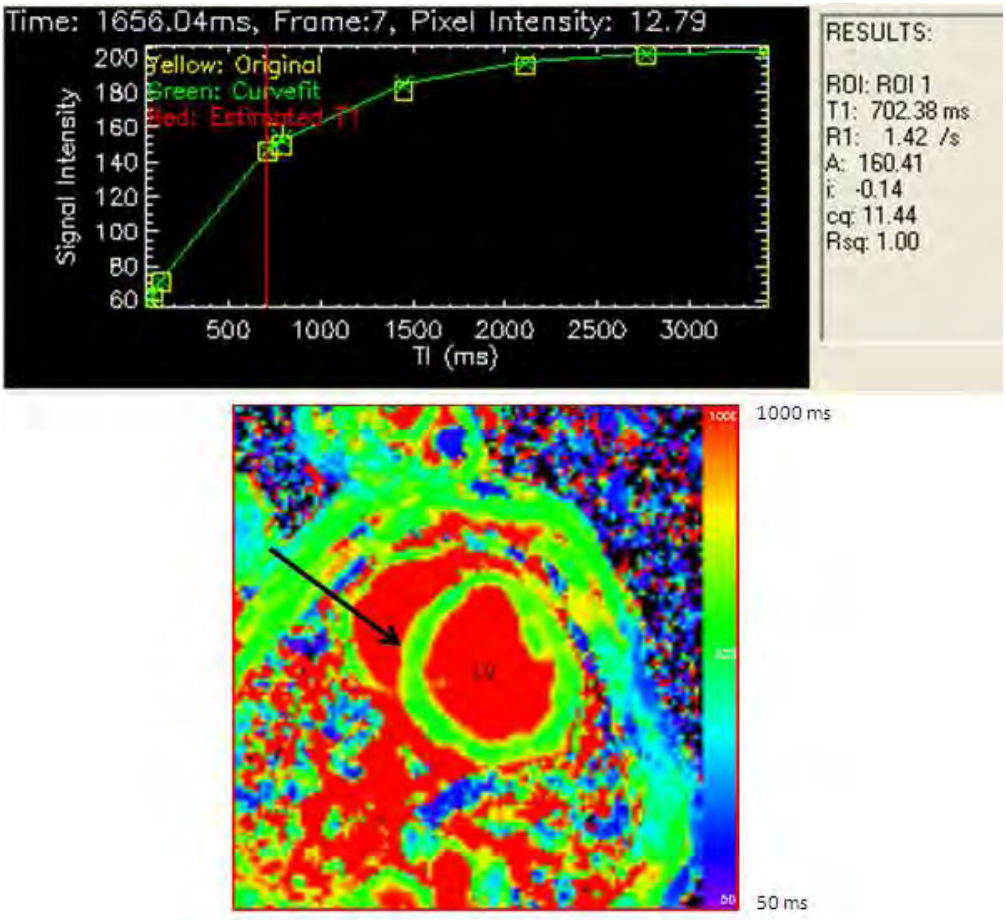

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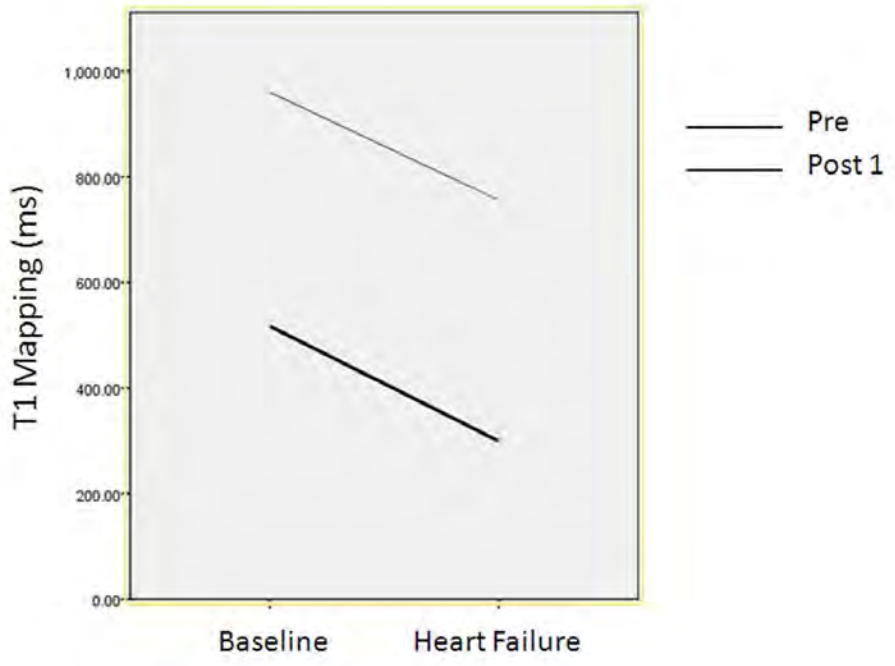

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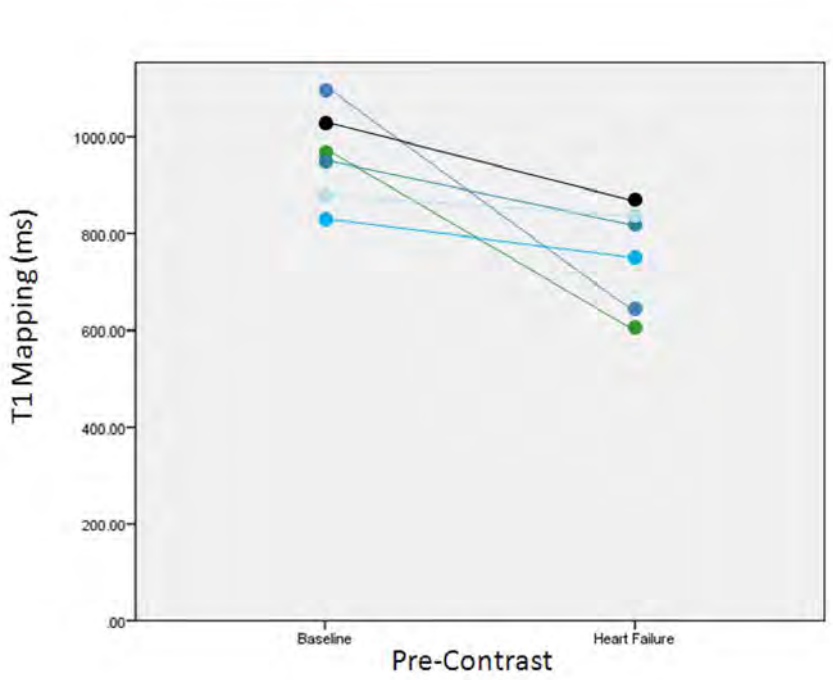

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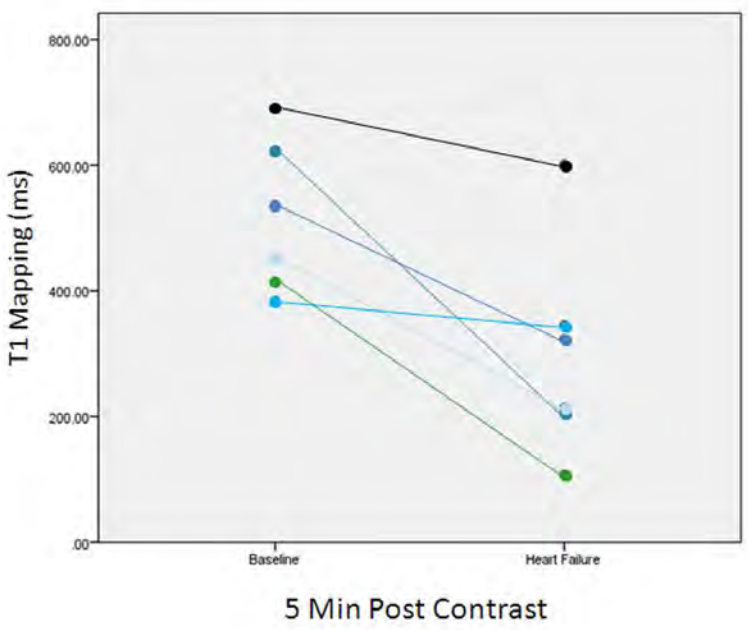

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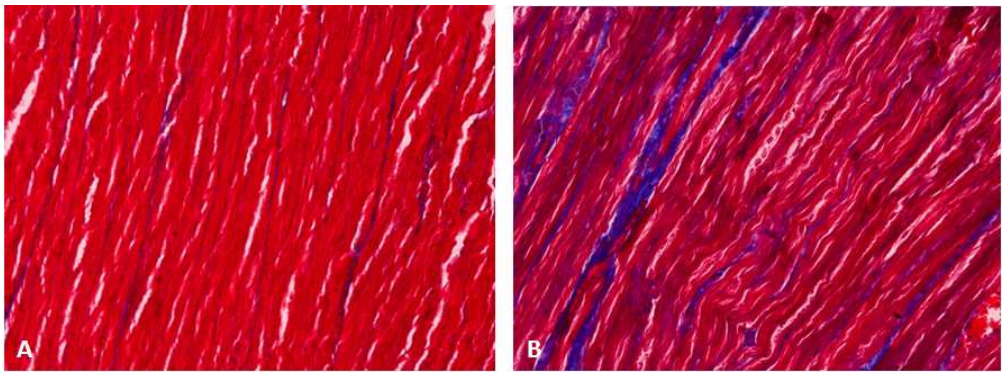

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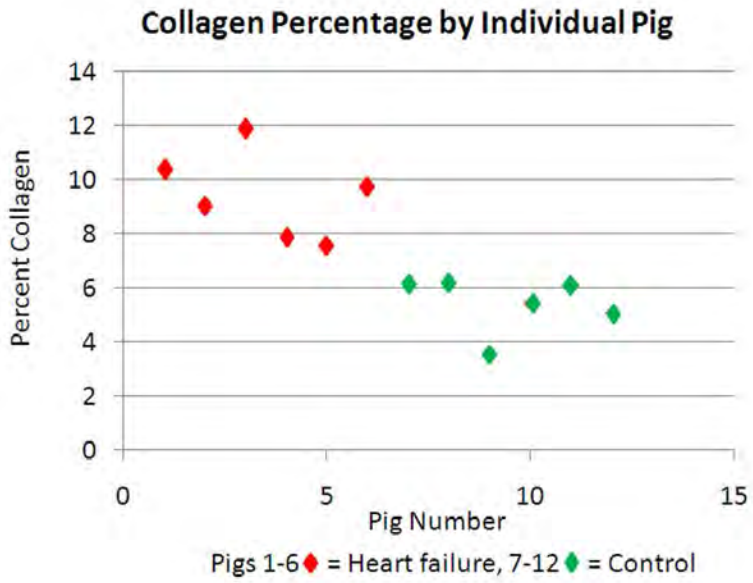

Figure 8
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Editorial Manager(tm) for Investigative Radiology
Manuscript Draft

Manuscript Number:

Title: MRI of a Swine Model of Heart Failure: Changes in Morphology, Function, Collagen and TGF β-1 Pathway Proteins

Article Type: Original Article

Keywords: Magnetic resonance imaging; heart failure; collagen; blotting, Western; Smad Proteins

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Abstract: Objectives: The functional and morphologic changes demonstrated on Magnetic Resonance Imaging (MRI) may be related to the increase in collagen Types I, III and VI in the heart as is remodels due to pathological forces such as tachycardia. The purpose of this study was to explore the changes in structure and function as seen on MRI and then compare the images to the changes in collagen between control and heart failure swine and Transforming Growth factor Beta-1 pathway proteins that are thought to be associated with fibrosis.
Materials and Methods: Yorkshire pigs (N=9) were implanted with a cardiac pacemakers and paced at 200 bpm for 3 to 6 weeks to induce heart failure. Animals were scanned using MRI twice, once at baseline and once at heart failure. Tissue from the septum and left ventricular free wall were collected from the MRI heart failure animals (N=6), and other heart failure animals (N=4) upon sacrifice, and also from control animals (N=10). Immunohistochemistry of collagens type I, III, and VI were performed with control and heart failure tissue. Western Blot analysis of Transforming Growth Factor Beta-1 Receptor, Smad2, Smad 3 and Smad 7 were completed. The bench science results were compared to the MRI findings.
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Suggested Reviewers: Michael Knopp MD
Radiology, Ohio State University
knopp.16@osu.edu
Dr. Knopp is a well respected Radiologist in the field of MRI and he is very experiences in scientific research. I feel he would give a fair review.

Opposed Reviewers:

Cover Letter

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6 March 2011

Dr. Val Runge, Editor
c/o Karen Harmon, Managing Editor
Lippincott Williams & Wilkins
1803 Research Blvd, Suite 300
Rockville, MD 20850

RE: Manuscript
Title: "MRI of a Swine Model of Heart Failure: Changes in Morphology, Function, Collagen and TGF β-1 pathway proteins"

Dear Dr. Runge,

Enclosed is my manuscript entitled "MRI of a Swine Model of Heart Failure: Changes in Morphology, Function, Collagen and TGF β-1 pathway proteins." I've been working on this research with my dissertation committee as a part of my doctoral thesis. We feel this manuscript would fit in well in Investigative Radiology as your journal strives for publishing not only imaging research, but also multidisciplinary research that involves imaging. I look forward to hearing comments from you and your reviewers regarding this manuscript.

Thank you for reviewing this manuscript.

Best Regards,

Maureen N. Hood, MS, RN

*Title Page

MRI of a Swine Model of Heart Failure:

Changes in Morphology, Function, Collagen and TGF β-1 Pathway Proteins

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Disclosures

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Changes in Morphology, Function, Collagen and TGF β-1 Pathway Proteins

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Conclusions: This MRI portion of this study found significant changes in the morphology and function of the heart after just 3 to 6 weeks of tachycardia. Western blots revealed increased trends in the fibrotic pathway proteins TGFBR1, Smad 2, Smad 3, and Smad 7. There was also an increase in collagen types I, III and VI between control tissue samples and heart failure tissue samples. The contractile dysfunction demonstrated on MRI is consistent with altered expression of collagen and TGFBR1, Smad 2, Smad 3 and Smad 7 ECM proteins in heart failure animals. More research needs to be done to elucidate the mechanisms involving the proteins associated with increased fibrosis in heart failure.

Key words: Magnetic resonance imaging; heart failure; collagen; blotting, Western; Smad Proteins

Introduction

Heart failure is one of the most common chronic conditions in the United States and can result from a number of causes (1). Changes in cardiac contractility occur during heart failure as the heart has been assaulted by some sort of change in preload filling or ventricular volume and as a result, the heart adapts to try to account for changes in altered function. The Frank-Starling principle describes a relationship between preload, and the corresponding cardiac contractility which is generally related to the fiber length of the myocardial muscle fibers (2). The heart will continue to try to respond to increasingly higher left ventricular volumes until it reaches a point in which the muscle fibers cannot stretch any further. Once this upper threshold of muscle fiber length has been reached, function starts to decline as the myocardium can only sustain this maximum level of ventricular tension for a short period of time.

During prolonged tachycardia, the heart may not be able to fully supply the myocardium with the nutrients and oxygen needed to supply the myocytes with the energy needed to compensate for the increased physical stress (2, 3). Besides alterations in contractile and cytoskeletal proteins, the extracellular matrix (ECM) surrounding the cardiomyocytes also responds to changing mechanics. The ECM is a complex, highly ordered array of proteins, proteoglycans, and other supportive material that provide a fibrous network with biologically active molecules that work in conjunction with the myocytes to provide the overall structure and function of the heart(4). In recent years, the ECM has become an increased area of research as researchers try to better understand the pathological fibrosis that occurs in heart

failure. Many of the mechanisms and features related to these changes in fibrosis (myocardial remodeling) are still uncertain (5, 6).

There are a number of factors related to the remodeling process of the myocardium. The Transforming Growth Factor (TGF) Superfamily is well known to regulate a wide array of developmental and repair pathways in the ECM. In the heart, TGF-beta (β) is a regulator of cellular processes. It has been found to be associated with the fibrotic remodeling process (7, 8). The TGF-β receptor, when phosphorylated, signals the Small Mother Against Decapentaplegic (Smad) mediators (9). The receptor (R) Smads (Smads 1, 2, 3, 5, 8) receive the signal from TGF-β receptor through phosphorylation. This phosphorylation can cause the R-Smads to accumulate along with co-Smad 4. Together R-Smads and co-Smads can mediate various gene transcriptional activators or repressors, causing either up or down regulation of gene expression (9). Smads 6 and 7 are inhibitory Smads. They counter the TGF- β pathway signaling by interfering with the R-Smads (10). More specifically in the heart, when the TGF- β1 receptor (TGFBR1) is phosphorylated, TGFBR1 then signals R-Smads 2 and 3. Smads 2, 3 then work with co-Smad 4 to either up or down regulate genes, some of which are responsible for making extracellular matrix components such as collagen. Lastly, the inhibitory Smad 7 is thought to be an active protein that can interfere with the TGF- β pathway (11). Thus Smad 7 may be an important factor in the regulation of fibrosis.

The main fibrous proteins supporting the function of the left ventricle are the fibrillar collagens type I and type III (4). Collagen is a triple helical protein structure that can have very strong tensile strength through its characteristic cross-links with neighboring fibrils every 67 nm

(12). Type I collagen (molecular formula $\alpha1(I)_2\alpha2$ (I)) has exceptionally high tensile strength, and has been associated with myocardial stiffness (13-14). Type III collagen (molecular formula $\alpha1(III)_3$) is much thinner than type I collagen and has elastic type characteristics (15). Collagen has been found to increase in patients with ischemic heart failure, with type III collagen being the primary collagen that increases (16), lowering the ratio of type I to type III in the myocardium. Mukherjee and Sen (1991)(16) suggest that the increase in type III collagen and the lowering of the type I:III may play a role in myocardial dysfunction, but the functional relationship between type I and type III collagen and how this effects myocardial function are not completely understood.

The use of *in vitro* methods to determine altered ratios of ECM in patient populations of dilated cardiomyopathy is no usually possible; therefore, less invasive methods would be highly useful. Advances in magnetic resonance imaging (MRI) technology permits imaging of the structure, function and tissue characteristics of the heart *in situ*. In this project, a tachycardia-induced swine model was chosen to simulate dilated cardiomyopathy (DCM). MRI was used as the clinical imaging to demonstrate the differences in cardiac morphology and function between baseline and heart failure. Our hypothesis is that a change in the cardiac structure and function demonstrated by MRI will result in changes in the ECM. This project explores part of the TGF-β pathway along with collagen I, III and VI in the ECM of a tachycardia-induced swine model of heart failure. It is expected that the dysfunction demonstrated on MRI will be consistent with changes in collagen and TGFBR1, Smad 2, Smad 3 and Smad 7 ECM proteins between control and heart failure animals.

Methods

Tachycardia-induced Yorkshire swine were selected as the model of heart failure. Pig heart structure and function are remarkably similar to human hearts and therefore make adequate models for clinical study (17). Nine animals were included in the MRI portion of this study. Additional heart failure and control animals were also incorporated into this study to give a minimum of 10 heart failure and 10 control animals for protein and immunohistochemical comparisons.

Magnetic Resonance Imaging

After obtaining Institutional Animal Care and Use Committee (IACUC) approval, Yorkshire swine (N = 9) were implanted with pacemakers and paced for 3 to 5 weeks at 200 beats per minute. Each animal was scanned in a 1.5 T MRI scanner (GE Signa, Waukesha, WI) at baseline (non-paced pacemaker in situ) and then at heart failure (N = 6) (confirmed by echocardiography as half the fractional shortening as compared to baseline (from approximately 32 at baseline to 16 percent at failure), with pacer turned off). The animals were sedated and intubated for the MRI examinations. Animals were given oxygen, but were breathing spontaneously during the procedure. The animals received an intravenous ketamine/propofol drip to maintain anesthesia as approved by the IACUC. The animals were fully monitored by a porcine experienced anesthesia crew and then recovered after each MRI.

The animals received a comprehensive electrographically-gated cardiac MRI examination that included, short axis and long axis steady state free precession (SSFP), T2-weighted fast spin echo (FSE) with fat suppression, , cine inversion recovery (IR), 2D Inversion

Recovery (IR) Myocardial Delayed Enhancement (MDE) sequences. For the contrast administration, a standard 0.2 mmol/kg dose of gadolinium (Gd)-chelate contrast agent (Gadoteridol, Bracco, Milan, Italy) was given at a rate of 1 mL/sec. The MDE sequences were acquired between 6 and 12 minutes post-contrast with the optimal inversion time for each MDE determined from the cine IR sequence performed prior to the respective MDE sequence. MDE sequences used a Fast GRE sequence with an IR prep pulse, 20^0 flip angle, 31 cm field of view, ± 31.25 receive bandwidth, 1 R-R interval, 10 views per segment, 8 mm Slice thickness and 256x160 image matrix.

MRI Image Analysis

Ejection fraction was calculated using the short axis cine SSFP images with IDL volume analysis software (Cine Tool, GE Healthcare, Niskayuna, NY). The MDE images were read and graded by an experienced cardiac MRI Radiologist.

Western Blots

Myocardial tissue was acquired from the swine for use in laboratory analyses. Immediately post-euthanasia, tissue was cut from the left ventricular free wall and immediately immersed in liquid nitrogen. Frozen sections were stored at -80 centigrade. Protein extraction was performed on the heart failure (N = 10) and control (N = 10) samples using a single batch of lysing solution. Protein concentration was determined using the Bradford Assay method (Bradford 1976). Equal amounts of proteins were then loaded (20 µL/lane) for the heart failure and control samples. Samples were separated using 10% polyacrylamide gels (Invitrogen, Carlsbad, CA) and transferred using nitrocellulose membranes (Invitrogen, Carlsbad, CA). The

membranes were blocked in Odyssey Blocking Buffer solution (Li-cor Biosciences, Lincoln, NE) for 1 hour on a rotary shaker. Primary antibodies were incubated on a shaker overnight at 4 degrees Celsius (Anti-TGF Beta Receptor Type 1 at 1:250, Millipore (Billerica, MA); Smad 2/3 Antibody at 1:250), Cell Signaling (Danvers, MA); MADH7 Antibody at 1:500, abCam (Cambridge, MA); and MMP14 antibody at 1:500, abCam (Cambridge, MA)). The membranes were washed in phosphate buffered saline (PBS) with 0.1% Tween 4 times and then a conjugated secondary antibody (Odyssey Donkey anti-Mouse 800, or Odyssey Goat anti Rabbit, 680, Li-cor Biosciences, Lincoln, NE) (1:10,000) was applied for 1 hour. The membranes were washed 4 times again before signal visualization with an Odyssey-Infrared Imaging System (Li-cor Biosciences, Lincoln, NE).

Immunohistochemistry

Myocardial tissue was excised from septum and left ventricular free wall for histology and then was immediately fixed in 10% buffered formalin. The tissue was later embedded in paraffin and cut into 5 µm sections using standard methods. Slides were deparaffinized prior to immunohisotchemistry (IHC). The slides were then incubated in PBS with 0.6% Triton X-100 for one hour, followed by a one hour incubation period in 50 mM ammonium chloride to help reduce endogenous fluorescence. The tissue was rinsed three times in PBS and then blocked with 0.6% Triton X-100 and 10% normal goat serum. The tissue sections were then incubated with primary antibodies (Collagen I Antibody, (Abcam, Cambridge, MA), 1:100; Collagen III Antibody, (Abcam, Cambridge, MA), 1:100; Collagen VI Antibody, (Abcam, Cambridge, MA) 1:100), for 48 hours at 4 degrees Celsius. The tissue was washed in PBS three times and then

incubated with secondary antibodies (Alex Fluor Goat Anti Mouse IgG and/or Alex Fluor Goat Anti Rabbit IgG, (Invitrogen, Carlsbad, CA), 1:100) for 2 hours at room temperature. The tissue slides were again washed in PBS and then stained with DAPI (DAPI Nucleic Acid Stain, (Invitrogen, Carlsbad, CA) 1:1000) for 2 minutes. The tissue slides were washed in PBS one last time and then cover slipped with CC/Mount, (Sigma-Aldrich Corp, St. Louis, MO). After the slides dried for 24 hour hours, the images were then photographed using a Nikon Eclipse 80i microscope with digital DXM 12000c camera and analyzed using Nikon NIS Elements SW, vs. 3.1 (Nikon Instruments Inc., Melville, New York).

Results

The MRI results from the swine demonstrated a significant difference in several indicators of heart structure and function (Table 1, Figure 1), demonstrating that the tachycardic pacing of the animals provided a sufficient model of heart failure (dilated cardiomyopathy). Baseline ejection fractions were 46.6 ± 1.1% Standard Error of the Mean (SEM) (N = 9) and for the heart failure group, 15.6 ± 2.7% SEM (N = 6). In addition, key functional parameters such as end systolic and end diastolic volumes were significantly larger at heart failure (127.4 mL), than at baseline (48.4 mL). On T2-weighted fast spin echo and MDE imaging, the left ventricular myocardium appeared normal on all MRI exams performed at baseline and at heart failure (Figure 2).

Western Blots assessed the protein levels of several proteins related to the TGF-β1 pathway using an infrared imaging system with computerized quantification (Li-cor Biosciences, Lincoln, NE). TGFBR1 (Figure 3) and the receptors Smad 2 and Smad 3 (Figure 4) showed a

slight increase in the heart failure animals as compared to the control animals. In addition, inhibitory Smad 7 (Figure 5) showed a slight increase in protein concentration in the heart failure animals as compared to controls. No drift in protein weight in the control group of the heart failure group was noted.

Immunohistochemistry of the myocardial tissue sections demonstrated an increase in all collagens between the control and heart failure animals. The control tissue shows a small percentage of collagen, whereas the heart failure tissue shows a visible increase in Types I, III and VI (Figures 6 and 7). A comparison of the ratio between Type I and Type III collagen obtained by double staining shows not only an increase in both collagens, but an increase in Type III collagen in comparison to the increase in Type I collagen (Figure 6).

Discussion

MRI has been shown to reliably demonstrate myocardial morphology, function, blood flow, myocardial perfusion, and myocardial injury or infiltrative/fibrotic changes non-invasively during a single session (18). Cardiac MRI is the single modality that can offer a comprehensive set of tools to evaluate and monitor the heart failure patient. Cine steady state free precession (SSFP) “bright blood” images provide structural and function images in any plane needed. Cine SSFP can assess for wall motion abnormalities, thickness of myocardium, volume size, ejection fraction, valvular disorders, and global function (19-28). Measurement of wall thickness and movement during contraction and relaxation help to determine the functional aspects of the heart, which is another advantage of using the SSFP sequence for evaluation of the heart.

T2 weighted fast spin echo (FSE) with fat saturation sequences are able to help assess for areas of acute infection and lesions that are high in water content. T2 FSE with fat saturation in theory can discern a difference in the left ventricular myocardium when compared before and after heart failure induction as areas that have in increase in water or focal fibrosis will have a higher signal, however, in this study, there was no discernable difference between baseline and heart failure which probably are related to either the global nature of the change, the lack of sufficient contrast-to-noise and/or spatial resolution of current MRI techniques.

The finding of hyper-enhancement within the wall of the myocardium for patients with dilated cardiomyopathy can be significant and have an impact on the course of their care. A study by Nazarian, Bleumke, and Lardo, et al (2005)(29), found that those patients with 26% to 75% thickness of mid-wall enhancement (scar) were at significantly greater risk of inducible ventricular tachycardia (VT). Although this is only one study, it is consistent with the coronary artery disease study by Bello, Fieno, and Kim , et al (2005)(30), that found infarct size based on MDE cardiac MRI measurements to be a stronger predictor of the risk of VT than left ventricular ejection fraction. Non-sustained VT is associated with an increased risk of mortality from sudden death (31). In addition, it is generally accepted that persons with dilated cardiomyopathy are at risk for sudden death (32). The animals in this study clearly have an increase in fibrosis as demonstrated by the IHC, but did not have enough fibrosis or any focal fibrosis to be able to be found with MDE imaging. This clearly shows a weakness in the current commercially available T2-weighted FSE and MDE for identification of myocardial tissue changes.

Although MDE and T2-weighted sequences in MRI could not see the fibrotic changes, MRI did find a number of morphological and functional changes that can be seen in the tissue with histological and protein analysis procedures. The collagen matrix in the heart is a complex weave of collagen strands and cross-linking fibers (33). In the normal heart, Type I collagen makes up the primary strands and cross linkages. The normal heart contains a small percentage of collagen as collagen is an important part of the supporting structure for the cardiomyocytes. In conditions such as dilated cardiomyopathy and cardiomegaly, the thinner, more elastic Type III collagen increases as supported by the IHC results in this study. Weber and colleagues (1988)(34) speculate that since the Type III collagen has a much lower tensile strength than Type I collagen, the increase in Type III collagen may allow the heart to deform over time as it cannot hold the myocytes in place as well as the Type I collagen cross linkages. The animals in this study experienced a significant widening of the left ventricular diameter, an increase in ventricular volume and a thinning of the heart wall, all parameters consistent with dilated cardiomyopathy. The greater elasticity may allow the heart to stretch, but may be to the point where collagen type I cross linkages are breaking (34-36)).

Although most of the focus on collagen in heart failure has been on Types I and III, Type VI is also a collagen that deserves attention. Collagen Type VI has large globular N- C-terminals which make the collagen have a bead-like appearance (37, 38). However, the role of Type VI collagen is not entirely clear. Naugle, et al (2005)(39) found collagen Type VI to be associated with inducing myofibrobalst differentiation in pathologic myocardial remodeling. This study also found an increase in collagen Type VI for the heart failure animals, similar to results found in a post-infarct model by Bryant and colleagues (2009)(40). It is clear from these studies that

collagen Type VI is playing a role in the pathologic remodeling process of the heart. Collagen Type VI displays a branching network on muscle tissue that intertwines with collagen fibers and is close to basement membranes (41). Bryant and colleagues report that collagen Type VI may be linked to α3 integrin receptor (40). Furthermore, Collagen Type VI plays an essential role as an ECM component of adipocytes in the fibrosis of obesity (42), and may play a similar role in the ECM of the myocardium; however, more research is needed to elucidate these connections and mechanisms.

This study found a slight increase in the TGF-β 1pathway proteins. TGFBR1 is a protein that directly activates the receptor Smads 2/3. We found that TGFBR1 was slightly elevated, but not to a significant level. However, Smads 2, 3, and 7 were all also elevated, with Smad 3 elevated to a significant level. Since Smad 7 was elevated, it may be trying to inhibit TGFBR1, acting as a negative feedback loop (43), but Smad 7 may not have been able to block the entire TGFBR1 signaling. The animals in this study developed heart failure with increased levels of fibrosis. Thus, there was enough signaling of the fibrotic pathway happening for the pathologic remodeling process to progress. Part of the remodeling may have been through the TGF-β 1pathway signaling the Smads, but some of the fibrosis may also be happening through the Bone Morphogenic Protein (BMP) pathway as well. It is clear that more research is needed to tease out the influencing factors surrounding the pathway(s) of fibrosis.

This was a small study using a model animal of heart failure and the time frame to failure was short (3 to 6 weeks). In the human experience, heart failure encompasses a wide array of causes, both acute and chronic. This model is meant to demonstrate the function and

structural difference in one type of heart failure. None the less, the tachycardic swine model was able to produce DCM and demonstrate changes in the proteins of the ECM. This study did not assess changes in the cardiomyocytes, which is another area that needs to be investigated along with the other changes in the ECM. The combination of imaging and bench science is an approach that needs more attention in the realm of clinical research.

Summary

Magnetic resonance imaging can demonstrate morphological and functional changes in a tachycardic swine model of heart failure. As tachycardia stress the heart, the myocardium remodels itself in an attempt to retain function. In this study we found increases in collagen that were not depicted onT2 weighted or myocardial delayed enhancement images during the MRI. The functional and morphologic changes demonstrated on MRI may be related to the increase in collagen Types I, II and VI. As collagen type I linkages break, and the more elastic type III collagen increase, the heart is allowed to expand and the muscle also, thus creating DCM and a decrease in cardiac function as seen on MRI. In addition, an up regulation of TGFBR1, the receptor Smads 2 and 3, and the inhibitory Smad 7 is related to increased fibrosis and were slightly up regulated in this study, but still need further investigation.

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Tables

Table 1. MRI measurements obtained for the 9 animals (Baseline N = 9, Failure N = 6). Each animal was scanned twice, once at baseline and the second time at heart failure. Dependent T-test significance level was based on a two-tailed analysis of < .025.

Parameter	Baseline	Heart Failure	Significance
Ejection fraction	46.6 ± 1.1 % SEM	15.6 ± 2.7% SEM	.000*
LV End Diastolic Diameter	34.8 mm	52.0 mm	.000*
LV End Systolic Diameter	27.1 mm	46.6 mm	.000*
LV End Diastolic Volume	48.4 cc	127.4 cc	.000*
LV End Systolic Volume	25.8 cc	108.8 cc	.000*
LV Stroke Volume	22.6 cc	18.6 cc	.211
LV End Diastolic Wall Thickness	4.8 mm	4.9 mm	.001*
LV End Systolic Wall Thickness	7.3 mm	5.8 mm	.608
Heart rate	103 bpm	95 bpm	.248

Table 2. Western Blot results of proteins comparing control animals (N = 10) to heart failure animals (N = 10). Independent T-test significance based on a two tailed result of p <.025 due to the small sample size.

Protein	Control	Failure	Std Error	Significance
TGFBR1	3.44	4.15	.83	.405
Smad2	0.38	.54	.18	.412
Smad3	0.09	0.21	.03	.002*
Smad7	0.36	0.99	.33	.065

Figure Legends

Figure 1. Short axis Steady State Free Precession images of a single animal. The baseline images showing end systole (A) and end diastole (B) show a normal left ventricular chamber. At heart failure, end systole (C) and end diastole (D) show a dilated chamber and little difference between systole and diastole.

Figure 2. Delayed enhancement images of all animals both at baseline and heart failure were negative. Short axis MDE from an animal is shown at baseline (A) and heart failure (B).

Figure 3. Western blot results for transforming growth factor beta-1 receptor (TGFBR1). TGFBR1 was found in slightly higher amounts than in control tissue samples. However, there was not a significant difference found between the two groups.

Figure4. Western blots results for the receptor Smads 2 and 3. A difference between control and heart failure tissue samples was found, but only Smad 3 showed a significant difference (*) between the two groups.

Figure5. Western blot results for Smad 7. The results show an increase in the inhibitor Smad 7 protein in the heart failure tissue, but not at a statistically significant level.

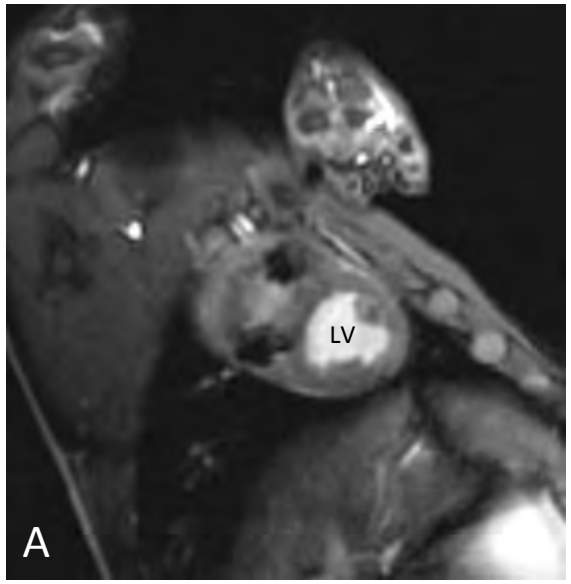
Figure6. Images of fluorescent immunohistochemistry showing control tissue (A), and heart failure tissue (B). Collagen type I is stained red, collagen type III is green and the nuclei are stained blue. These examples show that the overall amount of collagen between heart failure and control is up regulated.

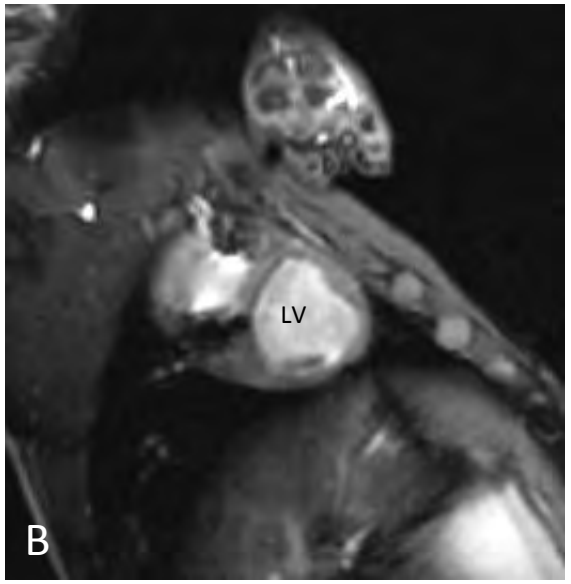
Figure 7. Fluorescent immunohistochemistry images of collagen type VI. Very little collagen VI is found in control animal tissue (A). Heart failure animal tissue had much more type VI collagen (B) as depicted in these images.

Figure (.tif, .eps, or .ppt files)

Figure 1

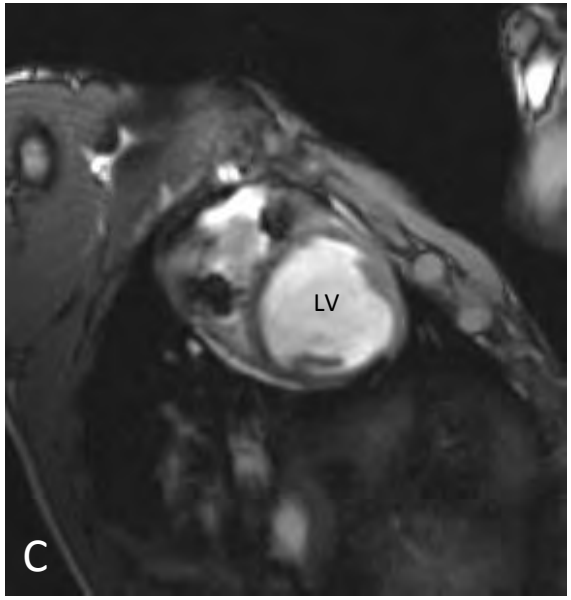
Baseline
End Systole

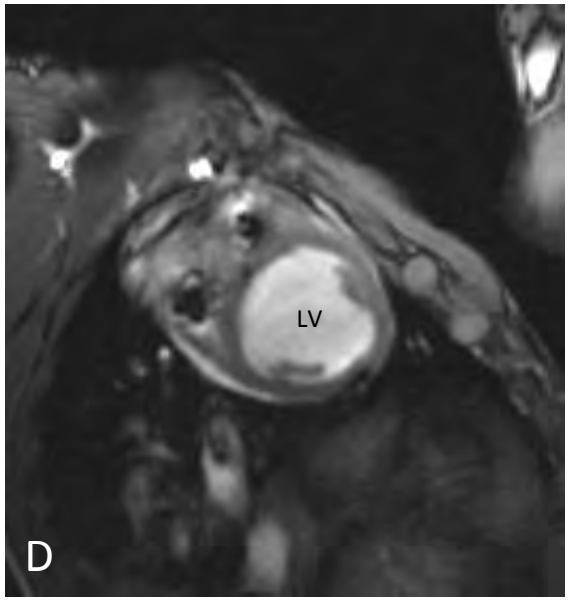




Baseline
End Diastole

Failure
End Systole

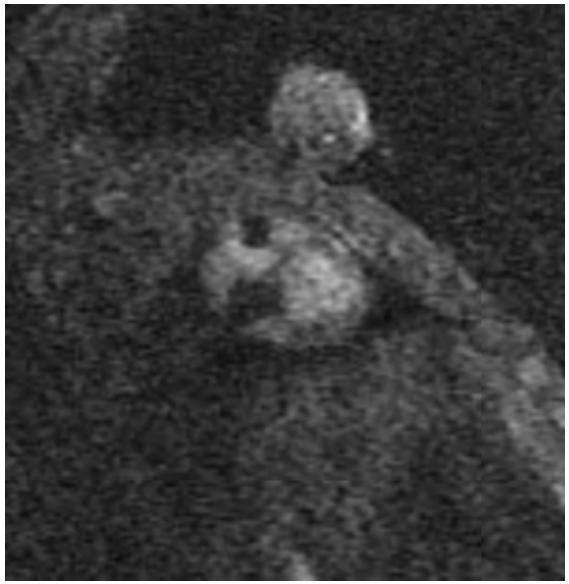




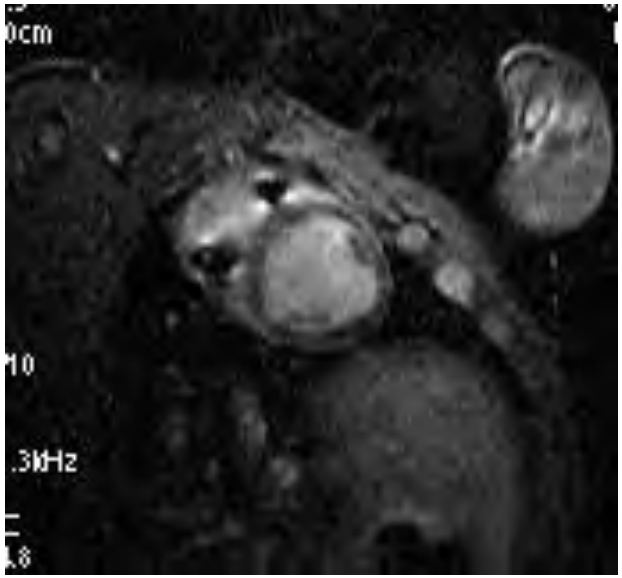
Failure
End Diastole

Figure 2

Baseline MDE

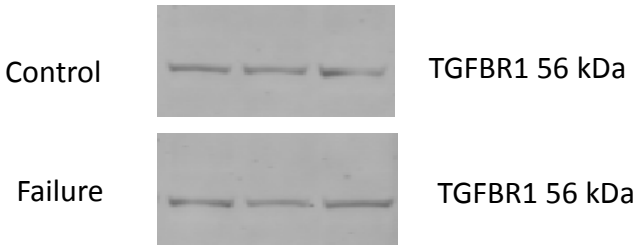


Failure MDE



Animal #8

Figure 3


Control
TGFBR1 56 kDa
Failure
TGFBR1 56 kDa

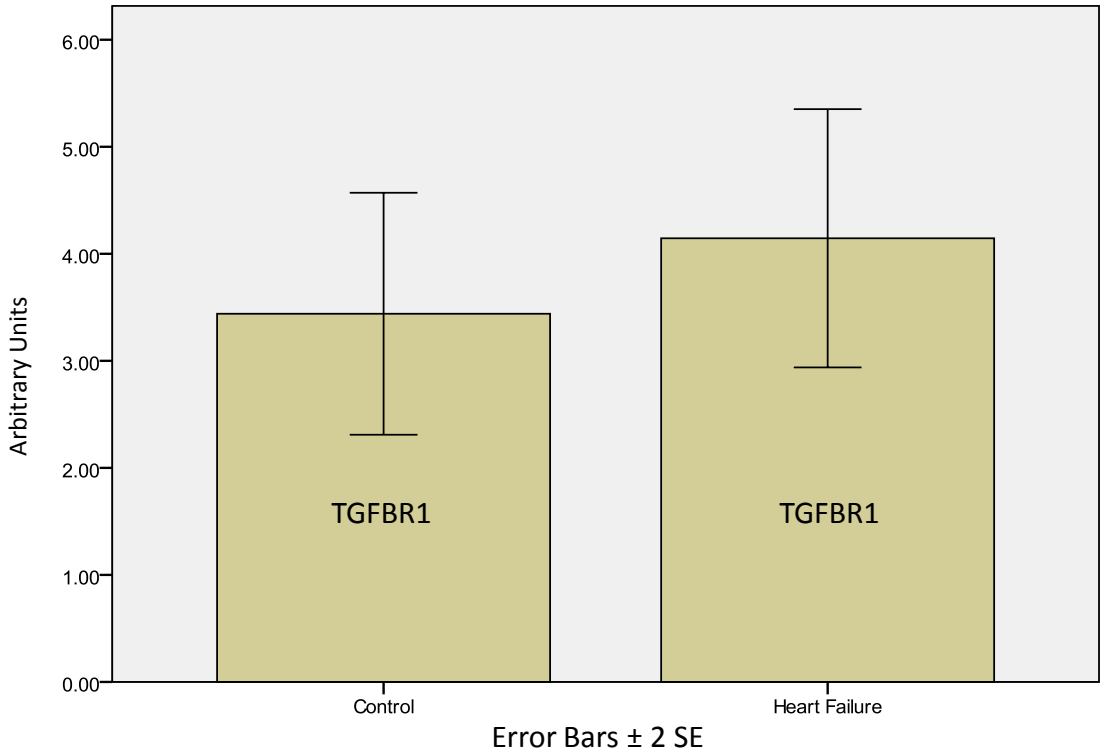
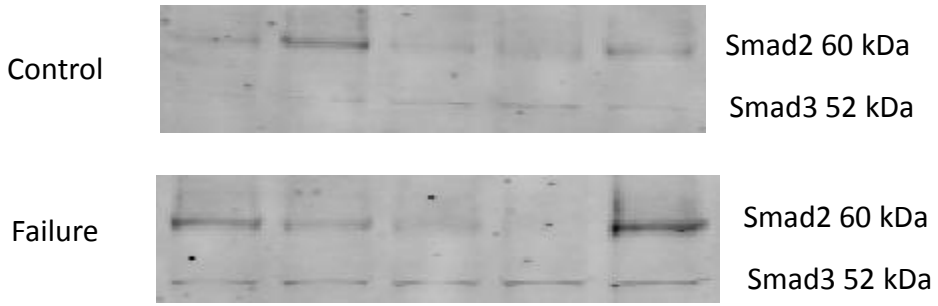
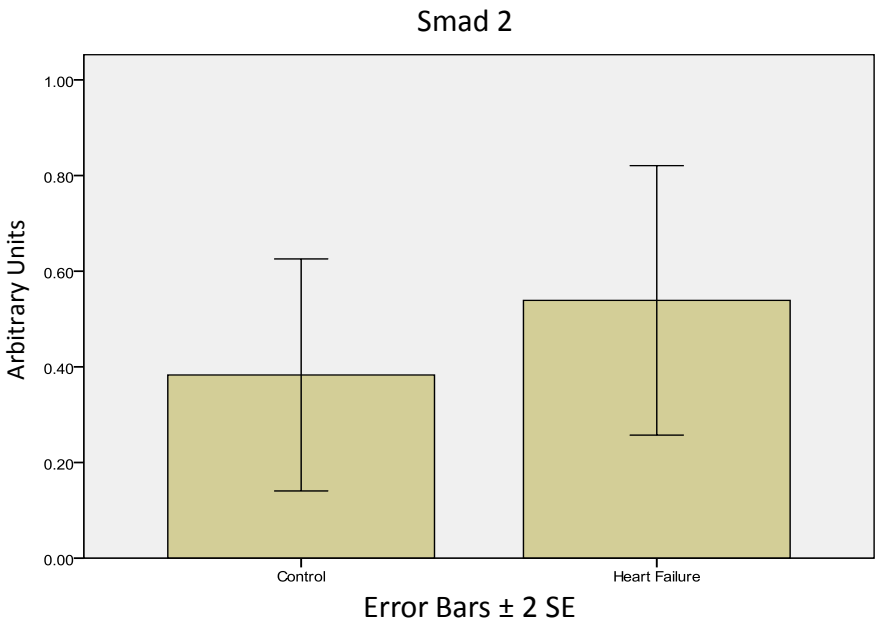

6.00
5.00
4.00
3.00
2.00
1.00
0.00
Arbitrary Units
TGFBR1
TGFBR1
Control
Heart Failure
Error Bars ± 2 SE

Figure 4


Control
Smad2 60 kDa
Smad3 52 kDa
Failure
Smad2 60 kDa
Smad3 52 kDa


Smad 2
Arbitrary Units
1.00
0.80
0.60
0.40
0.20
0.00
Control
Heart Failure
Error Bars ± 2 SE

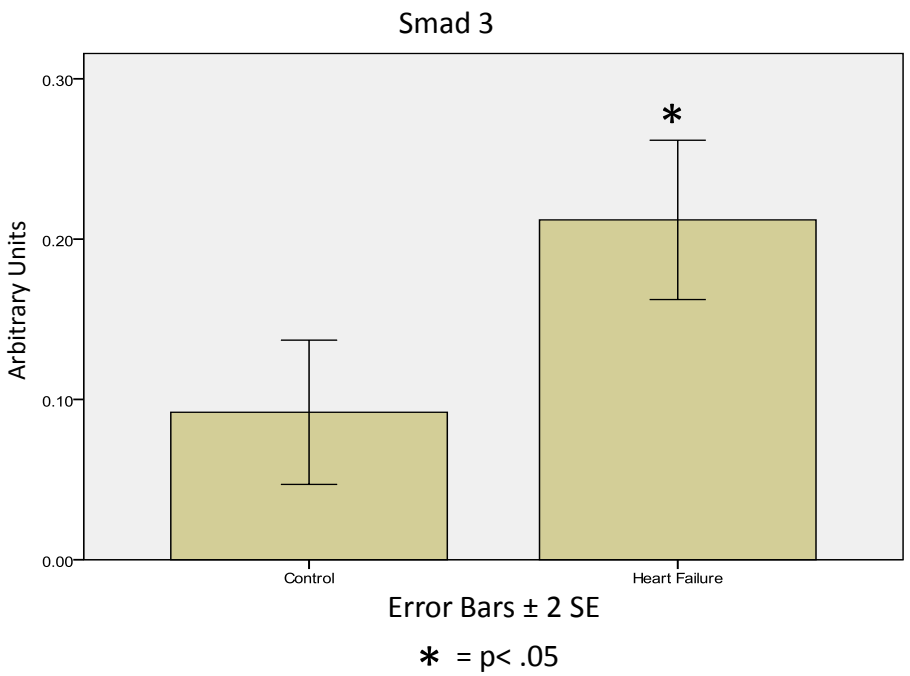

Smad 3
Arbitrary Units
0.30
0.20
0.10
0.00
*
Control
Heart Failure
Error Bars ± 2 SE
* = p< .05

Figure 5

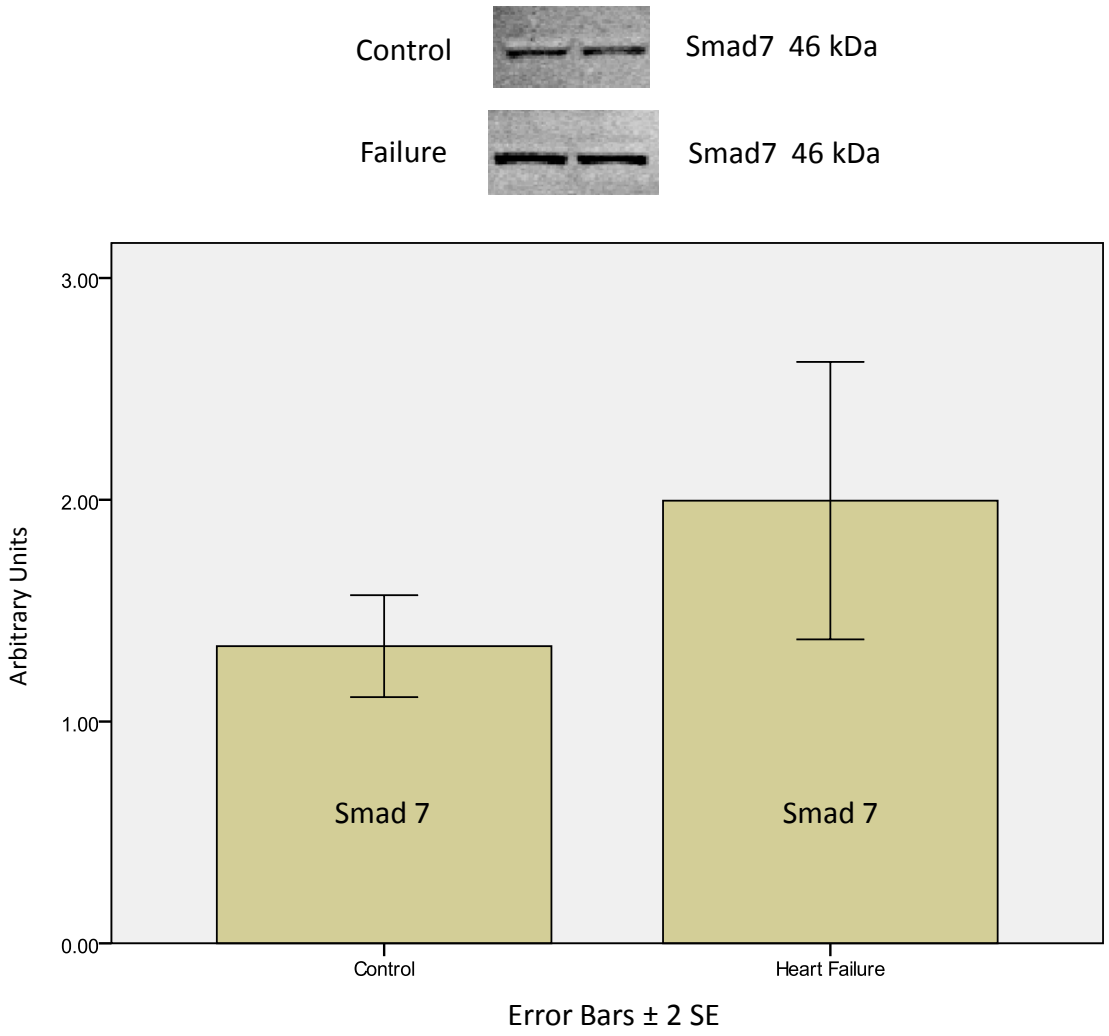

Control
Smad7 46 kDa
Failure
Smad7 46 kDa
3.00
2.00
1.00
0.00
Arbitrary Units
Smad 7
Smad 7
Control
Heart Failure
Error Bars ± 2 SE

Figure 6

Control Myocardium

60 X Power

Heart Failure Myocardium

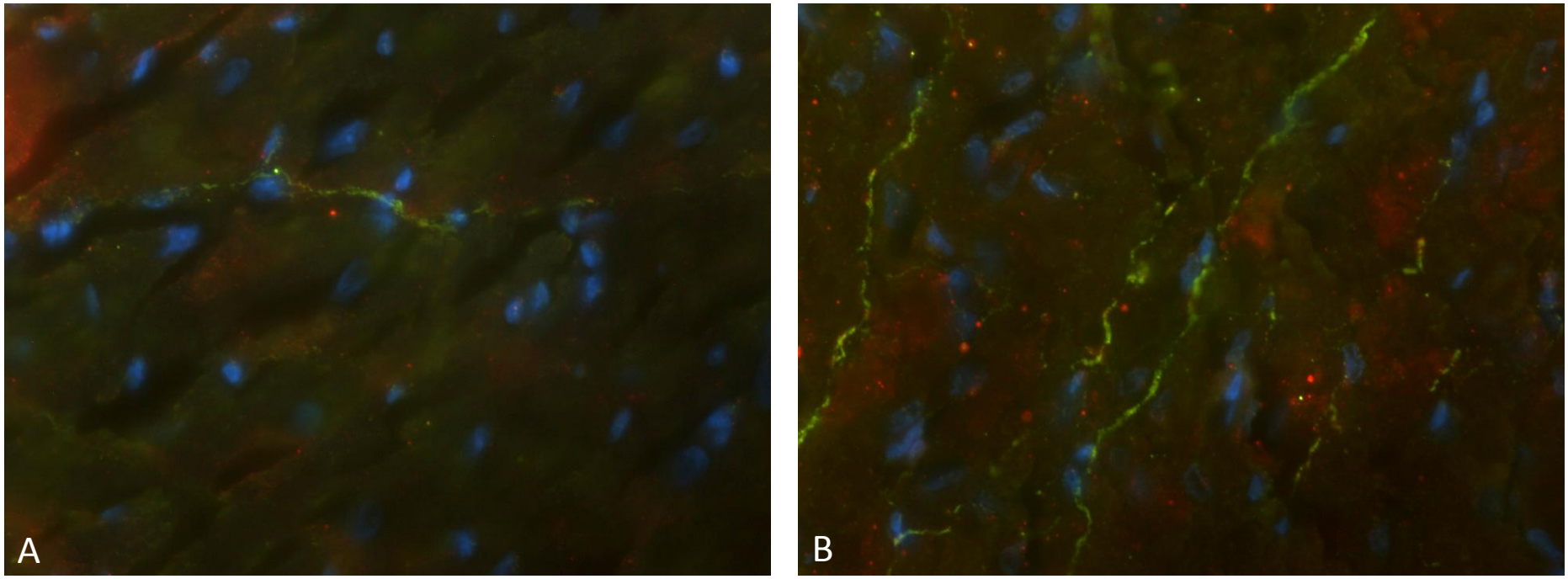
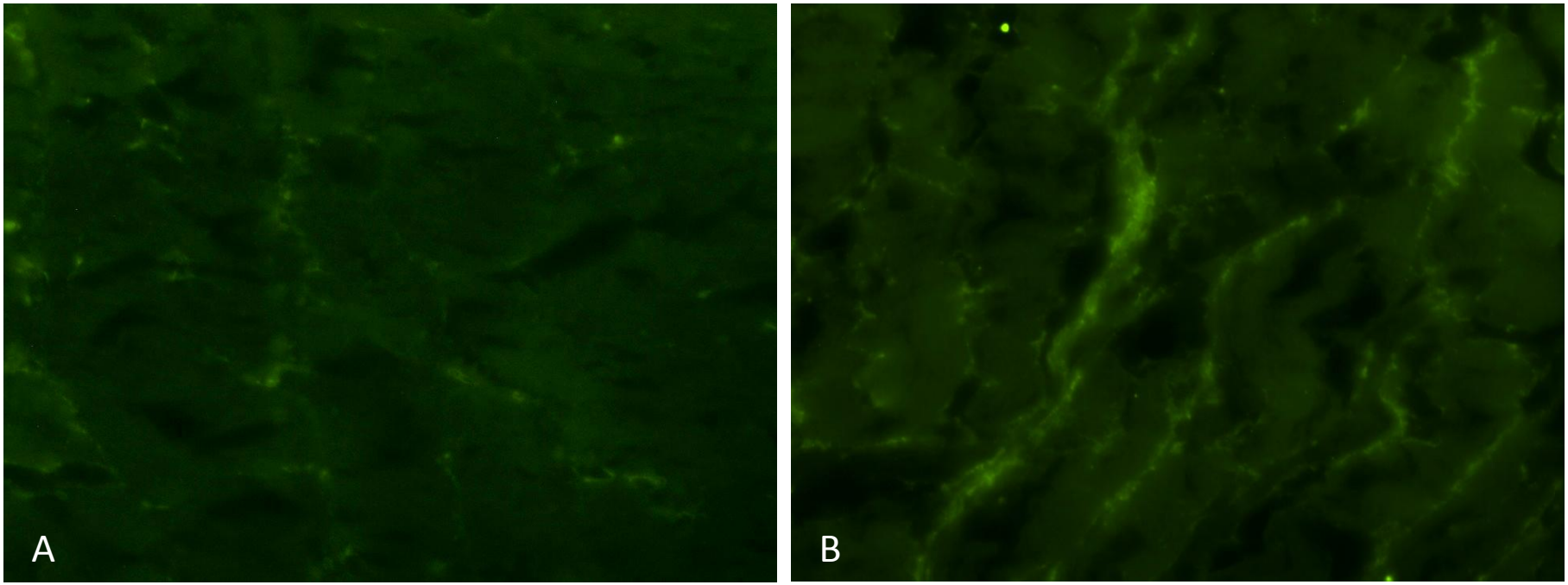


Figure 7

Control Collagen VI

60 X Power

Heart Failure Collagen VI



Abstract for Dissertation Research

Background: Heart failure (HF) is one of the leading causes of morbidity and mortality in the United States. The impending nursing shortage may impact care of the HF patient. Finding improved methods to stage and manage HF may enhance quality of care.

Hypothesis: The main hypothesis is that free-breathing T_1 mapping can quantify myocardial T_1 changes in myocardium between healthy and tachycardia-induced heart failure in swine. For aim 1, the pattern of fibrosis in post-mortem heart failure histological specimens will correlate with MRI T1 mapping values. For aim 2, the TGF-β 1 pathway proteins that relate to the development of fibrosis will be elevated in the myocardium of swine in heart failure as compared to controls using quantitative Western blotting from post-mortem tissue specimens.

Methods: Yorkshire swine (N=9) were implanted with pacemakers and paced for 3 to 5 weeks at 200 beats per minute. Animals were scanned in a 1.5 T MRI scanner (GE Signa, Waukesha, WI) at baseline (non-paced pacemaker in situ) and then at heart failure (N=6) (confirmed by echocardiography, pacer off).

T_1 mapping used a Modified Look-Locker with Saturation Recovery SSFP sequence [Song, ISMRM 2010], with 1-2 min free breathing using 3 Look-Locker imaging blocks (2, 2, and 6 heartbeats respectively). T_1 mapping sequences were performed before contrast and at 5 minutes post intravenous administration of a 0.2 mmol/kg dose of gadolinium (Gd)-chelate contrast agent (Gadoteridol).

Myocardial tissue from the left ventricle was fixed in 10% formalin, embedded in paraffin, and cut into 5 µm sections using standard methods. Histology slides were stained using Accustain Masson Trichrome Blue and for immunohisotchemistry using antibodies for Collagen Types I, III, and VI.

Results: Mean T_1 for the pre-contrast T_1 mapping was 960 ±96 ms, baseline; and 726 ±94 ms, HF, (Paired t-test, $p = .020$). Five min post contrast T_1 at baseline was 546 ±180 ms; and HF 300 ±171 ms ($p = .005$). Histology of collagen showed a difference between the control and HF animals (5.4 = ±1%, 9.4 = ±1.6%, respectively, $p < .001$). Immunohisotchemistry found increases in all collagen at HF. All proteins related to the fibrotic pathway of TGF-β1 were found to have an increased trend, with Smad 3 being significantly elevated.

Discussion and Conclusions: This study demonstrates that free breathing T_1 mapping is a promising technique to quantify myocardial changes in HF. Myocardial T_1 values are reduced in HF, with and without Gd-chelate contrast agent. The reduction in myocardial T_1 value is consistent with an increase in collagen and the elevation of fibrotic pathway proteins. More research is needed to further correlate the T1 mapping values with the range of percentage change in collagen in the myocardium.

Magnetic Resonance Imaging of Heart Failure Using a Swine Model

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Graduate School of Nursing, Doctoral Program

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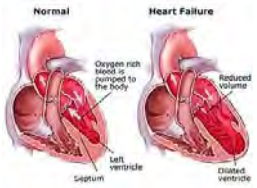
Introduction

- Heart Failure Overview
- Why MRI
- Animal Model
- Physics – MRI and T1 Mapping
- Research
- Summary
- Future Research

3

What is Heart Failure?

- American College of Cardiology/American Heart Association Definition
- "Heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood."



Hunt, et al, 2009 ACF/AHA Heart Failure Guidelines

Background

- The prevalence and incidence of heart failure is rising
- There are an estimated 5 million people in the USA with HF.*
- In 2007, >$33 Billion spent on HF.
  - Heart failure is the number one cause of hospitalization in the Medicare population.
- 550,000 new cases are diagnosed each year. *
- People are living longer with HF, but we still have an estimated 300,000 deaths a year. ‡
- **The nursing shortage**
  - Estimated shortage by 2025 = 260,000 nurses†
  - Nursing shortage poses a threat to quality patient care

*AHA Scientific Statement , Prevention of Heart Failure, 2008

5 ‡National Health, Lung, Blood Institute, 2009. † American Association of Colleges of Nursing, 2010

Background MRI Study

- Dilated cardiomyopathy (Grimm 2004; Peters 1988)
  - most common type of non-ischemic heart failure
  - degree of fibrosis may be related to risk of sudden death
- MRI may offer a non-invasive tool to quantify the degree of fibrosis in patients with diffuse disease by taking advantage of the inherent T_1 values of tissues (Iles, 2008; Messroghli 2007).
  - Cardiac structure and function*
  - The only modality that can quantify fibrosis – detect scarring in the myocardium*

*Stork, et al, 2007; Knopp, et al, 2002; Assomull, et al, 2007; Assomull , et al, 2006; Dewey , et al, 2004; McCrohon, et al, 2003; Nazarian, 2005; Teraoka et, al, 2004; Vohringer, et al, 2007; Brodoefel, et al, 2007; Soriano, et al, 2007; Casolo, et al, 2006; White, et al, 2006; Soriano, et al, 2005; Mahrholdt, et al, 2005; Tandri, et al, 2005; De Cobelli, et al, 2006; Weinsaft, et al, 2007; Sechtem, et al, 2006; Moon, et al, 2004; Moon, et al, 2003; Jackson , et al, 2007; Gupta, et al, 2004.

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Animal Model

- The pig is a large animal model that more closely matches the heart and circulatory system of humans than do rats, mice or dogs
- "In anatomy and physiology the pig is remarkably like man … its heart and circulatory system, its diet…. it has a tendency to be sedentary." *
- This study used Yorkshire pigs

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*Bustad LK. Pigs in the Laboratory. *Scientific American*, 1966.

Overview of MRI

- Nuclear Magnetic Resonance Imaging
- Focuses on the nucleus of an atomic species (e.g. ^{1}H, ^{19}F, ^{31}P)
- Tissue is placed in a powerful magnetic field.
- Specific radiofrequencies are transmitted to excite the tissue and perform the NMR sequence
- Radio-receivers (MR coils) detect the signal
- The signal is mathematically converted into images or spectra to be interpreted.

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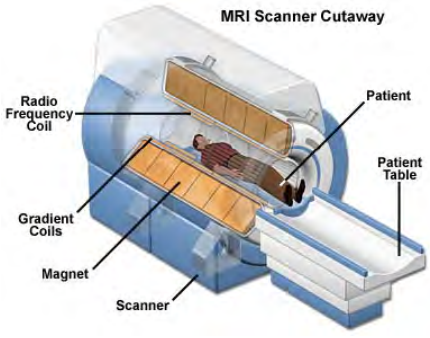
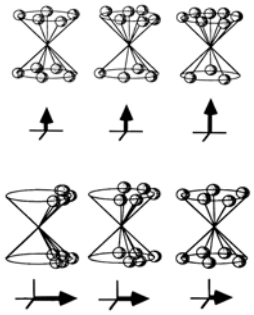
What is MRI?



MRI Signal - 2 Types of Relaxation



T_1 Relaxation:
Spins return to equilibrium with respect to the main magnetic field. (T_1 = rate where 63% nuclei return to equilibrium)

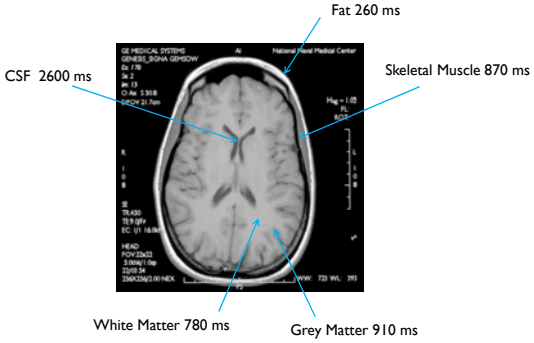
T_2 Relaxation:
Spins return to randomness with respect to the other atoms in the tissue.

T_1 Relaxation Time

- The T_1 relaxation time reflects the relationship between the frequency of molecular motions and the resonance (Larmor) frequency - (field strength dependent)

1. **Solids** ..... **short T1 times (Bright)**
2. Mobile liquids ..... long T1 times (dark)
3. **Fats** ..... **short T1 times (Bright)**
4. Very large proteins ..... long T1 times (dark)

Typical T1 values at 1.5 Tesla



Short T1 values = bright, LongT1 values = dark

Hypothesis and Aims

- **MRI will be able to quantify changes induced by myocardial remodeling from tachycardic pacing in a swine model**
  - Aim 1: MRI can quantify myocardial T_1 changes in a tachycardia-induced heart failure model using a new free-breathing T_1 Mapping pulse sequence
    - T_1 mapping values without the use of a contrast agent
    - T_1 mapping values after administration of a gadolinium (Gd)-chelate.

Note: T_1 Mapping is a research sequence – NOT FDA approved

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Hypothesis and Aims

- MRI will be able to quantify changes induced by myocardial remodeling from tachycardic pacing in a swine model
  - Aim 2: Correlate the pattern of fibrosis in post-mortem heart failure histological specimens with the tissue characterizing MRI techniques of T1 mapping values and myocardial delayed enhancement (MDE)
    - Masson's Trichrome Blue Histology
    - Immunohistochemistry of Col I, III, VI

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Hypothesis and Aims

- MRI will be able to quantify changes induced by myocardial remodeling from tachycardic pacing in a swine model
  - Aim 3: Measure the quantity of protein changes in the TGF-Beta 1 pathway of fibrosis in the myocardium of swine in heart failure as compared to controls using quantitative Western blotting from post-mortem tissue specimens

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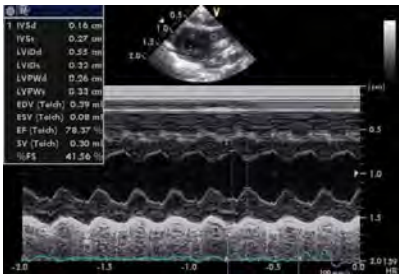
Methods

- Yorkshire swine (N=9) were implanted with pacemakers
  - paced for 19 to 41 days at 200 bpm
- Animals were scanned twice in a 1.5 T GE Signa MRI Scanner (Waukesha, WI):
  - 1. baseline (non-paced, pacemaker in situ)
  - 2. heart failure (50% reduction in fractional shortening per weekly echocardiogram), (Survivors =6)
    - HF sequences were performed after tachypacing was discontinued (spontaneous HR 90-130)

16

Determining End Point

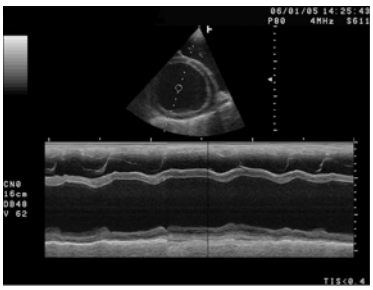
- Echo M-mode Normal Rat heart



17

Determining End Point

- Echo M-mode of a dog with DCM



18

Methods

- Standard and investigational cardiac gated MRI sequences were performed before and after the use of a gadolinium-chelate (Gadoteridol) contrast injection (0.2 mmol/kg).
- Scouts, parallel imaging calibration, Short axis cine SSFP, Radial long axis cine SSFP, T2 FSE with FS, T2 Grid mapping, perfusion, Cine IR, 2D and 3D IR Delayed Enhancement
  - Total exam time 60-90 minutes

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Methods

- T_1 Mapping: Modified Look-Locker with Saturation Recovery (MLLSR) pulse sequence
  - Uses three Look-Locker imaging blocks (2, 2, and 6 heartbeats respectively)
  - Initial Inversion Time (TI) of 50 ms, then TI increments of 40 ms if the heart rate is 100 bpm (RR interval is 600ms) thus TIs of 50, 90, 130, 650, 690, 730, 1330, 1930, 2530, and 3130ms (50, 50+40, 50+2x40, 50+RR, 50+40+RR, 50+40x2 +RR, 50+40x2 +2xRR, 50+40x2 +3xRR, 50+40x2 +4xRR, 50+40x2 +5xRR).
    - Collecting 8 to 10 T_1 data points at pre-selected TI times.
  - SSFP imaging was performed at each of the TI times (TE/TR 1.9/4.3ms, 45° flip angle, 256x160matrix, 3 NEX, 20 VPS, 8mm slice thickness)
  - 1-2 min free breathing scan duration

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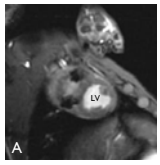
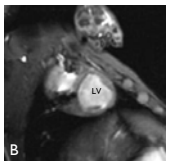
Song, ISMRM Annual Scientific Meeting, 2009

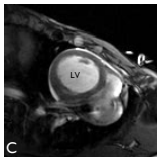
Results – Short axis Cine SSFP



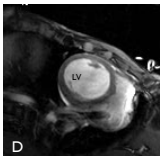
Baseline End Systole



Baseline End Diastole

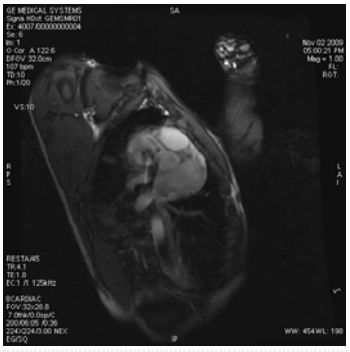


Failure End Systole



Failure End Diastole

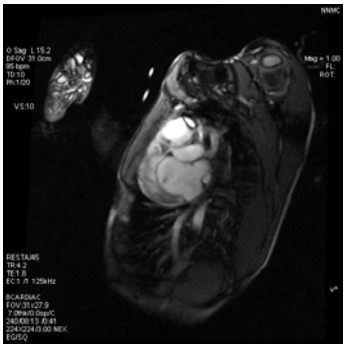
Results - 2D Cine SSFP (FIESTA)



22

Baseline

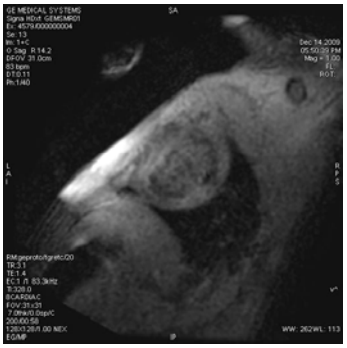
Results - 2D Cine SSFP (FIESTA)



23

Failure

Results - Heart Failure, Perfusion



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Results

Parameter	Baseline	Heart Failure	Significance
Ejection fraction	46.6 ± 1.1 % SEM	15.6 ± 2.7% SEM	.000*
LV End Diastolic Diameter	34.8 mm	52.0 mm	.000*
LV End Systolic Diameter	27.1 mm	46.6 mm	.000*
LV End Diastolic Volume	48.4 cc	127.4 cc	.000*
LV End Systolic Volume	25.8 cc	108.8 cc	.000*
LV Stroke Volume	22.6 cc	18.6 cc	.211
LV End Diastolic Wall Thickness	4.8 mm	4.9 mm	.608
LV End Systolic Wall Thickness	7.3 mm	5.8 mm	.001*
Heart rate	103 bpm	95 bpm	.248

*T-test significance level was based on a two-tailed analysis of < .025.

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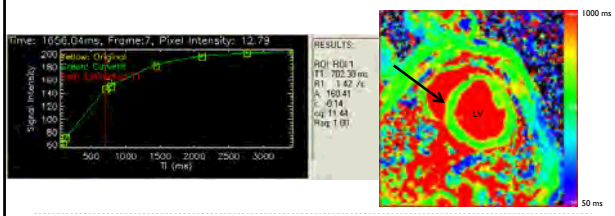
Results

Baseline T_1 Mapping	Heart Failure T_1 Mapping
Pre Contrast = 960 ±96 ms	Pre Contrast = 726 ±94 ms
5 Min Post Contrast = 546 ±180 ms	5 Min Post Contrast = 300 ±171 ms
12 Min Post Contrast = 509 ±129 ms	12 Min Post Contrast = 295 ±89 ms



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Results – T_1 Mapping

Paired Samples T-Test	2-tailed Sig. (.05)
Pre Contrast: Baseline – HF (N=6)	p = .020
5 min Post Contrast: Baseline – HF (N=6)	p = .005
12 Min Post Contrast: Baseline – HF (N=5)	p = .060

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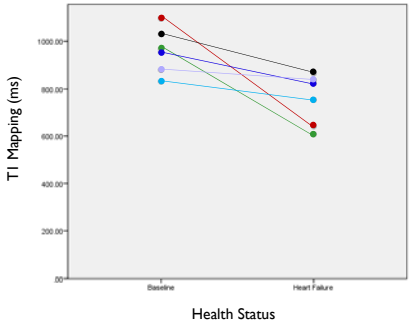
Results – T_1 Pre Contrast



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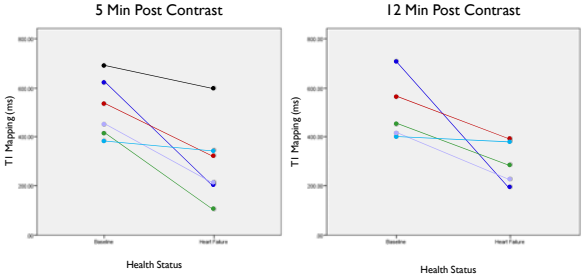
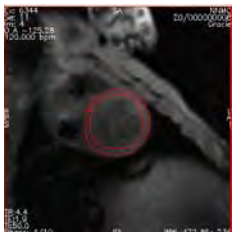
Results – T_1 Post Contrast

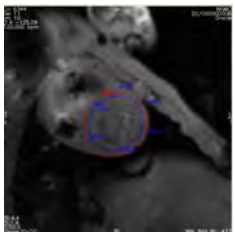


29

Results



Global Myocardium Measurement



6 Sector Myocardium Measurement

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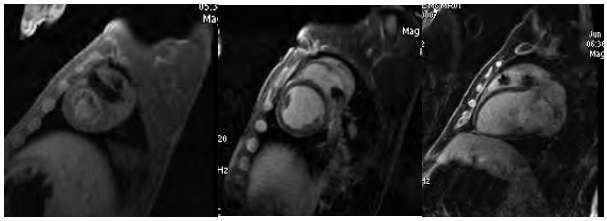
Results - MDE

•Myocardial Delayed Enhancement Technique = All negative.



Baseline Short Axis Long Axis

Heart failure

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Myocardial Delayed Enhancement

- MDE sequences use an inversion recovery (IR) pulse that can be adjusted to nullify the signal from normal myocardium.
- Areas of myocardial fibrosis or inflammation will demonstrate differences in the 'wash-in' and 'wash-out' kinetics, thereby showing areas of hyper-enhancement.
- Pitfall – The IR pulse is variable and is intended to suppress the signal from the myocardium in order to see focal areas of abnormality, therefore diffuse pathology will be hard to detect if at all.

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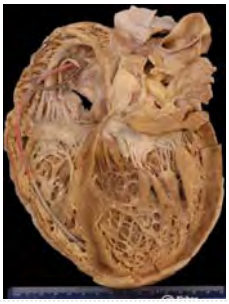
After the Imaging is Over….

- **Gross heart specimen from a patient with dilated cardiomyopathy who died in end-stage heart failure. Defibrillator leads are in the right heart. The ventricles are dilated with normal ventricular wall thicknesses, imparting an appearance of thin ventricular walls. The ventricles are dilated more than the atria.**



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Preparing for Lab Work - SGI

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Histopathology

- At least 29 known types of collagen
- Triple helix formation
- Col I and III are thought to be the most abundant and significant in the heart
- Col I – high tensile strength (stronger than steel)
- Col III – highly elastic
- Col VI – ball-like collagen, structural component of myofibrils

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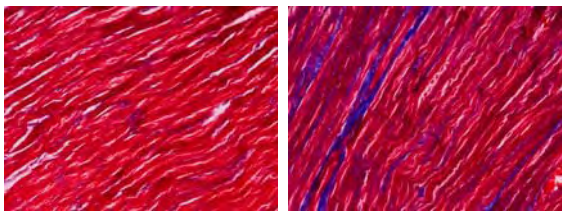
Histology

Paraffin tissue sections, stained with Masson's Trichrome Blue

Normal **HF**



Blue – Collagen

Red - Myocytes

Heart failure myocardium has more collagen/fibrosis than normal tissue.

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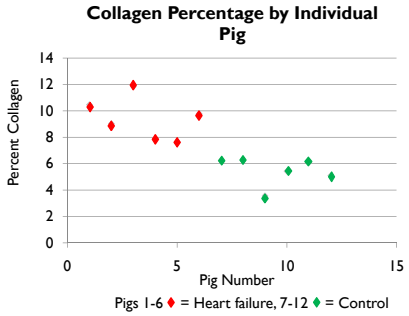
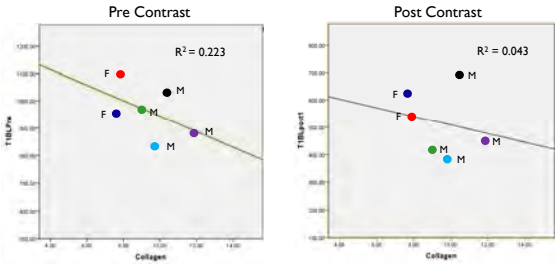
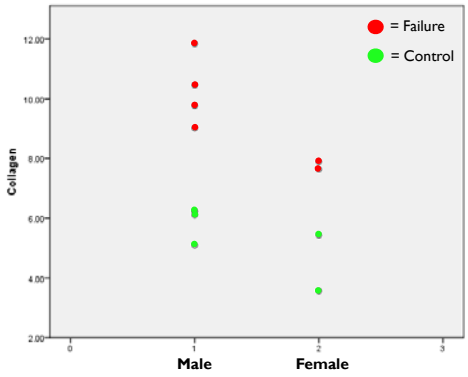
Histology Statistics



Results: T1 Mapping Versus Histology



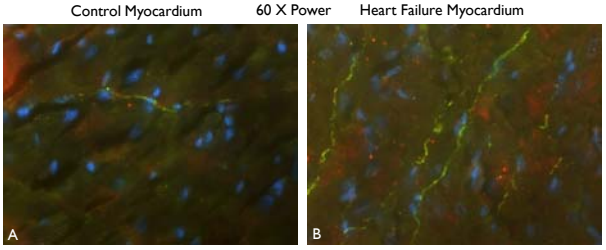
Results: T1 Mapping Versus Histology



Immunohistochemistry – Col I and III

IHC-P. Double stained with Primary Col-I Antibody, Col-III Antibody, Both Abcam, 1:100, and Secondary Alex Fluor Goat Anti Mouse IgG and Alex Fluor Goat Anti Rabbit IgG, Invitrogen, 1:100)

Control Myocardium 60 X Power Heart Failure Myocardium



Red – Collagen I Green – Collagen III Blue - Nuclei

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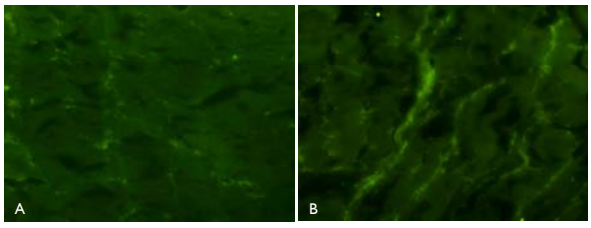
Immunohistochemistry – Col VI

IHC-P. Stained with Primary Col-VI Antibody, Abcam, 1:100, and Secondary Alex Fluor Goat Anti Mouse IgG Invitrogen, 1:100, 60 X Power.

Control Collagen VI Heart Failure Collagen VI



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Collagen Discussion

- Collagen is increased in the heart failure subjects
- Hearts remodel in heart failure – DCM, HCM
- All Collagen is increased in HF, with Col III increasing the most*
  - Col I is strong and is the primary structural collagen
    - Stronger than steel, but cross linkages may be breaking when heart is under assault
  - Col III is an elastic collagen
    - May be responsible for allowing the stretch and dilation
  - Col VI function in heart not clear
    - ?plays role in structural component of microfibrils
    - Associated with basement membrane proteins

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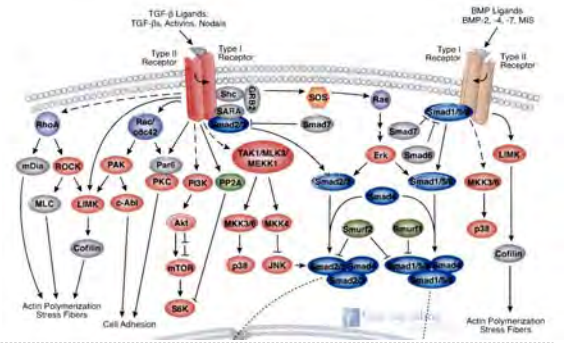
*Marijianowski, JACC 1995;25:1263-1272

Proteins in Fibrosis



Cell Signaling Technology

TGF Beta 1 Signaling Pathway

- TGF β-1 is involved in the development and regeneration of cells.
- TGF β-1 pathway is associated with the formation of fibrosis in heart disease.
- TGFBR1 – signaling protein that activates Smads
- Smad 2 and Smad3: receptor Smads
  - complex with Smad 4 and
  - regulate gene expression
- Smad 7 – Inhibitor Smad of TGF β-1 Pathway
  - TGFBR1
  - Smad 2 and Smad 3

Figure 3

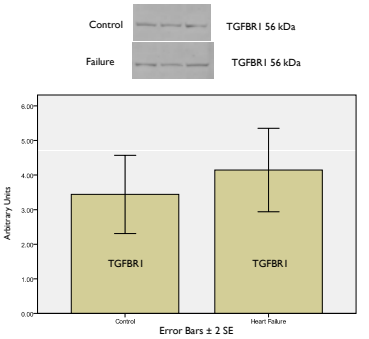


Figure 4

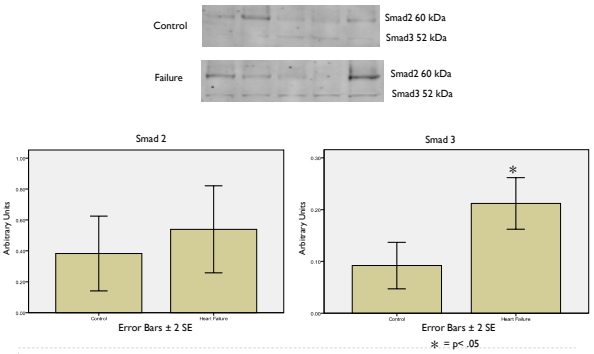
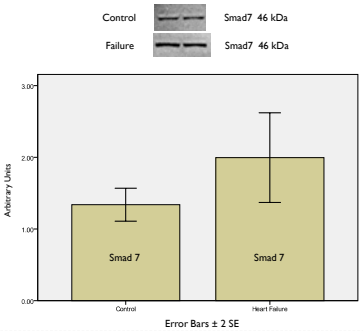


Figure 5



Results

Protein	Control	Failure	Std Error
TGFBR1	3.44	4.15	.83
Smad2	0.38	.54	.18
Smad3	0.09	0.21	.03*
Smad7	0.36	0.99	.33

Up regulation trend in the Proteins related to TGF Beta 1 pathway of fibrosis.

*T-test significance level was based on a two-tailed analysis of < .05.

Overall Conclusions

- This study demonstrates that T_1 mapping is a promising technique to quantify the T_1 value of the myocardium using a free/spontaneously breathing technique.
  - Able to scan higher acuity pts
  - Able to scan sedated pts easily
- T_1 mapping is also a promising alternative to contrast injection techniques (MDE) in non-ischemic subjects.
- Histology confirms the increase in collagen in the heart failure subjects.
- TGF-β1 pathway proteins upward trends support the increase in fibrosis seen.

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Conclusions

- Free breathing cardiac T_1 Mapping
  - Small study, but consistent findings
  - Similar to breath hold technique in humans*
- More research needs to be done with MRI
  - More subjects due to variability (rhythm & respiratory)
- More research is needed to better understand the changes in collagen, especially in the specific types of collagen and their developmental pathways.
  - Western Blots
  - Collagen IV and VI

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*Iles, 2008; Messroghli 2007

Summary

- MRI can be used as a non-invasive tool to help manage heart failure patients more frequently and accurately
  - Identify those patients at greater risk for sudden cardiac death
  - Help select mode of therapy or device support
- Improved treatment and prevention approaches for HF
  - Improve nursing case management of the HF patient
  - Improve case specific needs for optimal functioning
  - Improve quality of life

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Future

- Continuing on with current projects
- Increasing samples for correlation of histology to T1 Mapping
- Working on Quantifying Collagens
- Adding PIIINP to current tests
- Skeletal Muscle change in HF
- Pacemaker lead tip tissue changes from MRI
- Gap Junction proteins
- More Proteins related to fibrotic pathways
- Collagens Type IV and VI

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Current Successes

- T1 Mapping abstract was a finalist in the American Heart Association Young Investigator Award Competition in the Cardiovascular Imaging Section in 2010 at the North American Society for Cardiovascular Imaging in Seattle.
  - Awarded Honorable Mention for oral presentation
- Abstract accepted to the International Society for Magnetic Resonance in Medicine Annual Scientific Meeting in Montreal, Canada, May 2011.
- Abstract submitted to National Institutes of Nursing Research, 2011 Symposium

53

Papers while in PhD Program

- Ho VB, Corse WR, **Hood MN**, Rowedder AM. Magnetic resonance angiography of the thoracic vessels. *Magn Reson Imaging Clin N Am* 2004;12(4):727-47.
- Ho,VB, Bakalov,VK, Cooley M,Van PL, **Hood MN**, Burklow TR, Bondy CA. Major vascular anomalies in turner syndrome: prevalence and magnetic resonance angiographic features. *Circulation*. 2004;110(12):1694-700
- **Hood MN**, Ho VB. Contrast agents: innovations and potential applications for body MR angiography. *Magn Reson Imaging Clin N Am*. 2005 13(1):189-203.
- **Hood MN**, Gugerty B, Levine R, Ho VB. Computerized information management for institutional review boards. *CIN Comput Inform Nurs* 2005; 23(4): 190-198, also accepted as CE feature.
- **Hood MN,** Scott HB. Introduction to Picture Archive and Communication Systems: PACS. *Journal of Radiology Nursing 2006;* 25(3): 69-74. Winner of the 2006 Linda Strangio Editor's Award.
- **Hood MN**, Kasper CE. Skeletal Muscle Physiology and Damage Models from Exercise. Submittted to Biological Research for Nursing. Not published.
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- **Hood MN**. Chapter 19: MRI Safety. In: VB Ho, G Reddy, Eds. *Imaging of the Cardiovascular System*. Elsevier, 2010.
- **Hood MN**, Song T, Bedocs P, Capacchione JF, Kasper CE, Haigney MC, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure. *Radiology*, submitted 2011.
- **Hood MN**, Song T, Bedocs P, Capacchione JF, Kasper CE, Ho VB, Haigney MC. MRI of a Swine Model of Heart Failure: Changes in Morphology, Function, Collagen and TGF β-1 Pathway Proteins. *Investigational Radiology*, submitted 2011.

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Acknowledgements

Dissertation Committee

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Joseph DeSoto, MD, PhD

Consultants:
Edward Miller, MD, PhD - Western Blots
Cara Olsen, PhD - Power Analysis
Frank Shellock, PhD - MRI Safety

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Cathy Harrison
Martha Corcoran
Walter Tombini
Denis and Gail Krupa
And others- you know who you are – thank you!

Vintage Lightning/United Lightning

57

The "Volunteers"

- Seaman Hornsby
- Jimmy Dean
- Wilbur
- Arnold Ziffle
- Rita Pigstein
- Maltida
- Sir Francis Bacon
- Neils Boar
- Gracie
- Lulu
- CAPT Link Hogthrob
- Dr. Julius Strangepork
- Miss Piggy
- Piggy Sue
- Porkchop
- The Grinch
- Frank Swineatra
- The Controls

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Questions?

1. Reference list available upon request

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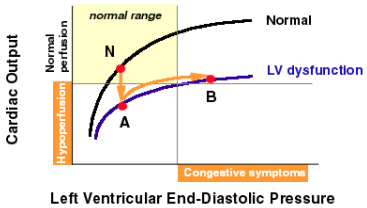
Frank Starling Curves



The ability of the heart to change its force of contraction and therefore stroke volume in response to changes in venous return is called the Frank-Starling mechanism. The curves represent ventricular function in a normal subject and one with left ventricular dysfunction. Line N to A represents the initial reduction in cardiac output due to CHF. Line A to B represents the Frank-Starling mechanism of compensation; an increase in left ventricular end-diastolic pressure needed to maintain cardiac output. Notice that this point (B) puts the subject into the area of congestive symptoms such as dyspnea.

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http://www.gcrweb.com/HeartDSS/epipump.htm

Uniformed Services University of the Health Sciences
Graduate School of Nursing

Report of Dissertation Defense for the Doctor of Philosophy Degree (Form G)

Student Name: **Maureen N. Hood**

Title of the dissertation: **Magnetic Resonance Imaging of the Heart Failure Using a Swine Model**

The decision of the Dissertation Committee is:

PASS
A. Both the dissertation and the oral defense are satisfactory: ✓
B. Minor changes are recommended by the Dissertation Advisory Committee that is to be made to the satisfaction of the Dissertation Chairperson: _____

DEFER
A. Major changes in the dissertation are required. Changes must be made to the satisfaction of the Dissertation Chairperson: _____
B. Major changes in the dissertation are required. Changes must be made to the satisfaction of the Dissertation Advisory Committee and at that time the oral defense will be rescheduled: _____

FAIL
Neither the oral performance nor the dissertation is adequate: _____

Signatures of the Committee
Chairperson: _____
Member: _____
Member: _____
Member: _____

Approval/Disapproval
Signature: _____ Date: 21 March 2011
Director, Doctoral Program

Approval/Disapproval
Signature: _____ Date: 4-4-11
Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing

Certification of Dissertation (Form H)

Name of Student: **_Maureen N. Hood__**

This is to certify that the accompanying copies of the doctoral dissertation of the student named above are completed and correct copies as approved by the Dissertation Advisory Committee.

Title of the dissertation:
Magnetic Resonance Imaging of the Heart Failure Using a Swine Model

Signature, Chairperson — Dr. Christine E. Kasper, PhD, RN, FAAN, FACSM (Printed Name) — Date: 3/21/11

Approval/Disapproval

Signature: _____ Date: 3/21/11
Dr. Penny F. Pierce, PhD, RN, FAAN

Approval/Disapproval

Signature: _____ Date: 4-4-11
Dean, Graduate School of Nursing, USUHS

Abstracts from Dissertation research

1. **Hood MN**, Song T, Bedocs, P, Capacchione C, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure. Oral Presentation. American Heart Association Young Investigator Finalist at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
2. **Hood MN**, Song T, Bedocs P, Capacchione J, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 Pre and Post Contrast in Swine with Non-ischemic Heart Failure. *Proc. Intl. Soc. Mag. Reson. Med. 19 (2011),* Traditional Poster, Montreal, Canada, May 7-13, 2011.
3. **Hood MN**, Song T, Bedocs P, Capacchione J, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 Pre and Post Contrast in Swine with Non-ischemic Heart Failure. Submitted to NINR 2011 Symposium.

Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure

Maureen Hood, MS, RN, Ting Song, PhD, Peter Bedocs, MD, John Capacchione, MD, Christine E. Kasper, PhD, RN, Mark Haigney, MD, Vincent Ho, MD, MBA

Introduction: Dilated cardiomyopathy is the most common type of non-ischemic heart failure, with persistent tachycardia being a common cause [1-2]. The degree of myocardial fibrosis is thought to play a critical role in determining the risk of sudden death. Cardiac T_1 mapping MRI can enable signal quantification on a standard scale [3]. The purpose of this study was to investigate a new free-breathing pulse sequence to quantify myocardial T_1 changes in tachycardia-induced heart failure swine model.

Methods: After obtaining IACUC approval, Yorkshire swine (N=6) were implanted with pacemakers and paced for 3 to 5 weeks at 200 beat per minute. Each animal was scanned in a 1.5 T MRI scanner at baseline (non-paced pacemaker in situ) and then at heart failure (confirmed by echocardiography).

For the T_1 Mapping, we used a modified look-locker with saturation recovery SSFP sequence [4] (TE/TR 1.9/4.3ms, 45° FA, 256x160, 3 NEX, 20 VPS, 8mm slice, with 1-2 min free breathing) with three Look-Locker imaging blocks (2, 2, and 6 heartbeats respectively). The sequences start with an initial TI of 50 ms, then TI increments of 40 ms.

Results: Baseline ejection fractions were 47% ±4%; and for the heart failure group, 15% ±9%. The mean T_1 value for the pre-contrast T_1 mapping sequence was 936 ±126 at baseline (n=6); and for the heart failure group, 800 ±201 (n=4), ($p > .05$). The initial post contrast T_1 value for the baseline pigs was 475 ±88 baseline; and 213 ±88, for the heart failure group ($p = .003$). The delayed post contrast T_1 value for the baseline pigs was 533 ±105 baseline; and 275 ±87, for the heart failure group ($p = .003$).

Discussion: This study demonstrates that free breathing T_1 mapping is a promising technique to quantify myocardial changes in heart failure, especially when contrast media is administered.

References:
1. Grimm W, Alter P, Maisch B. Herz 2004;29(3):348-352. 2. Peters KG, Kienzle MG. Am J Med 1988;85(2):242-244. 3. 3. Iles L, et al. J Am Coll Cardiol 2008;52:1574-11580. 4. Song T, et al, ISMRM Annual Scientific Meeting 2009, pp483.

Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 Pre and Post Contrast in Swine with Non-ischemic Heart Failure

Maureen Hood, MS, RN, Ting Song, PhD, Peter Bedocs, MD, John Capacchione, MD, Mark Haigney, MD, Christine Kasper, PhD, RN, Vincent Ho, MD, MBA

Synopsis: MRI may offer a non-invasive tool to quantify the degree of fibrosis in patients with diffuse disease by taking advantage of the inherent T_1 values of tissues. Purpose of this study is to investigate a new free-breathing pulse sequence to quantify myocardial T1 changes in tachycardia-induced heart failure in swine and investigate T_1 value changes after administration of a gadolinium-chelate. T1 mapping results were compared to traditional myocardial delayed enhancement and to control and heart failure histological tissue samples.

Introduction: Dilated cardiomyopathy is the most common type of non-ischemic heart failure, with persistent tachycardia being a common cause [1, 2]. The degree of myocardial fibrosis is thought to play a critical role in determining a patient's risk for sudden cardiac death. Cardiac T_1 mapping MRI enables myocardial signal quantification on a standard scale [3, 4]. The purpose of this study was to investigate a new free-breathing T_1 mapping pulse sequence to quantify myocardial T_1 changes in a tachycardia-induced heart failure swine model and to compare the T_1 mapping values to histological sections.

Methods: After obtaining IACUC approval, Yorkshire swine (N=9) were implanted with pacemakers and paced for 3 to 5 weeks at 200 beats per minute. Each animal was scanned in a 1.5 T MRI scanner (GE Signa, Waukesha, WI) at baseline (non-paced pacemaker in situ) and then at heart failure (N=6) (confirmed by echocardiography, with pacer turned off).

For the T_1 mapping, a Modified Look-Locker with Saturation Recovery SSFP sequence [5] (TE/TR 1.9/4.3ms, 45° FA, 256x160, 3 NEX, 20 VPS, 8mm slice, with 1-2 min free breathing) with three Look-Locker imaging blocks (2, 2, and 6 heartbeats respectively). The sequences starts with an initial TI of 50 ms, then adds TI increments of 40 ms (e.g. if the heart rate is 100 bpm (RR interval is 600ms), thus acquiring ten TIs of 50, 90, 130, 650, 690, 730, 1330, 1930, 2530, and 3130ms (50, 50+40, 50+2x40, 50+RR, 50+40+RR, 50+40x2 +RR, 50+40x2 +2xRR, 50+40x2 +3xRR,50+40x2 +4xRR, 50+40x2 +5xRR)). SSFP imaging was performed at each of the TI times with the following parameters: TE/TR 1.9/4.3ms, 45° flip angle, 256x160matrix, 3 NEX, 20 VPS, 8mm slice thickness, with 1-2 min free breathing scan duration. T_1 mapping sequences were performed before contrast, as well as at 5 and 12 minutes post intravenous administration of a 0.2 mmol/kg dose of gadolinium (Gd)-chelate contrast agent (Gadoteridol). Myocardial delayed enhancement sequences were acquired between 6 and 12 minutes post-contrast. The images were then post-processed using specially designed software.

Immediately post-euthanasia, myocardial tissue was collected from septum and left ventricular free wall and then was fixed in 10% formalin, embedded in paraffin, and cut into 5 µm sections using standard methods. Slides were stained using Accustain Masson Trichrome Blue. Ten images per tissue slide were captured with a Nikon Eclipse 80i microscope with digital DXM 12000c camera and analyzed using Nikon NIS Elements SW, vs. 3.0 (Nikon Instruments Inc., Melville, New York). Descriptive statistics performed using SPSS Statistical Software, v 16, (SPSS Inc., Chicago, IL).

Results: All baseline and all heart failure myocardial delayed enhancement images were negative. Baseline ejection fractions were 45 ± 4% and for the heart failure group, 14 ±7%. The mean T_1 value for the pre-contrast T_1 mapping sequence was 960 ±96 ms at baseline; and for the heart failure group, 726 ±94 ms, (Paired t-test, N= 6, p = .020). The 5 min post contrast T_1 value for the baseline pigs was 546 ±180 ms; and 300 ±171 ms, for the heart failure group (p = .005). The 12 min post contrast T_1 value for the baseline pigs was 509 ±129 ms; and 295 ±89 ms, for the heart failure group (N = 5, p = .060). Histological analysis showed an average of 0.88% fibrosis for the control animals and 2.60% (p < .01) for the heart failure animals.

Table 1. Statistical results for T_1 mapping using paired T-tests. Pre contrast and 5 minute post comparisons had 6 animals; the 12 minute post only had 5 animals.

Acquisition Period	Baseline T1 Mapping	Heart Failure T1 Mapping	Paired T-tests 2-tailed Sig. (.05)
Pre Contrast	960 ±96 ms	726 ±94 ms	p = .020
5 Min Post Contrast	546 ±180 ms	300 ±171 ms	p= .005
12 Min Post Contrast	509 ±129 ms	295 ±89 ms	p = .060

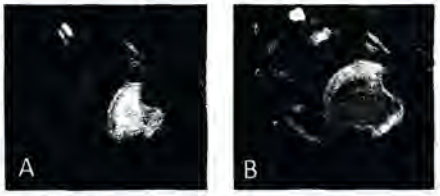

Figure 1. 2D Cine SSFP End Systolic Images at baseline (A), and heart failure (B).

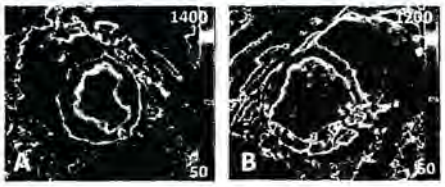

Figure 2. Short axis T1 maps acquired pre-contrast. Baseline map (A) has a higher range of values, 50-1400 ms, than the heart failure map (B) 50-1200 ms.

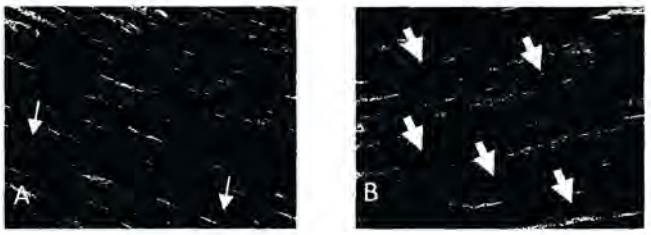

Figure 3. Myocardium stained with Masson trichrome Blue. Myocytes are red and collagen is blue. A small amount of structural collagen (small arrow) is seen in the control tissue (A), whereas, considerably more fibrotic (collagen) blue streaks (bold arrows) are seen in heart failure (B).

Discussion: This study demonstrates that free breathing T_1 mapping is a promising technique to quantify myocardial changes in heart failure and myocardial T_1 values are significantly reduced in heart failure, with or without Gd-chelate contrast agent. The changes in myocardial T_1 values appear to reflect an increase in collagen in the heart failure myocardium as compared to control animals. In addition, myocardial changes on T_1 mapping appear to pre-date changes seen on MDE. More research is needed to further correlate the T1 mapping values with the degree of change in collagen levels in the myocardium and to improve the free breathing T_1 mapping technique.

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1. Grimm W, Alter P, Maisch B. *Herz* 2004;29(3):348-352. 2. Peters KG, Kienzle MG. Am J Med 1988;85(2):242-244. 3. Iles L, Pflugr H, Phrommintikul A, et al. *J Am Coll Cardiol* 2008;52:1574-1580. 4. Messroghli DR. Greiser A, Frohlich M, et al. JMRI 2007;26:1081-1086. 5. Song T, Ho VB, Slavin G, et al. *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010)*,pp483.

Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 Pre and Post Contrast in Swine with Non-ischemic Heart Failure

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"The opinions or assertions contained herein are the private views of the author and are not to be construed as official or reflecting the views of the Department of Defense, the Veterans Administration or the Uniformed Services University of the Health Sciences."

Background: Heart failure (HF) is a leading cause of morbidity and mortality in the US. The impending nursing shortage may impact care of the HF patient. Finding improved methods to stage and manage HF will enhance quality of care.

Hypothesis: Free-breathing T_1 mapping MRI can quantify myocardial T_1 changes in myocardium between healthy and tachycardia-induced heart failure in swine.

Methods: Yorkshire swine (N=9) were implanted with pacemakers and paced for 3 to 5 weeks at 200 beats per minute until severe systolic dysfunction detected by echocardiography. Animals were scanned in a 1.5 T MRI scanner (GE Signa, Waukesha, WI) at baseline (pacer implanted but not activated) and then at HF (N=6).

T_1 mapping used a Modified Look-Locker with Saturation Recovery SSFP sequence [Song, ISMRM 2010], with 1-2 min free breathing using 3 Look-Locker imaging blocks (2, 2, and 6 heartbeats respectively). T_1 mapping sequences were performed before contrast and at 5 minutes post intravenous administration of a 0.2 mmol/kg dose of gadolinium (Gd)-chelate contrast agent (Gadoteridol).

Myocardial tissue from the left ventricle was fixed in 10% formalin, embedded in paraffin, and cut into 5 μm sections. Histology slides were stained using Accustain Masson Trichrome Blue and for immunohistochemistry (IHC) using antibodies for Collagen Types I, III, and VI.

Results: Mean T_1 for the pre-contrast T_1 mapping was 960 ±96 ms, baseline; and 726 ±94 ms, HF, (Paired t-test, $p = .020$). Five min post contrast T_1 at baseline was 546 ±180 ms; and HF, 300 ±171 ms ($p = .005$). Histology of collagen showed a difference between the control and HF animals (5.4 = ±1%, 9.4 = ±1.6%, respectively, $p < .001$). ICH found increases in all collagen at HF.

Discussion and Conclusions: This study demonstrates that free breathing T_1 mapping MRI is a promising technique to quantify myocardial changes in HF. Myocardial T_1 values are reduced in HF, with and without Gd-chelate contrast agent. The reduction in myocardial T_1 value is consistent with an increase in collagen. More research is needed to further correlate the T1 mapping values with the range of percentage change in collagen in the myocardium.

The Section for Magnetic Resonance Technologists
hereby recognizes and designates as a Fellow

Maureen N. Hood, M.S., R.T., (R)(MR)R.N.

for her significant and substantial contributions
to the mission of the SMRT.

Presented this 2nd day of May 2010

Pamela Vincent

Pamela Vincent
SMRT President 2009 - 2010

Julia Lowe

Julia Lowe
SMRT President 2010 - 2011

Papers while in PhD Program

Peer Reviewed:

1. Ho VB, Corse WR, **Hood MN**, Rowedder AM. Magnetic resonance angiography of the thoracic vessels. *Magn Reson Imaging Clin N Am* 2004;12(4):727-47.
2. Ho, VB, Bakalov, VK, Cooley M, Van PL, **Hood MN**, Burklow TR, Bondy CA. Major vascular anomalies in turner syndrome: prevalence and magnetic resonance angiographic features. *Circulation*. 2004;110(12):1694-700
3. **Hood MN**, Ho VB. Contrast agents: innovations and potential applications for body MR angiography. *Magn Reson Imaging Clin N Am*. 2005 13(1):189-203.
4. **Hood MN**, Gugerty B, Levine R, Ho VB. Computerized information management for institutional review boards. *CIN Comput Inform Nurs* 2005; 23(4): 190-198, also accepted as CE feature.
5. **Hood MN,** Scott HB. Introduction to Picture Archive and Communication Systems: PACS. *Journal of Radiology Nursing 2006;* 25(3): 69-74. Winner of the 2006 Linda Strangio Editor's Award.
6. Foo, TKF, Slavin GS, Bleumke DA, Montequin M, **Hood MN**, Ho VB. Simultaneous Myocardial and Fat Suppression in Magnetic Resonance Myocardial Delayed Enhancement Imaging. J Magn Reson Imaging, 2007, 26(4):927-33.
7. **Hood MN.** A Review of Cohort Study Design for Nursing Research. Journal of Cardiovascular Nursing, 2009;24(6):E1-9.
8. **Hood MN**, Song T, Bedocs P, Capacchione JF, Kasper CE, Haigney MC, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure. *Radiology*, submitted 2011.
9. **Hood MN**, Song T, Bedocs P, Capacchione JF, Kasper CE, Ho VB, Haigney MC. MRI of a Swine Model of Heart Failure: Changes in Morphology, Function, Collagen and TGF β-1 Pathway Proteins. *Investigational Radiology*, submitted 2011.

Invited:

1. Momeni A, **Hood MN,** Carter WR, Ho VB. Radiology Corner: Intralobar Bronchopulmonary Sequestration. *Military Medicine* 2007; 172:viii-ix.
2. Gumboc RD, **Hood, MN**, Ho VB. Radiology Corner: Coronary Fistula. *Military Medicine* 2007; 172(4):xi-xii.

Book Chapters:

1. **Hood MN,** Kaar JF, Ho VB. Chapter 11: Review Boards. In: Shayne C. Gad, Ed. *Clinical Trials Handbook. 2009.* John Wiley & Sons, Inc.: Hobocken, NJ.
2. **Hood MN**. Chapter 19: MRI Safety. In: VB Ho, G Reddy, Eds. *Imaging of the Cardiovascular System*. Elsevier, 2010.

Abstracts/Presentations while in PhD Program

1. Ho VB, **Hood MN**, Montequin M. Foo TKF. Cine Inversion Recovery (IR): Rapid Tool for Optimized Myocardial Delayed Enhancement Imaging. Scientific Poster at 2005 ISMRM Annual Meeting, Miami Beach FL, May 2005.
2. **Hood MN,** Ho VB, Bakalov VK, Cooley M, Van PL, Burklow TR, Bondy CA. Turner Syndrome: Characterization and Prevalence of Cardiovascular Anomalies by MR. Scientific Poster at 2005 SMRT Annual Meeting, Miami Beach FL, May 2005. First Place - research poster category.
3. **Hood MN**, Foo TKF, Ho VB. MRI of anomalous coronary arteries. Oral scientific poster presentation at the 17th Annual Workshop of the International MR Angio Club in Beijing, China, September 2005.
4. **Hood MN.** Stress Imaging Patient Care. Oral education presentation at the 34th Annual North American Society of Cardiovascular Imaging, Las Vegas, NV, October 2206.
5. Stanley D, Comeau C, **Hood MN.** Cardiac Jeopardy for Associated Sciences. Oral education presentation at the 34th Annual North American Society of Cardiovascular Imaging, Las Vegas, NV, October 2006.
6. **Hood, MN**. Patient Preparation and Care for Cardiac CT and MR. Round Table Discussion on How to Run a Cardiac Imaging Service for CT and MRI. Society of Interventional Radiology Annual Meeting, Seattle, WA, March 2007.
7. **Hood, MN**. Patient Care in MRI. John Koveleski Memorial SMRT Regional Meeting at the Penn State Milton S Hershey Medical Center in Hershey, PA on August 25, 2007.
8. Fung M, Schmidt EJ, **Hood MN**, Ho VB. Robust Coronary Artery Imaging using Multiphase-Multislab Free breathing 3D SSFP. Oral presentation at the19th Magnetic Resonance Angiography Workshop which will be held in Istanbul Turkey Oct 3- 5, 2007.
9. Fung M, Schmidt EJ, Kwong R, Holmvang G, **Hood MN**, Ho VB. Novel Approach for ECG-Gated Dynamic Contrast Enhanced MRA. Oral presentation at the19th Magnetic Resonance Angiography Workshop which will be held in Istanbul Turkey Oct 3- 5, 2007.
10. Comeau C, **Hood MN**, Strunk R, Stanley D. Cardiac Jeopardy for Allied Health Professionals. The North American Society for Cardiac Imaging's 35th Annual Meeting & Scientific Sessions, October 5-9, 2007 at the J.W. Marriott Pennsylvania Avenue in Washington DC.
11. **Hood MN**. How to write for publication/presentation. The North American Society for Cardiac Imaging's 35th Annual Meeting & Scientific Sessions, October 5-9, 2007 at the J.W. Marriott Pennsylvania Avenue in Washington DC.
12. Fung MM, Schmidt EJ, **Hood MN**, Golmvang G, Kwong RY, Ho VB. Clinical Applications of Contrast Inflow Dynamics MRA (CIDA): Novel Approach for ECG-Gated Dynamic Contrast Enhanced MRA (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;104. *Oral presentation* (3-9 May 2008; Toronto, Canada).
13. Fung MM, Ho VB, **Hood MN**, Schmidt EJ. Multi-Phase Fat-Suppressed 3D SSFP for Robust Coronary Artery Imaging: Improvements Over the Single-Phas Technique (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;313. *Oral presentation* (3-9 May 2008; Toronto, Canada)
14. Aksit P, Shankaranarayanan A, Gupta SN, Beatty PJ, Aletras AH, Fung MM, Schmidt EJ, **Hood MN**, Ho VB. Externally Calibrated ARC Parallel Imaging Reconstruction for DENSE Imaging: Initial Experience (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;700. *Oral presentation* (3-9 May 2008; Toronto, Canada).

15. Fung MM, Aksit P, Gupta SN, Shankaranarayanan A, Beatty PJ, Aletras AH, Schmidt EJ, **Hood MN**, Ho VB. Respiratory Triggered DENSE Imaging with Navigator Echoes: Initial Experience (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;701. *Oral presentation* (3-9 May 2008; Toronto, Canada).
16. Gupta SN, Aletras A, **Hood MN**, Ho VB, Schmidt EJ, Aksit P. Improved Cardiac Strain Estimation from DENSE Using Automatic Outlier Rejection(abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;989. *Oral poster presentation* (3-9 May 2008; Toronto, Canada).
17. **Hood, MN**. Ethics in Healthcare and MRI Research. John Koveleski Memorial SMRT Regional Meeting at the Penn State Milton S Hershey Medical Center in Hershey, PA, August 9, 2008.
18. **Hood MN.** Advances in Cardiac Imaging: Coronary CT and MRI. Advances in the Care of Hospitalized Cardiac Patient: Third Annual Cardiovascular Nursing Symposium. American Heart Association Scientific Sessions 2008, New Orleans, November 8-12, 2008.
19. Song T, Fung MM, Stainsby J, **Hood MN**, Ho VB. A Novel Cardiac MR Chamber Volume Model for Mechanical Dyssynchrony Assessment. Society of Photographic Instrumentation Engineers Annual Meeting, February 2009 in Orlando, FL, USA.
20. Song T, Aletras A, Gupta SN, Fung MM, Rettmann D, **Hood MN,** Ho VB, Schmidt E. Long Axis Cardiac DENSE Image Analysis and Visualization. North American Society of Cardiovascular Imaging Annual Meeting, October 2008, Scottsdale, Arizona.
21. Song T, Stainsby J, **Hood MN**, Ho VB. Cardiac T1 mapping: A comparison of methodologies for quantifying cardiac T1 values. International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
22. Song T, Stainsby J, **Hood MN**, Ho VB. Quantification of Global Hypokinesis in Left Ventricle using Center Point Trajectory (CPT). International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
23. Song T, **Hood MN**, Ho VB, Gupta S, Stainsby J. Optimization and Validation of a Modified Look-Locker Saturation-Recovery (MLLSR) Sequence Applied To Cardiac T1 mapping. International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
24. Song T, Stainsby J, **Hood MN,** Ho VB. Quantification of Focal Left Ventricular Wall Motion Abnormalities using Center Point Trajectory (CPT) Mapping . International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
25. Fung MM, Ho VB, **Hood MN**, Zurs Y, Schmidt EJ. Automatic detection of Quiescent Cardiac Phases using Navigator Echoes with Adjacent Complex Correlation Algorithm. International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
26. **Hood MN**, Song T, Ho VB. Review of Delayed Myocardial Enhancement in Cardiac MRI. Scientific Poster. Uniformed Services University of the Health Sciences Research Week, May 11-13, 2009 in Bethesda, MD, USA.
27. Song T, Ho VB, Slavin G, **Hood MN**, and Stainsby JA. "Varied Sampling Patterns in Modified Look-Locker with Saturation Recovery for Flexible Cardiac T1 Mapping", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* oral presentation, Stockholm, Sweden, May 1-7, 2010.
28. Song T, Stainsby JA, **Hood MN**, Ho VB. " A Novel Centerline Model for Cardiac Long Axis Wall Motion Analysis", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.

29. Song T, Ho VB, Slavin G, **Hood MN**, Stainsby JA. "Clinical Evaluation of a Cardiac T1 Mapping Method Using a Reduced Number of Sample Times", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.
30. Song T, Bustamante AI, Stainsby JA, **Hood MN**, Ho VB. "Center Point Trajectory Model for Cardiac Wall Motion Abnormality Assessment Compared with Echocardiography Strain", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.
31. Song T, Ho VB, Slavin G, **Hood MN**, Stainsby JA. " Reducing the Sensitivity to Respiratory Motion of Modified Look-Locker with Saturation Recovery for Cardiac T1 Mapping", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),*E-poster session, Stockholm, Sweden, May 1-7, 2010.
32. Song T, Bustamante AI, Stainsby JA, **Hood MN**, Ho VB.. "Evaluating Left Ventricular Wall Motion Abnormalities using Centerline Trajectory Mapping", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.
33. Dainer H, **Hood M**, Ho V. Myocardial Delayed Enhancement MR Imaging: A Pattern Approach. Poster at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
34. Song T, Ho VB, Slavin G, Hood MN, Stainsby JA. Clinical and Phantom Evaluations of a Cardiac T1 Mapping Method with Half of the Scan Time. Poster at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
35. Song T, Lai P, Stainsby JA, Hood MN, Ho VB. Myocardial Perfusion Imaging using Kat Autocalibrating Reconstruction for Cartesian Sampling: Acceleration Factor of Eight. Poster at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
36. Song T, Stainsby JA, **Hood MN**, Ho VB. Three-segment Center Point Trajectory Model for Segmental Tracking and Quantification of Cardiac Wall Motion. Poster Honorable Mention Award. North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
37. **Hood MN**, Song T, Bedocs, P, Capacchione C, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure. Oral Presentation. American Heart Association Young Investigator Finalist at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
38. **Hood MN**, Song T, Bedocs P, Capacchione J, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 Pre and Post Contrast in Swine with Non-ischemic Heart Failure. *Proc. Intl. Soc. Mag. Reson. Med. 19 (2011),* Traditional Poster, Montreal, Canada, May 7-13, 2011.
39. **Hood MN**, Song T, Bedocs P, Capacchione J, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 Pre and Post Contrast in Swine with Non-ischemic Heart Failure. Submitted to NINR 2011 Symposium.

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July 28, 2006

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Maureen Hood, MS, RN
Hugh Scott, MS

For their article

Introduction to Picture Archive and Communication Systems

Presented by the
American Radiological Nurses Association

March 2006
Seattle, WA


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Presents to
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Who's Who
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This is to certify that

MAUREEN HOOD

has been elected to
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in recognition of outstanding merit and
accomplishment as a student at

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES GRADUATE SCHOOL OF NURSING

2010

Janet LaFay

Director

Curriculum Vitae

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CURRENT POSITION

NMR Practitioner - (1996-present)
Instructor - (1997-2003)
Assistant Professor - (2003-present)
Department of Radiology & Radiological Sciences
Uniformed Services University of the Health Sciences
F. Edward Hébert School of Medicine

Current Secondary Appointments
Guest Researcher/Nursing Staff – (2000-2008), Diagnostic Radiology Department, National Institutes of Health, Clinical Center, Bethesda, Maryland
Nursing Staff – (2000-present), Magnetic Resonance Clinic, Radiology Department, National Naval Medical Center, Bethesda, Maryland

EDUCATION

1986 **Bachelor of Science in Biology, Phi Sigma**
Minor in Mathematics
University of Puget Sound, Tacoma, Washington

1989 **Associate of Technical Arts in Radiologic Technology, High Honors**
Tacoma Community College, Tacoma, Washington

2000 **Associate of Science in Nursing, Phi Theta Kappa**
Montgomery College, Takoma Park, Maryland

2002 **Bachelor of Science in Nursing, High Honors**
University of Maryland, School of Nursing, Baltimore, Maryland

2003 **Master of Science in Nursing Informatics, Sigma Theta Tau, Phi Kappa Phi**
University of Maryland, School of Nursing, Baltimore, Maryland

2011 (Projected)
Doctor of Philosophy in Nursing Research
Uniformed Services University of the Health Sciences, Graduate School of Nursing, Bethesda, Maryland 20814

SPECIALIZED EDUCATION AND TRAINING

1996 **Fellowship in Magnetic Resonance Imaging for Technologists**,
Siemens Medical Systems.

2006 **Certificate in Molecular Genetics in Research, Health and Society**
National Institutes of Health, National Institute of Nursing Research,
Summer Genetics Institute
Georgetown University – School of Nursing and Health Studies, 12 credits

PREVIOUS EXPERIENCE

1983-1985 **Docent, Internist and Researcher**
Point Defiance Zoo and Aquarium, Tacoma, Washington

1986 **Foreign Fisheries Observer**
University of Washington, School of Fisheries, Seattle, Washington

1987 **Foreign Fisheries Observer**
Frank Orth & Associates, Bellevue, Washington
Joint-Venture Representative
ProFish International, Seattle, Washington

1989-1994 **Magnetic Resonance Imaging Technologist**
Tacoma Magnetic Imaging, Tacoma, Washington

1993-1994 **Magnetic Resonance Imaging Technologist**
Advanced Medical Imaging, Bremerton, Washington

1994-1996 **Magnetic Resonance Imaging Technologist**
American Shared Hospital Services, Western Washington Division
Franciscan Health Services NW, St. Joseph Hospital, Tacoma, Washington
Senior Magnetic Resonance Imaging Technologist
Mobile Technology Incorporated, Western Washington Division

1996 **Clinical Coordinator - MRI Research**
Madigan Army Medical Center, Dept of Clinical Investigation, Tacoma, Washington
Magnetic Resonance Imaging Research Technologist
First Hill Diagnostic Imaging Center, Seattle, Washington

TEACHING EXPERIENCE

1985 University of Puget Sound, Biology Department
Biology 101 Laboratory Instructor

1992-1993 Tacoma Community College, Radiologic Sciences Program
Cross-sectional anatomy of the extremities

1997-present USUHS, Department of Radiology and Radiological Sciences, MS IV Elective
Magnetic Resonance Safety and Bio-effects,
Magnetic Resonance Physics, (2004-5)

1998-2000 National Naval Medical Center, Nuclear Medicine Physics Course for Residents
Magnetic Resonance Image Optimization

1998-present National Naval Medical Center, Nuclear Medicine Physics Course for Residents
Magnetic Resonance Safety and Bio-effects

1999 SMRT Regional Seminars
Introduction to MR Physics for Technologists, Collinsville, Illinois

2001 SMRT Regional Seminars
MR Physics for Technologists, Bethlehem, Pennsylvania

2001 DeWitt Health Care Network
MR Safety for Hospital Employees

2002 National Naval Medical Center,

Technologist Training, MR Physics for Technologists
2003-2005 National Naval Medical Center,
Patient Falls Prevention Training, Nursing Staff
2006 National Naval Medical Center, Anesthesiology Department Grand Rounds, Magnetic Resonance Safety
2007 MRI Safety for the Radiology Department, National Naval Medical Center.
2009 - present MRI Safety and Patient Care. National Naval Medical Center, Radiology Department – Annual training for Technologists and Administrative Staff.
2010 MRI Safety and Patient Care. Walter Reed Army Medical Center - MRI Safety Stand down Course.

UNIVERSITY COMMITTEES

2004-2006 USUHS, GSN, PhD Student Advisory Council, Steering Committee & Interim Board Member
2007-2009 USUHS, GSN, PhD Student Advisory Council, President
2008-2009 USUHS, Combined Student Council, GSN, Doctoral Student Representative
2009-present USUHS Faculty Senate, Nominations Committee
2011-present USUHS Steering Committee for the Middles States Commission on Higher Education Self-study Program

DEPARTMENTAL COMMITTEES

2000-2007 Department of Risk Management, National Naval Medical Center, Patient Fall Prevention Evidence Based Practice Committee
2002-2008 Department of Radiology and Radiological Sciences, USUHS, Medical Student IV Elective Curriculum Committee. Coordinator of Radiological Physics and Risk

EXTRAMURAL ACTIVITIES

ISMRM/SMRT
- 1997-2004 Newsletter Committee
- 1998-2001 Home-Study Educational Program Committee
- 1998-2001 Policy Board,
- 1999-2001 *Chair, Regionals Education Committee*
- 2000-present Abstract Reviewer, Section for Magnetic Resonance Technologists Annual Scientific Meeting Program
- 2001-present Education Committee
- 2001-2004 Executive Committee
- 2001-2004 *Chair, External Liaison Committee,*
- 2002-present Recognized Continuing Education Evaluation Mechanism (RCEEM) Steering Committee and Board Member. Awarded RCEEM status 2004
- 2009-present Awards Committee

2001-2004 Radiological Society of North America, Associated Sciences Consortium, Educational Planning Committee
2003-2004 Assisted with installation of Computerized Radiography at the US Capital, OAP
2004 Reviewer, *Biomedical Engineering Online*
2005-present Reviewer, *Military Medicine*

2005-present North American Society of Cardiac Imaging Annual Scientific Meeting, Technologist/Nurses Program Committee, Communications committee, Allied Health Program Chair 2006 and 2007
2007-Present Editorial Board member for *Journal of Radiology Nursing.*

PROFESSIONAL SOCIETIES

1992-present International Society for Magnetic Resonance in Medicine (ISMRM), Section for Magnetic Resonance Technologists (SMRT)
2000-present American Radiological Nurses Association (ARNA)
2001-2004 Radiological Society of North America, Associated Sciences Consortium
2001-2004 Capital Area Roundtable on Informatics in Nursing
2002-present Sigma Theta Tau International
2002-2005 Healthcare Information and Management Systems Society
2004-present American Heart Association
2005-present North American Society of Cardiac Imaging
2007-present American Association for the Advancement of Science

PROFESSIONAL CERTIFICATIONS

1980-present CPR & Basic Life Support
1989 Intravenous Procedures Certification
1990-present American Registry of Radiologic Technologists, Radiography, #237974
1992 Registry of MRI Technologists, #1048
1995-present American Registry of Radiologic Technologists, Magnetic Resonance Imaging, #237974
2000-present Registered Nurse License, State of Maryland, #R149359
2006-present Advanced Cardiac Life Support

ACADEMIC AND RESEARCH EXPERIENCE

Publications – Peer Reviewed

1. **Hood MN**, Ho VB, Smirniotopoulos JG, Szumowski J. Chemical Shift: The Artifact and Clinical Tool Revisited. *RadioGraphics* 1999;19:357-371. Also accepted as a CME feature.
2. Ho VB, Choyke PL, Foo TKF, **Hood MN**, Miller DL, Czum JM, Aisen AM. "Automated Bolus Chase Peripheral MR Angiography: Initial Practical Experiences and Future Directions of this Work-In-Progress." *JMRI* 1999;10:376-388.
3. Czum JM, Ho VB, **Hood MN**, Foo TKF, Choyke PL. Bolus-Chase Peripheral 3D MRA Using a Dual-Rate Contrast Media Injection. *J Magn Reson Imaging* 2000;12:769-775.
4. Foo TKF, Ho VB, **Hood MN**. Vessel-Tracking: Prospective Adjustment of Section-Selective MR Angiographic Locations for Improved Coronary Artery Visualization over the Cardiac Cycle. *Radiology* 2000; 214:283-289.
5. Saranathan M, Ho VB, **Hood MN**, Foo TKF, Hardy CJ. Adaptive Vessel Tracking: Automated Computation of Vessel Trajectories for Improved Efficiency in 2D Coronary MR Angiography. *J Magn Reson Imaging* 2001;14:368-373.
6. Foo TKF, Ho VB, **Hood MN**, Marcos HB, Hess SL, Choyke PL. High-Spatial-Resolution Multistation MR Imaging of Lower-Extremity Peripheral Vasculature with Segmented Volume Acquisition: Feasibility Study. *Radiology* 2001;219:835-841.

7. Foo TKF, Ho VB, **Hood MN**, Hess SL, Choyke PL. Preferential Arterial Imaging using Gated Thick-Slice Gadolinium-Enhanced Phase Contrast Acquisition in Peripheral MR Angiography. *J Magn Reson Imaging* 2001;13:714-721.
8. Ho VB, Meaney JF, Kent KC, Choyke PL, Watts R, **Hood MN**, Wang Y, Winchester P, Dong Q, Prince MR. Bolus-chase peripheral MR angiography: technical considerations. *Applied Radiology* 2002, 31:11-19.
9. Ho VB, Allen SF, **Hood MN**, Choyke PL. Renal masses: quantitative assessment of enhancement with dynamic MR imaging. *Radiology* 2002, 224:695-700.
10. **Hood MN**, Ho VB, Corse WR. 3D Phase Contrast MR Angiography: A Useful Clinical Adjunct to Gadolinium-Enhanced 3D Renal MRA? *Military Medicine* 2002; 167:343-349.
11. **Hood MN**, Ho VB, Foo TKF, Marcos HB, Hess SL, Choyke PL. "High-Resolution Gd-Enhanced 3D Elliptical Centric MRA of the Infrapopliteal Arteries: Lessons for Improving Bolus-Chase Multi-Station Peripheral MRA." *Magnetic Resonance Imaging,* 2002, 20:543-549.
12. Foo TKF, Ho VB, Marcos HB, **Hood MN**, Choyke PL. MR Angiography using Steady State Free Precession. *Magn Reson Med* 2002, 48:699-706.
13. Ho VB, Corse WR, **Hood MN**, Rowedder AM. MRA of the thoracic vessels. *Semin Ultrasound CT MR*. 2003;24(4):192-216.
14. Ho VB, Corse WR, **Hood MN**, Rowedder AM. Magnetic resonance angiography of the thoracic vessels. *Magn Reson Imaging Clin N Am* 2004;12(4):727-47.
15. Ho, VB, Bakalov, VK, Cooley M, Van PL, **Hood MN**, Burklow TR, Bondy CA. Major vascular anomalies in turner syndrome: prevalence and magnetic resonance angiographic features. *Circulation*. 2004;110(12):1694-700
16. **Hood MN**, Ho VB. Contrast agents: innovations and potential applications for body MR angiography. *Magn Reson Imaging Clin N Am*. 2005 13(1):189-203.
17. **Hood MN**, Gugerty B, Levine R, Ho VB. Computerized information management for institutional review boards. *CIN Comput Inform Nurs* 2005; 23(4): 190-198, also accepted as CE feature.
18. **Hood MN,** Scott HB. Introduction to Picture Archive and Communication Systems: PACS. *Journal of Radiology Nursing 2006;* 25(3): 69-74. Winner of the 2006 Linda Strangio Editor's Award.
19. Foo, TKF, Slavin GS, Bleumke DA, Montequin M, **Hood MN**, Ho VB. Simultaneous Myocardial and Fat Suppression in Magnetic Resonance Myocardial Delayed Enhancement Imaging. J Magn Reson Imaging, 2007, 26(4):927-33.
20. **Hood MN.** A Review of Cohort Study Design for Nursing Research. Journal of Cardiovascular Nursing, 2009;24(6):E1-9.
21. **Hood MN**, Song T, Bedocs P, Capacchione JF, Kasper CE, Haigney MC, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure. *Radiology*, submitted 2011.
22. **Hood MN**, Song T, Bedocs P, Capacchione JF, Kasper CE, Ho VB, Haigney MC. MRI of a Swine Model of Heart Failure: Changes in Morphology, Function, Collagen and TGF β-1 Pathway Proteins. *Investigational Radiology*, submitted 2011.

Invited and Other Publications

1. Baron KD, Faulkner W, Foo TKF, Kean M, Ho VB, **Hood, MN**, Posh J. The basics of magnetic resonance angiography. *SMRT Educational Seminars*, 1999, 2(4).
2. **Hood MN**, Ho VB, Smirniotopoulos JG, Szumowski J, Mirowitz SA, Baron KD, Cruz R, Faulkner W, Fletcher DW. Artifacts encountered in Abdominal MRI. *SMRT Educational Seminars*, 2000, 3(4).

3. **Hood MN**, Ho VB, Schweikert M, Corse WR. Contrast-Enhanced Peripheral MRA: Practical Aspects of Bolus Chasing. *Diagnostic Imaging: Advanced Strategies for CT & MR Contrast Imaging.* 2003;December:2-6.
4. Momeni A, **Hood MN,** Carter WR, Ho VB. Radiology Corner: Intralobar Bronchopulmonary Sequestration. *Military Medicine* 2007; 172:viii-ix.
5. Gumboc RD, **Hood, MN**, Ho VB. Radiology Corner: Coronary Fistula. *Military Medicine* 2007; 172(4):xi-xii.
6. **Hood MN,** Kaar JF, Ho VB. Chapter 11: Review Boards. In: Shayne C. Gad, Ed. *Clinical Trials Handbook. 2009.* John Wiley & Sons, Inc.: Hobocken, NJ.
7. **Hood MN**. Chapter 19: MRI Safety. In: VB Ho, G Reddy, Eds. *Imaging of the Cardiovascular System*. Elsevier, 2010.

Presentations

1. **Hood MN.** The Use of Gadolinium in Magnetic Resonance Imaging. Presented at WSRT Annual Conference April 1989, Alderbrook, WA. First Place Award.
2. Ho VB, Rovira MJ, Borke RC, **Hood MN**, Williams JB, Smirniotopoulos JG. Posterior Fossa Arachnoid Granulations: Radiographic Features. Presented at USUHS Research Day, March 1997, Bethesda.
3. **Hood MN**, Ho VB. Chemical Shift Magnetic Resonance Imaging: Applications in Body Imaging. Presented at USUHS Research Day, March 1997, Bethesda.
4. **Hood MN**, Ho VB, Smirniotopoulos JG, Peller PJ, Szumowski J. Chemical Shift: The Artifact and Clinical Tool Revisited. Scientific poster presentation at RSNA, November 1997, Chicago.
5. Foo TKF, Ho VB, McCann RA, Moalemi A, **Hood MN**. "Single Bolus Contrast enhanced peripheral 3D MRA using automated table motion integrated with Automated Bolus Detection and acquisition triggering (MR SMARTPREP)" at 1998 ISMRM Annual Meeting, Sydney, Australia.
6. Foo TKF, Ho VB, **Hood MN**. "A Novel Method for Improved Visualization of Coronary Arteries: Prospective Slice Selective Adjustment for Coronary Artery Positional Variation over the Cardiac Cycle (Vessel Tracking)" at 1998 ISMSM Annual Meeting, Sydney, Australia.
7. **Hood MN**, Ho VB, Haggerty MF, Corse WR. " Can 3D Phase Contrast MRA Aid Breath-hold Gd-enhanced 3D MRA for the Evaluation of Renal Artery Stenosis?" at 1998 SMRT Annual Meeting, Sydney, Australia.
8. Czum JM, Ho VB, **Hood MN**, Foo TKF, Choyke PL. Dual-Phase, Single Gadolinium Chelate Contrast For Peripheral 3D MRA Using Automated Table Motion And Variable K-Space Ordering." Scientific Poster at USUHS Research Day, 1999, Bethesda, MD.
9. **Hood MN**, Ho VB, Foo TKF, Czum JM, Choyke PL. "Bolus Pursuit Peripheral MRA: Timing Issues." Scientific Poster at USUHS Research Day, 1999, Bethesda, MD.
10. Foo TKF, Ho VB, **Hood MN**, Czum JM, Wolff SD, Zhang Y, Choyke PL. "A Novel Method for MR Arterial and Venous Discrimination Using Gated Phase Contrast and VENC Selection" at 1999 ISMRM Annual Meeting, Philadelphia, PA.
11. Czum JM, Ho VB, **Hood MN**, Foo TKF, Choyke PL. Dual-Phase, Single Gadolinium Chelate Contrast For Peripheral 3D MRA Using Automated Table Motion And Variable K-Space Ordering" at 1999 ISMRM Annual Meeting, Philadelphia, PA.
12. **Hood MN**, Ho VB, Foo TKF, Czum JM, Choyke PL. "Bolus Pursuit Peripheral MRA: Timing Issues" at 1999 SMRT Annual Meeting, Philadelphia, PA.
13. **Hood MN**, Ho VB, Foo TKF, Czum JM, Marcos HB, Hess SL, Choyke PL. "High-Resolution Gd-Enhanced 3D Elliptical Centric MRA of the Infrapopliteal Arteries: Lessons for Improving Bolus-Chase Multi-Station Peripheral MRA," Scientific Poster at 2000 ISMRM Annual Meeting, Denver, CO.

14. Choyke PL, Ho VB, Marcos HB, **Hood MN**, Foo TKF. "Shoot and Scoot: A method for improving temporal and spatial resolution of multistation peripheral MR Angiography. Oral Presentation, ISMRM 2001, Glasgow, UK.
15. Marcos HB, Choyke PL, Ho VB, **Hood MN**, Foo TKF. Automated Real-time Multi-station Projection MR Angiography ('Bolus Prep'). Oral Presentation, ISMRM 2001, Glasgow, UK.
17. Marcos HB, Ho VB, **Hood MN**, Choyke PL. 3D Steady State Free Precession (3D-FIESTA) Imaging of The Pancreaticobiliary Ductal System. Oral Presentation, ISMRM 2001, Glasgow, UK.
19. Sortur AG, Ho VB, Saranathan M, Foo TKF, Hood MN, Wolff SD, Comeau CR. Artifacts in Cardiac MR Imaging. Scientific Poster, RSNA 2001, Chicago, IL.
20. Sortur AG, Ho VB, **Hood MN**, Saranathan M, Foo TKF. Respiratory Compensation for Coronary Artery MR Angiography: A Review and Practical Guide. Scientific Poster, RSNA 2001, Chicago, IL.
21. Ho VB; Maniam S, Sortur AG, **Hood MN**, McLuckie AE, Haigney MC. EKG-Gating for Cardiac MRI: A Review and Practical Guide. Scientific Poster, RSNA 2001, Chicago, IL.
23. **Hood, MN**. MRI Safety for Healthcare Workers. DeWitt Army Community Hospital. Presenter, Oral Presentation. September 2001, Fort Belvoir, VA.
24. Marcos H, Ho V, Foo T, **Hood MN**, Choyke P. MR Venography with 3D FIESTA: A New Approach to Renal Vein Thrombosis (abst.). International Society for Magnetic Resonance in Medicine Tenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2002;1765. Poster presentation (18-24 May 2002; Honolulu, Hawaii).
25. Choyke P, Ho V, Marcos H, **Hood MN**, Hess S, Foo T. Continuously Acquired Moving Table MRI: A Method for Rapid Whole Body Scanning (abst.). International Society for Magnetic Resonance in Medicine Tenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2002;560. Oral presentation (18-24 May 2002; Honolulu, Hawaii).
26. Aksit P, Frigo FJ, Polzin J, Choyke P, Ho VB, **Hood MN**, Hess S, Montequin M, Foo T. Shoot and Scoot: A Segmented Volume Acquisition Method for High-Resolution Multi-station Imaging of Peripheral Vasculature (abst.). International Society for Magnetic Resonance in Medicine Tenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2002;208. Oral presentation (18-24 May 2002; Honolulu, Hawaii).
27. Shellock FG, Kanal E, Sawyer-Glover A, Falkner W, Kean M, **Hood MN**, Rohoman L, Lowe J, Hipps C. ISMRM/SMRT MR Safety Forum. ISMRM Annual Meeting, Honolulu, HI May 2002.
28. Hirsch JD, Ho VB, Noonan P, Miller D, Johnson M, **Hood MN**. Imaging of Carotid Artery Disease: 4-year Experience at One Teaching Hospital *Oral presentation* at the International MR Angio Club XV Annual International Workshop From Technology to Practice. (24-26 September 2003; Dublin, Ireland).
29. Ho VB, Foo TKF, Cheng LQ, **Hood MN**, Saranathan M, Montequin M. Breath-hold 3D SSFP Coronary MRA: Early Clinical Feasibility. *Oral presentation* at the International MR Angio Club XV Annual International Workshop: From Technology to Practice. (24-26 September 2003; Dublin, Ireland).
30. Aksit P, Ho VB, **Hood MN**, Choyke PL, Montequin MB, Foo TKF. Single-Injection Multi-station Bolus Timing for Planning of 3D Peripheral MR Angiography, International Society for Magnetic Resonance in Medicine Twelfth Scientific Meeting and Exhibition Program. Kyoto, Japan, May 2004.
31. Ho VB, **Hood MN**, Montequin M. Foo TKF. Cine Inversion Recovery (IR): Rapid Tool for Optimized Myocardial Delayed Enhancement Imaging. Scientific Poster at 2005 ISMRM Annual Meeting, Miami Beach FL, May 2005.
32. **Hood MN,** Ho VB, Bakalov VK, Cooley M, Van PL, Burklow TR, Bondy CA. Turner Syndrome: Characterization and Prevalence of Cardiovascular Anomalies by MR.

Scientific Poster at 2005 SMRT Annual Meeting, Miami Beach FL, May 2005. First Place - research poster category.

33. **Hood MN**, Foo TKF, Ho VB. MRI of anomalous coronary arteries. Oral scientific poster presentation at the 17th Annual Workshop of the International MR Angio Club in Beijing, China, September 2005.
34. **Hood MN.** Stress Imaging Patient Care. Oral education presentation at the 34th Annual North American Society of Cardiovascular Imaging, Las Vegas, NV, October 2206.
35. Stanley D, Comeau C, **Hood MN.** Cardiac Jeopardy for Associated Sciences. Oral education presentation at the 34th Annual North American Society of Cardiovascular Imaging, Las Vegas, NV, October 2006.
36. **Hood, MN**. Patient Preparation and Care for Cardiac CT and MR. Round Table Discussion on How to Run a Cardiac Imaging Service for CT and MRI. Society of Interventional Radiology Annual Meeting, Seattle, WA, March 2007.
37. **Hood, MN**. Patient Care in MRI. John Koveleski Memorial SMRT Regional Meeting at the Penn State Milton S Hershey Medical Center in Hershey, PA on August 25, 2007.
38. Fung M, Schmidt EJ, **Hood MN**, Ho VB. Robust Coronary Artery Imaging using Multiphase-Multislab Free breathing 3D SSFP. Oral presentation at the19th Magnetic Resonance Angiography Workshop which will be held in Istanbul Turkey Oct 3- 5, 2007.
39. Fung M, Schmidt EJ, Kwong R, Holmvang G, **Hood MN**, Ho VB. Novel Approach for ECG-Gated Dynamic Contrast Enhanced MRA. Oral presentation at the19th Magnetic Resonance Angiography Workshop which will be held in Istanbul Turkey Oct 3- 5, 2007.
40. Comeau C, **Hood MN**, Strunk R, Stanley D. Cardiac Jeopardy for Allied Health Professionals. The North American Society for Cardiac Imaging's 35th Annual Meeting & Scientific Sessions, October 5-9, 2007 at the J.W. Marriott Pennsylvania Avenue in Washington DC.
41. **Hood MN**. How to write for publication/presentation. The North American Society for Cardiac Imaging's 35th Annual Meeting & Scientific Sessions, October 5-9, 2007 at the J.W. Marriott Pennsylvania Avenue in Washington DC.
42. Fung MM, Schmidt EJ, **Hood MN**, Golmvang G, Kwong RY, Ho VB. Clinical Applications of Contrast Inflow Dynamics MRA (CIDA): Novel Approach for ECG-Gated Dynamic Contrast Enhanced MRA (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;104. *Oral presentation* (3-9 May 2008; Toronto, Canada).
43. Fung MM, Ho VB, **Hood MN**, Schmidt EJ. Multi-Phase Fat-Suppressed 3D SSFP for Robust Coronary Artery Imaging: Improvements Over the Single-Phas Technique (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;313. *Oral presentation* (3-9 May 2008; Toronto, Canada)
44. Aksit P, Shankaranarayanan A, Gupta SN, Beatty PJ, Aletras AH, Fung MM, Schmidt EJ, **Hood MN**, Ho VB. Externally Calibrated ARC Parallel Imaging Reconstruction for DENSE Imaging: Initial Experience (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;700. *Oral presentation* (3-9 May 2008; Toronto, Canada).
45. Fung MM, Aksit P, Gupta SN, Shankaranarayanan A, Beatty PJ, Aletras AH, Schmidt EJ, **Hood MN**, Ho VB. Respiratory Triggered DENSE Imaging with Navigator Echoes: Initial Experience (abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition Program. Berkeley, Calif: ISMRM, 2008;701. *Oral presentation* (3-9 May 2008; Toronto, Canada).
46. Gupta SN, Aletras A, **Hood MN**, Ho VB, Schmidt EJ, Aksit P. Improved Cardiac Strain Estimation from DENSE Using Automatic Outlier Rejection(abst.). International Society for Magnetic Resonance in Medicine Sixteenth Scientific Meeting and Exhibition

Program. Berkeley, Calif: ISMRM, 2008;989. *Oral poster presentation* (3-9 May 2008; Toronto, Canada).

47. **Hood, MN**. Ethics in Healthcare and MRI Research. John Koveleski Memorial SMRT Regional Meeting at the Penn State Milton S Hershey Medical Center in Hershey, PA, August 9, 2008.
48. **Hood MN.** Advances in Cardiac Imaging: Coronary CT and MRI. Advances in the Care of Hospitalized Cardiac Patient: Third Annual Cardiovascular Nursing Symposium. American Heart Association Scientific Sessions 2008, New Orleans, November 8-12, 2008.
49. Song T, Fung MM, Stainsby J, **Hood MN**, Ho VB. A Novel Cardiac MR Chamber Volume Model for Mechanical Dyssynchrony Assessment. Society of Photographic Instrumentation Engineers Annual Meeting, February 2009 in Orlando, FL, USA.
50. Song T, Aletras A, Gupta SN, Fung MM, Rettmann D, **Hood MN,** Ho VB, Schmidt E. Long Axis Cardiac DENSE Image Analysis and Visualization. North American Society of Cardiovascular Imaging Annual Meeting, October 2008, Scottsdale, Arizona.
51. Song T, Stainsby J, **Hood MN**, Ho VB. Cardiac T1 mapping: A comparison of methodologies for quantifying cardiac T1 values. International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
52. Song T, Stainsby J, **Hood MN**, Ho VB. Quantification of Global Hypokinesis in Left Ventricle using Center Point Trajectory (CPT). International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
53. Song T, **Hood MN**, Ho VB, Gupta S, Stainsby J. Optimization and Validation of a Modified Look-Locker Saturation-Recovery (MLLSR) Sequence Applied To Cardiac T1 mapping. International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
54. Song T, Stainsby J, **Hood MN,** Ho VB. Quantification of Focal Left Ventricular Wall Motion Abnormalities using Center Point Trajectory (CPT) Mapping . International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
55. Fung MM, Ho VB, **Hood MN**, Zurs Y, Schmidt EJ. Automatic detection of Quiescent Cardiac Phases using Navigator Echoes with Adjacent Complex Correlation Algorithm. International Society for Magnetic Resonance in Medicine Seventeenth Scientific Meeting and Exhibition Program. April 2009, Honolulu, HI, USA.
56. **Hood MN**, Song T, Ho VB. Review of Delayed Myocardial Enhancement in Cardiac MRI. Scientific Poster. Uniformed Services University of the Health Sciences Research Week, May 11-13, 2009 in Bethesda, MD, USA.
57. Song T, Ho VB, Slavin G, **Hood MN**, and Stainsby JA. "Varied Sampling Patterns in Modified Look-Locker with Saturation Recovery for Flexible Cardiac T1 Mapping", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* oral presentation, Stockholm, Sweden, May 1-7, 2010.
58. Song T, Stainsby JA, **Hood MN**, Ho VB. " A Novel Centerline Model for Cardiac Long Axis Wall Motion Analysis", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.
59. Song T, Ho VB, Slavin G, **Hood MN**, Stainsby JA. "Clinical Evaluation of a Cardiac T1 Mapping Method Using a Reduced Number of Sample Times", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.
60. Song T, Bustamante AI, Stainsby JA, **Hood MN**, Ho VB. "Center Point Trajectory Model for Cardiac Wall Motion Abnormality Assessment Compared with Echocardiography Strain", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.

61. Song T, Ho VB, Slavin G, **Hood MN**, Stainsby JA. " Reducing the Sensitivity to Respiratory Motion of Modified Look-Locker with Saturation Recovery for Cardiac T1 Mapping", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),*E-poster session, Stockholm, Sweden, May 1-7, 2010.
62. Song T, Bustamante AI, Stainsby JA, **Hood MN**, Ho VB.. "Evaluating Left Ventricular Wall Motion Abnormalities using Centerline Trajectory Mapping", *Proc. Intl. Soc. Mag. Reson. Med. 18 (2010),* E-poster session, Stockholm, Sweden, May 1-7, 2010.
63. Dainer H, **Hood M**, Ho V. Myocardial Delayed Enhancement MR Imaging: A Pattern Approach. Poster at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
64. Song T, Ho VB, Slavin G, Hood MN, Stainsby JA. Clinical and Phantom Evaluations of a Cardiac T1 Mapping Method with Half of the Scan Time. Poster at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
65. Song T, Lai P, Stainsby JA, Hood MN, Ho VB. Myocardial Perfusion Imaging using Kat Autocalibrating Reconstruction for Cartesian Sampling: Acceleration Factor of Eight. Poster at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
66. Song T, Stainsby JA, **Hood MN**, Ho VB. Three-segment Center Point Trajectory Model for Segmental Tracking and Quantification of Cardiac Wall Motion. Poster Honorable Mention Award. North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
67. **Hood MN**, Song T, Bedocs, P, Capacchione C, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 in Swine with Heart Failure. Oral Presentation. American Heart Association Young Investigator Finalist at the North American Society of Cardiovascular Imaging Annual Meeting, October 3-5, 2010, Seattle Washington.
68. **Hood MN**, Song T, Bedocs P, Capacchione J, Haigney M, Kasper C, Ho VB. Free-breathing T_1 Mapping MRI for Quantification of Myocardial T_1 Pre and Post Contrast in Swine with Non-ischemic Heart Failure. *Proc. Intl. Soc. Mag. Reson. Med. 19 (2011),* Traditional Poster, Montreal, Canada, May 7-13, 2011.

RESEARCH PROTOCOLS

1. *Clinical Coordinator.* Ho VB, Szumowski J. Magnetic Resonance Mammography (MRM): A Promising Application for Fat Suppression by Phase Unwrapping in the 3-Point-Dixon Method, 1996.
2. *Research Technologist.* Porter BA, Smith JP, Koch JE, Mate T, Tickman R, Lilly J, Gottesman JE. Computerized Pattern Recognition Analysis of Magnetic Resonance Imaging for Prostate Cancer Assessment and Staging, 1996.
3. *Research Technologist.* Porter BA, Smith JP, Borrow JW, Schultze-Haake H. Clinical Optimization of MR Imaging Pulse Sequence Software. 1996.
4. *Clinical Coordinator*. Ho VB, Corse WR, Hoffmeister K, Cash S. A Multicenter, Randomized, Double-Blind Study to Evaluate the Safety, Tolerability, and Efficacy of OptiMARK Compared to Magnevist in Patients with Liver Pathology, 1996-97. NNMC #B96-065.
5. *Clinical Coordinator.* Ho VB, Georgia J, Miller DL, Corse WR, Hood MN, Czum JM. An Open-label, Multicenter, Single-dose, Phase 2, Feasibility Study of NC100150 Injection for Evaluation of Peripheral MR Angiography. 1998. NNMC #B98-043. USUHS G18943-01.

6. *Volunteer Investigator.* Ho VB, Choyke PL, Summers R, Foo TKF, Zhang Y, Pastaskia B, Moalemi A, Lundergan CF. Contrast Enhanced Magnetic Resonance Angiography (MRA) in the Diagnosis of Atherosclerotic Disease: A Pilot Technical Development Study. NIH #99-CC-0061. USUHS G18946-01.
7. *Volunteer Investigator.* Ho VB, Choyke PL, Summers R, Patronas NJ, Dagher AP. Normal Volunteer Scanning on MR. NIH #98-CC-0019. USUHS G18945.
8. *Clinical Coordinator.* Ho VB, Donovan MS, McLuckie AE, **Hood MN**, Czum JM, Pace ME, Sortur A. A Phase II Feasibility Study of the Safety and Efficacy of Coronary Magnetic Resonance Angiographic (CMRA) Imaging with MS-325. NNMC #B99-055. USUHS #G18949-01.
9. *Associate Investigator.* Ho VB, McGuigan EA, **Hood MN**, Nayak, G. Observational MRI Protocol of New Pulse Sequences. NNMC.1999.038. USUHS #G18948-01.
10. *Volunteer Researcher.* Choyke PL, Wilcox CS, Marcos H, Chen C, Kopp J, Ho VB, Preas H, Talagala L, Altizer G, Jones S. Assessment of Renal Artery Stenosis and Renovascular Hypertension by Contrast Enhanced Magnetic Resonance Imaging: A Pilot Study. NIH #00-CC-0195. USUHS #G18953.
11. *Volunteer Researcher.* Choyke PL, Olan W, Dick B, Arai A, Zhang Y, Ho VB, Marcos H, Jones S. A Comparison of Contrast Enhanced Magnetic Resonance Angiography (MRA) and Conventional Angiography in the Diagnosis of Atherosclerotic Disease: A Pilot Study. NIH #00-CC-0028. USUHS #G18954.
12. *Associate Investigator.* Rowedder AM, Donovan MS, Ho VB, Ketron GD, Rothstein JH, Pace ME, La Corte-Gonzalez J, **Hood MN**. MR Phase-Contrast Flow Quantification: Useful Adjunct to MR Angiography? NNMC.2001.027. USUHS #G18959.
13. *Associate Investigator.* An In-depth Review of Imaging for Carotid Artery Diseases: One Teaching Hospital Experience. USUHS IRB # HU8971, NNMC.2003.050.
14. *Clinical Coordinator*. CAI Multi-center Clinical Feasibility Study with 2D Spiral Coronary MR Angiography, USUHS IRB #HU8976
15. *Clinical Coordinator*. CAI Multi-center Clinical Feasibility Study with 3D FIESTA Coronary MR Angiography, USUHS IRB #HU8977
16. *Associate Investigator.* Cassimatis D, Ho VB, Taylor A, **Hood MN**. ARBITER IV: Magnetic resonance images of the aorta to assess treatment effects of lowering cholesterol – A pilot study utilizing Cardiac Magnetic Resonance (CMR) to assess aortic plaque and pulse wave velocity in ARBITER II patients. USUHS IRB # HU 8978.
17. *Principal Investigator.* **Hood MN**, Ho VB, Ferguson M, Newman M, Dendall R, Sabatino M. Descriptive Evaluation of Anomalous Coronary Arteries: A Retrospective Review of an Early Experience Using MRI. #NNMC.2005.024. USU IRB # HU8990.
18. O'Connor FG & Deuster PA. AI's: Adams B. Team Leaders: Ricciardi R, **Hood MN**, Valenzuela R. Study 3 - Enlyten™ Electrolyte SportStrips™ for the Acute Treatment of Exercise Associated Muscle Cramps in a Mass Participation Event: A Double Blind Intervention Trial. Marine Corps Marathon, October 28, 2007.
19. *Associate Investigator.* Ho VB, **Hood, MN**, Bustamante A. Wall motion assessment on cardiac MRI: A retrospective review. NNMC.2009.0105.

AWARDS AND HONORS

1984	Phi Sigma Biological Honors Society
1986	Phi Sigma/Sigma Xi Recognition for Outstanding Undergraduate Research
1988	NWICCAA Scholar Athlete of the Quarter
1989	Allied Health Student of the Year-Tacoma Community College

1997 Certificate of Merit Award, RSNA 1997 for scientific poster exhibit: **Hood MN**, Ho VB, Smirniotopoulos JG, Peller PJ, Szumowski J. Chemical Shift: The Artifact and Clinical Tool Revisited, Chicago

1998 President's Award for outstanding paper: **Hood MN**, Ho VB, Haggerty MF, Corse WR. "Can 3D Phase Contrast MRA Aid Breath-hold Gd-enhanced 3D MRA for the Evaluation of Renal Artery Stenosis?" at the SMRT Annual Meeting in Sydney, Australia

1999 Phi Theta Kappa International Honors Society, Montgomery College

2002 Sigma Theta Tau, International Nursing Honors Society, Pi Chapter – University of Maryland, Baltimore

2003 Phi Kappa Phi Honor Society, University of Maryland, Baltimore

2005 First Place Research Poster for **Hood MN**, Ho VB, Bakalov VK, Cooley M, Van PL, Burklow TR, Bondy CA, "Turner Syndrome: Characterization and Prevalence of Cardiovascular Anomalies by MR" at the SMRT Annual Meeting in Miami Beach, Florida.

2006 SMRT Crues and Kressel Award for outstanding contributions to the education of MR technologists at the SMRT Annual Meeting in Seattle, May 2006.

2007 American Radiological Nurses Association, 2006 Linda Strangio Editor's Award, for *Introduction to Picture Archive and Communication Systems*, at the ARNA Annual Meeting in Seattle, March 2007.

2010 SMRT Fellow of the Section Award for outstanding contributions to the SMRT. Annual Meeting in Stockholm, Sweden, May 2010.

2010 Who's Who Among Students in American Universities and Colleges, Uniformed Services University of the Health Sciences, Graduate School of Nursing

2010 Council on Cardiovascular Radiology and Intervention of the American Heart Association, Young Investigator Competition. Honorable Mention Award for *Free-breathing T1 Mapping for Quantification of Myocardial T1 in Swine with Heart Failure*. Seattle, WA Oct, 2010.