Chapter 5:

Discussion

Study#1

This study found that some, but not all, peripheral skeletal muscles exhibited changes in MHC isoform expression during CHF in rats. The data also suggested that the MHC isoform changes were dependent upon the severity of CHF manifested in the animals. No significant differences in MHC isoform expression were detected in the soleus or white gastrocnemius (WG) muscles between treatments. However, there were significant differences found in MHC IIx and IIb expression of the plantaris between the SHAM and severe CHF group. Also, there were significant 7-9% elevations in MHC IIx and IIb expression at the expense of MHC I and IIa in the red gastrocnemius (RG) of both CHF groups compared to the SHAM group. Significant correlations were found between changes in MHC expression and various indicators of CHF.

This is one of the first studies to show that different muscles respond to CHF in different ways. These data agree with currently published data in that there are changes in MHC expression in peripheral musculature of rats inflicted with CHF. However, Vescovo et al. (1998) and Yamaguchi et al. (1999) both claim to see dramatic changes in rat skeletal muscle MHC after CHF, however their methodology they used to separate the MHC isoform was unable to detect MHC IIx. Therefore, it is unclear if the increases in MHC IIx expression in their studies resulted from contamination by the IIa band, because of the close proximities of the bands on the gel. Thus, this study provides the first data to identify changes in all four MHC isoforms in the plantar flexor muscles of the rat in response to CHF.

Many argue that skeletal muscles from humans or animals with CHF demonstrate significant changes in muscular phenotype (Delp et al. 1997, Peters et al. 1997) However,

upon careful examination of the literature it is evident that the data are somewhat ambiguous concerning changes in MHC isoform expression. One would expect that the soleus muscle, a predominately slow muscle, would be extremely susceptible to these changes in MHC isoform expression during CHF. For instance, Simonini et al. (1996a 1996b) suggested that the soleus muscle shows many dramatic (i.e., histological and molecular) changes, which would be detrimental to overall function of the animal. However, the MHC isoform changes they assessed were only at the mRNA level. They did not measure any changes in MHC isoform expression at the protein level. It is currently unclear as to why mRNA for MHC IIx and IIb was present without expression of the corresponding MHC protein. However, it should be noted that this phenomenon has been previously documented in skeletal muscle, in that Andersen et al. (1998) found muscle fibers which contain a certain MHC isoform mRNA which does not match the MHC protein expressed. In the present study, no significant changes in MHC protein isoform were found in the soleus muscle. The present data are consistent with that of Vescovo et al. (1998) who indicated that the rat soleus muscle exhibited a significant 4% increase in MHC IIa expression at the expense of MHC I during CHF. While their change was statistically significant, the present study found similar changes in magnitude (5%) that was non-significant. Based on these results, it seems that CHF is not sufficient to cause major changes in MHC expression of the soleus.

It is possible that a mixed muscle, one that contains all four MHC isoforms, may be more susceptible to changes in phenotype during CHF. According to the present data, this seems to occur in the plantaris and red gastrocnemius muscles, since both showed larger and statistically significant changes in MHC expression during CHF. It is unclear

why these muscles were affected, while others were not. It is possible that a mixed muscle is more adaptable to the stress of CHF due to its heterogeneous phenotype. Alternatively, it may be that muscles that experience considerable activity (*e.g.* soleus) or minimal activity (*e.g.* white gastrocnemius) during normal locomoter activity of the animal are less adaptable to the CHF-induced changes in muscle phenotype.

One of the interesting outcomes of this study was the finding that the changes in MHC expression correlated with various indicators of CHF morphology. For example, the decreases in RG MHC I or IIa showed strong correlations with changes in LVEDP, RV mass and lung mass (Table 3). However, this was not the first time this has been demonstrated. Sullivan et al. (1997) found similar results in humans afflicted with CHF. Their study suggested that the vastus lateralis, a mixed muscle, underwent significant decreases in MHC I expression with subsequent increases in MHC IIx expression. They also found that MHC I expression was related to $VO_{2\ peak}$ ($r=0.70$, $p<0.05$) of the patients. Thus, it appears that there are more pronounced changes in MHC expression in mixed muscles and they are related to disease severity.

Summary. Changes in MHC isoform expression have been shown to affect muscle contractile characteristics. The changes in MHC isoform expression could have an impact on overall muscle function and ultimately change exercise capacity of the CHF patient. However, there is no direct evidence to suggest how the shifts in MHC isoform expression directly cause reductions in exercise capacity. What is known though is that muscles which contain a higher percentage of fast MHC generally fatigue more rapidly than slow muscles. It is currently unclear why muscles, which express a higher percentage of fast MHC, fatigue more quickly than muscles with a high percentages of

slow MHC. Thus, it may not be the change in myosin isoform that is directly causing the increased development of fatigue during CHF, but it may be an indicator of a more fatigable muscle.

In summary, these results show that rats with CHF develop significant changes in MHC of mixed skeletal muscles. These changes tend to increase, as the severity of CHF increased. It is reasonable to suggest that the 'slow to fast' shift is indicative of a more fatigable muscle and that such changes may negatively impact exercise tolerance.

Study#2

Muscle mass. The major finding of this study was that skeletal muscle still retains its ability to adapt to exercise stress in the presence of CHF. This was evident in that the plantaris muscle of the CHF-FO group exhibited significant muscle hypertrophy without showing any significant differences in myocardial morphology when compared to the CHF group. Also, the skeletal muscle hypertrophy that occurred was similar between the CHF-FO and SHAM-FO suggesting that CHF did not inhibit or attenuate skeletal muscle growth resulting from increased activity and load bearing. Since FO did not affect myocardial performance, peripheral muscle adaptations, which occur with CHF, could be attenuated without reversing the condition of the heart.

MHC isoform expression. Curiously, there were no differences found in plantaris MHC isoform expression between the non-FO CHF and SHAM groups. This contradicts what was previously found. However, it may be explained by the fact that the severity of

CHF obtained in these animals was not as great as in Study #1, as changes in RV and lung mass were somewhat smaller than those observed in the first study. The plantaris muscles from SHAM-FO and CHF-FO group both showed signs of a fast to slow transformation as indicated by the significant whole muscle increases in MHC I at the expense of MHC IIx and IIb. This was an expected response, since it is known that with muscular overload there is an increase in slow muscle isoform proteins (Roy et al. 1997, Talmadge et al. 1994). The magnitude of the change was consistent with the observations of Roy et al. (1997) who suggested that in healthy animals with a ~30 % increase in plantaris mass there were only small (i.e. 5% increase) changes in MHC I expression. No differences were detected between the CHF-FO and SHAM-FO groups indicating that the animals inflicted with CHF still retained the ability to adapt their phenotype with the hypertrophic response. Also, it should be noted that the even in severe CHF changes in overall plantaris MHC reported here and throughout the literature are not large (<10% changes in MHC protein expression) compared to conditions of disuse.

This study was the first to examine muscles of animals inflicted with CHF by means of immunohistochemistry. The non-overloaded legs of the CHF animals showed no signs of specific muscle fiber atrophy when compared to the SHAM animals. This is consistent with the finding that CHF did not affect overall muscle mass. The data also show that CSA of type I fibers increased in both CHF-FO and SHAM-FO conditions, while only the IIa fibers from CHF-FO group showed increased size. This is not completely surprising since the plantaris muscles only showed about a 30% increase in total mass, and it is known that type I fibers adapt sooner than the other fiber types (Roy

et al. 1997). Roy et al. (1997) found in healthy animals that an increase in plantaris mass of 37% corresponded with increases in CSA of type I and IIa fibers but not IIx fibers. Therefore, these data suggest that individual fibers of the plantaris muscle adapt readily to FO during CHF.

Even though no whole muscle changes were detected in MHC isoform expression there is the possibility of regional changes occurring in the muscle during CHF. The plantaris consists of a deep region, which contains a high percentage of slow fibers, while the rest of the muscle contains mostly fast fibers. Regional changes in MHC isoform expression muscle were previously demonstrated to occur by Roy et al. (2000) in animals subjected to absolute inactivity. Their data suggest that fiber type transformations, based on MHC isoform expression, were larger in the region that contained a higher amount of slow fibers compared to fast fibers. The presented data here suggest that similar regional changes in MHC-based fiber proportions may also occur in the deep region of the plantaris muscle during CHF. In the CHF animals there were significantly more IIx fibers than the SHAM animals in the deep region of the plantaris. Thus, while whole muscle MHC expression was not significantly different between the CHF and SHAM groups, while there were differences in the proportion of fibers expressing fast MHC isoforms in specific regions.

The FO treatment seemed to be equally effective in producing a fast to slow transformation in both the SHAM-FO and the CHF-FO of this region. Expectedly, the SHAM-FO and CHF-FO contained significantly less IIx fibers than SHAM and CHF group. Interestingly, within this deep medial region the CHF-FO and SHAM group had a similar number of IIx fibers. This suggests that the FO treatment in CHF group

prevented or attenuated the CHF-induced rise in the percentage of IIx fibers from this region of the muscle. The data also show that SHAM-FO and CHF-FO had similar increases in type I fibers compared to the SHAM and CHF group, respectively. This indicates fiber type transformations occurred in the deep region of the plantaris in response to FO in both CHF and non-CHF rats. The extent of the changes were similar in both the CHF-FO and SHAM-FO group, again suggesting that CHF animals have the ability to adapt to increased levels of activity. It also suggests that changes in overall muscle MHC expression during severe CHF may be due to changes in MHC expression within this deep region.

SERCA expression. Surprisingly, the SR data suggested that altered functioning of the Ca^{2+}-ATPase during CHF was not due to changes in SERCA isoform expression. Specifically, there was a 44% increase in Ca^{2+} uptake rate in the CHF group compared to the other three groups, with no change in overall muscle SERCA expression. This conflicts with data from Siminoni et al. (1999) who suggested that there was a significant 16% reduction in SERCA 2 expression. Their study was lacking in functional measures, so it is unclear if such a reduction in SERCA 2 translates into alterations in Ca^{2+} pump activity. Also, the present data does not agree with Peters et al. (1997) who found significant reductions in SERCA 1 mRNA and protein expression. However, their study incorporated a genetic rat model of CHF, whose severity of CHF was greater than in the present study. This may explain why the differences exist, whereby as the animals begin to develop severe CHF there is a decrease in SERCA 1 expression.

Williams and Ward (1998) also found a 30% increase in Ca^{2+} uptake rates of isolated SR from CHF rats, without changes in isoform expression. This suggests that during CHF some unidentified regulatory mechanism is likely altered allowing for increases in Ca^{2+} pump activity. Some possibilities, which could affect Ca^{2+}-ATPase activity include, alterations in phospholamban, changes in calsequestrin content, changes in phosphorylation of the Ca^{2+}pump, or altered release channel function (Dux, 1993). More detailed analyses of the SERCA pump are needed to fully understand the CHF-induced changes in calcium pump function.

FO surgery was able to prevent the CHF-induced alterations in Ca^{2+} uptake, possibly by changing the isoform expression. It is clear that changes in Ca^{2+} uptake activity during CHF-FO were due, in part, to alterations in isoform expression since the functional and expression measurements were made on the same homogenate fraction and since qualitatively similar changes in expression and function were found. Kandarian et al. (1994) also found that FO in healthy rats caused a 15% reduction in Ca^{2+} uptake. They also suggested these changes resulted from alterations in SERCA isoform, since they found a large increase in SERCA 2 (130% increase) and were unable to detect any changes in phospholamban, a major regulatory protein of SERCA. In the present study, FO induced increases in plantaris SERCA 2 expression occurred only in the CHF animals and to a lesser extent than that shown by Kandarian et al. (1994). The differences in SERCA isoform expression may be related to differences in the hypertrophic response observed. Since an 80% increase in mass was observed by Kandarian et al. (1994), compared to the 30% increase in mass observed here.

Kandarian et al. (1994), also found that FO of the plantaris markedly increased SERCA 2 and slightly decreased Ca^{2+} uptake rates. Interestingly, the FO-induced changes in Ca^{2+} uptake rates in the CHF animals and in Kandarian et al. (1994) were similar in magnitude while changes in SERCA isoform expression differed. Taken together, the present study and that of Kandarian et al. (1994) indicate that there is a dissociation between SERCA isoform expression and Ca^{2+} pump function. This again raises the possibility that some unidentified mechanism influences SERCA activity during CHF.

It also seems that the SERCA protein may not be as adaptable during CHF as MHC. This was evident when the SHAM-FO muscles demonstrated a significant increase in the number of fibers (as determined by immunohistochemistry) expressing SERCA 2 in the deep region of the plantaris, while there was no change in any other group. The CHF-FO, CHF, and SHAM groups had nearly identical proportions of fibers expressing SERCA 2. Thus, SERCA 2 expression may not be as regionally adaptable during CHF as it is in healthy muscle. There was a 48% increase in the number of fibers expressing SERCA 2 in the SHAM-FO group, which is nearly identical to what was found by Talmadge et al. (1996). Even though the latter study was conducted in cats, it is the only study that has examined FO and changes in SERCA isoform expression in individual fibers. It is also interesting that no regional changes were detected in SERCA 2 expression in CHF-FO group, while there was a significant change in overall muscle expression of SERCA 2 (i.e. western blot). However, it should be noted that immunohistochemistry is not a quantitative measure, and can only determine if individual fibers are expressing the protein or not. Therefore, it is possible that even though there

was no increase in the number of fibers expressing SERCA 2, it could be that the individual fiber themselves contained more SERCA 2 protein. This is possible since the type I fibers, in which SERCA 2 is preferentially located were significantly larger in the CHF-FO than in the CHF group. This suggests that regional changes not only occurred in fiber type expression, but also in individual fiber SERCA expression. The data suggest that while FO is effective at altering SERCA function it does not cause regional adaptations of fibers expressing SERCA 2 during CHF.

Summary. In summary, these data suggest that skeletal muscle is quite plastic even during CHF. This was evident by changes in phenotype and muscle mass. The study also lends support to the idea that it is not necessary to reverse changes in the heart to alter the peripheral musculature. Numerous studies have suggested that changes in isoform expression within the skeletal muscle are major contributors to the reduction in exercise tolerance during CHF. Since, during CHF, Ca^{2+} handling rates change without changes in SERCA isoform expression, other regulatory mechanisms must be operative in regulating muscle function. Accordingly, FO is able to restore these changes without dramatic alterations in SERCA isoform expression. Therefore these data suggest that altered functioning of skeletal muscle may not be due solely to changes in isoform expression, but may involve alterations in other mechanisms which regulate the control of these functional proteins. Therefore, it is of importance to follow this study by examining possible changes in regulatory mechanisms within the skeletal muscle during CHF.

Overall conclusions

This is the first study to show clearly that MHC isoform, individual fiber type and SR adaptations occur in peripheral skeletal muscles during CHF. It is also the first to show that with a form of strength training that these peripheral alterations are reversible without adaptations in the heart. This suggests that skeletal muscle is capable of adapting without any improvement in cardiac function. It also lends to the idea that strength training may prove to beneficial for CHF patients by improving regulatory mechanisms of the ECC and contractile processes within skeletal muscle. Therefore, these data have provided new information that not only benefits scientists, but also clinicians who are dealing with CHF patients.

Future Research

Current literature suggests that alterations in peripheral muscle function play a significant role in the reduction in exercise tolerance. Since the data here indicates that reductions in muscle function are not simply due to changes in muscle phenotype, it indicates that the alteration in function must involve other factors. It seems logical to suggest that regulatory mechanisms of various ECC and contractile proteins may be altered during CHF. Therefore, it seems reasonable to suggest that future studies should attempt to determine if other regulatory mechanisms within skeletal muscle are altered during CHF.

It is very possible that there are other key proteins involved in skeletal muscle contractions, which are affected by CHF. For example, ryanodine receptor, dihydropyridine receptor, and/or myosin light chain. Therefore it would be of interest to

try and screen the muscle in an attempt to determine if there are changes in other proteins, which may have a significant impact on skeletal muscle performance.

Lastly, knowing that skeletal muscle adapts favorably to strength training during CHF, it would be of interest to determine if this type of training could improve the overall function of the CHF patient. This could hopefully help to restore some of the functional capacity of the patient and ultimately improve their quality of life.