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# DOES CHRONIC SWIM TRAINING ALTER THE MYOCARDIAL HANDLING OF AN INCREASE IN EXTRACELLULAR CALCIUM LOAD

by

Eric Querbach

A Thesis
Submitted to the Faculty of Graduate Studies and Research
through the Department of Kinesiology
in Partial Fulfillment of the Requirements for
the Degree of Masters of Human Kinetics at the
University of Windsor

Windsor, Ontario, Canada

1997

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## ABSTRACT

The purpose of this study was to examine: (1) if chronic swim training improves cardiac performance, (2) if chronic swim training alters sarcoplasmic reticulum (SR) calcium ($Ca^{2+}$) storage, the fractional release (FR) of SR $Ca^{2+}$ and SR $Ca^{2+}$ uptake during relaxation and (3) if exercise training alters the handling of an increase in extracellular $Ca^{2+}$ load in cardiac muscle.

Chronic swim training produced a significant increase in the peak developed tension (PDT) and in the maximum rates of contraction and relaxation in cardiac muscle. Utilizing rapid cooling contractures we found that improvements in cardiac performance were associated with significant increases in both SR $Ca^{2+}$ stores and FR of SR $Ca^{2+}$ in the exercised animals. The fraction of cytoplasmic $Ca^{2+}$ removed by the SR during relaxation was not altered by swim training. However, the significant increase in SR $Ca^{2+}$ content in the exercised animals suggests an increase in the absolute amount of SR $Ca^{2+}$ uptake during relaxation in these animals.

Elevating the extracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_o$) led to an increase in PDT, SR $Ca^{2+}$ storage, and FR of SR $Ca^{2+}$ in both the sedentary and exercised groups. In the exercise group elevating $[Ca^{2+}]_o$ had a more significant effect on cardiac muscle PDT but a less significant effect on SR $Ca^{2+}$ storage and FR compared to the sedentary group.

In summary chronic swim training improved cardiac performance by increasing both the total SR $Ca^{2+}$ available for release and the fraction of this SR $Ca^{2+}$ that is released during contraction. Exercise training also altered the inotropic response of cardiac muscle to an increase in $[Ca^{2+}]_o$.

# DEDICATION

To my parents. Thankyou for always believing in me.

## ACKNOWLEDGEMENTS

I would like to thank Dr. Kenji Kenno for giving me an opportunity to pursue graduate studies and pushing me to make the most of my education. His concern, friendship and support through the hard times both academically and personally are greatly appreciated and will never be forgotten. I would like to thank Dr. Paul Taylor for allowing me to work in his laboratory. His expert assistance and guidance will always be greatly appreciated. I would like to thank Dr. Wayne Marino for his help with the statistical analysis. I would also like to thank Dr. Warner for letting me use the refrigerated water bath.

I would like to thank my parents for all there support and understanding throughout this endeavor. Finally I owe a great deal of gratitude to my girlfriend Tara, without her I could have never finished this project. Her love, patience, encouragement and undying support during this radical time of life will never be forgotten. Mere thanks is not enough for her.

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## CHAPTER ONE: INTRODUCTION

Excitation and contraction of the myocardial cell are linked by the movement of calcium ($Ca^{2+}$) ions (**Fig. 1**). When the cell is depolarized **(1)** $Ca^{2+}$ flows from the extracellular to the intracellular space via the L-type $Ca^{2+}$ channels of the sarcolemma (SL) **(2)** and the transient rapid reversal of the sodium calcium exchanger (Na-Ca exchanger) **(3)** (Bers, 1985). This inward flow of extracellular $Ca^{2+}$ can directly activate the myofilaments **(4)** (Delbridge et al., 1996, Lee and Tsien, 1982) and more importantly initiate a further release of $Ca^{2+}$ from the sarcoplasmic reticulum (SR) **(5)** called calcium-induced calcium-release (CICR) (Beuckelmann and Weir, 1988, Fabiato and Fabiato, 1975). This results in a rapid transient increase in the intracellular $Ca^{2+}$ concentration $[Ca^{2+}]_i$ . The $[Ca^{2+}]_i$ reacts with the myofilaments (MF) of the cardiac cell **(6)** by binding to troponin C (TnC). This allows actin and myosin to form crossbridges and generate tension through shortening.

Relaxation results from a decline in the $[Ca^{2+}]_i$. The majority of $[Ca^{2+}]_i$ is resequestered by the SR via the SR Ca-Adenosine triphosphatase (ATPase) pump **(7)** (Bers and Bridge, 1989, Schouten, 1985). The remaining $[Ca^{2+}]_i$ leaves the cell via the Na-Ca exchanger **(8)** and to a small extent via the SL Ca-ATPase pump **(9)** (Bassani et al., 1992, Bers & Bridge, 1989). The decrease in $[Ca^{2+}]_i$ causes dissociation of $Ca^{2+}$ from TnC and a decrease in muscle contraction.

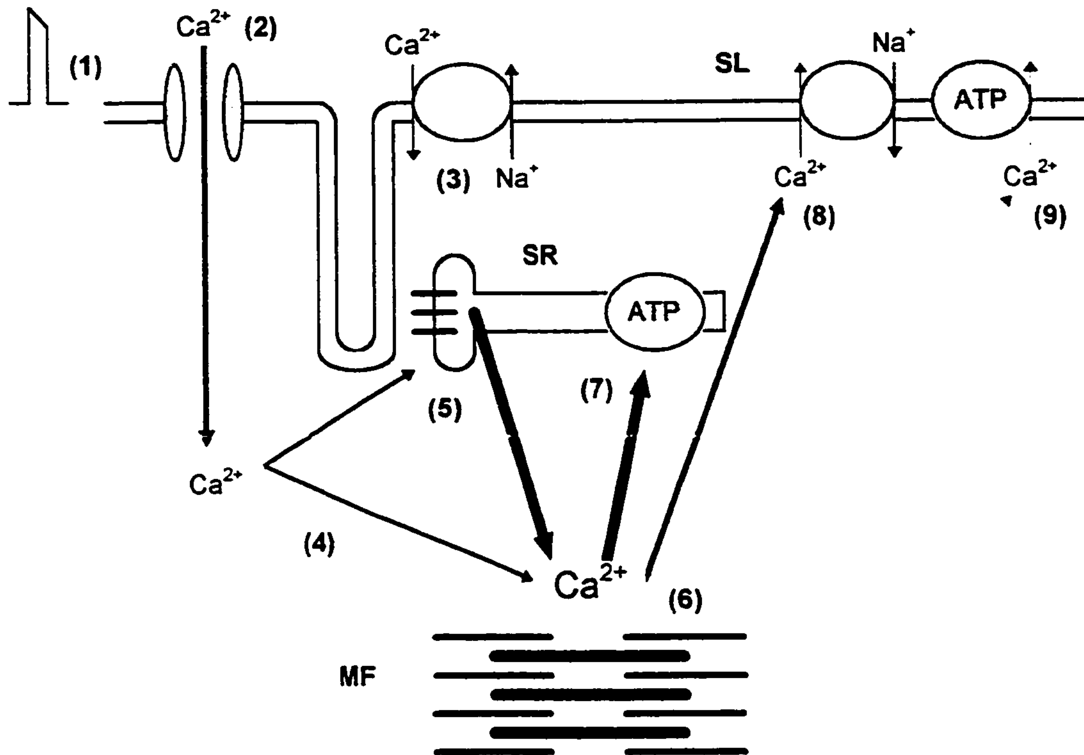


Figure 1: Schematic diagram of myocardial excitation-contraction coupling. SL = sarcolemma, SR = sarcoplasmic reticulum, MF = myofilaments, ATP = adenosine triphosphate, $Ca^{2+}$ = calcium, $Na^{+}$ = sodium. The width of the arrows represents the relative amounts of $Ca^{2+}$ and $Na^{+}$ movement. Each number is explained in the text.

The movement of $Ca^{2+}$ between the extracellular and the intracellular spaces is critical in linking the electrical and mechanical events associated with muscle contraction. One way to examine the importance of the handling of $Ca^{2+}$ during excitation-contraction coupling (ECC) in cardiac muscle is to determine the inotropic (contractile) response of the tissue to changes in the extracellular calcium concentration ($[Ca^{2+}]_o$). To examine this Snow and associates (1980), using whole heart preparations from sedentary rats, found that an increase in the $[Ca^{2+}]_o$ from 1.3 to 3.9 mM

produced a 243% increase in left ventricle developed pressure. Schouten (1985), using trabeculae muscle, found that force increased curvilinearly as $[Ca^{2+}]_o$ was increased from 0.2 mM to 2.5 mM where maximal force was reached. Further increases in $[Ca^{2+}]_o$ resulted in a small decrease of peak force, often accompanied by after contractions. This suggests that one of the $Ca^{2+}$ handling systems in the muscle maybe saturated at a $[Ca^{2+}]_o$ of 2.5 mM. Recently, Tang (1993), using trabeculae muscle, also demonstrated that steady-state developed force increased in a curvilinear fashion as $[Ca^{2+}]_o$ was increased from 0.25 mM to 2.0 mM, in sedentary rats. These previous studies indicate that cardiac performance, characterized as peak force development, is in part regulated by the $[Ca^{2+}]_o$.

Research indicates that elevating $[Ca^{2+}]_o$ can lead to an increase in SR $Ca^{2+}$ content (Kurihara and Sakai, 1985, Orchard and Lakatta, 1985). Given that $Ca^{2+}$ released from the SR accounts for approximately 90% of the total activator $Ca^{2+}$ available for contraction in the rat myocardium (Bers, 1985), the reported increases in force production associated with elevated levels of $[Ca^{2+}]_o$ (Schouten, 1985, Snow et al., 1980, Tang, 1993) may be a result of an increase in SR $Ca^{2+}$ content. In fact, Orchard and Lakatta (1985), using rat papillary muscles, found that as $[Ca^{2+}]_o$ was increased from 2.0 mM to 4.0 mM the SR $Ca^{2+}$ content (determined by the photoprotein aequorin) and the corresponding peak tension were both increased. Furthermore, Kurihara and Sakai (1985), using guinea-pig papillary and trabeculae muscle, demonstrated that increasing $[Ca^{2+}]_o$ from 2.5 mM to 5.0

mM significantly increased peak tension development and rapid cooling contractures (RCCs). Rapid cooling of muscle to near 0°C activates a contracture commonly termed an RCC whose amplitude is an indirect representation of the total SR $Ca^{2+}$ content (Banijamali et al., 1991). Bers (1989), also found using rabbit trabeculae muscle, that progressively increasing $[Ca^{2+}]_o$ from 0.2 mM to 10.0 mM resulted in a progressive increase in RCCs and peak developed force. These studies suggest that the increase in force production associated with an elevation in $[Ca^{2+}]_o$ is a result of an increased SR $Ca^{2+}$ content.

Another variable associated with force production in cardiac muscle is the amount of $Ca^{2+}$ released from the SR during contraction. All of the $Ca^{2+}$ stored in the SR is not released during a contraction. The amount of SR $Ca^{2+}$ that is released is defined as the fractional release. It has been reported that fractional release (determined from RCCs) was approximately 23% in trabeculae muscle from sedentary rats (Losito et al., 1996). Also, Bassani and associates (1993) have reported that fractional release (determined by caffeine contractures) is approximately 50% in cardiac myocytes from sedentary rabbits. Recently, the effects of elevating $[Ca^{2+}]_o$ on the fractional release of $Ca^{2+}$ from the SR have been investigated. Bassani and associates (1995), using single cardiac myocytes from sedentary ferrets, found that the fractional release of SR $Ca^{2+}$ was increased as $[Ca^{2+}]_o$ and SR $Ca^{2+}$ content were elevated. As $[Ca^{2+}]_o$ was progressively increased from 0.5 to 8.0 mM the fractional release of SR $Ca^{2+}$

increased from 10 to 59% while the SR $Ca^{2+}$ content was held constant (by varying $[Ca^{2+}]_o$ during the test twitch only). Also as the SR $Ca^{2+}$ content was increased from low to normal to high (55 to 91 to 95 umol/l cell water) the fractional release increased from 4 to 35 to 59% respectively. This data illustrates that the amount of SR $Ca^{2+}$ released, the fractional release, is regulated by the size of the incoming $Ca^{2+}$ trigger, determined by the $[Ca^{2+}]_o$ and the SR $Ca^{2+}$ content. Thus, the increase in cardiac muscle force production associated with elevated $[Ca^{2+}]_o$ may also be associated with an increase in the fractional release of SR $Ca^{2+}$.

The reported increase in SR $Ca^{2+}$ content associated with an increased $[Ca^{2+}]_o$ (Bers, 1989, Kurihara and Sakai, 1985, Orchard and Lakatta, 1985) may be due in part to an increase in the amount of $Ca^{2+}$ resequestered by the SR during relaxation. Normally during and following contraction the removal of cytoplasmic $Ca^{2+}$ is dependent on two mechanisms: the SR Ca-ATPase pump and the SL Na-Ca exchanger. In rat myocardium the SR Ca-ATPase pump removes 60-80% of cytoplasmic $Ca^{2+}$ and the SL Na-Ca exchanger 20-40%, following a contraction (Bers et al., 1990, Schouten, 1985). The amount of $[Ca^{2+}]_i$ resequestered by the SR Ca-ATPase pump following each contraction is referred to as the recirculation fraction. The recirculation fraction has been reported to be approximately 60% in sedentary rats (Schouten, 1985, Tang, 1993). Schouten (1985),using trabeculae muscle from sedentary rats, found that the recirculation fraction was increased as $[Ca^{2+}]_o$ was elevated. As $[Ca^{2+}]_o$

was increased from 0.5 to 0.8 mM the recirculation fraction (determined from the decay of post-extrasystolic potentiation) rose slightly from 64% to 67%. An increase in the recirculation fraction with an elevation in $[Ca^{2+}]_o$ may explain the reported increases in SR $Ca^{2+}$ content associated with elevated $[Ca^{2+}]_o$.

The movement of $Ca^{2+}$ between the extracellular and intracellular spaces is critical for contraction of cardiac muscle. While the $Ca^{2+}$ handling mechanisms associated with cardiac excitation-contraction coupling have been extensively studied in sedentary rats (Bers, 1991) there is very little research pertaining to exercised-trained animals. This is surprising considering that chronic exercise has long been recognized as beneficial to the cardiovascular system as demonstrated by improved cardiac performance and increased maximal oxygen capacity (Blomquist and Saltin, 1983). In the rat, chronic treadmill running studies have produced equivocal results concerning improvements in cardiac function with exercise (Bowles and Starnes, 1994, Cutilletta et al., 1979, Deblieux et al., 1993, Fuller and Nutter,1981 , Nutter and Fuller, 1977, Schaible and Scheuer, 1979, Schaible et al., 1981, Tibbits et al., 1978, 1981). In contrast to this, chronic swim training improves myocardial function in the rat as indicated by an increased cardiac output , stroke volume, stroke work, ejection fraction (Penpargkul and Scheuer, 1970, Schaible and Scheuer, 1979, Scheuer, 1977), peak developed force, rate of force development and rate of relaxation (Crews

and Aldinger, 1967, Losito et al., 1996, Mole, 1978, Schaible and Scheuer, 1981) in exercised compared to sedentary rats.

However, the $Ca^{2+}$ handling mechanisms responsible for the improvement in cardiac performance associated with exercise-training are poorly understood. Evidence suggests that chronic swim training has no effect on myosin isoenzyme population (Takeda et al., 1985). Pierce and associates (1989) investigated the effects of chronic swim training on cardiac SL function. They found no significant improvement in Na-Ca exchanger activity and passive $Ca^{2+}$ binding to SL sites following chronic swim training. However, chronic swim training is associated with an increase in calmodulin-stimulated $Ca^{2+}$ uptake by SR vesicles (Levine and Kinasewitz, 1986) and the uptake of $Ca^{2+}$ in SR enriched membrane fractions (Tate et al., 1990). Recently, in our laboratory, we have demonstrated that chronic swim training improved cardiac performance (force production), and significantly increased both the total SR $Ca^{2+}$ content and the fractional release of SR $Ca^{2+}$ compared to sedentary animals (Losito et al., 1996).

The effect of chronic swim training is similar to the research demonstrating that elevating $[Ca^{2+}]_o$ leads to an increase in force production (Schouten, 1985, Snow et al., 1980, Tang, 1993), SR $Ca^{2+}$ content (Bers, 1989, Kurihara and Sakai, 1985, Orchard and Lakatta, 1985), fractional release of SR $Ca^{2+}$ (Bassani et al., 1995) and the recirculation fraction (Schouten, 1985) of cardiac muscle from sedentary animals. Collectively,

this research suggests that there are similiar responses in cardiac muscle to two different interventions, chronic swim training and elevations in $[Ca^{2+}]_o$. However, there are no reports to date that have examined if exercise training alters the handling of an elevation in $[Ca^{2+}]_o$.

Therefore the general purpose of this study was to examine if chronic swim training alters the myocardial handling of an increase in extracellular $Ca^{2+}$ load . The specific objectives of this study were to determine the effects of increases in $[Ca^{2+}]_o$ on trabeculae muscle peak force development, the SR $Ca^{2+}$ content, the recirculation fraction and the fractional release of SR $Ca^{2+}$ in chronic swim trained versus sedentary rats.

## CHAPTER TWO: METHODS

### 1. Animal Maintenance

Adult female Wistar rats (Charles River, Quebec) were used for both experimental groups. The animals were housed 3 per cage in a temperature and light controlled room and were provided with Purina lab chow and water ad libitum. The exercise group had an initial body weight of 200 to 220 grams. Weight-matched sedentary animals were purchased following the training progam and their initial weight was 300-350 grams. The exercise and sedentary animals were weight-matched to reduce possible growth differences (Schaible and Scheuer, 1979, Tang, 1993).

### 2. Exercise Training Protocol

The exercised rats swam 60 min/day, 5 days/wk for 40-44 weeks. To increase exercise intensity and ensure that the rats did not float 3-5% of group mean body weight was attached to each animal (Pierce et al., 1989). The rats swam in plastic bins at a water temperature of 32 °C. There were four animals in each bin because rats swim more vigorously in groups (Schaible and Scheuer, 1979). The rats continued to train until they were sacrificed. To determine if the exercise protocol elicited cardiac hypertrophy heart weight-to-body weight ratio was measured.

### 3. Buffer

A modified Krebs-Henseleit (KH) buffer, pH 7.4, composed of the following in millimoles (mM) was used: NaCl, 117; KCL, 5.0; $MgCl_2$, 1.2;

$Na_2SO_4$, 1.2; $NaH_2PO_4$, 2.0; $NaHCO_3$, 27; glucose, 10.0; and an amount of $CaCl_2$ necessary for each different $[Ca^{2+}]_o$. The buffer was bubbled with 95% $O_2$ and 5% $CO_2$ and maintained at 26 °C. See Appendix A for a detailed review of the buffer preparation. A thermocouple located in the muscle bath was used to monitor temperature. The flow rate of the KH buffer through the muscle bath was 40 ml/min and the muscle was initially exposed to a 0.5mM $[Ca^{2+}]_o$.

**4. Muscle Preparation**

After anesthesia the abdominal cavity was opened by a midline incision and heparin (200 units) was injected into the inferior vena cava to avoid blood clotting. The heart was quickly removed, placed in cold KH buffer and transferred to a dissection dish. The aorta was cannulated and the heart was perfused retrogradely via the Langendorff technique with KH buffer containing 2,3-Butane Dione Monoxime (BDM) (3mg/ml) to stop contractions (Armstrong and Ganote, 1991). The right ventricle wall was cut open and free running trabeculae muscles were dissected. Only muscles with a diameter of $< 0.2$ mm were used. After dissection the trabeculae was placed in a 0.6 ml muscle bath containing KH buffer (without BDM) attached to the stage of an inverted microscope. The base of the muscle was attached to a cradle at one end of the bath which was connected to a force transducer (Aker 801). Changes in force, displayed by the muscle as it contracted, were measured by the transducer, converted to a voltage and amplified by a Grass low level D. C. amplifier (model 7P122E). The signals

were simultaneously displayed on an oscilloscope and digitized by a 12 bit A/D converter at a rate of 200 Hz and stored on computer disks for future analysis. The data was analyzed using the data acquisition computer software Unklescope version 3.10b (Unklescope, MIT, USA). The valve end was attached to a tungsten wire hook connected to a micromanipulator (Stoelting, Chicago, USA).

Following horizontal positioning, the trabeculae was electrically stimulated at a voltage 50% above threshold with a 4ms pulse duration delivered via two platinum wire electrodes attached to a stimulator (Grass S6 C). To test the viability of the preparation the muscle was potentiated to ensure that it was responding properly. Viable preparations did not display any spontaneous contractions. The trabeculae was then equilibrated for 60 min at a pacing frequency of 1.0 Hz to allow the muscle to recover from surgery (Schouten, 1985). After recovery, the muscle was adjusted using a micromanipulator (Stoelting, Chicago, USA) to a sarcomere length of 2.0 um and checked using a video imaging system (see Appendix B) and given an additional 30 min to equilibrate at the baseline pacing frequency of 0.2 Hz. We have previously found in our laboratory, using muscle preps of a similiar muscle length and using similar experimental protocols, that a sarcomere length of 2.0 um allows the muscle to maintain steady-state force production throughout the entire experimental protocol (Tang, 1993, Losito et al., 1996). Also, Takeda and associates (1985) found no significant differences in the

length-tension relation between chronic swim trained and sedentary rats suggesting that sarcomere length did not effect the experimental data., The cross-sectional area of each trabeculae muscle was estimated from width and thickness measurements obtained with an ocular micrometer (see Appendix C).

### 5. Experimental Protocol

To determine if chronic swim training improved myocardial performance in the exercised rats, twitch function curves were collected and used to determine peak developed tension, the maximum rate of tension development and the maximum rate of tension decay (see Appendix D) at the baseline pacing frequency of 0.2 Hz.

To indirectly examine the total SR $Ca^{2+}$ content, fractional release and recirculation fraction, a buffer switching technique called paired rapid cooling contractures (PRCCs) (Tang, 1993) was used ( **Fig. 2**) (see Appendix E). Rapid cooling of the muscle was achieved by rapidly exchanging the warm KH buffer (26°C) with a cold KH buffer (0-0.5°C). This was done by turning off the stimulator and switching from one perfusion solution to another using a pair of solenoid valves (Cole Parmer) located near the inlet of the muscle bath. The solenoid valves were manually triggered with a 12 DC power supply with the first RCC being initiated at the same time interval as the baseline pacing frequency. The exchange of

warm buffer to cold was completed in < 1 second and the temperature was monitored by a thermocouple near the surface of the muscle.

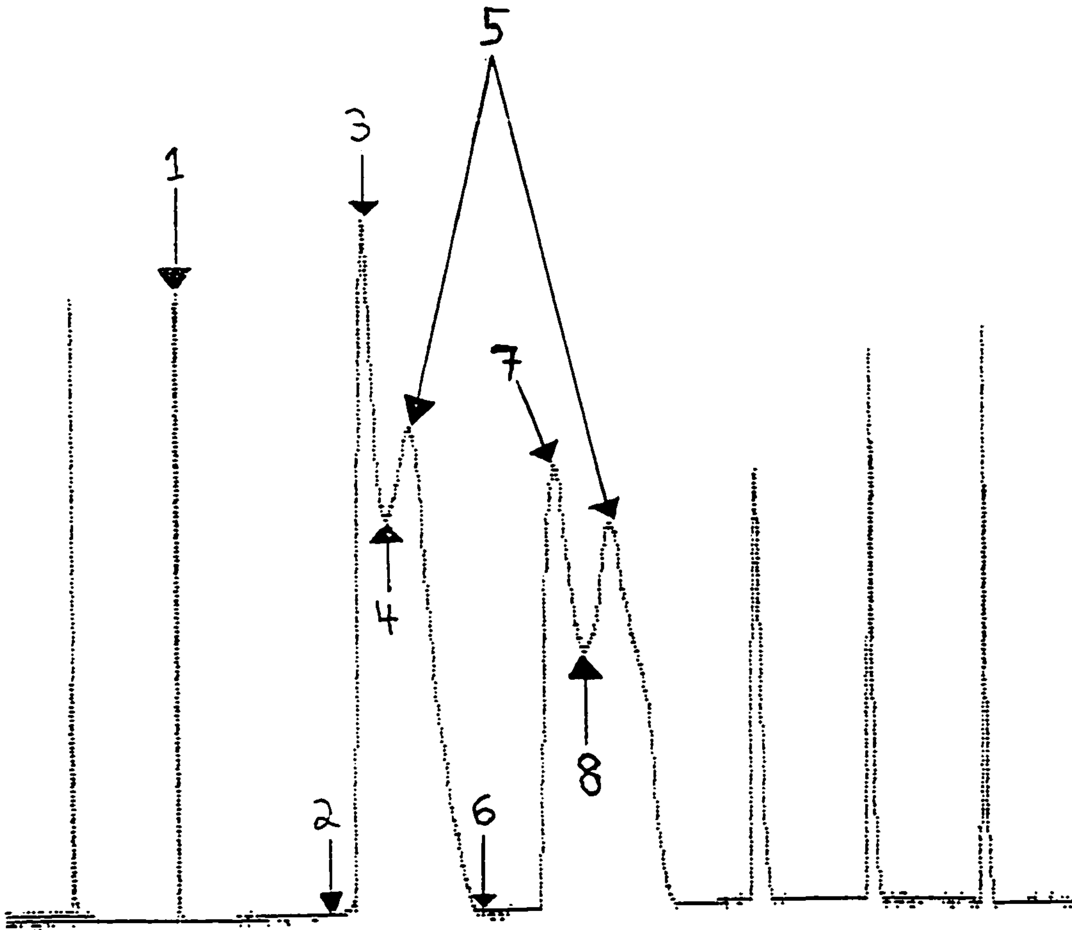


Figure 2. Actual tracing of a paired rapid cooling contracture obtained from a right ventricular trabeculae muscle stimulated at 0.2 Hz, $[Ca^{2+}]_o$ of 0.5mM, and sarcomere lenght of 2.0 *u*m. $\uparrow_1$ = steady-state PDT. $\uparrow_2$ = the point at which the cold buffer was induced for the first RCC. $\uparrow_3$ = peak tension produced by $RCC_1$. $\uparrow_4$ = the first switch back to the warm buffer. $\uparrow_5$ = rewarming spikes for $RCC_1$ and $RCC_2$. $\uparrow_6$ = the point at which the cold buffer was induced for the second RCC. $\uparrow_7$ = peak tension produced by $RCC_2$. $\uparrow_8$ = the second switch back to the warm buffer which is followed by a second rewarming spike.

The total SR $Ca^{2+}$ content was estimated indirectly as the peak tension of the first RCC ($RCC_1$) (arrow 1) (Banijamali et al., 1991, Bers et al., 1990). Following the peaking of $RCC_1$ the cold KH buffer was rapidly exchanged with the 26°C KH buffer (arrow 2). Rewarming after a RCC leads to a transient increase of force (rewarming spike) that has been attributed to an increase in myofilament $Ca^{2+}$ sensitivity at higher temperatures while $[Ca^{2+}]_i$ is elevated (Harrison and Bers, 1990). Relaxation, upon the rewarming of $RCC_1$ (from the peak of the rewarming spike to baseline), represents the removal of $[Ca^{2+}]_i$ by the SR Ca-ATPase pump and the Na-Ca exchanger which were both inhibited by the cold buffer. Upon reaching baseline, a second RCC ($RCC_2$) was immediately evoked. The peak tension of $RCC_2$ is also an indirect measure of the SR $Ca^{2+}$ content and represents the amount of $Ca^{2+}$ resequestered by the SR during relaxation from $RCC_1$(Bers et al., 1989). Recirculation fraction is the amount of $Ca^{2+}$ that is resequestered by the SR following a contraction and was estimated indirectly from the ratio of peak $RCC_1$ tension to peak $RCC_2$ tension.

Fractional release is a measure of the amount of $Ca^{2+}$ released into the cytoplasm by the SR following a normal electrical stimulation. The fractional release was indirectly estimated from the ratio of peak baseline twitch tension to peak $RCC_1$ tension. Peak $RCC_1$ tension represents the total SR $Ca^{2+}$ content, while peak baseline twitch tension represents the

amount of SR $Ca^{2+}$ that was released during the electrically stimulated contraction.

To examine if chronic swim training altered the handling of an increase in extracellular $Ca^{2+}$ load, all twitch characteristics and RCCs were collected, from sedentary and swim trained rats in 3 progressively increasing $[Ca^{2+}]_o$ (0.5, 1.0, and 1.5mM). $[Ca^{2+}]_o$ was progressively increased by adding appropriate amounts of a 1M $CaCl_2$ stock solution to the KH buffer. Following the addition of $CaCl_2$ to the KH buffer the trabeculae muscle was equilibrated until a new steady-state baseline tension was achieved.

**6. Statistical Analysis.**

A two-way analysis of variance of a 2 x 3 design with repeated measures on $Ca^{2+}$ concentrations was utilized to determine if significant differences existed. A Tukey's post hoc test was used to identify the location of any statistically significant differences. P-values $\leq 0.05$ were considered to be statistically significant.

# CHAPTER THREE: RESULTS

## 1. Animal Morphology

The morphological characteristics of the animals, at the time of sacrifice, are displayed in **Table 1**. In this study the sedentary group was weight-matched to the exercise group in order to more clearly determine if the swimming program produced cardiac hypertrophy. As a result there were no significant differences in the mean body weight between the exercised and the sedentary groups.

Table 1: Morphological Characteristics of Exercised and Sedentary Animals

| Characteristics | Sedentary | Exercise |
|---|---|---|
| Body Weight (g) | 332.7 ± 7.5 | 356.8 ± 14.1 |
| Heart Weight (mg) | 1055.7 ± 43.6 | 1417.1 ± 49.0 [a] |
| Heart to Body Weight (mg/g) | 3.2 ± 0.1 | 4.0 ± 0.2 [a] |
| Dry to Wet Heart Weight (mg/mg) | 0.22 ± 0.006 | 0.22 ± 0.003 |
| Cross-Sectional Area ($mm^2$) | 0.06 ± 0.01 | 0.05 ± 0.01 |

All values are the mean ± SE (n = 7). Significance level $p < 0.05$.
[a] Exercise significantly different from Sedentary

It has been suggested that cardiac hypertrophy is a physiological adaptation to chronic swim training and may be associated with exercise induced improvements in myocardial performance. To determine if the swim training protocol used in this study was of sufficient intensity and duration to induce cardiac hypertrophy, I measured heart weight and the ratio of heart weight to body weight in the exercised and sedentary animals.

Following chronic swim training, the mean heart weight was significantly (34%, $p < 0.05$) greater in the exercised group compared to the sedentary group. The increased heart weight in the exercised group was not due to an increase in the water content of the heart as there was no significant difference in the ratio of dry heart weight to wet heart weight (see Appendix F) in the exercised and sedentary groups. Similar to previous studies utilizing swim training (Crews and Aldinger, 1967, Hickson et al., 1983, Mole, 1978, Oscai et al., 1971b, Schaible and Scheuer, 1981) but in contrast with others (Geenen et al., 1988, Penpargkul and Scheuer, 1970, Scheuer, 1977) my results demonstrate that chronic swim training led to a significant increase in heart weight and therefore produced cardiac hypertrophy in the exercised group.

The ratio of heart weight to body weight is another widely used method in determining if exercise training produces cardiac hypertrophy. The heart weight was normalized by body weight to ensure that cardiac hypertrophy was not due to subtle growth differences in the exercised and sedentary animals. In order for the ratio of heart weight to body weight to be

a valid measure of cardiac hypertrophy the mean body weights of the exercise and sedentary groups must be similar. Previous studies, utilizing age matched animals, have reported that exercise training produced a significant increase in the ratio of heart weight to body weight (Burgess et al., 1996, Fuller and Nutter, 1981, Nutter et al., 1981, Oscai et al., 1971a, Penpargkul and Scheuer, 1970, Schaible et al., 1981, Schaible and Scheuer, 1979, Scheuer, 1977) suggesting exercise induced cardiac hypertrophy. However, in these studies the increase in heart weight to body weight ratio was a result of a significant decrease in the body weight of the exercise groups due to the training protocols and not an increase in heart weight. In these reports the significant increase in heart weight to body weight ratio does not represent a true cardiac hypertrophy. In this study, I used a weight-matched sedentary group to ensure that heart weight to body weight ratio would determine if swim training produced cardiac hypertrophy. Like previous studies using sedentary and exercised animals with similar body weights (Mole, 1978, Oscai et al., 1971b, Schaible and Scheuer, 1981) I found that chronic swim training led to a significant (25%, $p<0.05$) increase in the ratio of heart weight to body weight in the exercised group compared to the sedentary group. This was due to a significant increase in the heart weight of the exercised animals. These results clearly demonstate that the swim training protocol used in this study produced cardiac hypertrophy.

Although there was significant cardiac hypertrophy in the exercised group there was no significant difference in the mean cross-sectional area of

the trabeculae muscles used for the experiments in the exercised and sedentary groups. This suggests that any change in cardiac performance in the exercise group was not a function of muscle size but associated with an intrinsic change in excitation-contraction coupling.

## 2. Effects of Exercise and $[Ca^{2+}]_o$ on Cardiac Twitch Performance

Chronic swim training for 40 - 44 weeks resulted in a significant ($p<0.05$) 340% increase in peak developed tension (PDT) in the exercised group compared to the sedentary group (**Fig. 3**) at the baseline $[Ca^{2+}]_o$ of 0.5mM. This significant increase in PDT, as a result of chronic swim training was also maintained when the $[Ca^{2+}]_o$ perfusing the trabeculae muscle was elevated to 1.0mM and 1.5mM as PDT was increased by 264% and 285% respectively in the exercised compared to the sedentary group at these $[Ca^{2+}]_o$.

Chronic swim training also resulted in a significant (246% and 247% respectively, $p<0.05$, **Table 2**) increase in the rate of tension development (+dT/dt) and the rate of tension decay (-dT/dt) in the exercised group compared to the sedentary group at the baseline $[Ca^{2+}]_o$ of 0.5mM. This significant increase in $\pm$ dT/dt as a result of swim training was maintained when the $[Ca^{2+}]_o$ was elevated to 1.0mM and 1.5mM.

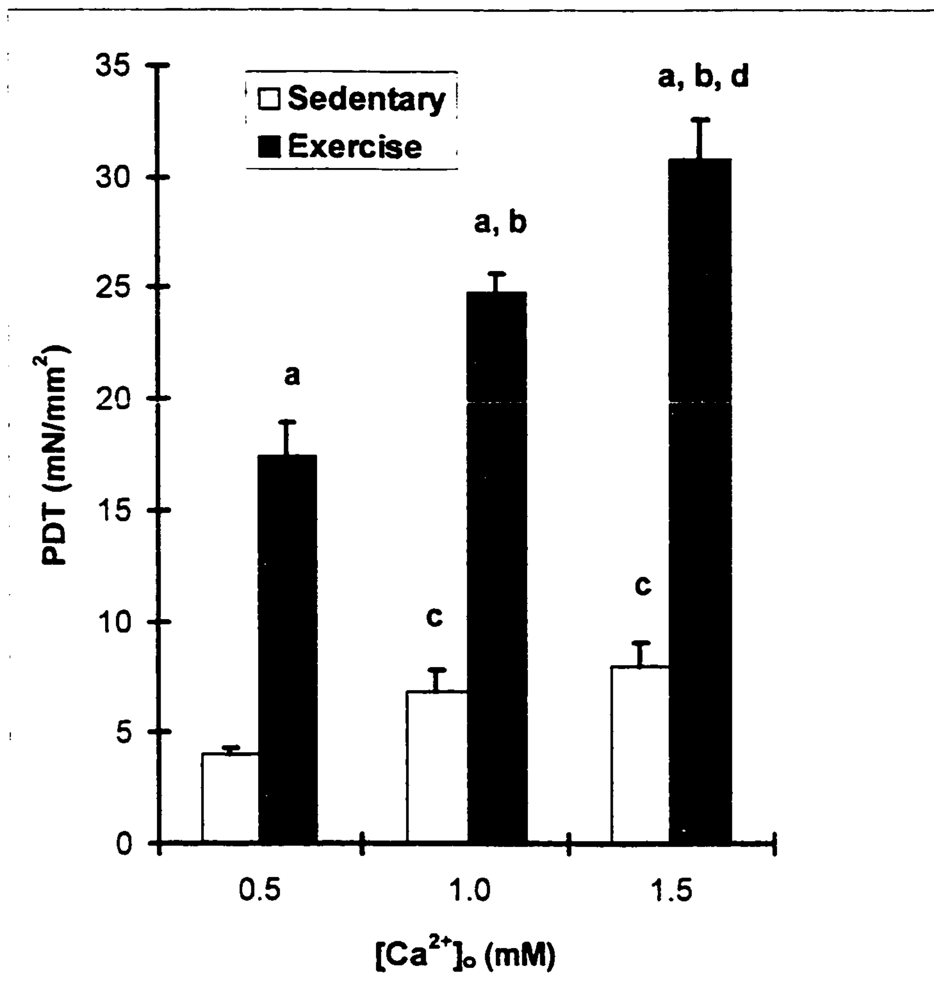


Figure 3: Effects of exercise and $[Ca^{2+}]_o$ on peak developed tension. All values are means ± SE (n=7). Significance level $p < 0.05$.
EX = Exercise, SD = Sedentary.
[a] EX significantly different from SD
[b] significantly different from EX at 0.5mM
[c] significantly different from SD at 0.5mM
[d] significantly different from EX at 1.0mM

Table 2: Mean Rates of Tension Development and Decay

| | $[Ca^{2+}]_o$ (mM) | Sedentary | Exercise |
|---|---|---|---|
| Rate of Tension Development ($mN/mm^2 \bullet sec^{-1}$) | 0.5 | 53.0 ± 4.1 | 183.6 ± 22.0 [a] |
| | 1.0 | 93.2 ± 8.4 [c] | 251.3 ± 22.8 [a,b] |
| | 1.5 | 109.0 ± 11.4 [c] | 309.9 ± 23.3[a,b] |
| Rate of Tension Decay ($mN/mm^2 \bullet sec^{-1}$) | 0.5 | 32.8 ± 1.2 | 113.7 ± 19.7 [a] |
| | 1.0 | 63.7 ± 6.1 [c] | 131.2 ± 20.1 [a] |
| | 1.5 | 69.3 ± 8.8 [c] | 150.9 ± 23.1 [a] |

All values are the mean ± SE (n=7). Significance level $p < 0.05$.
EX = Exercise, SD = Sedentary.
[a] EX significantly different from SD
[b] significantly different from EX at 0.5mM
[c] significantly different from SD at 0.5mM

To determine if chronic swim training altered the inotropic characteristics of trabeculae muscle to an elevation in extracellular $Ca^{2+}$ load I examined PDT with progressively increasing $[Ca^{2+}]_o$ loads. In the sedentary group there was a significant ($p<0.05$, **Fig 3**) 71% increase in PDT when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and a moderate but not statistically significant 18% increase in PDT when $[Ca^{2+}]_o$ was increased from 1.0 to 1.5 mM. Although I did not directly elevate $[Ca^{2+}]_o$ from 0.5 to 1.5mM it should be noted that compared to 0.5mM at an $[Ca^{2+}]_o$ of 1.5mM PDT was significantly ($p<0.05$) increased by 102%.

In the exercise group there was a significant ($p<0.05$) 42% increase in PDT when the $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and a significant

($p<0.05$) 25% increase when the $[Ca^{2+}]_o$ was raised from 1.0 to 1.5mM. Also, compared to the baseline $[Ca^{2+}]_o$ of 0.5mM PDT was significantly ($p<0.05$) increased by 76% at an $[Ca^{2+}]_o$ of 1.5mM in the exercised animals.

To further examine the inotropic effects of $[Ca^{2+}]_o$ on trabeculae muscle performance I measured +dT/dt and -dT/dt in the exercise and sedentary groups at progressively increasing $[Ca^{2+}]_o$ **(Table 2)**. In the sedentary group there were significant increases (76% and 94% respectively, $p<0.05$) in both +dT/dt and -dT/dt as $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM. There were moderate but not statistically significant (17% and 9% respectively) increases in +dT/dt and -dT/dt when $[Ca^{2+}]_o$ was increased from 1.0 to 1.5mM.

In the exercised group there was a significant ($p<0.05$) 37% increase in +dT/dt when the $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and a modest but not significant 23% increase in +dT/dt when $[Ca^{2+}]_o$ was elevated from 1.0 to 1.5 mM. Elevating $[Ca^{2+}]_o$ produced no significant change in -dT/dt in the exercise group.

### 3. The Effects of Exercise and $[Ca^{2+}]_o$ on Cardiac SR $Ca^{2+}$ Content

In rat cardiac muscle the force of contraction is closely related to the amount of $Ca^{2+}$ stored in the SR (Bers, 1985). The amplitude of a RCC can be used to indirectly estimate the absolute amount of $Ca^{2+}$ stored in the SR (Banijamali et al., 1991, Bers et al., 1990). Following chronic swim training

there was a significant ($p<0.05$, **Fig. 4**) 237% increase in peak RCC tension in the exercised group compared to the sedentary group at the baseline $[Ca^{2+}]_o$ of 0.5mM. These results indirectly demonstrate that chronic swim training produced a significant increase in the cardiac SR $Ca^{2+}$ content of the exercised group. The effect of chronic swim training on SR $Ca^{2+}$ stores was also maintained when $[Ca^{2+}]_o$ was elevated to 1.0mM and 1.5mM as RCC was increased by 202% and 199% respectively in the exercised animals compared to the sedentary animals.

To examine if chronic swim training modified the response of the SR to an increase in extracellular $Ca^{2+}$ load I collected RCCs at progressively increasing $[Ca^{2+}]_o$ (**Fig. 4**). In the sedentary group there was a significant ($p<0.05$) 35% increase in peak RCC tension when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and a moderate but not statistically significant 12% increase in RCC tension when $[Ca^{2+}]_o$ was elevated from 1.0 to 1.5mM. While the change in RCC tension between 0.5 and 1.5mM $[Ca^{2+}]_o$ was not directly measured it should be noted that compared to 0.5mM, RCC tension was significantly ($p<0.05$) increased by 52% at 1.5mM $[Ca^{2+}]_o$.

In the exercised group there were moderate but statistically insignificant increases (21% and 11% respectively) when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and 1.0 to 1.5mM. However, when RCC tension at the baseline $[Ca^{2+}]_o$ of 0.5mM was compared to that at 1.5mM $[Ca^{2+}]_o$ there was a significant ($p<0.05$) 35% increase in the exercised group. These results indicate that elevating $[Ca^{2+}]_o$ produces significant

increases in cardiac SR $Ca^{2+}$ content in both groups but in the exercised animals a cumulative increase in $[Ca^{2+}]_o$ is required to see a signifcant change.

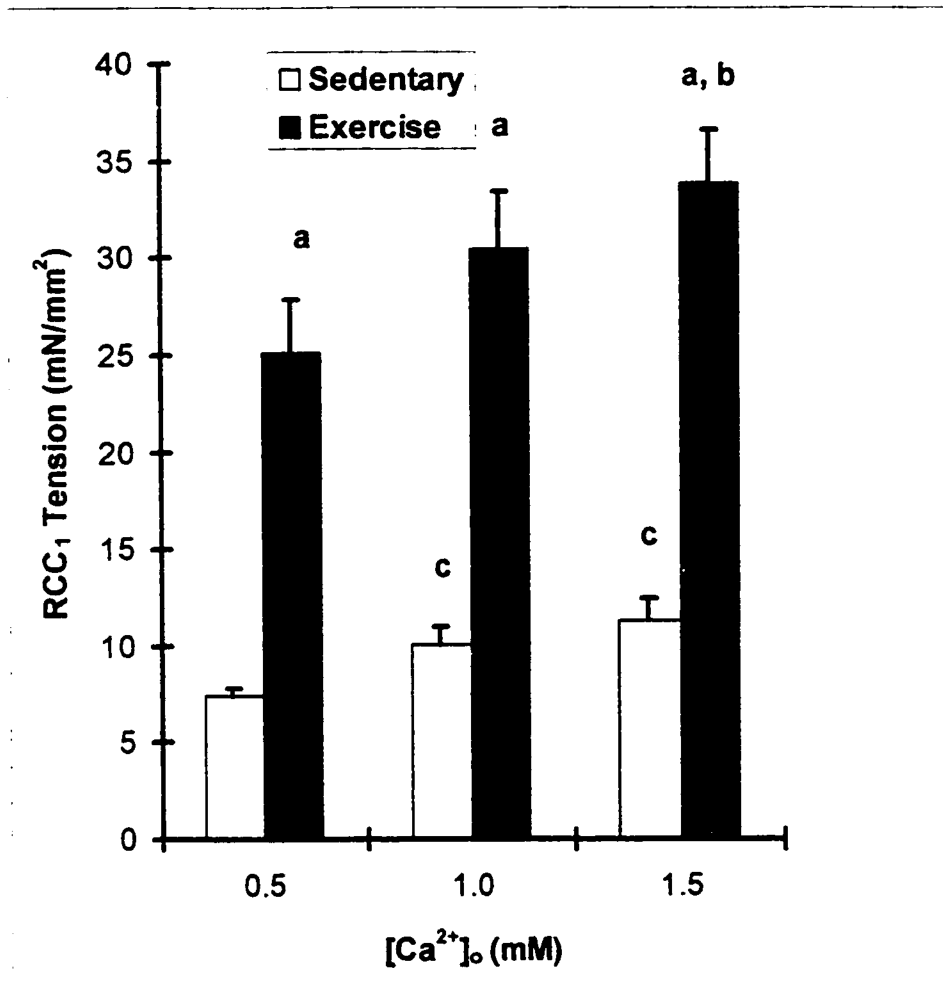


Figure 4: Effect of exercise and $[Ca^{2+}]_o$ on peak $RCC_1$ tension. All values are means ± SE (n=7). Significance level $p < 0.05$.
EX = Exercise, SD = Sedentary.
[a] EX significantly different from SD
[b] significantly different from EX at 0.5mM
[c] significantly different from SD at 0.5mM

## 4. Effects of Exercise and $[Ca^{2+}]_o$ on Fractional Release of SR $Ca^{2+}$ in Cardiac Muscle

In mammalian cardiac muscle not all of the $Ca^{2+}$ stored in the SR is released during a normal electrically stimulated contraction. The amount that is released is referred to as the fractional release (FR) of SR $Ca^{2+}$. In rat cardiac muscle FR is a very important mechanism since the force of contraction is highly dependent on SR $Ca^{2+}$ (Bers, 1985). In sedentary animals the FR of SR $Ca^{2+}$ was 54% at the baseline $[Ca^{2+}]_o$ of 0.5mM. In the exercised group, chronic swim training significantly increased ($p<0.05$, **Fig.5**) the FR to 72% at 0.5mM $[Ca^{2+}]_o$. The significant ($p<0.05$) increase in FR was maintained in the exercised animals (84% and 93% respectively) compared to the sedentary animals (67% and 70% respectively) at 1.0mM and 1.5mM $[Ca^{2+}]_o$.

To examine if exercise altered the effects of extracellular $Ca^{2+}$ load on the FR of SR $Ca^{2+}$ I estimated FR at three $[Ca^{2+}]_o$ in both the sedentary and exercised animals (**Fig.5**). In the sedentary group there was a significant (0.24-fold, $p<0.05$) increase in FR when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and a moderate but not significant 0.06-fold increase in FR when $[Ca^{2+}]_o$ was increased from 1.0 to 1.5mM. While the change in FR between 0.5 and 1.5mM $[Ca^{2+}]_o$ was not directly measured it should be noted that compared to 0.5mM, FR was significantly (0.31-fold, $p<0.05$) increased at 1.5mM $[Ca^{2+}]_o$. In the exercise group there were moderate but

not statistically significant (0.17-fold and 0.10-fold respectively) increases in FR when $[Ca^{2+}]_o$ was increased from 0.5 to 1.0mM and 1.0 to 1.5mM. However, when FR at 0.5mM was compared to that at 1.5mM $[Ca^{2+}]_o$ there was a significant (0.29-fold, $p<0.05$) increase in FR in the exercised group.

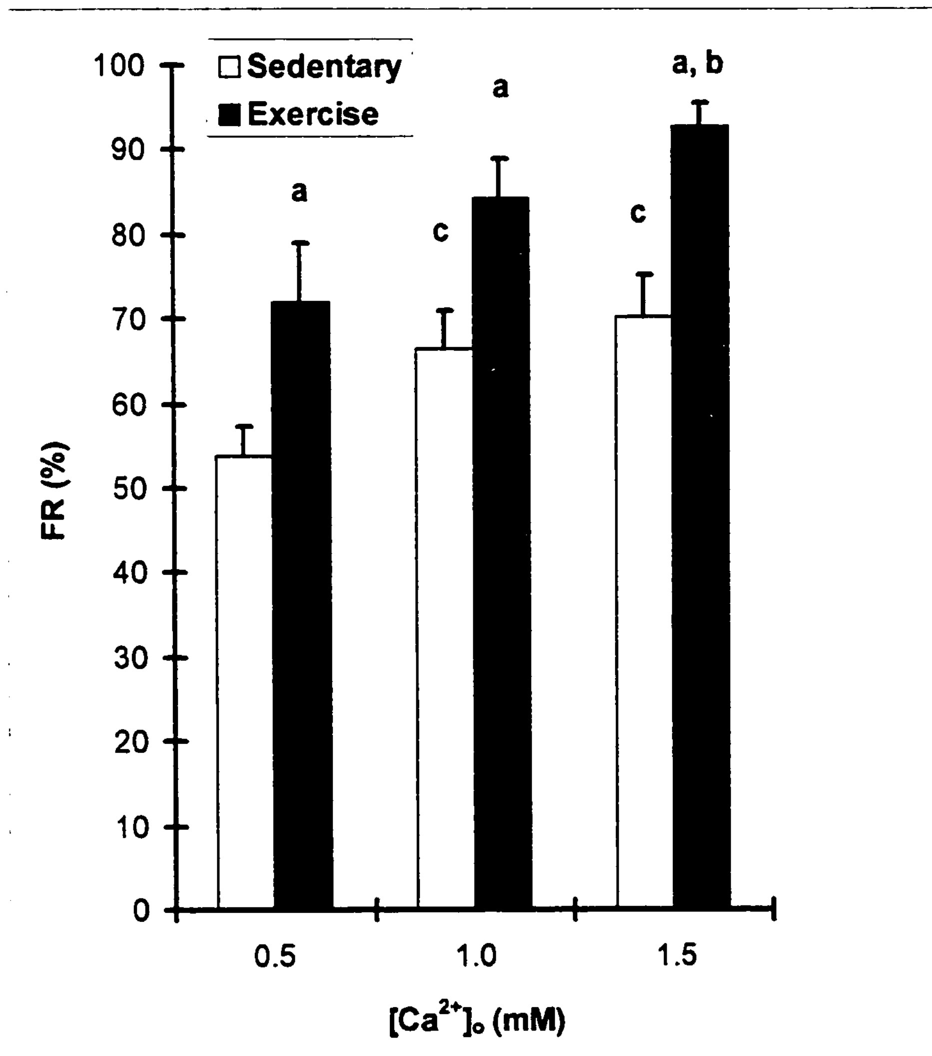


Figure 5: Effects of exercise and $[Ca^{2+}]_o$ on fractional release of SR $Ca^{2+}$. All values are means ± SE (n=7). Significance level $p < 0.05$. EX = Exercise, SD = Sedentary.
[a] EX significantly different from SD
[b] significantly different from EX at 0.5mM
[c] significantly different from SD at 0.5mM

### 5. Effects of Exercise and $[Ca^{2+}]_o$ on Recirculation Fraction of $Ca^{2+}$

During relaxation in rat cardiac muscle $Ca^{2+}$ is primarily removed by two mechanisms: the SR Ca-ATPase pump and the SL Na-Ca exchanger (Bers and Bridge, 1989). The amount of $Ca^{2+}$ that is resequestered by the SR Ca-ATPase pump is referred to as the recirculation fraction (RF). In the sedentary group the RF was 56% at the baseline $[Ca^{2+}]_o$ of 0.5mM, 71% at 1.0mM $[Ca^{2+}]_o$, and 69% at 1.5mM $[Ca^{2+}]_o$. In the exercised animals there was a moderate but not statistically significantly increase in RF to 71% at the baseline $[Ca^{2+}]_o$ of 0.5mM, 76% at 1.0mM $[Ca^{2+}]_o$, and 83% at 1.5mM $[Ca^{2+}]_o$ (**Fig. 6**) suggesting that swim training had no significant effects on the RF of $Ca^{2+}$ in cardiac trabeculae muscle.

To determine if exercise altered the effects of increasing extracellular $Ca^{2+}$ load on the RF of $Ca^{2+}$ I estimated RF at three $[Ca^{2+}]_o$ in both the sedentary and exercised animals (**Fig. 6**). In the sedentary group RF increased (0.27-fold) when the $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and decreased (0.03-fold) when $[Ca^{2+}]_o$ was elevated from 1.0 to 1.5mM, these changes were not statistically significant. When RF at 0.5mM was compared to that at 1.5mM $[Ca^{2+}]_o$ there was a moderate but not significant 0.24-fold increase. In the exercise group there were moderate but not significant (0.08-fold and 0.09-fold respectively) increases in RF when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and 1.0 to 1.5mM . When RF at

0.5mM was compared to that at 1.5mM $[Ca^{2+}]_o$ there was a modest but not significant (0.17-fold) increase in the exercised animals. These results demonstrate that $[Ca^{2+}]_o$ had only minimal effects on the RF of $Ca^{2+}$.

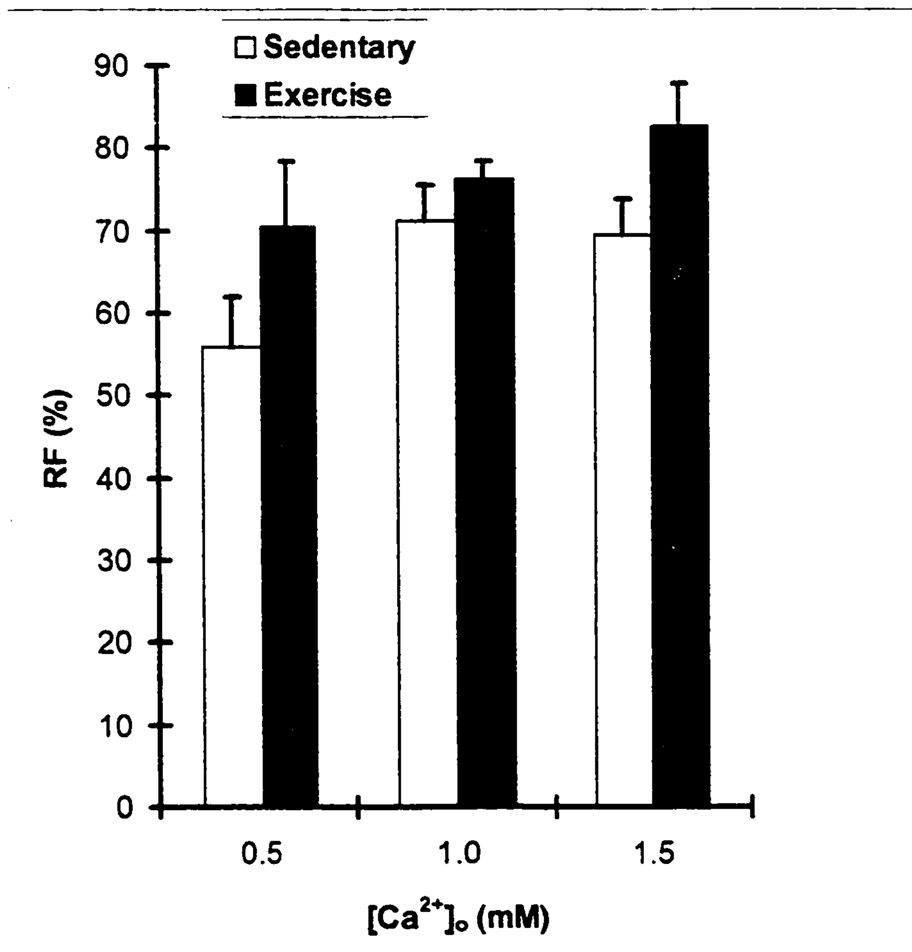


Figure 6: Influence of exercise and $[Ca^{2+}]_o$ on recirculation fraction. All values are means ± SE (n=7). Significance level $p < 0.05$.

# CHAPTER FOUR: DISCUSSION

## 1. Effects of Exercise and $[Ca^{2+}]_o$ on Cardiac Twitch Performance

This study clearly demonstrated that chronic swim training improves cardiac performance. Following an extended period of chronic swim training (40-44 weeks) there were significant improvements in peak developed tension (PDT), the rate of tension development (+dT/dt) and the rate of tension decay (-dT/dt) in the exercised compared to the sedentary animals. These results are in agreement with previous reports demonstrating that chronic swim training improves cardiac performance in isolated whole hearts (Penpargkul and Scheuer, 1970, Schaible and Scheuer, 1979, Schaible and Scheuer, 1981, Scheuer, 1977), isolated left ventricular papillary muscle (Mole, 1978), isolated left ventricular trabeculae muscle (Kammereit et al., 1975, Steil et al., 1975) and isolated right ventricular trabeculae muscle (Losito et al., 1996). It should be noted that, studies using treadmill running to investigate the effects of chronic aerobic exercise on cardiac performance have produced equivocal results. While some research has demonstrated improvements in cardiac performance() other studies have found no changes ()(Bowles and Starnes, 1994, Burgess et al., 1996, Cox et al., 1986, Cutilletta et al., 1979, Deblieux, et al., 1993, Fuller and Nutter, 1981, Nutter et al., 1981, Ritzer et al., 1980, Schaible and Scheuer, 1979, Schaible et al, 1981, Tibbits et al., 1978, 1981).

While twitch characteristics are a useful means of examining cardiac performance, they themselves cannot expose any particular cellular mechanisms that may have been altered by chronic swim training. Yet, the significant improvement in cardiac performance seen in this study suggests that chronic swim training altered some cellular mechanism associated with ECC and thus the handling of $Ca^{2+}$ in the trained myocardium.

To determine if swim training altered $Ca^{2+}$ handling in trabeculae muscle I examined the effect of increasing $[Ca^{2+}]_o$ on PDT for both the sedentary and exercised animals. Elevating $[Ca^{2+}]_o$ reportedly increases $Ca^{2+}$ entry through the SL L-type $Ca^{2+}$ channels in sedentary animals (Mitchell et al., 1985, Schouten, 1990) leading to an increase in the amount of $Ca^{2+}$ stored in (Bers 1989, Kurihara and Sakai, 1985, Orchard and Lakatta, 1985) and released (Bassani et al., 1995) from the SR and thus an increase in the intracellular $Ca^{2+}$ transient during contraction. My data indicated that there was a significant (71%) increase in PDT in the sedentary animals as $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM and suggests there was an increase in the $Ca^{2+}$ available to the myofilaments for contraction.

A further increase in $[Ca^{2+}]_o$ from 1.0 to 1.5mM produced an insignificant (18%) increase in PDT suggesting that a component of cardiac ECC sensitive to an increasing extracellular $Ca^{2+}$ load was or may have been saturated and no longer responsive to the increase in extracellular $Ca^{2+}$ load above 1.0mM $[Ca^{2+}]_o$.

Similar to the sedentary animals in the exercised animals there was a significant 42% increase in PDT when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM. However, there was also a significant (25%) increase in PDT when $[Ca^{2+}]_o$ was elevated again from 1.0 to 1.5mM. This data demonstrates that the processes involved in $Ca^{2+}$ handling for cardiac muscle contraction in the swim trained animals are better able to utilize a rise in $[Ca^{2+}]_o$ than those in the sedentary animals.

My results are comparable to other studies examining the effect of $[Ca^{2+}]_o$ on cardiac performance in sedentary animals using trabeculae muscle (Schouten, 1985, Tang, 1993) and exercised animals using papillary muscle (Tibbits et al., 1981). Tibbits and associates (1981) found that elevating $[Ca^{2+}]_o$ led to a curvilinear increase in PDT in exercised animals.

This data suggests that the improvement in PDT associated with exercise may be related to an increase in the $Ca^{2+}$ available to the myofilaments since increasing $[Ca^{2+}]_o$ further enhances the significant increase in cardiac performance in the exercised compared to the sedentary animals. Consistent with this, Hazel and associates (1996) have recently reported that exercise training did not effect the sensitivity of the myofilaments to $Ca^{2+}$ again indicating that the improvements seen with exercise may be related to an increase in $Ca^{2+}$ availability to the myofilaments.

## 2. Effects of Exercise and $[Ca^{2+}]_o$ on Cardiac SR $Ca^{2+}$ Content

To determine if chronic swim training produced an increase in SR $Ca^{2+}$ content I used a well documented technique called a rapid cooling contracture (RCC). This technique has been used extensively in our laboratory to indirectly assess SR $Ca^{2+}$ content (Losito et al., 1996, Tang, 1993). Rapidly cooling cardiac muscle to 0.5 - 1.0 °C produces a contracture which has been attributed to $Ca^{2+}$ release from the SR (Banijamali et al., 1991, Bers, 1987, Bridge, 1986, Hryshko et al., 1989, Kurihara and Sakai, 1985). Rapid cooling is not accompanied by an action potential and does not depend on $Ca^{2+}$ influx across the sarcolemma since changes in the $[Ca^{2+}]$ and $[Na^{+}]$ of the cold buffer do not affect the amplitude of the RCC (Bers et al., 1988, Bridge, 1986, Kurihara and Sakai, 1985). Thus, the amplitude of RCCs most likely reflects the amount of $Ca^{2+}$ stored in the SR as has been shown using the fluorescent $Ca^{2+}$ indicator, Indo-1 (Bers et al., 1989, 1993).

There has been extensive research done to determine if RCCs are an appropriate assay of SR $Ca^{2+}$ content. Rapid cooling contractures are abolished by caffeine (Bers, 1987, Bers et al., 1987, 1989, Bridge, 1986, Hryshko et al., 1989) a substance that prevents the SR from accumulating $Ca^{2+}$ (Blayney et al., 1978, Fuchs, 1969, Weber and Herz, 1968). The

amplitude of RCCs change in a similar manner to the amplitude of contractions that are highly dependent on SR $Ca^{2+}$ content such as rest potentiation and rest decay (Bers, 1989, Bridge, 1986). Also, Bers and associates (1989) discovered that evoking RCCs in the presence of the fluorescent $Ca^{2+}$ indicator Indo-1 led to an increase in $[Ca^{2+}]_i$ that was large enough to saturate indo-1. In the cardiac cell saturation of indo-1 is reached at approximately 13-32 uM-$Ca^{2+}$. It has been reported that a release of 140-235 umol $Ca^{2+}$/kg wet weight from the SR would be necessary to increase free $[Ca^{2+}]_i$ to a value of 13-32 uM (Pierce et al., 1985). This amount is similar to various estimates of SR $Ca^{2+}$ content from mammalian cardiac muscle (Dani et al., 1979, Hunter et al., 1981, Levitsky et al., 1981, Solaro and Briggs, 1974). This research suggests that RCCs are dependent on SR $Ca^{2+}$ stores and are a useful means of assessing total SR $Ca^{2+}$ content.

My experiments determined that following 40 - 44 weeks of chronic swim training there was a 237% increase in the SR $Ca^{2+}$ content of the exercised group compared to the sedentary group at an $[Ca^{2+}]_o$ of 0.5mM. These results confirm those found previously in our laboratory by Losito and associates (1996) who using rat right ventricular trabeculae reported that 30 weeks of swim training produced a 253% increase in SR $Ca^{2+}$ content at a $[Ca^{2+}]_o$ of 0.5mM.

Studies using interventions that alter SR $Ca^{2+}$ content such as: post-rest contractions (Bers 1989) and force-frequency responses (Tang 1993) have demonstrated (using RCCs) that in the rat changes in PDT are

closely related to changes in the SR $Ca^{2+}$ content. With this in mind, the data suggests that the significant improvement in PDT in the swim trained animals was a direct result of an increase in the cardiac SR $Ca^{2+}$ content of this group.

This increase in SR $Ca^{2+}$ content observed indirectly using RCCs might be the result of an increase in SR $Ca^{2+}$ uptake as has been suggested by Levine and Kinasewitz (1986), and Penpargkul and associates (1977). Consistent with this Tate and associates (1995) recently reported that the activity of the SR Ca-ATPase pump (mechanism responsible for SR $Ca^{2+}$ uptake) and the expression of the gene encoding this pump (SERCA2a) are increased following exercise training albeit in older animals.

Interestingly, in contrast to my data, studies utilizing treadmill training to examine cardiac SR $Ca^{2+}$ handling in rat cardiac myocytes (using Fura-2) (Laughlin et al., 1992), cardiac SR $Ca^{2+}$ binding and uptake in dogs (Sordhal et al., 1977), and cardiac SR $Ca^{2+}$ ATPase activity in pigs (Laughlin et al., 1991) did not report any significant changes in SR $Ca^{2+}$ handling in the trained compared to the sedentary animals.

It should be noted that Schaible and Scheuer (1979) using isolated whole hearts, reported that when treadmill training and swim training are compared, swim training produces greater improvements in cardiac performance than treadmill training suggesting that cardiac adaptations to running and swimming may be qualitatively different. Also, my exercise training protocol of 40-44 weeks was much longer then previously

mentioned treadmill studies (22 weeks). Therefore, the discrepancy in results on SR function might be attributed to the type of exercise but more importantly to the duration of my exercise swimming protocol. Finally, to date no one has examined SR $Ca^{2+}$ content using RCCs following a treadmill training protocol so the difference in results may be attributed to experimental methods.

To further examine if swim training altered $Ca^{2+}$ handling in cardiac muscle I investigated the effects of progressively increasing $[Ca^{2+}]_o$ on the SR $Ca^{2+}$ content in both groups.

In the sedentary animals increasing $[Ca^{2+}]_o$ from 0.5 to 1.0mM produced a significant increase in SR $Ca^{2+}$ content but a further increase from 1.0 to 1.5 mM led to no further significant elevation in SR $Ca^{2+}$ stores. This increase in SR $Ca^{2+}$ content (Fig. 4) is similar to findings from previous reports using RCC to estimate SR $Ca^{2+}$ content when $[Ca^{2+}]_o$ was elevated (Bers, 1989, Kurihara and Sakai, 1985).

It has been reported that increasing $[Ca^{2+}]_o$ enhances $Ca^{2+}$ influx through the SL L-type channels (Mitchell et al., 1985, Schouten, 1990) and also increases SR $Ca^{2+}$ content (Bers, 1989). Given that $Ca^{2+}$ released from the SR accounts for approximately 90% of the total activator $Ca^{2+}$ available for contraction in the rat myocardium (Bers, 1985), the observed increases in PDT (Fig. 3) associated with elevated levels of $[Ca^{2+}]_o$ in the sedentary group may be a result of the observed increases in SR $Ca^{2+}$ content (Fig. 4) when $[Ca^{2+}]_o$ was increased. In fact, I found that the significant changes in

SR $Ca^{2+}$ content (Fig. 4) mimicked the significant changes in PDT (Fig. 3) in the sedentary group suggesting that the increases in PDT as $[Ca^{2+}]_o$ was elevated were directly associated with increases in SR $Ca^{2+}$ load.

For the first time I also discovered that in swim trained animals, where SR $Ca^{2+}$ stores are significantly greater at the baseline $[Ca^{2+}]_o$ of 0.5mM compared to sedentary animals (Fig. 4), increasing $[Ca^{2+}]_o$ led to a gradual increase in the SR $Ca^{2+}$ stores. In this group there were no significant changes in SR $Ca^{2+}$ content when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM or 1.0 to 1.5mM. However, when SR $Ca^{2+}$ content at 0.5mM was compared to that at 1.5mM this cumulative increase was significant. It should be noted that while SR $Ca^{2+}$ stores increased gradually until becoming significant at 1.5mM $[Ca^{2+}]_o$ in the exercised group, the PDT rose significantly ($p<0.05$) at both 1.0mM and 1.5mM $[Ca^{2+}]_o$ in these animals.

The data suggests that the mechanisms responsible for SR $Ca^{2+}$ storage are initially more responsive to the rise in extracellular $Ca^{2+}$ load in the sedentary compared to the exercised animals. The blunted response in the exercised animals may be a result of the already exercised induced increase in SR $Ca^{2+}$ stores at 0.5mM $[Ca^{2+}]_o$. Previously, Kurihara and Sakai (1985) examining the effect of $[Ca^{2+}]_o$ on SR $Ca^{2+}$ stores in guinea-pig papillary muscle and Tang (1993) examining the effect of $[Ca^{2+}]_o$ on the force-frequency relationship in rat trabeculae muscle demonstrated that SR $Ca^{2+}$ storage is sensitive to the magnitude of the steady state SR $Ca^{2+}$ content. Therefore, in the exercised animals, the insignificant change in the

SR $Ca^{2+}$ content when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM may be a result of the already significantly (237%) greater cardiac SR $Ca^{2+}$ content at the baseline $[Ca^{2+}]_o$ of 0.5mM. This suggests that in swim trained rats the intra SR $Ca^{2+}$ stores may play a more critical role in regulating the responsiveness of $Ca^{2+}$ handling to an increase in $[Ca^{2+}]_o$. Furthermore, the data also suggests that there is a distinction between how sedentary and in particular how exercise trained cardiac tissue handles an increase in $[Ca^{2+}]_o$ given the already increased SR $Ca^{2+}$ stores in this tissue. Therefore the dramatic improvements in PDT in the exercised group as $[Ca^{2+}]_o$ was elevated may not have been solely a result of the progressive increase in SR $Ca^{2+}$ content following an increase in $[Ca^{2+}]_o$.

### 3. Effects of Exercise and $[Ca^{2+}]_o$ on the Fractional Release of SR $Ca^{2+}$

Not all of the $Ca^{2+}$ stored in the SR is released during an electrically stimulated contraction in mammalian cardiac muscle. As previously mentioned the amount that is released is referred to as the fractional release of SR $Ca^{2+}$ (FR). In this study FR was estimated indirectly as the ratio of PDT to peak RCC tension (Bouchard and Bose, 1991).

In the sedentary group, the FR was 54% when $[Ca^{2+}]_o$ was 0.5mM. This is similiar to the FR of SR $Ca^{2+}$ reported in cardiac myocytes from sedentary rabbits (~50%) (Bassani et al., 1993) and cardiac myocytes from sedentary ferrets (35%) (Bassani et al., 1995). Chronic swim training

significantly increased FR in the exercised animals to 72% at an $[Ca^{2+}]_o$ of 0.5mM. These results confirm those of Losito and associates (1996) who found that following 30 weeks of swimming, FR was approximately 74% in the exercised animals at an $[Ca^{2+}]_o$ of 0.5mM.

This increase in FR might be attributed to an increase in trans-sarcolemmal $Ca^{2+}$ entry but, the data examining changes in trans-sarcolemmal $Ca^{2+}$ movement due to exercise training are equivocal at this time (Laughlin et al., 1991, Mokelke et al., 1997, Pierce et al., 1989, Saborido et al., 1995, Tibbits et al., 1989, Tibbits et al., 1981). However, given our swimming protocol of 40-44 weeks an alteration in trans-sarcolemmal $Ca^{2+}$ movement cannot be completely eliminated.

The data now suggests that chronic swim training improves cardiac performance by increasing not only the SR $Ca^{2+}$ stores but also the amount of SR $Ca^{2+}$ that is released to the myofilaments for contraction.

To further examine if swim training altered $Ca^{2+}$ handling in cardiac muscle I investigated the effects of progressively increasing $[Ca^{2+}]_o$ on the FR of SR $Ca^{2+}$ stores in both groups.

In the sedentary animals, elevating $[Ca^{2+}]_o$ from 0.5mM to 1.0mM produced a significant increase in FR but a further increase from 1.0mM to 1.5mM led to no further significant elevation in the FR of SR $Ca^{2+}$ stores (Fig. 5). These results are similar to those of Bouchard and Bose (1991) who examining rat right ventricular trabeculae muscle reported that FR was increased when $[Ca^{2+}]_o$ was elevated from 1.25 to 2.5mM.

For the first time I also found that in swim trained animals where FR is already significantly greater at an $[Ca^{2+}]_o$ of 0.5mM compared to the sedentary animals, increasing $[Ca^{2+}]_o$ led to only a gradual increase in the FR of the SR $Ca^{2+}$ stores. In swim trained animals there were moderate increases in FR when $[Ca^{2+}]_o$ was elevated from 0.5 to 1.0mM or 1.0 to 1.5mM but when the FR at 0.5mM $[Ca^{2+}]_o$ was compared to that at 1.5mM the cumulative increase in FR was significant.

These increases in FR due to an elevation in $[Ca^{2+}]_o$ may be related to enhanced $Ca^{2+}$ flux through the SL L-type $Ca^{2+}$ channels (Bassani et al., 1995) and the increase in SR $Ca^{2+}$ content (Bassani et al., 1995) associated with an increase in $[Ca^{2+}]_o$ . Previously, Bassani and associates (1995), using ferret cardiac myocytes demonstrated using changes in pacing frequency and $[Ca^{2+}]_o$ to alter SR $Ca^{2+}$ load, that FR increased from 3.6 to 35 to 59% for the low, normal and high SR loading conditions respectively (with the same $Ca^{2+}$ trigger) thereby demonstrating that FR was sensitive to the SR $Ca^{2+}$ content.

My data demonstrated that when an increase in $[Ca^{2+}]_o$ significantly increased SR $Ca^{2+}$ content, there was also a concommitant significant increase in FR in both the sedentary and exercised groups. Thus, the dramatic increase in FR in the sedentary group when the $[Ca^{2+}]_o$ was initially increased from 0.5mM to 1.0mM may be related to the substantial increase in SR $Ca^{2+}$ content. Furthermore, the insignificant change in FR in the exercised group when $[Ca^{2+}]_o$ was initially elevated from 0.5 to 1.0mM may

be a result of the insignificant change in SR $Ca^{2+}$ content with the same increase in $[Ca^{2+}]_o$.

In summary, my data indirectly suggests that FR may be regulated by the SR $Ca^{2+}$ content in both the sedentary and exercised animals. Furthermore, this may account for the difference in the response of the mechanisms associated with FR to the intial increase in $[Ca^{2+}]_o$ from 0.5 to 1.0mM between the sedentary and exercised groups.

## 4. Effects of Exercise and $[Ca^{2+}]_o$ on the Recirculation Fraction of $Ca^{2+}$

Myocardial relaxation results from the lowering of cytoplasmic $Ca^{2+}$. The majority of this $Ca^{2+}$ is either resequestered by the SR (60-80%) or extruded from the cell via the SL Na/Ca exchanger (20-40%) (Bers and Bridge, 1989, Schouten, 1985). $Ca^{2+}$ can also be removed by the SL Ca-ATPase pump but this mechanism is to slow to significantly effect relaxation (Bers et al., 1989). The amount of $Ca^{2+}$ resequestered by the SR during relaxation is defined as the recirculation fraction (RF) and plays an important role in the magnitude of the SR $Ca^{2+}$ content.

To measure the RF I used paired rapid cooling contractures (see methods). The first RCC is used to release the total SR $Ca^{2+}$ content. A second RCC is then used to assess the fraction of $Ca^{2+}$ resequestered by the SR during relaxation from the first RCC (Bers et al., 1989, Hryshko et

al., 1989) Consequently the recirculation fraction was indirectly estimated as the ratio of $RCC_1$ to $RCC_2$ (Tang, 1993).

The RF was 56% in the sedentary animals at the base line $[Ca^{2+}]_o$ of 0.5mM and is similar to the RF of 59% estimated by Tang (1993) and 60% estimated by Losito and associates (unpublished results) both from rat right ventricular trabeculae muscle using paired RCCs and an $[Ca^{2+}]_o$ of 0.5mM.

Following the swimming protocol the RF was increased but not significantly to 71% in the exercise groups at the baseline $[Ca^{2+}]_o$ of 0.5mM. This data indicates that swim training did not significantly alter either the portion of $Ca^{2+}$ resequestered by the SR or the portion extruded from the cell by the Na-Ca exchanger during relaxation.

In the exercised group the SR $Ca^{2+}$ content was significantly elevated and the total intracellular $Ca^{2+}$ transient (SR $Ca^{2+}$ release) was enhanced during contraction as evidenced by an increased PDT in the exercised animals. This data infers that there was increased SR $Ca^{2+}$ uptake during relaxation in these animals. However the RF did not increase significantly suggesting that there was an increase in the absolute amount of SR $Ca^{2+}$ uptake and $Ca^{2+}$ extrusion via the Na-Ca exchanger in the exercised animals. Thus while I was surprised to find no significant changes in RF the results infer that there was a physiological adaptation resulting in increased SR $Ca^{2+}$ uptake in the exercised group. In fact, Tate and associates (1990) found an increase in ATP dependent SR $Ca^{2+}$ uptake with

exercise and Tibbits and associates (1989) found an exercise induced increase in Na-Ca exchanger activity suggesting that both these $Ca^{2+}$ removal mechanisms are improved following treadmill training.

To further investigate if swim training altered the RF in cardiac muscle I examined the effects of progressively increasing $[Ca^{2+}]_o$ on RF of $Ca^{2+}$ in both groups. There were no significant changes in RF when $[Ca^{2+}]_o$ was increased from 0.5mM to 1.0mM and then to 1.5mm in the sedentary or exercised groups. However, the significant increase in SR $Ca^{2+}$ content (Fig. 4) in both groups as a result of elevated $[Ca^{2+}]_o$ suggests an increase in SR $Ca^{2+}$ uptake during relaxation. Also, the significant increase in PDT (Fig. 3) observed in both groups as a result of an increase in $[Ca^{2+}]_o$ suggests that the total amount of $Ca^{2+}$ available to the myofilaments was increased during contraction and consequently that more $Ca^{2+}$ had to be removed during relaxation. This suggests that while elevating $[Ca^{2+}]_o$ produced no significant elevation in RF there may have been an increase in the absolute amount of SR $Ca^{2+}$ uptake in cardiac muscle in both groups as $[Ca^{2+}]_o$ was increased.

# CHAPTER FIVE: CONCLUSION

In this study I found that chronic swim training improved cardiac performance as witnessed by a significant increase in PDT and ±dT/dt in the exercised compared to the sedentary animals. Using RCCs I discovered that chronic swim training produced a significant increase in the SR $Ca^{2+}$ content and the FR of $Ca^{2+}$ from the SR. These results suggest that the improvement in cardiac performance associated with exercise is due to an increase in the $Ca^{2+}$ available to the contractile machinery for contraction. This improved $Ca^{2+}$ availability is due to an increase in: (1) the SR $Ca^{2+}$ available for release and (2) the amount of SR $Ca^{2+}$ that is released for contraction. Chronic swim training did not significantly affect the fraction of $Ca^{2+}$ reseqestered by the SR during relaxation but appears to have increased absolute SR $Ca^{2+}$ uptake following contraction.

Another objective of this study was to examine if chronic swim training altered the handling of an increase in $[Ca^{2+}]_o$. Elevating $[Ca^{2+}]_o$ produced an increase in PDT, SR $Ca^{2+}$ content, and the FR of SR $Ca^{2+}$ in both sedentary and exercised cardiac muscle. However in the exercised group an elevation of $[Ca^{2+}]_o$ produced a more significant effect on PDT and a less significant effect on SR $Ca^{2+}$ storage and FR of SR $Ca^{2+}$ compared to the sedentary animals. These results demonstrated that swim training did alter the inotropic response of cardiac muscle to an increase in $[Ca^{2+}]_o$ in the exercised animals.

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## APPENDIX A: MODIFIED KREBS-HENSELEIT BUFFER

**Chemical Composition:**

**Table 1: 10 x Stock Buffer**

| Name | Millimole | Molecular Weight | Grams/Litre | 10 x Stock (G/L) |
|---|---|---|---|---|
| NaCl | 112 | 58.44 | 6.54 | 65.45 |
| KCl | 5 | 74.55 | 0.37 | 3.73 |
| $MgCl_2 \bullet 6H_2O$ | 1.2 | 203.3 | 0.24 | 2.44 |
| $Na_2SO_4$ | 1.2 | 142.04 | 0.17 | 1.7 |
| $NaH_2PO_4 \bullet H_2O$ | 2 | 137.99 | 0.28 | 2.76 |

**Table 2: Working Buffer**

| Name | Millimole | Molecular Weight | Grams/Litre |
|---|---|---|---|
| $NaHCO_3$ | 27 | 84.01 | 2.3 |
| Glucose | 10 | 180.16 | 1.8 |
| $CaCl_2$ | 1 | | |

**Table 3: Required Amount of 2N CaCl**

| Working Buffer (ml) | Desired $[Ca^{2+}]$ (mM) | Amount of 2N $CaCl_2$ (*ul*) |
|---|---|---|
| 500 | 0.25 | 125 |
| 500 | 0.5 | 250 |
| 500 | 1.0 | 500 |

A one litre stock buffer was prepared using the chemicals and amounts outlined in the 10 x Stock (grams/litre) column (see Table 1). The stock buffer was kept refrigerated (for up to 2 weeks). To prepare a 1.0 litre working buffer, 100ml of the 10 x stock buffer was removed and diluted with 800ml of double distilled water. To this solution, the appropriate amounts of $NaHCO_3$ and glucose (see Table 2) were then added and the buffer was diluted with another 100 ml of double distilled water to produce a final volume of 1000 ml. The working buffer was then bubbled with 95% $O_2$ and 5% $CO_2$ for 30 minutes to establish pH. This results in a $Ca^{2+}$ - free perfusate.

Perfusion buffer :

To prepare a calcium perfusion buffer, 500 ml of the $Ca^{2+}$ - free perfusate was removed and the appropriate volume of 2N calcium chloride (BDH, analytical grade) was added (see Table 3).

Dissection buffer :

To prepare the dissection buffer 500 ml of the $Ca^{2+}$ - free perfusate was used. This buffer contained low calcium (0.5 mM) to reduce intracellular calcium overload during the microsurgery. In addition, 2,3-Butane Dione Monoxime was added at 3.0 mg/ml of buffer (to arrest cardiac contractions) and the perfusate was continously gassed with 95% $O_2$ and 5% $CO_2$.

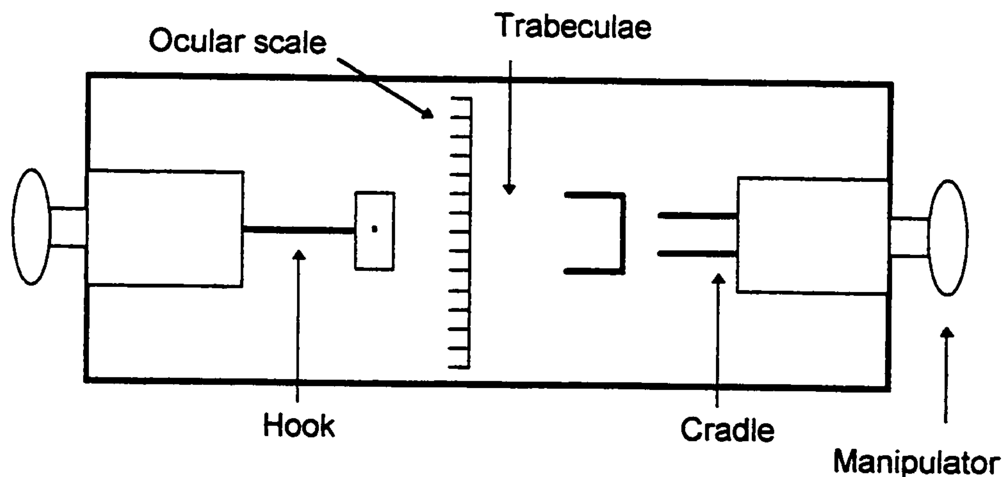
## APPENDIX B: VIDEO IMAGING SYSTEM

It has been demonstrated that the average sarcomere length of the trabeculae muscle plays a critical role in tension generation. Therefore, it is critical that every experiment be performed at a fixed sarcomere length. In these experiments a sarcomere length of 2.0 *um* was used. A video imaging system was utilized to ensure that the sarcomere length was maintained at 2.0 *um*. An inverted microscope was modified to allow a video camera (Panasonic CCTV Camera, Model WV-1410) to be attached. The muscle was visualized with a 40x long working distances objective lens. The camera projected the image onto a video monitor (Panasonic Video Monitor Model TR-930 CB). At this magnification individual sarcomeres could be observed on the monitor. Using a calibrated acetate grid we were able to measure the average sarcomere length of runs of 5 sarcomeres at various locations across the trabaculae muscle. The average sarcomere length was measured at a least three locations on the muscle to ensure that the average sarcomere length was 2.0 *um* along the entire trabeculae.

## APPENDIX C: CROSS-SECTIONAL AREA

The cross-sectional area (CSA) of each trabeculae muscle was estimated from width and thickness measurements. At the end of each experiment the muscle was placed in a muscle bath designed specifically for measuring cross-sectional area (see illustration). The width and thickness were measured using an ocular scale installed into the eye piece of a dissecting microscope. It is important to measure the width and thickness from the same area on each muscle. In this study measurements were always obtained from the mid-section of the muscle. Since a trabaculae is rectangular in shape the cross-sectional area was calculated as follows:

$$\textbf{CSA (mm}^2\textbf{) = width (mm) x thickness (mm)}$$




Schematic illustration of a muscle bath designed for measuring CSA.

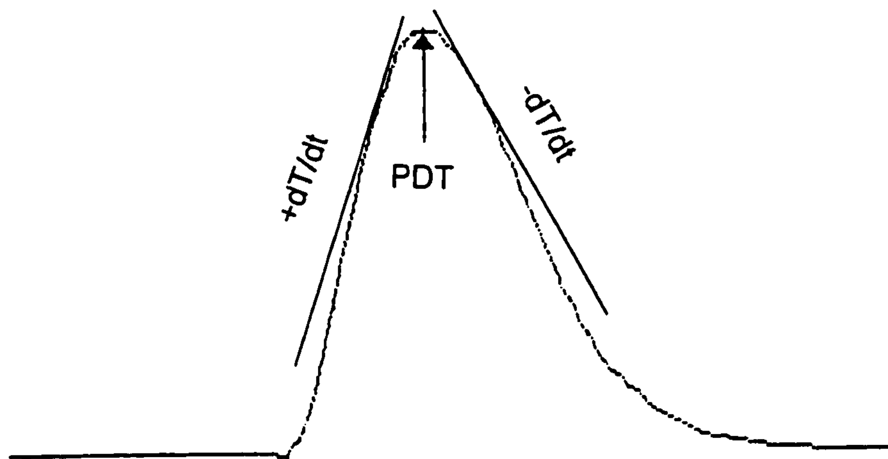
## APPENDIX D: TWITCH CURVE DEFINITIONS

Twitch function curves were collected from each trabeculae muscle at all $[Ca^{2+}]_o$ in both the exercise and sedentary groups. The following twitch characteristics were determined:

1. Steady-state peak developed tension (PDT). The peak amount of force produced by a muscle measured in $mN/mm^2$

2. Maximum rate of tension development (+dT/dt). The maximum rate of force production measured in $mN/mm^2 \bullet sec^{-1}$

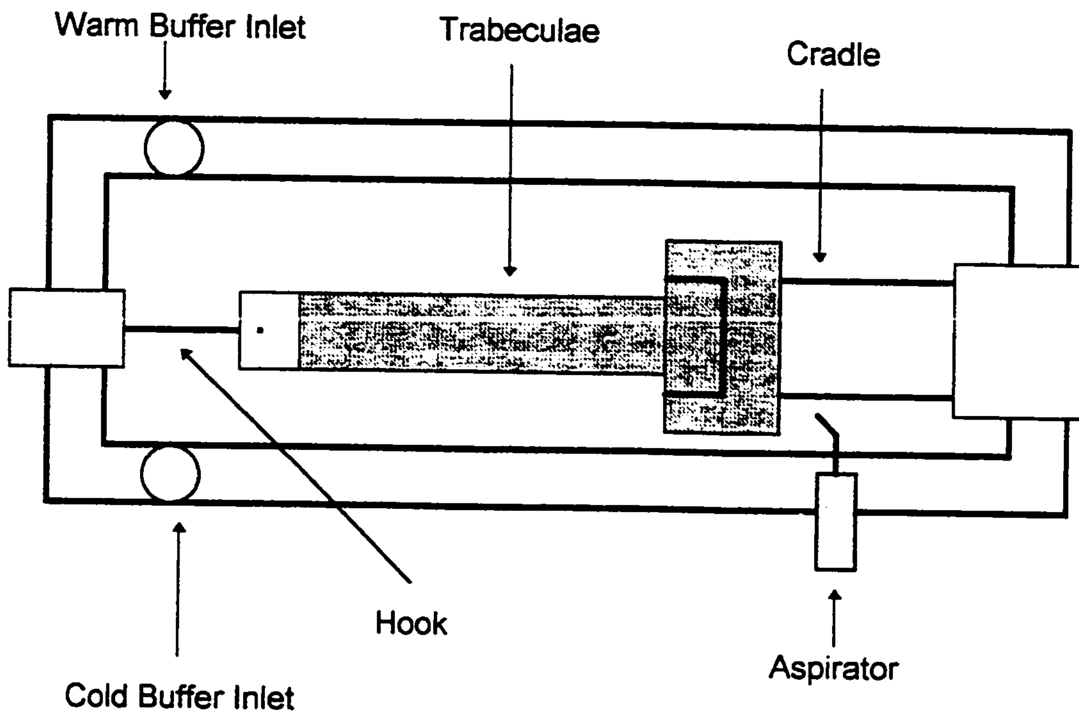
3. Maximum rate of tension decay (-dT/dt). The maximum rate of force relaxation measured in $mN/mm^2 \bullet sec^{-1}$




Actual tracing of a steady-state twitch curve from a right ventricle trabeculae muscle. PDT = peak developed tension, +dT/dt = maximum rate of tension development, -dT/dt = maximum rate of tension decay. Stimulation rate = 0.2 Hz and $[Ca^{2+}]_o$ = 0.5mM.

## APPENDIX E: RAPID COOLING CONTRACTURE MUSCLE BATH

In order to produce consistent rapid cooling contractures (RCCs) it is very important to prevent recirculation of the cold buffer in the muscle bath. Recirculation may result in a gradual rewarming of the buffer and a depressed RCC. To prevent recirculation, cold buffer inflow should equal buffer outflow via the aspirator. In order to rapidly cool and rewarm the muscle, buffer inflow (warm and cold) and buffer outflow were set to 60ml/min. This fast rate of flow ensured that the muscle was cooled and rewarmed quickly and was continuously perfused with oxygen, glucose and other essential nutrients. Also to prevent recirculation of the cold buffer, the aspirator was placed directly behind the muscle. If recirculation of cold buffer still occurs another option is to use a second aspirator. HINT: to produce proper RCCs position the trabeculae muscle as close to the cold buffer inlet as possible. This ensures that there is no warming of the cold buffer before it encounters the muscle. On the next page there is an illustration of a muscle bath designed specifically for RCCs. Notice that the muscle is positioned near the buffer inlets and that the aspirator is placed directly behind the muscle.




Schematic illustration of a muscle bath designed specifically for RCCs.

## APPENDIX F: DRY TO WET HEART WEIGHT RATIO

The ratio of dry heart to wet heart weight was used to determine if chronic swim training affected the water content of the heart. Following removal of the trabeculae muscle the heart was quickly frozen in a -70 °C freezer. At a later time the hearts were removed from the freezer and thawed. After thawing all excess tissue (valves and adipose) were removed. The ventricles were then cut open. Any excess water was removed using a small piece of paper. Next, a 400-500 mg piece of the ventricle was dissected. This piece of tissue was weighed using a precision mechanical balance (Federal Pacific Electrical Company). This measurement is the WET weight. The piece of tissue was then placed in an oven at a temperature of 80 °C for 72 hours. Following this the tissue was again weighed. This measurement is the DRY weight. The ratio of dry to wet heart weight is then obtained by dividing the dry weight of the tissue by the wet weight of the tissue. This ratio represents the percentage of the heart weight that is not made up of water.

## APPENDIX G: CONVERSION OF VOLTAGE TO FORCE

The raw data from each muscle was collected and stored in the computer in volts. To properly interpret the data it must be converted to a physiological measure of force and normalized by the size of the muscle. In this study, all raw data collected in volts was converted to force in milliNewtons (mN) and normalized by the cross-sectional area of each muscle in millimetres$^2$ (mm$^2$). In order to do this, the force transducer must be calibrated so that voltage can be defined in milligrams (see illustration below). Note that 1 mg = 0.009807046 mN. The following equation was used to convert voltage to force in mN/mm$^2$ :

$$F = V / K * N / C$$

where:

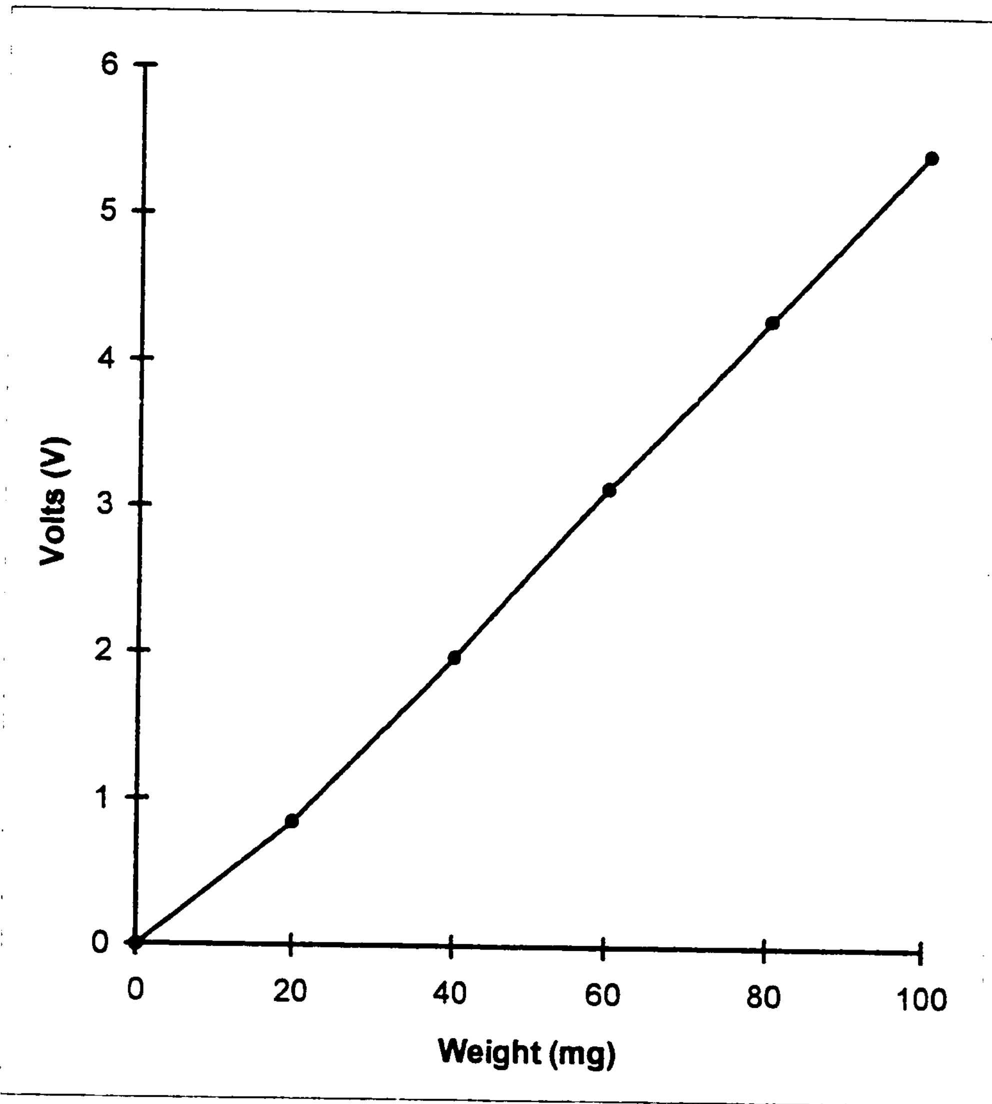
V = data measured in volts

K = calibration constant (this was derived from the calibration curve for the force transducer)

N = 0.009807046 mN

C = cross-sectional area (mm$^2$)

Note that K is the slope of the linear regression from the calibration curve for the force transducer. In this case K = 0.0578 Volts / milligrams.




An illustration of a transducer calibration curve at a sensitivty of 20 mV/cm. A linear regression was fit to the curve and the slope of this line was used as the calibration constant K. The slope of this curve is 0.0578 V/mg.

## APPENDIX H: MORPHOLOGICAL RAW DATA, MEANS AND STANDARD ERROR

**Body Weight (grams)**

| Animal No. | Sedentary | Exercise |
|---|---|---|
| 1 | 302.8 | 330.8 |
| 2 | 336.2 | 384.2 |
| 3 | 337 | 372.5 |
| 4 | 306.9 | 358.7 |
| 5 | 346.6 | 388.4 |
| 6 | 350.8 | 284.6 |
| 7 | 348.9 | 378.5 |
| Mean | 332.7 | 356.8 |
| S.E. | 7.5 | 14.1 |

**Heart Weight (milligrams)**

| Animal No. | Sedentary | Exercise |
|---|---|---|
| 1 | 980 | 1470 |
| 2 | 1060 | 1410 |
| 3 | 1290 | 1220 |
| 4 | 960 | 1390 |
| 5 | 1070 | 1650 |
| 6 | 960 | 1370 |
| 7 | 1070 | 1410 |
| Mean | 1055.7 | 1417.1 |
| SE | 43.6 | 49 |

**Heart Weight / Body Weight Ratio (milligrams / grams)**

| Animal No. | Sedentary | Exercise |
|---|---|---|
| 1 | 3.24 | 4.44 |
| 2 | 3.15 | 3.67 |
| 3 | 3.83 | 3.28 |
| 4 | 3.13 | 3.88 |
| 5 | 3.09 | 4.25 |
| 6 | 2.74 | 4.81 |
| 7 | 3.07 | 3.73 |
| Mean | 3.18 | 4.01 |
| SE | 0.12 | 0.2 |

**Dry Heart Weight / Wet Heart Weight Ratio (milligrams / milligrams)**

| Animal No. | Sedentary | Exercise |
|---|---|---|
| 1 | 0.224 | 0.221 |
| 2 | 0.196 | 0.231 |
| 3 | 0.196 | 0.222 |
| 4 | 0.23 | 0.23 |
| 5 | 0.213 | 0.219 |
| 6 | 0.23 | 0.207 |
| 7 | 0.237 | 0.229 |
| Mean | 0.218 | 0.223 |
| SE | 0.006 | 0.003 |

**Right Ventricular Trabeculae Muscle Cross-sectional Area (millimeters$^2$)**

| Animal No. | Sedentary | Exercise |
|---|---|---|
| 1 | 0.071 | 0.125 |
| 2 | 0.093 | 0.04 |
| 3 | 0.053 | 0.053 |
| 4 | 0.107 | 0.044 |
| 5 | 0.027 | 0.036 |
| 6 | 0.036 | 0.027 |
| 7 | 0.027 | 0.018 |
| Mean | 0.059 | 0.049 |
| S.E. | 0.012 | 0.014 |

## APPENDIX I: MORPHOLOGICAL STATISTICAL ANALYSIS

**Body Weight**

| Source | Sum of Squares | Degrees of Freedom | Mean Squares | f-score | Main Effect p< 0.05 |
|---|---|---|---|---|---|
| Between | 2028.02 | 1 | 2028.02 | 2.27 | 4.75 |
| Within | 10732.79 | 12 | 894.40 | | |
| Total | 12760.80 | 13 | | | |

**Heart Weight**

| Source | Sum of Squares | Degrees of Freedom | Mean Squares | f-score | Main Effect p<0.05 |
|---|---|---|---|---|---|
| Between | 457207.14 | 1 | 457207.14 | 30.77 | 4.75 |
| Within | 178314.29 | 12 | 14859.52 | | |
| Total | 635521.43 | 13 | | | |

**Heart Weight / Body Weight Ratio**

| Source | Sum of Squares | Degrees of Freedom | Mean Squares | f-score | Main Effect p< 0.05 |
|---|---|---|---|---|---|
| Between | 2.42 | 1 | 2.42 | 12.64 | 4.75 |
| Within | 2.30 | 12 | 0.19 | | |
| Total | 4.72 | 13 | | | |

**Dry Heart Weight / Wet Heart Weight Ratio**

| Source | Sum of Squares | Degrees of Freedom | Mean Squares | f-score | Main Effect $p < 0.05$ |
|---|---|---|---|---|---|
| Between | 9.609E-05 | 1 | 9.609E-05 | 0.57 | 4.75 |
| Within | 0.002 | 12 | 0.0002 | | |
| Total | 0.002 | 13 | | | |

**Cross-Sectional Area**

| Source | Sum of Squares | Degrees of Freedom | Mean Squares | f-score | Main Effect $p < 0.05$ |
|---|---|---|---|---|---|
| Between | 0.0004 | 1 | 0.0004 | 0.32 | 4.75 |
| Within | 0.0138 | 12 | 0.0011 | | |
| Total | 0.0141 | 13 | | | |

## APPENDIX J: TWITCH CHARACTERISTICS RAW DATA, MEANS AND STANDARD ERROR

### Peak Developed Tension ($mN/mm^2$)

**Sedentary**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 5.46 | 8.85 | 9 |
| 2 | 4.04 | 6.78 | 8.35 |
| 3 | 3.85 | 11.34 | 13.61 |
| 4 | 3.39 | 4.87 | 5.75 |
| 5 | 3.05 | 4.68 | 6.44 |
| 6 | 3.22 | 5.96 | 7.33 |
| 7 | 4.81 | 5.09 | 5.7 |
| Mean | 3.98 | 6.8 | 8.02 |
| S.E. | 0.33 | 0.96 | 1.09 |

**Exercise**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 17.1 | 24.19 | 27.33 |
| 2 | 21.2 | 24.8 | 29.28 |
| 3 | 19.87 | 20.61 | 25.86 |
| 4 | 21.99 | 27.01 | 36.9 |
| 5 | 17.53 | 24.96 | 33.87 |
| 6 | 12.51 | 25.28 | 27.61 |
| 7 | 12.3 | 26.5 | 35.08 |
| Mean | 17.5 | 24.76 | 30.85 |
| S.E. | 1.5 | 0.86 | 1.76 |

**Rate of Tension Development ($mN/mm^2 \bullet sec^{-1}$)**

**Sedentary**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 50.04 | 104.98 | 125.36 |
| 2 | 73.9 | 93.35 | 108.68 |
| 3 | 54.68 | 124.85 | 145.83 |
| 4 | 49.18 | 84.78 | 104.38 |
| 5 | 51.59 | 83.23 | 94.92 |
| 6 | 38.18 | 102.66 | 127.42 |
| 7 | 53.65 | 58.47 | 56.4 |
| Mean | 53.03 | 93.19 | 109 |
| S.E. | 4.12 | 8.35 | 11.41 |

**Exercise**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 144.34 | 279.54 | 306.95 |
| 2 | 204.52 | 243.5 | 288.9 |
| 3 | 211.86 | 161.65 | 230.09 |
| 4 | 173.36 | 219.93 | 260.46 |
| 5 | 286.15 | 319.17 | 360.44 |
| 6 | 104.73 | 222.35 | 348.23 |
| 7 | 159.93 | 312.63 | 374.54 |
| Mean | 183.55 | 251.25 | 309.94 |
| S.E. | 22.02 | 22.76 | 23.29 |

**Rate of Tension Decay (mN/mm$^2$ • sec$^{-1}$)**

**Sedentary**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 30.18 | 65.00 | 67.07 |
| 2 | 38.96 | 47.76 | 52.67 |
| 3 | 32.67 | 90.80 | 103.87 |
| 4 | 33.36 | 60.53 | 75.49 |
| 5 | 31.64 | 55.72 | 55.72 |
| 6 | 28.89 | 76.35 | 91.83 |
| 7 | 33.70 | 49.53 | 38.52 |
| Mean | 32.77 | 63.67 | 69.31 |
| S.E. | 1.23 | 6.09 | 8.80 |

**Exercise**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 73.08 | 78.56 | 80.39 |
| 2 | 121.06 | 138.03 | 157.29 |
| 3 | 109.71 | 88.73 | 103.18 |
| 4 | 127.64 | 88.83 | 104.36 |
| 5 | 218.05 | 228.37 | 245.56 |
| 6 | 72.22 | 151.67 | 208.94 |
| 7 | 74.29 | 144.45 | 156.83 |
| Mean | 113.72 | 131.24 | 150.94 |
| S.E. | 19.72 | 20.12 | 23.06 |

## APPENDIX K: TWITCH CHARACTERISTICS STATISTICAL ANALYSIS

**Peak Developed Tension ($mN/mm^2$)**

| Source | Sum of Squares | Degrees of Freedom | Variance Estimate | f-score | f-value p<0.05 |
|---|---|---|---|---|---|
| Rows | 3441.5 | 1 | 3441.5 | 255.5 | 4.75 |
| S/R | 161.6 | 12 | 13.5 | | |
| Columns | 534.1 | 2 | 267 | 40.9 | 3.4 |
| R x C | 151.5 | 2 | 75.7 | 11.6 | 3.4 |
| SC/R | 156.8 | 24 | 6.5 | | |
| Total | 4445.6 | 41 | | | |

**Rate of Tension Development ($mN/mm^2 \bullet sec^{-1}$)**

| Source | Sum of Squares | Degrees of Freedom | Variance Estimate | f-score | f-value p<0.05 |
|---|---|---|---|---|---|
| Rows | 279576.5 | 1 | 279576.5 | 93.89 | 4.75 |
| S/R | 35733.8 | 12 | 2977.8 | | |
| Columns | 58844.2 | 2 | 29422.1 | 24.15 | 3.4 |
| R x C | 8815.4 | 2 | 4407.7 | 3.62 | 3.4 |
| SC/R | 29237.33 | 24 | 1218.2 | | |
| Total | 412207.3 | 41 | | | |

**Rate of Tension Decay ($mN/mm^2 \bullet sec^{-1}$)**

| Source | Sum of Squares | Degrees of Freedom | Variance Estimate | f-score | f-value $p<0.05$ |
|---|---|---|---|---|---|
| Rows | 61794.8 | 1 | 61794.8 | 15.98 | 4.75 |
| S/R | 46393.2 | 12 | 3866.1 | | |
| Columns | 9828.2 | 2 | 4914.1 | 9.36 | 3.4 |
| R x C | 440.1 | 2 | 220.1 | 0.42 | 3.4 |
| SC/R | 12597.6 | 24 | 524.9 | | |
| Total | 131053.8 | 41 | | | |

## APPENDIX L: RCC RAW DATA, MEANS AND STANDARD ERROR

### Peak RCC ($mN/mm^2$)

**Sedentary**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 8.22 | 10.61 | 11.39 |
| 2 | 7.51 | 11.19 | 11.32 |
| 3 | 6.58 | 13.77 | 15.20 |
| 4 | 7.19 | 7.35 | 7.81 |
| 5 | 5.67 | 7.98 | 9.69 |
| 6 | 8.75 | 11.33 | 13.89 |
| 7 | 8.14 | 8.23 | 9.88 |
| Mean | 7.44 | 10.06 | 11.31 |
| S.E. | 0.4 | 0.94 | 1.07 |

**Exercise**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 19.07 | 27.19 | 27.70 |
| 2 | 24.58 | 30.23 | 30.78 |
| 3 | 21.70 | 22.80 | 26.28 |
| 4 | 39.80 | 46.52 | 46.57 |
| 5 | 26.30 | 28.34 | 34.15 |
| 6 | 18.90 | 25.96 | 30.23 |
| 7 | 25.30 | 31.66 | 40.78 |
| Mean | 25.09 | 30.38 | 33.78 |
| S.E. | 2.76 | 2.97 | 2.84 |

**Fractional Release (%)**

**Sedentary**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 66.37 | 83.46 | 78.99 |
| 2 | 53.82 | 60.65 | 73.75 |
| 3 | 58.58 | 82.32 | 89.50 |
| 4 | 47.13 | 66.29 | 73.59 |
| 5 | 53.88 | 58.62 | 66.48 |
| 6 | 36.79 | 52.64 | 52.75 |
| 7 | 59.12 | 61.87 | 57.66 |
| Mean | 53.67 | 66.55 | 70.39 |
| S.E. | 6.08 | 4.53 | 4.82 |

**Exercise**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 89.66 | 88.98 | 98.68 |
| 2 | 86.27 | 82.04 | 95.11 |
| 3 | 91.57 | 90.40 | 98.43 |
| 4 | 55.25 | 58.06 | 79.23 |
| 5 | 66.63 | 88.06 | 99.19 |
| 6 | 66.16 | 97.38 | 91.33 |
| 7 | 48.61 | 83.70 | 86.03 |
| Mean | 72.02 | 84.09 | 92.57 |
| S.E. | 6.88 | 4.75 | 2.94 |

**Recirculation Fraction (%)**

**Sedentary**

| Animal No. | $[Ca^{2+}]$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 71.14 | 75.68 | 85.05 |
| 2 | 66.70 | 69.57 | 68.75 |
| 3 | 63.18 | 71.83 | 51.67 |
| 4 | 32.12 | 89.61 | 72.62 |
| 5 | 71.36 | 75.52 | 63.64 |
| 6 | 39.86 | 55.92 | 80.68 |
| 7 | 46.62 | 60.20 | 63.23 |
| Mean | 55.86 | 71.19 | 69.38 |
| S.E. | 6.08 | 4.3 | 4.28 |

**Exercise**

| Animal No. | $[Ca^{2+}]_o$ | | |
|---|---|---|---|
| | 0.5mM | 1.0mM | 1.5mM |
| 1 | 82.38 | 71.24 | 96.04 |
| 2 | 74.85 | 83.86 | 84.33 |
| 3 | 91.76 | 78.09 | 93.98 |
| 4 | 46.79 | 78.89 | 81.11 |
| 5 | 95.82 | 76.99 | 92.59 |
| 6 | 50.22 | 77.74 | 68.81 |
| 7 | 52.04 | 67.28 | 62.85 |
| Mean | 70.55 | 76.3 | 82.81 |
| S.E. | 7.82 | 2.05 | 4.99 |

## APPENDIX M: RCC STATISTICAL ANALYSIS

**Peak RCC ($mN/mm^2$)**

| Source | Sum of Squares | Degrees of Freedom | Variance Estimate | f-score | f-value $p<0.05$ |
|---|---|---|---|---|---|
| Rows | 4262.92 | 1 | 4262.92 | 52.56 | 4.75 |
| S/R | 973.29 | 12 | 81.11 | | |
| Columns | 282.40 | 2 | 141.20 | 37.82 | 3.40 |
| R x C | 323.13 | 2 | 161.56 | 43.27 | 3.40 |
| SC/R | 89.61 | 24 | 3.73 | | |
| Total | 5648.95 | 41 | | | |

**Fractional Release (%)**

| Source | Sum of Squares | Degrees of Freedom | Variance Estimate | f-score | f-value $p<0.05$ |
|---|---|---|---|---|---|
| Rows | 3934.12 | 1 | 3934.12 | 11.93 | 4.75 |
| S/R | 3955.61 | 12 | 329.63 | | |
| Columns | 2523.64 | 2 | 1261.82 | 20.81 | 3.40 |
| R x C | 43.14 | 2 | 21.57 | 0.36 | 3.40 |
| SC/R | 1455.56 | 24 | 60.65 | | |
| Total | 11912.07 | 41 | | | |

**Recirculation Fraction (%)**

| Source | Sum of Squares | Degrees of Freedom | Variance Estimate | f-score | f-value p<0.05 |
|---|---|---|---|---|---|
| Rows | 1288.86 | 1 | 1288.86 | 4.83 | 4.75 |
| S/R | 3202.17 | 12 | 266.85 | | |
| Columns | 1320.33 | 2 | 660.17 | 4.41 | 3.40 |
| R x C | 189.97 | 2 | 94.98 | 0.63 | 3.40 |
| SC/R | 3590.28 | 24 | 149.59 | | |
| Total | 9591.61 | 41 | | | |

## VITA AUCTORIS

| | |
|---|---|
| NAME: | Eric Anthony Querbach |
| PLACE OF BIRTH: | Windsor, Ontario |
| YEAR OF BIRTH: | 1970 |
| EDUCATION: | Kennedy Collegiate Institute, Windsor, Ontario<br>1984-1989 |
| | University of Windsor, Windsor, Ontario<br>1989-1994, B.H.K. |
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