**Chapter 1:**
**Introduction**

Congestive Heart Failure (CHF) is one of the most debilitating and commonly presented conditions in the middle aged and older populations. This condition currently accounts for billions of dollars spent on medical care each year. One of its hallmark symptoms is a severe reduction in exercise capacity (*i.e.* peak oxygen consumption ($VO_{2\ peak}$)). The primary mechanism, which causes or contributes to this reduction, is currently unclear. Many scientific investigations have suggested that CHF causes many detrimental alterations in various tissues throughout the body (Coats, 1996). Therefore, it is of great interest to determine which specific tissues within the body must be restored to the original function in order to improve or return the functional capacity of the CHF patients. To understand how to combat this condition, one must understand what the limiting factors are within the CHF patient.

In order to understand how to prevent or alleviate the symptoms of CHF, one must first understand what occurs within the condition. Any single cause of cardiovascular dysfunction (i.e. irregular heartbeat, obstructed flow, and/or contractile dysfunction) will ultimately lead to irregular cardiac function and prevent the heart from meeting the metabolic requirements of the peripheral tissues (Schoen, 1994). When describing pathological changes occurring during CHF, one must first consider the myocardium. The compensation of the heart to maintain normal work output is evident by the hypertrophy of the ventricular tissue. Hypertrophy is any increase in the size of the muscle cells, which ultimately contribute to increases in overall mass. The characterization of this increase in mass is dependent upon the cause of failure in the heart. For example, during myocardial infarction (MI), there is a death of myocytes, causing the remaining elements in the non-infarcted region to hypertrophy due to compensation of a

reduced amount of myocardium and no changes in the level of work. Cardiac hypertrophy

is an adaptive response to a changing environment, however it must be balanced in that

too much hypertrophy evolves into cardiac failure due to a decreased myocyte to capillary

ratio and/or increased fibrous tissue (Schoen, 1994). When attempting to balance between

cardiac compensation and cardiac failure, there are multiple changes in the functional

measurements of the myocardium. For example there are significant reductions in the

stroke volume (i.e. amount of blood ejected by the ventricles), ejection fraction (i.e. the

proportion of blood ejected by a ventricular contraction compared to the total filling of the

ventricle) and cardiac output (i.e. amount of blood ejected by the heart times the heart

rate). It would seem logical that alterations in any or all of these functions could severely

restrict exercise performance. However, many other morphological changes which occur

during CHF are distant from the heart, and could also contribute to a reduced functional

capacity (Coats, 1996).

While it is apparent that the heart changes structurally and functionally, one should

realize that there are multiple changes occurring in other systems. For instance, with

CHF, there are significant pathophysiological changes to the lung tissue due to pooling of

the blood within the pulmonary circulation (Schoen, 1994). This results in pulmonary

edema and congestion often described pathologically as 'heavy wet lungs' (Schoen, 1994).

These changes in the lungs can cause increased occurrences of breathlessness or severe

coughing, often following exertion. Dyspnea is usually the earliest sign of CHF seen in

patients and is often attributed as the cause for the early onset of fatigue. Therefore, when

discussing CHF it would be very easy to conclude that the reduction in exercise capacity

of the CHF patients is due to changes in myocardium and lung structure/function.

However, as often is the case when discussing a complex condition, nothing is ever so simple.

Current evidence suggests that cardiovascular function may not be the primary limiting factor of the reduction in exercise tolerance in CHF. Simply, comparing correlations of exercise capacity (i.e. $VO_{2\,peak}$) and indices of cardiovascular function (i.e., ejection fraction or cardiac output) show no significant relations (i.e., ejection fraction and exercise capacity (r=0.04)), even though the correlations are significant in healthy populations (Franciosa et al., 1981). This suggests that the limiting factor may lie elsewhere. In fact, using the same type of correlations, it has been found that $VO_{2\,peak}$ correlates well with measures of skeletal muscle performance (i.e., muscular strength (r=0.65) and endurance (r=0.63)) (Volteranni et al., 1994). This indicates that a possible limiting factor in exercise performance of CHF patients is the function of their skeletal muscle.

It is very possible that skeletal muscle function is a limiting factor during exercise. This idea is supported by wealth of evidence showing that skeletal muscle strength and endurance are significantly altered in CHF patients (Minnoti et al. 1991, Minnoti et al. 1993). In fact, multiple studies were conducted, all suggesting that CHF patients are susceptible to skeletal muscle weakness and fatigue, both of which may contribute to reductions in exercise tolerance (Sullivan et al. 1991, Buller et al. 1991, Okita et al. 1998). However, what is unclear is the cause of this reduced performance capacity of the skeletal muscle. In the past, various investigators have speculated that intrinsic alterations within the cellular environment of the muscle contribute to the development of muscle fatigue within healthy tissue (Williams and Klug 1993, Allen et al. 1995). For example, it has

been suggested that the function of various intracellular proteins (i.e. ryanodine receptor and/or contractile apparatus) are altered during fatigue thereby possibly preventing normal force production of the muscle (Ward et al. 1998, Williams et al. 1998). Similar arguments have been made for chronic conditions of increased and decreased use. Therefore, it seems reasonable to suggest that these changes may be exacerbated in CHF patients and may contribute to the dysfunction of the skeletal muscle.

Existing data demonstrate that skeletal muscle of CHF patients exhibits atrophy and an increased reliance on glycolytic metabolism, at the expense of oxidative metabolism (Okita et al. 1998, Sullivan et al. 1990, Massie et al. 1987, Mancini et al.1992). However, neither of these changes is sufficient to account for the dramatic changes in exercise tolerance that accompanies CHF. For example, investigations have shown that the reductions in knee extensor strength and endurance are not related to muscle size and seem to result from intrinsic changes within the muscle fibers themselves (Minotti et al. 1993). Also, there are cases of CHF patients not exhibiting muscular atrophy, who still have significant reductions in force production. These reductions in muscle strength could not be due to alterations in metabolism (Minotti et al. 1993). There is also evidence to suggest that during CHF skeletal muscle exhibits a change in overall muscle fiber type expression, in that there is a shift in expression of fiber types from slow to fast (Sullivan et al. 1990). It is well known that fast glycolytic fibers are more susceptible to fatigue, and it has been proposed that this may be a contributing factor to the early onset of muscular fatigue in CHF patients (Delp et al. 1997).

It should be noted that these changes resemble muscular alterations seen during reduced activity. However, there is evidence to suggest that these alterations are specific

to CHF and not to reduced activity that is associated with CHF. For example, Vescovo et al. (1996) compared changes in skeletal muscle myosin heavy chain (MHC) between CHF patients and bed-ridden patients. It was determined that the changes in MHC expression were more pronounced (*i.e.* greater slow to fast transition) in the CHF patients compared to the bed-ridden patients. Also, Bigard et al. (1998) found that reduced muscle activity causes a similar transition in MHC as CHF, but reduced activity does not cause the changes in the muscle mitochondrial capacity that is seen during CHF. This suggests that skeletal muscle changes that occur during CHF are not specific to reduced activity, and seem specific to the condition.

The excitation-contraction coupling (ECC) process ultimately limits muscular strength. Further changes in ECC play important roles in both acute and chronic changes in muscle force production and endurance. That is, force production by the muscle fiber is determined by the level of activation of the contractile apparatus, which in turn, is determined by kinetics of sarcoplasmic reticulum (SR) $Ca^{2+}$ release and uptake. Recent investigations indicate that there are multiple changes in SR function during the development of CHF. For example, the first study by Perrault et al. (1993) demonstrated that skeletal muscle ECC, more specifically $Ca^{2+}$ release, was severely comprised during activation of muscle fibers taken from CHF rats. Also, Williams & Ward (1998) demonstrated that sarcoplasmic reticulum (SR) $Ca^{2+}$ release and uptake are accelerated during moderate heart failure, which seemed to contribute to alterations in force production. Peters et al. (1997) found decreased expression of $Ca^{2+}$-ATPase pump, which is directly involved in $Ca^{2+}$ uptake. They also showed decreases in specific activity of the $Ca^{2+}$-ATPase. Therefore, it is not clear if these changes in ECC are due to alterations in

protein expression associated with the ECC process or due to changes in intrinsic properties of the proteins.

In summary, skeletal muscle demonstrates significant alterations during CHF that are not related to reduced activity and in fact seem to be specific to CHF. These changes may include alterations in phenotypic expression of various structures, which directly control ECC and force production within or by the muscle. Therefore, it is very likely that these changes directly contribute to the reduction in exercise tolerance exhibited by people afflicted with CHF.

**Statement of Problem**

*Study #1*

Upon examination of the literature, there is some ambiguity between research studies related to changes in skeletal muscle MHC isoform expression during CHF. This is partly due to the fact that all the studies used various models and differing levels of severity to mimic CHF. Therefore, upon examining these studies one can find that there are inconsistent changes in MHC isoform expression in various muscles. For example during CHF, Simonini et al. (1996) clearly demonstrate increases in IIx and IIb mRNA levels in the soleus muscle, which normally would only contain I and IIa MHC. Unfortunately, the study did not measure changes in MHC protein levels during CHF, making it difficult to interpret the results. Additionally, Vescovo et al. (1998) examined MHC changes in heart failure of the soleus muscle. They only found increases in fast MHC isoform (i.e. IIa) expression, however it should be noted that they used a different model of CHF. The model used was a pharmacologically-induced CHF, which caused the

animal to develop pulmonary hypertension and subsequently develop CHF. This raises the question of whether the changes were specific to the models being used or specific to the degrees of failure being produced. It also suggests that the changes in mRNA levels in the previous study might have not occurred at the protein level. Therefore, it is important to first determine which muscles demonstrate changes in MHC isoform expression and second to determine if these changes are specific to the severity of the disease. In this study, changes in hindlimb skeletal muscle MHC isoform expression were determined using a rat surgical myocardial infarction model to induce CHF. Measures of myocardial function were determined to evaluate the severity of disease and to determine if they had any relationship to changes in muscle CHF expression.

*Study #2*

Currently, it is known that exercise training will improve the functional capacity of CHF patients (Minotti and Massie, 1992). Traditionally, endurance training is utilized to reverse the effects of CHF on patients. Many have argued that qualitatively similar training-induced improvements in parameters such as muscle oxidative capacity directly result in marked improvements in exercise capacity (Minotti and Massie, 1992). However, endurance training also results in increased muscle blood flow and improved myocardial function, making the interpretation of the results somewhat difficult. That is, are the changes due to alterations in the cardiovascular system or because of changes to the skeletal muscle itself? As a result, firm conclusions regarding the role of skeletal muscle in CHF-induced exercise intolerance have been difficult to make using an endurance-training model. Therefore, the purpose of this study was to gain insight into

the changes that occur during CHF by employing a technique that is known to alter SR $Ca^{2+}$ handling, MHC, and SERCA expression in healthy muscle without affecting cardiovascular performance.  This technique is gastrocnemius muscle ablation, which causes a functional overload (FO) of the remaining musculature (i.e. soleus and plantaris). Interestingly, this overload model produces muscular effects which are opposite to which is seen during CHF.  For example, functional overload of the plantaris muscle is known to increase slow phenotype protein expression and also slow SR $Ca^{2+}$ handling.

This study used FO in an effort to reverse or prevent CHF-induced changes in MHC, SERCA expression, and $Ca^{2+}$ handling by the SR.  This study is perhaps the first to provide direct evidence that it may be possible to restore the original muscle phenotype and function without changing the function of the heart. Also, it will provide evidence as to whether or not the muscle becomes less adaptable during CHF.  For example, it has been suggested by some that aged muscle has a decreased ability to undergo the hypertrophy process compared to normal muscle (Blough and Linderman, 2000). Therefore, it may be that skeletal muscle of animals inflicted with CHF is unable to adapt as well as healthy muscle.  If FO is ineffective in altering the skeletal muscle during CHF this would suggest that the heart and skeletal muscle are somehow linked, and in order to return the function of the skeletal muscle one must first restore the function of the heart.

### *Significance of the study*

These studies should have a profound impact on what is currently understood about skeletal muscle during CHF.  First, it will help to clarify the existing data that have been published concerning the changes in muscle phenotype during CHF.  It will help to

reveal whether the changes are muscle specific and specific to the severity of the disease. Secondly, it provides evidence concerning whether or not the muscle has a loss in its ability to adapt to exercise during CHF. Also, it provides evidence as to whether or not the heart and peripheral muscle exhibit a form of cross talk, in that one cannot change without the other changing during CHF.

The study has clinical applications in that it helps to shed light on the idea of whether or not strength training may be beneficial for CHF patients. That is, strength training may prove to be more beneficial than endurance training in restoring exercise capacity after the onset of CHF. This study therefore sought to improve our understanding of how the heart and skeletal muscle may be linked during CHF and whether skeletal muscle is capable of adaptation during CHF.

**Research Hypotheses**

The following null hypotheses were tested during this investigation:

_Study#1_

Ho$_1$: There will be no effect of the MI surgery on myocardial morphology in rats.

Ho$_2$: There will be no effect of CHF on MHC expression in the plantaris, white gastrocnemius, red gastrocnemius, and soleus muscles of rats.

Ho$_3$: There will be no relationship between expression of fast MHC and the severity of CHF in rats.

$Ho_1$: There will be no effect of CHF, FO or their combination on myocardium

morphology in rats.

$Ho_2$: There will be no effect of CHF, FO or their combination on plantaris muscle mass in

rats.

$Ho_3$: There will be no effect of CHF, FO or their combination on plantaris muscle MHC

protein expression in rats.

$Ho_4$: There will be no effect of CHF, FO or their combination on plantaris muscle SERCA

protein expression in rats.

$Ho_5$: There will be no effect of CHF, FO or their combination on plantaris muscle $Ca^{2+}$

uptake rates in rats.

$Ho_6$: There will be no effect of CHF, FO or their combination on plantaris muscle fiber

type expression in rats.

**Delimitations**

The investigator set the following delimitations:

1.  The subjects were 60 adult female Sprague-Dawley rats.

2.  Only one leg was subjected to functional overload and the contralateral leg

    served as a control muscle.

3.  The coronary ligation technique was used to model a model of CHF in rats.

4.  Isolated homogenate SR $Ca^{2+}$ uptake mimics SR $Ca^{2+}$ uptake *in vivo.*

5.  Four and 11 weeks of coronary ligation were sufficient to develop CHF.

6. Nine weeks were used to develop muscle hypertrophy after the functional overload surgery.

7. Only the plantar flexor muscles were studied.

**Limitations/Assumptions**

The following limitations were inherent in this experimental design:

1. The removal of a portion of the gastrocnemius will not have any effect on cardiac function.

2. There was no underlying factor within the muscle, unknown to the investigator that would affect normal function.

3. The animals were infected by and recovered from an exposure to the sialodacryoademitis (SDA) virus, which was beyond the investigators control.

4. The investigation was limited to effects observed 11 weeks post MI and included only one species of animal.

5. Myocardial infarction model of CHF successfully mimics human CHF.