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AUTHOR – AUTEUR

Full Name of Author – Nom complet de l'auteur
René Albert Turcotte

Date of Birth – Date de naissance
August 12, 1952

Canadian Citizen – Citoyen canadien
☑ Yes / Oui ☐ No / Non

Country of Birth – Lieu de naissance
CANADA

Permanent Address – Résidence fixe
32 Rand Ouest
Kirkland Lake, Ontario
P2N 2S9

THESIS – THÈSE

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University – Université
University of Alberta

Name of Supervisor – Nom du directeur de thèse
A. N. Belcastro

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Time Course of Training Responses on Myofibril ATPase

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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YEAR THIS DEGREE GRANTED Fall, 1985

(SIGNED) René Turcotte

PERMANENT ADDRESS:

32 Rand Crest

Kirkland Lake, Ontario

P2N 3S9

DATED :.................... 19

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The effects of training intensity on myofibril ATPase submitted by Rene Turcotte in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

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Supervisor

....

....

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External Examiner

Date 27/05/85

ABSTRACT

The purpose of the present study was to investigate the time course of training effects on myofibrillar ATPase activity in cardiac and skeletal muscle.

The myofibril ATPase activity was measured in the cardiac, soleus and plantaris muscles of female rats after 3,6 and 9 weeks of running training. Cardiac ATPase activity was elevated after 3 and 6 weeks (28-40%) ($p<.05$) but was similar to controls after 9 weeks. In contrast, the ATPase activity was changed in skeletal muscle in the latter part of the training programs with an increase in the soleus after 9 weeks and a decrease in the plantaris after 6 and 9 weeks ($p<.05$). The regulatory aspects of the myofibril ATPase activity were also investigated by deriving a pCa50 and Hill n from the classical Hill equation. The changes in the cardiac muscle appear to be related to a change in the regulatory aspects of the actomyosin cycle but these changes were not noted in skeletal muscle. In summary, it is suggested that running training can result in changes in myofibril ATPase activity in the heart if this parameter is evaluated early in the training. Furthermore, the time course of the training effect results in different responses depending on the muscle investigated.

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Where do I begin? I've been here so long that not only have I seen graduate students come and go, but many professors have come and gone as well.

I must mention all the roommates I have had over the years in my little house in Bonnie Doone. Many of them were graduate students and are now professors.

To all the graduate students without whom I'd have left for Rio a long time ago. Each one has touched me in a special way. I cannot mention all their names because there are over 200 of them.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

Functional overload of cardiac and skeletal muscle results in physiological adaptations that are specific to the demand placed upon the organism. With endurance training the overload or demand situation over time is accomplished by varying three essential factors; intensity, frequency and duration. The precise contribution of each variable to the physiological adaptation process that occurs with endurance training is uncertain, however, intensity has been identified by some as the primary factor for improvement (Roskamm, 1967; Faria, 1970; Davies and Knibbs, 1971; Hollering et al, 1971; Harger et al, 1971; Gledhill and Eynon, 1972; Knuttgen et al, 1973; Fox et al, 1973; Fox and Mathews, 1974; Chaloupka, 1975; Wenger and Macnab, 1975; Fox et al, 1975, 1977a, 1977b; Hickson et al, 1977; Whitten and Painter, 1977; Lesmes et al, 1978; Dietrick and Ruhling, 1978; Atomi and Miyashita, 1980). The precise nature of the intensity is uncertain. When classic cardiovascular (cv) variables such as heart rate, cardiac output (Q) and maximal oxygen consumption(VO_2 max) are measured to compare high and low intensity training programs, minimal improvements have been reported regardless of whether an interval or continous protocol was employed. (Henriksson and Reitman, 1976; Houston and Thomson, 1977; Eddy et al, 1977; Cunningham et al, 1979). Furthermore, a preliminary study has demonstrated that high intensity training (ie. 125-140% VO_2 max) was inferior to more traditional intensities (70-80% VO_2 max) endurance training in humans (Robinson and Sucec, 1980). Potential problems with this area include lack of control groups, lack of sound experimental designs and lack of characterization of the training programs (ie. quantification and equating of workloads of different types of training programs). In addition, the precise contribution of frequency and duration to training remains obscure for the same reasons (American College of Sports Medicine Position Statement, 1978).

Endurance training results in a variety of cardiac functional changes. These changes include enhanced myocardial contractility and mechanical function under normal, hypoxic and ischemic conditions (Penpargkul and Scheuer, 1970; Scheuer and Stezoski, 1972; Hepp et al, 1974; Scheuer et al, 1974; Williams and Potter, 1976; Dowell et al, 1976; Behrson and Scheuer, 1977; Dowell et al, 1977; Stone, 1977; Tibbits et al, 1978; Behrson and Scheuer, 1978; Noakes et al, 1979; Schaible and Scheuer, 1979; Ritzer et al, 1980; Meerson et al, 1980; Barnard et al, 1980; Tibbits et al, 1981), cardiac hypertrophy (Liere and Northrup, 1957; Astrand et al, 1963; Oscai et al, 1971a; Scheuer, 1973; Crews and Aldinger, 1974; Wyatt and Mitchell, 1974; Carey et al, 1976; Codini et al, 1977; Scheuer and Tipton, 1977; Allen et al, 1977; Baldwin et al, 1977; Carew and Covell, 1978; DeMaria et al, 1978; Ehsani et al, 1978; Wolfe et al, 1979; Rerych et al, 1980; Stein et al, 1980) and greater maximal cardiac output (Astrand and Rodahl, 1971; Clausen, 1976) in both humans and animals.

Interestingly, the cellular alterations that account for the enhanced myocardial contractility are uncertain. Tibbits et al, (1981) have suggested that an increased contractility in their running experiments with rats was due to greater Ca^{2+} transport across the sarcolemma with the contribution of the contractile element being negligible. Others have reported significant changes in the contractile (myosin or actomyosin) element of rats as a result of run training programs (Baldwin and Terjung, 1975; Baldwin et al, 1977; Penpargkul et al, 1980; Resink et al, 1981a, 1981b). The discrepancies with regard to changes in the myofibrillar ATPase may be due to a number of factors. Baldwin et al, (1977) have reported that with continous endurance training the cellular events involved with cardiac contraction (ie. myofibrillar ATPase) do not change. On the other hand, when the endurance training program was undertaken with intermittent exercise at a greater intensity, the cardiac myofibrillar ATPase activity was elevated (15%) and cardiac hypertrophy was maintained. It was suggested that the reduced exercise overload on the cv system with lower intensity training programs results in only transient changes in cardiac tissue of rats (Baldwin et al, 1977). The transient nature of adaptations has been reported and it appears that following 2 to 4 weeks the cardiac adaptations that occur with a conventional training program may be lost (Pierce et al, 1979). The small number of training groups (Baldwin et al, 1977) and the lack of quantification of exercise duration and total work done in animal experiments, add to the uncertainty of the effects of endurance exercise on the contribution of the contractile component (eg.myofibrillar ATPase) to the changes in myocardial contractility.

Thus, the purpose of the present study was to:

1- examine the effects of endurance training on myofibrillar ATPase activity of skeletal and cardiac muscle in female rats.

2- to see wether there was a relationship between the training and the time course of development of the adaptation.

3- to examine if the Ca^{2+} regulatory characteristics of the myofibrillar ATPase played a role in the alteration of the enzyme's activity.

Analysis of cardiac myofibrillar ATPase and cardiac morphological measures were evaluated at 3, 6, and 9 weeks since it appears that this time course would be most appropriate to investigate the transient changes in cardiac tissue (Baldwin et al, 1977). Furthermore, indirect investigation of the possibility that the transient cardiac changes are a function of the physiological overload accompanying endurance training was done by studying the magnitude of the training adaptations for fast and slow skeletal muscles. This approach is warranted, since it has been suggested that the improved effectiveness of the skeletal muscles to undertake the workload following training is an important feature of endurance training. Furthermore, it has been suggested that the cv adaptations partly are dependent on skeletal muscle adaptations (Gleser, 1973; Davies and Sargeant, 1975; Clausen, 1976; Mitchell et al, 1977; Holloszy, 1977; Mckenzie et al, 1978; Saltin and Rowell, 1980). This evidence suggests that training with specific muscle groups leads to cardiovascular adaptations (ie. cardiac output (Q) and stroke volume (SV)) that are not carried over to contralateral untrained muscle. Thus, it is possible that alterations in physiological overload and subsequent sympathetic activity is one mechanism responsible for the transient nature of the cardiac adaptations (Hollander and Bouman, 1975;Clausen, 1977; Mitchell et al, 1977), and the investigation of skeletal muscle myofibrillar ATPase was done to establish a possible relationship between cardiac and skeletal adaptation.

CHAPTER 2

Methods and procedures

Animals

A total of 116 female Sprague-Dawley rats, 180-200 g. were housed in cages in pairs. Females were used since others had reported a tendency to maintain body weight with training (Baldwin et al, 1977). The diet consisted of Purina rat chow and water ad libitum. The animals were kept on a reverse day-night cycle. Environmental temperature was kept at 20°C. Ten of the rats were used as initial controls and the remaining assigned to one of three experimental groups.

Exercise training programs

The animals assigned to the 3, 6 or 9 week groups were partitioned into one of two training groups or a control (CONT) group. The animals were randomly assigned to exercise groups and underwent a specific program on a motor-driven rodent treadmill (Appendix B and Table 2-1). In the present study, most of the rats ran spontaneously so that random assignment was not a problem and classification into runners and non-runners was not necessary. However, on occasion there were some animals that would not run and these were excluded from the experiment. Attrition due to the inability to run or through disease or death amounted to 15%. Briefly, program A (PROG) was a classical type of exercise training program consisting of a progressive increase in duration and intensity up to 35 meters per minute, for one hour. Program B(HINT) was considered a program of high intensity and consisted of exercise training at 35 meters per minute from the onset of training with the duration progressively increased to one hour per session.

Table 2-1

Exercise Training Programs

for P(progressive) and H(high intensity) groups

Variables	3 Week		6 Week		9 Week	
Group	P	H	P	H	P	H
Intensity m/min.	25	35	30	35	35	35
Duration min.	(15-60)	(10-30)	(30-45)	(30-45)	(30-60)	(45-60)
Frequency days/week	5	5	5	5	5	5
Total Work meters	Equated		Equated		Equated	

Analysis of tissue was sometimes not done until several

Tissue sampling and sacrifice

Animals were sacrificed by stunning and subsequent decapitation. Following exsanguination hearts were excised, the ventricles were removed, rinsed with cold saline and blotted dry. After morphological measures were determined, the ventricles were frozen in isopentane, pre-cooled with liquid nitrogen and stored for subsequent analysis (-70 C.). In addition, soleus and plantaris muscles were excised frozen and stored for biochemical analysis. Animals were sacrificed 40-50 hours after the last exercise bout, at the end of 3, 6 or 9 weeks of training. months after storage. All samples of a particular muscle were analyzed with a sample from each group on a particular day. The activities in each muscle were representative of values reported in the literature regardless of storage time. However, this does not rule out the possibility that storage time had an effect on the activity of the ATPase enzyme in the present study.

Morphological assessments

The hearts were trimmed of excess fat and a number of morphological measurements recorded. All weights were determined on a Sartorius balance and diameter measures were done according to the method of Crews and Aldinger(1974) with a Coastal inside-outside depth guage. The morphological measures taken included total heart weight (including atria), ventricular weight, left ventricular weight and right ventricular weight. All measures were expressed in milligrams(mg). To measure right ventricular weight a scalpel was used to trim the right ventricular wall away from the rest of the heart. Ratios between heart, ventricular and left and right ventricular and body weights were subsequently derived and analyzed statistically.

All anthropometric determinations were performed by two people. In all cases the measures were obtained in a totally independent manner. Once practice data was collected a correlation coefficient was derived to determine the reliability of these measures. Correlation coefficients exceeded 0.99 on all measures.

Biochemical analysis

Muscles were homogenized with a Polytron Pt-10 in a borate-KCl buffer at pH 7.1(39 mM Na borate, 50 mM Tris, 5 mM Ethylenediamine Tetraacetic Acid (EDTA). Briefly, the homogenate was centrifuged at 1000xg for 10 minutes. The pellet was resuspended in 39 mM Na borate and 50 mM Tris (pH 7.1) and centrifuged. In addition to the removal of EDTA, the myofibril fraction was washed twice with 50 mM Tris,5 mM Na azide, 100 mM KCl and 0,5% Triton x-100 (pH 7.4). This procedure allows for the isolation of myofibril proteins with minimal contamination from membrane ATPases (Perry and Corsi, 1958; Belcastro et al, 1980). Following the isolation procedure, the myofibril fraction was adjusted to a concentration of 2 mg/ml with 50 mM KCl and 5 mM Tris (pH 7.0) by the method of Lowry et al (1951).

The yields for each muscle were between 40-55 mg· gm.$^{-1}$ for heart, 55-70 mg· gm^{-1} for plantaris and 30-40 mg· gm^{-1} for soleus. If a protein determination fell outside this range they were respun and another Lowry assay was done.

The myofibril ATPase activity was determined in a reaction mixture as described by Godno et al (1978). The myofibril protein (0.5 mg/ml) was pre-incubated for 5 minutes with 50mM KCl, 2mM Tris and 1mM MgCl2 (pH 7.0). The free Ca^{2+} concentrations used in the incubations varied from a pCa of -8 to -4 and were calculated by the method of Katz et al (1970), with the binding constants of Ca^{2+} and Mg^{2+} for ATP as derived by Vianna (1975). The binding constant of 4.47 x 10 to the 6th was used for Ca^{2+}/EGTA. The Ca^{2+} concentrations were checked for accuracy with an Orion Ca^{2+} sensitive electrode by the method of Bers (1982). An Ethyleneglycol-bis-N,N'-Tetraacetic Acid (EGTA) concentration of 0.1 mM was used for all Mg^{2+} activated conditions (Katz et al, 1970). Three Mg^{2+} concentrations, 0.04, 1.0 and 10 mM were used in the presence of physiological Ca^{2+}(pCa -5) and in Mg^{2+} activated (5mM EGTA without Ca^{2+}) conditions. The reaction was initiated with 5 mM Mg.ATP and terminated with 12% TCA. All samples were centrifuged at 1000xg for 10 minutes and an aliquot of the supernatant was used for phosphate determination (Taussky and Shorr, 1953). Phosphate activity was expressed as umol Pi/mg/min and was read at a wavelength of 700 nM on a Unicam SP1800 Ultraviolet Spectrophotemeter.

Before experiments were begun assays were practised until the coefficient of variation was less than 5% for the Lowry, and ATPase assays and also for pipetting. The coefficient of variation was calculated by the following equation: Standard Deviation/ Mean x 100.

Data Manipulation and Statistical Analysis

The classical Hill equation was used to determine Hill plots (Rupp, 1980). From these data the slope of the Ca^{2+} activation curve (Hill n) and its pCa50 were derived. A Least squares method(Interpolation with a cubic spline) was then used to fit the Ca^{2+} activation curves. The equation used is as follows:

$$V = Vmax/1+Q/(Ca^{2+})n$$

n-was calculated by a plot of the

$$LOG\ of\ V/Vmax-Vvs\ -LOG\ (Ca^{2+})$$

pCa50-was calculated as LOG of the point that corresponds to 0 on the X axis of the Hill plot

Q-is the activation energy was allowed to vary with the Ca^{2+} concentration.

Once these data were reduced an analysis of variance with unequal n's was used to determine the significance of the data extracted from the study. When statistical significance was achieved, a post hoc Tukey, or a Least squares difference test was used to identify a significant difference between a pair of means. difference between a particular pair of means. Values were considered significant if they achieved a p level of 0.05 or less.

CHAPTER 3

Results

Results reported deal directly with the Ca^{2+} and Mg^{2+} activation characteristics of plantaris soleus and cardiac muscle in trained and control animals. Anthropometric data on the heart is also presented. Results were significant if they reached $p<0.05$. An initial control group was compared to the three week control group to see if there was any difference between these two. A t-test on the myofibril ATPase revealed no differences between this group and the three week control group $p<0.05$ (Appendix D).

Anthropometric data

Body weight increased for all groups throughout the time course of this study. Since the rate of body weight gain with time was different in some groups (Table 3-2a). and since the absolute body weight among the groups differed, the heart weight/body weight ratios for total ventricular weight (TV), right (RV) and left ventricular (LV) weight to body weight ratios were used to assess cardiac hypertrophy with training (Table 3-2). Both trained groups had significantly greater ratios when compared to the control values with the HINT group having the greater elevation in the ratios ($p<0.05$).

Table 3-1

Body and Heart Weights of Rats at 3, 6,

and 9 Weeks for CONT, PROG and HINT Programs

Training	Body Wt.	SEM	Heart Wt.	SEM	TV Wt.	SEM	RV Wt.	SEM	LV Wt.	SEM
CONT-3 n=11	215	2	607	11	549	9	116	3	402	8
PROG-3 n=12	231a	4	720a	15	639a	15	131	7	466a	8
HINT-3 n=8	228a	3	[illegible]	15	653a	14	125	4	500a	10
CONT-6 n=11	240	4	703	23	640	23	150	10	444	13
PROG-6 n=11	261a	3	872a	24	767a	22	170	8	539a	14
HINT-6 n=8	246	7	793a	25	698	22	154	8	534a	11
CONT-9 n=10	253	5	747	25	656	20	149	10	465	11
PROG-9 n=10	288a	13	874a	20	781	18	172	7	548a	14
HINT-9 n=9	250	6	811	21	719	8	150	6	541a	15

Body Weight is in grams and heart weights in mg.

a= significant difference at $p < .05$ for CONT, PROG and HINT

Table 3-2a

Growth rates and body weights of rats

	CONT	PROG	HINT
Growth -3	15%	18%	8%
Initial wt.	187g.	196g.	212g.
Final wt.	215g.	231g.	228g.
Growth -6	47.8%	58%	20.3%
Initial wt.	166g.	165g.	205g.
Final wt.	240g.	261g.	246g.
Growth -9	24.3%	40.7%	23.5%
Initial wt.	204g.	205g.	202g.
Final wt.	253g.	288g.	250g.

Table 3-2

Weight ratio measures on hearts

of rats in CONT, PROG, and HINT programs

Training	Heart Body	SEM	TV Body	SEM	LV RV	SEM	LV Heart	SEM	RV Heart	SEM
CONT-3 n=11	2.83	.03	2.56	.03	3.48	.10	.66	.01	.19	.01
PROG-3 n=12	3.12a	.04	2.77a	.04	3.63a	.09	.65	.01	.18	.01
HINT-3 n=8	3.22a	.04	2.84a	.03	4.02a	.09	.68a	.01	.17a	.01
CONT-6 n=11	2.89	.03	2.65	.03	3.07	.09	.63	.01	.21	.01
PROG-6 n=11	3.33a	.04	2.90a	.04	3.24	.09	.62	.01	.19	.01
HINT-6 n=8	3.22a	.04	2.88a	.03	3.50a	.09	.67	.01	.19	.01
CONT-9 n=10	2.87	.03	2.52	.03	3.23	.2	.62	.01	.20	.01
PROG-9 n=10	3.06	.04	2.73a	.04	2.70	.15	.62	.01	.20	.01
HINT-9 n=9	3.32a	.04	2.97a	.03	3.63a	.16	.67	.01	.19	.01

Ratios are in mg., body weight is in grams

a= significant difference at $p<.05$ for CONT, PROG and HINT

Cardiac Muscle

Figure 3-1 is a typical regression plot of the classical Hill equation for the myofibril complex as described by Rupp, (1980). The Hill n for the cardiac muscle of the control group was consistent with time and a mean value of 1.31 was observed. The training programs resulted in significant changes compared to control, however, the time course of the adaptations in Hill n were unique to the type of training program (Table 3-3).

Table 3-3

Hill-n and pCa50 of cardiac muscle

for CONT, PROG and HINT groups

Time	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Parameter	CONT		PROG		HINT	
	n=9		n=10		n=4	
3 week n	1.36	.05	1.19a	.02	1.31	.12
3 week Km	6.53	.06	6.79a	.04	6.51	.13
	n=12		n=8		n=8	
6 week n	1.29	.03	1.40a	.03	1.47a	.03
6 week Km	6.51	.04	6.51	.04	6.46	.13
	n=11		n=10		n=8	
9 week n	1.29	.04	1.33	.06	1.35a	.03
9 week Km	6.54	.05	6.49	.07	6.39	.04

a= denotes significance at $p<.05$ for Cont, PROG and HINT programs

The PROG program resulted in a decrease and increase in Hill n at 3 and 6 weeks, respectively, compared to control. In contrast, the HINT group had Hill n values of 1.47 and 1.35, at 6 and 9 weeks, compared to 1.29 and 1.29, for the CONT over the same period (Table 3-3)($p<.05$).

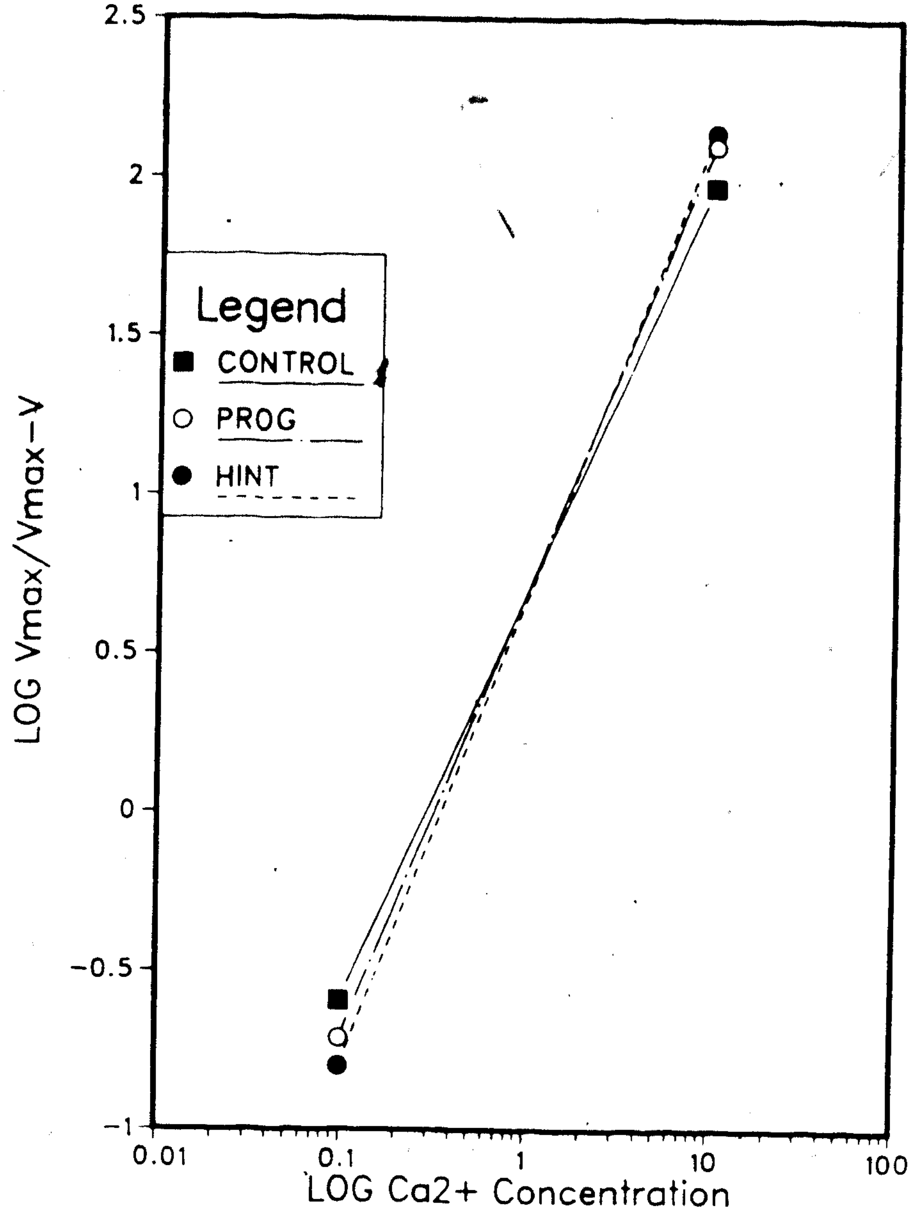

Figure 3-1 Linear regression plot of Hill n and Km of hearts after six weeks of training. Hill n is slope and Km is Ca^{2+} point at 0 point on the Y-axis of the regression line.

The mean pCa50, for the control group over time was 6.53. Different responses in pCa50 were noted for the two training programs. At 3 weeks, an increase in pCa50 was noted over control levels in the PROG group with no difference at 6 or 9 weeks (Table 3-3)($p<.05$). The HINT group did not show any differences in the pCa50 at any time (Table 3-3)($p<.05$).

<u>Ca^{2+} activation curves</u>

The Ca^{2+} ATPase relationship of the myofibril complex is seen in figure 3-2. After 9 weeks of training there are minimal differences between either training group and the control ($p>.05$) At 3 and 6 weeks, the maximal myofibril ATPase activity (Vmax) is greater for the PROG and HINT groups over their respective control ($p<.05$) Figure 3-3 demonstrates the time-course of the development of changes in myofibril ATPase activity at pCa5 only. Control values were not significantly different at 3, 6 or 9 weeks. At 3 and 6 6 weeks the trained groups are significantly elevated above their respective controls ($p<.05$).

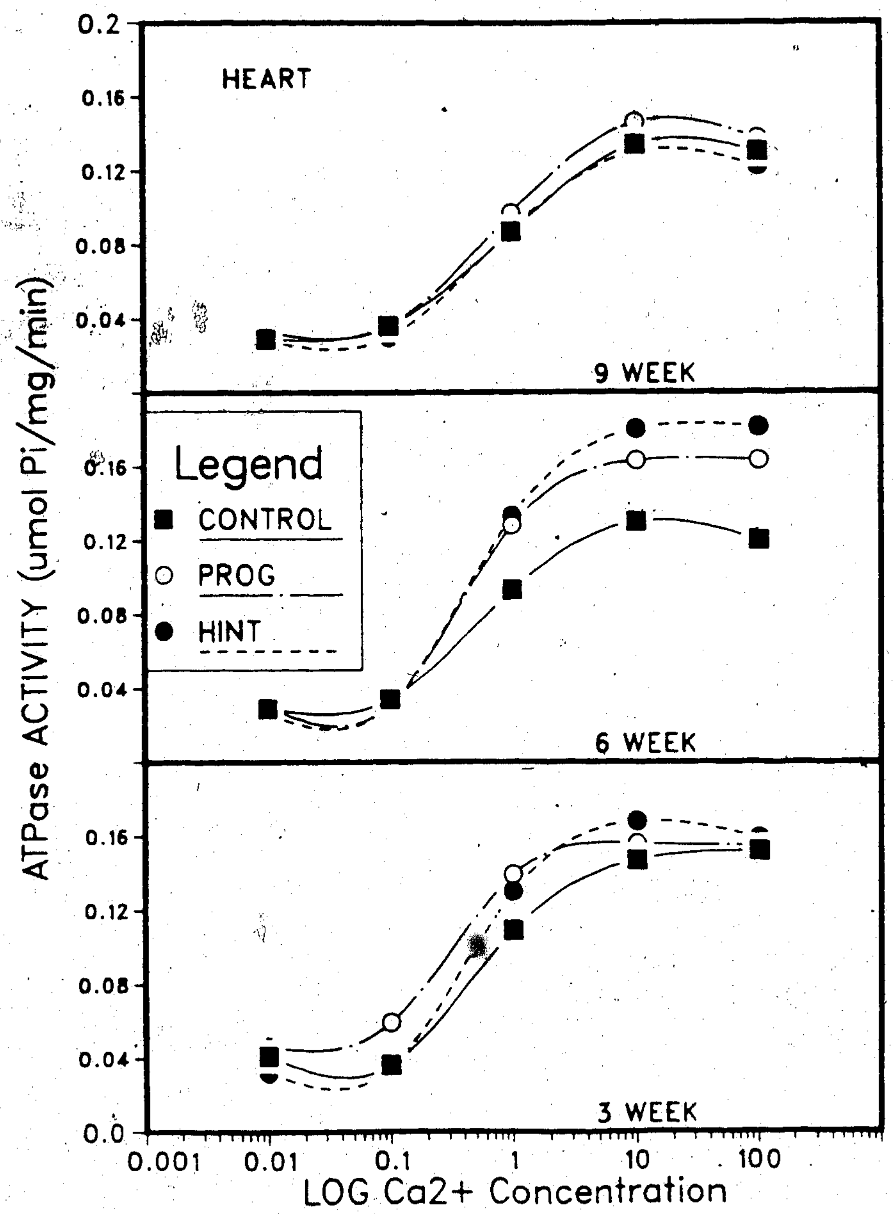

Figure 3-2 Ca^{2+} ATPase relationship of cardiac myofibrils from CONT, PROG and HINT groups.Curves were fitted by Interpolation. Typical S.E.M. range from 0.006 to 0.018.

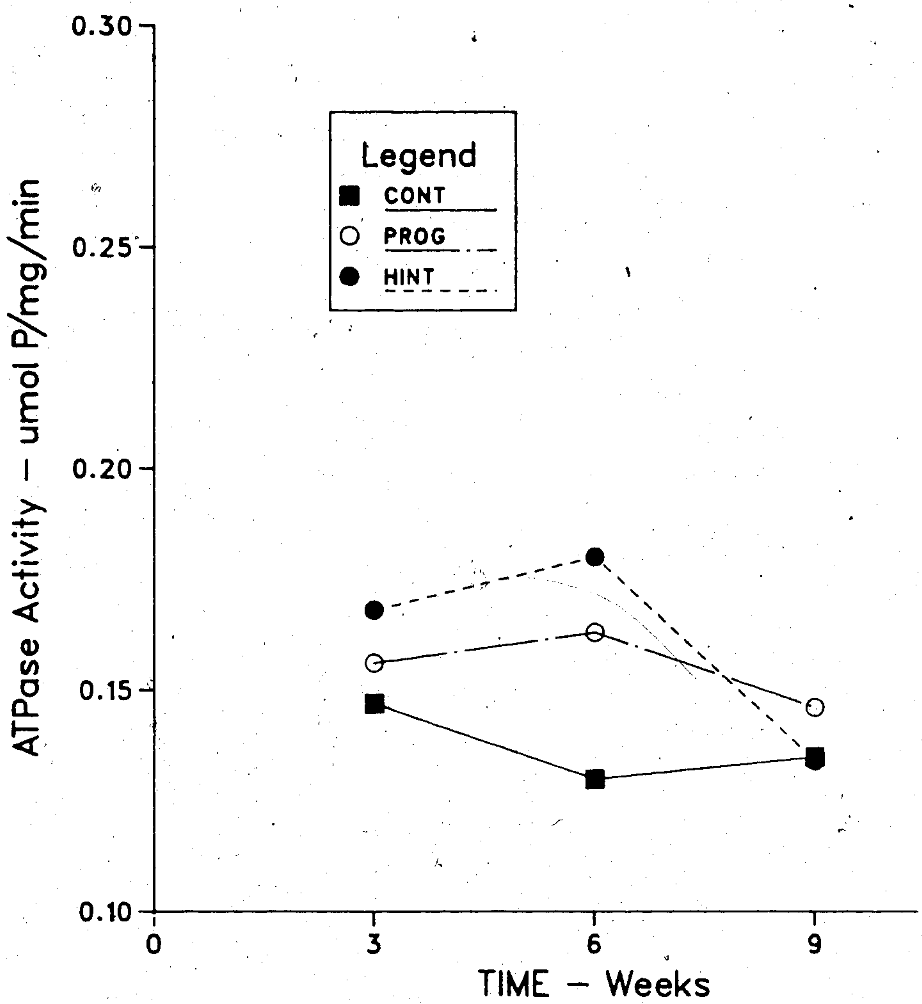

Figure 3-3 Myofibril ATPase activity at pCa5 at 3, 6 and 9 weeks in hearts. Typical S.E.M. range from 0.006 to 0.018.

<u>Skeletal muscle</u>

The Hill n and pCa50 data for soleus muscle for all groups is presented in Table 3-4. The training programs did not result in significant changes in the Hill n values or the pCa50 values in the trained groups at any time ($p < .05$).

Table 3-4

Hill-n and pCa50 of soleus muscle for

CONT, PROG and HINT

Group	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
	CONT		PROG		HINT	
	n=7		n=8		n=8	
3 week n	1.55	.10	1.58	.02	1.57	.03
3 week Km	6.36	.06	6.35	.02	6.33	.03
	n=9		n=8		n=8	
6 week n	1.51	.05	1.59	.05	1.55	.02
6 week Km	6.38	.04	6.37	.03	6.33	.02
	n=8		n=9		n=9	
9 week n	1.40	.057	1.52	.14	1.58	.02
9 week Km	6.50	.07	6.46	.06	6.39	.03

a= significant difference p<.05 for

Control, Prog and Hint groups

The soleus muscle of the trained groups revealed an increase in the myofibril ATPase activity at high Ca^{2+} concentrations after 9 weeks (Figure 3-4)($p<0.05$). The activities noted for 3 and 6 weeks did not result in any significant differences ($p>0.05$). Figure 3-5 depicts the myofibril ATPase activity for the soleus muscle at pCa5 at all time points. The six week control was higher than the 3 and 9 week CONT groups ($p<0.05$).

The mean Hill n value for control plantaris muscle was determined at 1.75. The training programs showed varying responses with regard to the time course of changes in plantaris muscle, but there were no differences between the PROG and HINT with respect to the direction and the magnitude of change (Table 3-5). The PROG program did not result in changes in the Hill n for either training group ($p>0.05$).

The Ca^{2+} ATPase relationship of the myofibril complex increased in a typical sigmoidal fashion for all groups, at all time points in plantaris muscles (Figure 3-6). The PROG and HINT training programs resulted in depressed maximal myofibril ATPase activities after 6 and 9 weeks ($p<0.05$). Training did not alter the expression of the myofibril ATPase earlier in the training program (3 weeks)($p>0.05$). Figure 3-7 shows the myofibril ATPase activity of the plantaris at pca5 for all time points. No differences were seen between control groups at any time points ($p>0.05$).

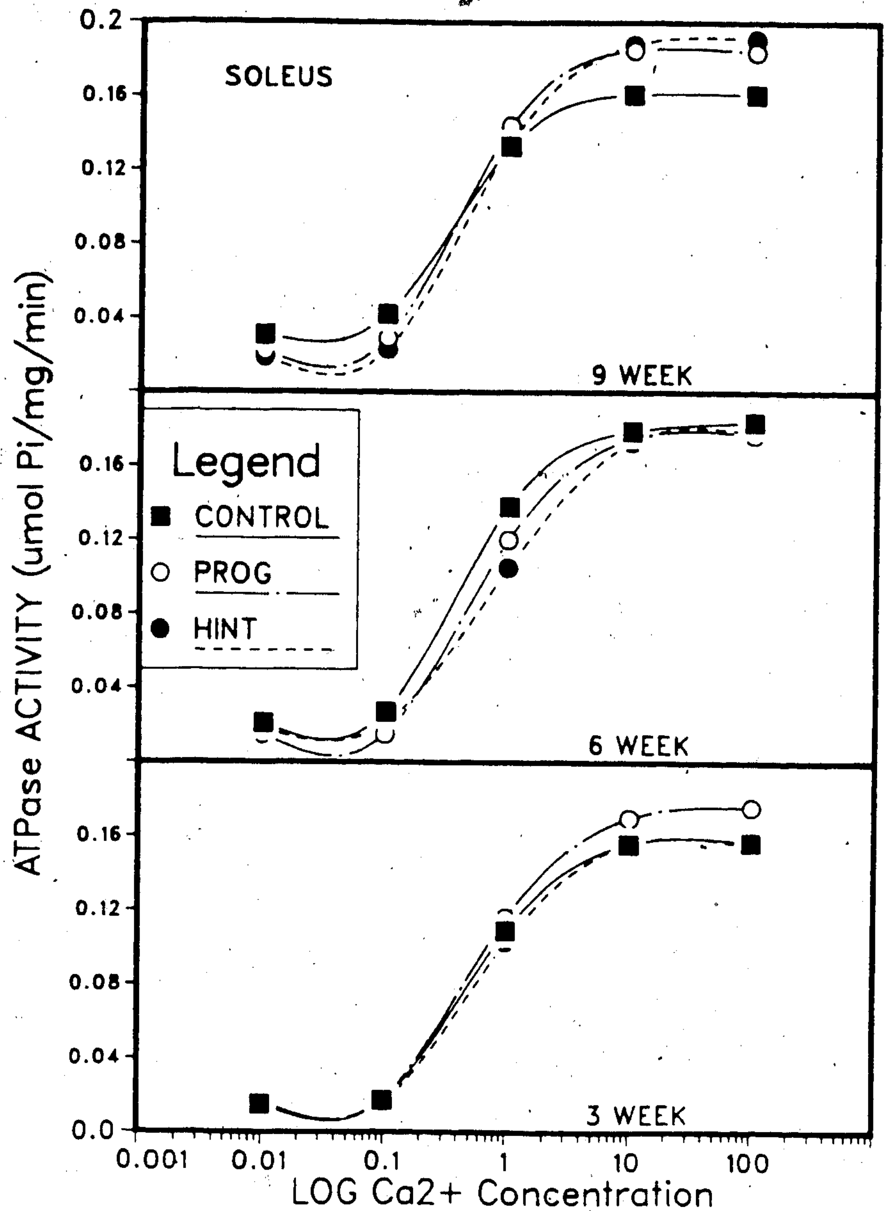

Figure 3-4 Ca^{2+} ATPase relationship of soleus muscle for CONT, PROG and HINT groups. Curves were fitted by Interpolation. Typical S.E.M. ranged from 0.002 to 0.019.

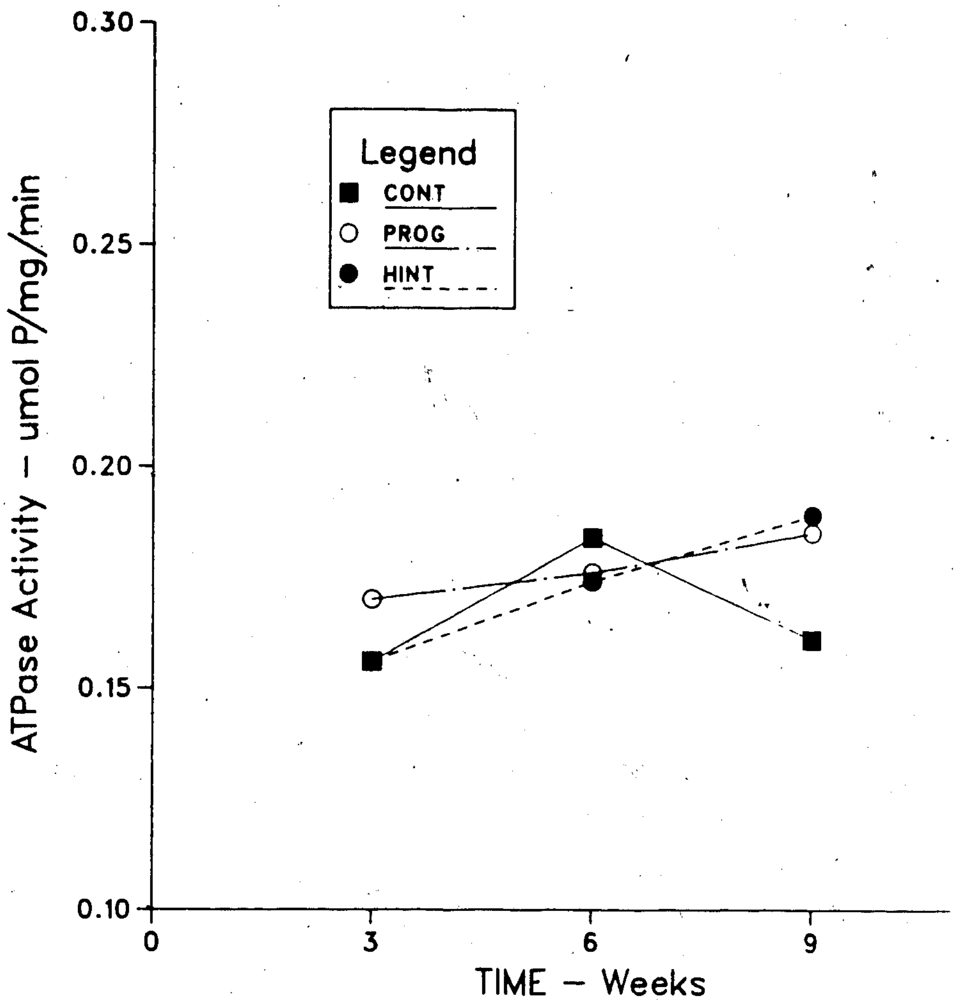

Figure 3-5 Myofibril ATPase activity at pCa5 after 3, 6 and 9 weeks of training in soleus muscle. Typical S.E.M. ranged from 0.002 to 0.019.

Table 3-5

Hill-n and pCa50 of plantaris muscle

for CONT, PROG and HINT programs

Time	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
	CONT		PROG		HINT	
	n=7		n=7		n=7	
3 week n	1.75	.04	1.70	.04	1.71	.11
3 week Km	6.49	.03	6.53	.03	6.56	.05
	n=9		n=8		n=8	
6 week n	1.72	.02	1.60	.07	1.74	.04
6 week Km	6.50	.03	6.59	.07	6.44	.04
	n=8		n=8		n=0	
9 week n	1.78	.06	1.70	.05	.00	.00
9 week Km	6.59	.06	6.56	.06	.00	.00

a= significant difference at $p<.05$ for

CONT, PROG and HINT groups

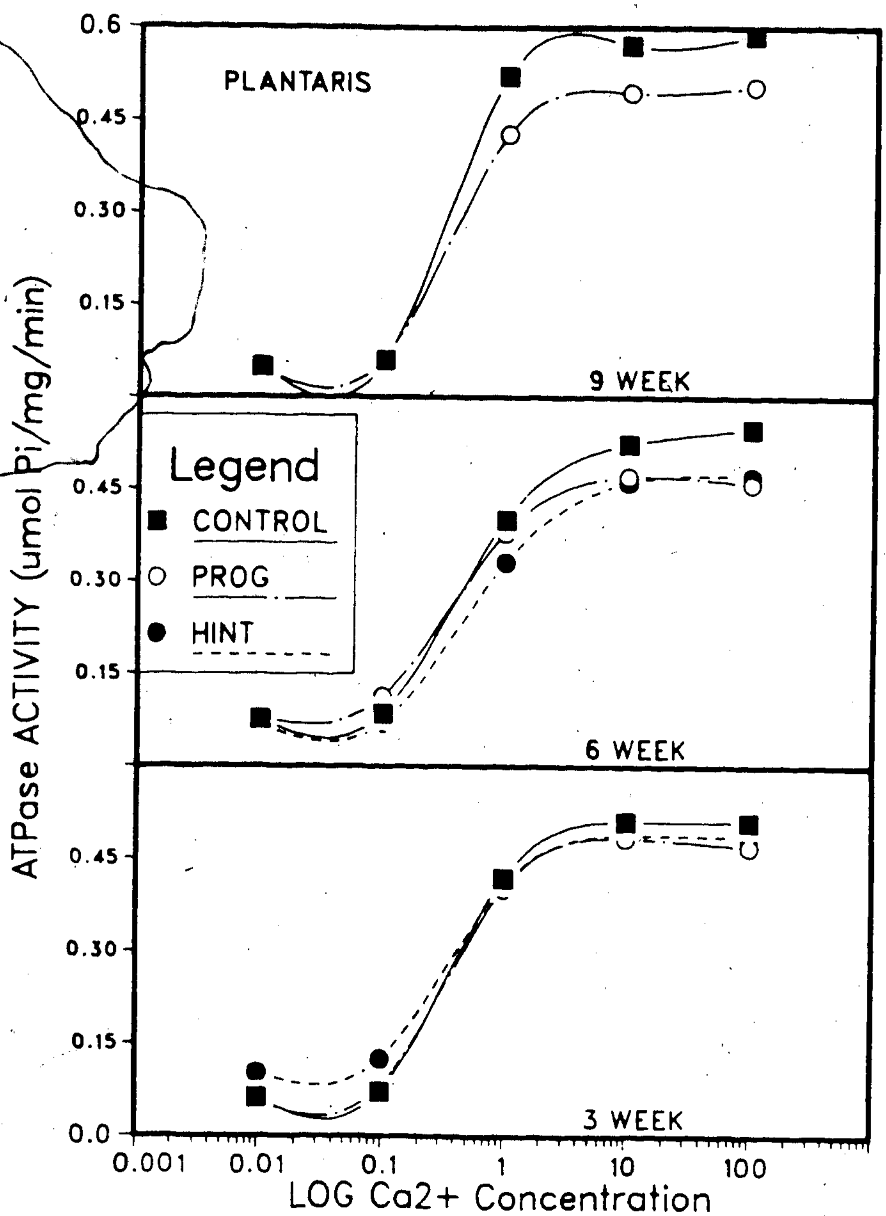

Figure 3-6 Ca^{2+} ATPase relationship of plantaris in CONT, PROG and HINT groups. Curves were fitted by interpolation. Typical S.E.M. ranged from 0.017 to 0.088.

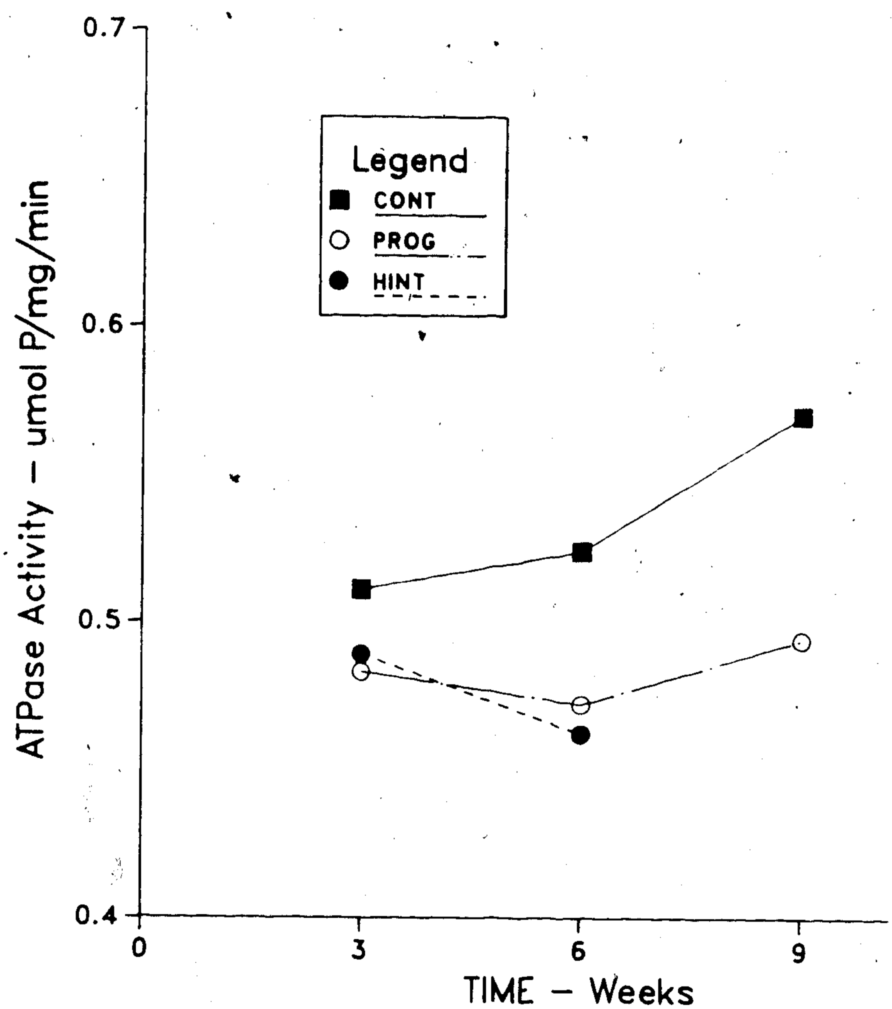

Figure 3-7 Myofibril ATPase activity at pCa5 after 3, 6 and 9 weeks of training in all groups. Typical S.E.M. ranged from 0.017 to 0.088.

Mg^{2+} influence on Ca^{2+} ATPase activity

The effects of varying the Mg^{2+} levels on the myofibril ATPase activity in the presence of Ca^{2+} in cardiac muscle is presented in Table (3-6). Following 6 weeks of training, the Ca^{2+} ATPase activities were elevated at all Mg^{2+} levels in both training groups ($p<.05$). The differences noted at 3 and 9 weeks were not significant ($p>.05$).

Table 3-6

Cardiac Ca^{2+} ATPase activity (pCa5)

with varying Mg^{2+} levels

Training	0.04 mM	S.E.M.	1.0 mM	S.E.M.	10.0 mM	S.E.M.
CONT3n=9	.143	.012	.147	.011	.113	.009
PROG n=10	.154	.011	.156	.010	.119	.006
HINT n=4	.142	.018	.168	.030	.116	.016
CONT6n=11	.121	.009	.130	.011	.105	.008
PROG n=11	.145a	.017	.163a	.018	.119a	.015
HINT n=8	.189a	.008	.180a	.013	.134a	.014
CONT9n=10	.132	.006	.134	.009	.110	.005
PROG n=10	.141	.007	.146	.007	.126a	.006
HINT n=9	.128	.010	.130	.010	.098	.007

a= significant difference at $p<.05$

between CONT, PROG, HINT groups

In the soleus and plantaris muscles no differences were found at any time with either training program ($p>.05$). In addition, the Mg^{2+} activated conditions did not result in a significant difference in any muscles between any groups at any time point ($p > .05$)(Appendix E). These experiments were identical to the previous Mg^{2+} experiments except that EGTA was used instead of Ca^{2+}.

CHAPTER 4

Discussion

Anthropometric data

The body weights of the trained females (both PROG and HINT) were higher than CONT at 3 weeks and the PROG trained groups at 6 and 9 weeks were greater than both the CONT and HINT groups. This resulted from a different rate of growth among the three groups. The finding of differing growth rates in rats is not uncommon and has been noted by others (Schaible et al, 1981; Leblanc et al, 1982). These differences could be due to individual variation and/or dietary differences. The latter factor must be considered since training results in a decrease in body fat and body weight because of a reduced caloric intake both in females and males (Leblanc et al, 1982; Hickson et al, 1983). Cardiac growth is linearly related to body weight (Beznak, 1954), therefore unless body weight at the end of a training period was equivalent in all groups, cardiac weight/body weight ratios were used rather than absolute cardiac weights. The use of ratios is traditionally an accepted method of evaluating hypertrophy. Although ratios are less sensitive than absolute heart mass in determining cardiac hypertrophy in animals, others have noted cardiac hypertrophy despite a lower body weight in trained animals (Baldwin et al, 1977; Anversa et al, 1982, 1983; Hickson et al, 1983). When body weight is factored out, both the HW/BW (heart weight/body weight), TV/BW (total ventricular/ body weight), were increased by 10-13% after 3, 6 and 9 weeks of training. This is in agreement with others who have used ratios to evaluate cardiac hypertrophy (Bloor and Leon, 1968; Bloor et al, 1970; Oscai et al, 1971 a,b; Javeed et al, 1974; Scheuer and Tipton, 1977; Schaible et al, 1981) after endurance training. A 10-13% increase in these ratios represents a significant but modest increase in cardiac hypertrophy, a finding that is consistent with results from previously mentioned rat studies and results from studies with other species (Carew and Covell, 1978; deMaria et al, 1978; Ehsani et al, 1978; Wolfe et al, 1979). No further increase is seen as training continues from 3 to 9 weeks indicating that the effect was maximized early in the training program. Baldwin et al (1977) have noted an increase in ventricular weight in female rats in the early stages of an endurance training program. After 8 weeks, however, these changes regressed to normal values again. These changes were attributed to the fact that the initially high intensity of training in their study was lost in the later stages of training. In the present study, the intensity of training was higher than in the study of Baldwin et al (1977) and this may possibly account for the maintenance of this hypertrophic effect. Nevertheless, the continued training is likely responsible for the anthropometric changes since others have shown that detraining, after exercise-induced cardiac hypertrophy in rats, led to a regression of cardiac hypertrophy (Hickson et al, 1983).

The HINT group also increased the LV/RV (left ventricular/ right ventricular) and the LV/ heart ratios significantly at all the time points but this was not seen in the PROG group. It could be argued that the HINT program was more intense in the first 3 and even the first 6 weeks of training, which again would lend support to the contention that higher intensity training would lead to a greater degree of cardiac hypertrophy (Baldwin and Terjung, 1975; Baldwin et al, 1977; Hickson et al, 1983). But after 9 weeks of training, the intensity was the same and the total work was equated in both groups, negating any effects of intensity. No explanation for this difference at 9 weeks is possible at this time.

The depressed RV/ heart ratio is a subtle but significant change that occurred only in the 3 week HINT group and is related to a greater LV mass in relation to the total mass of the heart. Since the RV/HW ratio remained approximately the same in both trained groups at other time points the right ventricle growth was in proportion to the LV growth indicating that the magnitude of the stimulus imposed on the right ventricle was as great as for the the left ventricle. This is in agreement with two studies (Van Liere et al, 1965; Crews and Aldinger, 1974) who found a proportional degree of cardiac hypertrophy in both the left and the right ventricle as a result of endurance training, but in direct opposition to the work of Anversa et al (1982, 1983) who have shown larger increases in right ventricular mass after training rats in their studies. This discrepancy may be due to the animals used, since Anversa's group studied these effects in much younger and smaller (80 grams) animals than in the present investigation. Further investigation is needed to evaluate the effects of exercise on the right ventricle.

Ca^{2+} activation and myofibril ATPase activity in cardiac muscle

The myofibril ATPase activity in cardiac muscle was not significantly elevated in either of the training groups after 9 weeks of training (Figure 3-2 $p<.05$). These findings are in agreement with the results of other running studies who found minimal changes in ATPase activity after 8 weeks or more of endurance training (Baldwin et al, 1975, 1977; Sordhal et al, 1977; Tibbits et al, 1978, 1981; Penpargkul et al, 1980; Nutter et al, 1981). The myofibril ATPase activity was only slightly increased (10%) in the PROG trained group and this non-significant finding is also comparable to that reported by others who trained rats for 8 to 12 weeks (Baldwin and Terjung, 1975; Watras and Gollnick, 1979; Penpargkul et al, 1980; Resink et al, 1981a,1981b).

At the end of 3 and 6 weeks of training, there was a significant increase in myofibril ATPase activity in both the HINT and PROG trained groups over the CONT at activating Ca^{2+} levels (Figure 3-2, $p>.05$) These increases ranged from 28% to 40% and are similar in magnitude to changes found with swimming training after 10-12 weeks in rats (Scheuer and Stezoski, 1972; Bhan and Scheuer, 1972; Scheuer, 1973; Scheuer et al, 1974; Medugorac, 1975; Bhan and Scheuer, 1975; Bhan et al, 1975; Malhotra et al, 1976; Schaible et al, 1981; Rupp, 1981; Rupp and Jacob, 1982; Rupp et al, 1984). In swimming training studies, These changes were not as great at 3 weeks and this maybe due to the fact that the 3 week CONT was more elevated than the 6 and 9 week CONT although it was not significantly greater than values from the initial CONT.No explanation except than of normal variation can be given for this result in the 3 week CONT. Rupp (1981), Rupp and Jacob (1982), and Rupp et al (1984), have attributed increases in activity to an observed isoenzyme shift but this possibility has not been investigated in cardiac muscle with running training studies. If myosin isoenzyme shifts are occurring with running training, the role of thyroid hormone must be considered since swimming training in rats has shown that thyroid deficient rats cannot shift isoenzymes from the slow V3 to the fast V1 as in normal animals (Pagani and Solaro, 1983). Other possibilities for the explanation of increased ATPase activity have been suggested by Resink et al (1981a), who have reported that run training results in an increase cardiac myosin light chain phosphorylation as evidenced by an increase in Pi content in the LC2. This hypothesis, however, remains unresolved since a physiological function has not been ascribed to light chain phosphorylation in cardiac or skeletal muscle (Winegrad, 1984).

In the present study the Hill n, which reflects the co-operativity of the ATPase versus pCa curve was increased at 6 and 9 weeks in the HINT and at 6 weeks in the PROG group. Both the myosin and the regulated thin filament (including tropomyosin (Tm) and troponin (Tn)), are necessary for full activation of the ATPase. For example, recent experiments have shown that the myosin ATPase activity is higher in the presence of Tm and Tn than with pure actin alone (Lehrer and Morris, 1982). These results are not yet fully understood, however, the kinetics of this enzyme involve a very complex series of reactions. Changes in the myosin concentration or the removal of different structural components of the thin filament greatly affect the binding characteristics and the kinetics of the actomyosin cycle. The thin filament is thought to enhance product release in the cycle which could theoretically enhance the rate of turnover of the cycle. The present experiments do not provide sufficient evidence to confirm a role of the thin filament in the adaptive process but an increase in co-operativity such as the one found in the present study warrants further investigation of the role of the thin filament in endurance training adaptations. This agrees with the report of Pagani and Solaro (1983), which suggests a role for the thin filament regulation. This suggestion was based on the fact that an increase in V1 isoenzyme (20%) resulted in an increase in myosin Ca^{2+}-ATPase activity, but no change in actomyosin Mg^{2+}-ATPase activity. This is in disagreement with the work of Rupp

(1981) who did find changes in myofibrillar ATPase activity with swimming training in parallel with isoenzyme shifts. Pagani and Solaro's (1983) findings were attributed to the fact that a 20% shift in V1 isoenzyme was not sufficient to account for myofibrillar changes in ATPase activity. This implies that the thin filament may have had a damping effect on the activity despite the shift in isoenzymes and/or changes in myosin ATPase activity. Studies on the thin filament are needed to clarify its role in the actomyosin cycle (Leavis and Gergeley, 1984).

Pagani and Solaro (1983) suggest that mechanical Vmax may be a more sensitive index of the training adaptations. Indeed it has been shown that physiological function does improve with training. Increase in rate of rise of pressure (dP/dt), peaked developped tension (PDT), peaked systolic pressure (PSP), stroke volume (SV), ejection fraction (EF), cardiac output (Q), stroke power (SP), velocity of shortening (Vcf) and isometric tension and a decrease in -dP/dT, time to peak tension (TPT) and 1/2 relaxation time (1/2 RT) have been noted with swimming training (Penpargkul and Scheuer, 1970; Scheuer and Stezoski, 1972; Carey et al, 1976; Behrson and Scheuer, 1977; Giusti et al, 1978; Schaible and Scheuer, 1979; Schaible et al, 1981). The biochemical changes that have resulted from the HINT and PROG training programs after 3 and 6 weeks of training agree with results from swimming training. It is tempting to ascribe a physiological improvement in parallel with the biochemical changes that have occurred in the early stages of training in the present investigation. However, after 10-12 weeks of running training, some investigators have shown indices of improved contractility in the hearts of rats without a concomitant change in myofibril ATPase activity (Dowell et al, 1977; Tibbits et al, 1978, 1981). In one study (Schaible et al, 1981), male and female rats underwent a running program of a fairly intense nature (31 $m \cdot min^{-1}$ for 2 hours/day) and no improvements in the contractile function of female hearts after training suggesting that a males and females respond differently to training. Other running studies have not been able to show increases in physiological parameters but the intensity of these training programs was perhaps not sufficient (Dowell et al, 1976; Nutter and Fuller, 1981).

Several points regarding the training paradigm for physiological and biochemical parameters should be made. The present study has examined the time-course of adaptation in the hearts and skeletal muscle of rats with training. A few studies have adopted this approach to evaluate training, however, in many of the running studies the training intensity and duration are quite varied (Baldwin et al, 1975; Baldwin and Terjung, 1975; Terjung, 1976; Baldwin et al, 1977; Tibbits et al, 1978; Penpargkul et al, 1980; Schaible et al, 1980; Resink et al, 1981a, 1981b; Tibbits et al, 1981; Dudley et al, 1981; Harms et al, 1983). It has been shown that intensity was critical, at least in skeletal muscle, in maximizing training benefits (Dudley et al, 1981; Harms et al, 1983). Generally, a protocol of 25 m·min^{-1} and 10% grade with continous running is sufficient to stress a rat to 70% of its Vo2max (Shepherd and Gollnick, 1976; Brooks and White, 1978; Patch and Brooks, 1980). Furthermore, Giusti et al (1978) have shown that detraining of only two weeks can result in the regression of cardiac myofibril ATPase activity and physiological function again showing that daily exercise overload itself is necessary for these changes to be maintained. Although the work of Giusti et al (1978) and of Hickson et al (1983) indicate the need for the maintenance of daily exercise, for training adaptations to be maintained, in some cases little concern is expressed over the progressiveness of the training program. With adaptations during the relative intensity of exercise (eg. treadmill speed)may become lower in magnitude centrally (Clausen, 1976). Since the relative intensity of the exercise is critical for maximizing training benefits, it may be that quite different responses of physiological and biochemical parameters are occuring as the organism adapts to a functional overload. It has been shown that specific cardiac biochemical adaptations may take place with only a few exercise bouts (Pierce et al, 1979) or a few weeks of training (Pierce et al, 1984) Thus it appears, that the time-course of adaptation and its relationship to the manipulation of variables such as intensity and duration or even mode of exercise need to be evaluated very early in a training program. Inconsistent results from previous studies are posssibly the result of the manipulation of particular variables in a training protocol (ie. intensity, duration and frequency) or as has been shown, a particular training method (swimming versus running).

The use of time course evaluation has not often been used as a method of monitoring the development of a training effect in rats and it is not possible in many cases to compare different studies. Most studies have employed 8-12 weeks as an end point to their training programs claiming that a steady-state of training has been achieved. The present study is a good example of the need for a more thorough evaluation of the development of a training effect in response to a particular exercise overload. Such evaluations are needed in order to piece together a model of endurance training.

Another factor that was not taken into account in the present study and in most other studies is the fact that measuring parameters during resting conditions may not be the best way to evaluate an organism's ability to respond to exercise. Logically there is merit in evaluating the organism as it responds to an exercise overload after the training period. Previous work in this laboratory has noted large differences in cardiac and skeletal muscle function in response to exhaustive exercise after endurance training (Maybank et al, 1982; Turcotte et al, 1982). Therefore, many factors that have not been previously considered need to be included in future studies to evaluate the effects of chronic exercise.

Soleus Muscle Adaptations

In the present study, a significant increase (15%) in myofibril ATPase activity was found at pCa levels of 4 and 5 in the soleus muscle of the PROG and HINT trained rats after 9 weeks of training. The increase in Ca^{2+}/Mg^{2+} myofibril ATPase is consistent with the findings of others who have examined contractile function in the rat soleus muscle (Syrovy et al, 1972; Baldwin et al, 1975; Belcastro and Wenger, 1982; Saltin and Gollnick, 1984). In addition, the biochemical results are in agreement with the physiological response of soleus muscle with training. For example, a 15% decrease in contraction time (CT) and increased peak rate of rise tension (dT/dt) during a twitch and during tetanic contraction, an increase in Vmax, a decrease in 1/2 relaxation time (1/2 RT) and an increase in maximum tetanic tension have been reported (Staudte et al, 1973; Fitts and Holloszy, 1977). Although physiological function has not been evaluated in the present study, the good relationship established between myosin ATPase and the velocity of muscle shortening (Barany, 1967) suggests that the soleus muscle did adapt its function after 9 weeks in both the PROG and HINT training programs. The time course measurements suggest that the adaptation only occurs at the later stages of training. However, after 6 weeks of training the CONT group's activities are elevated compared to the 3 and 9 week CONT. Although these results cannot be contested Figure 3-5 does show a linear increase in the activities in the trained groups from three to nine weeks. Nevertheless, it must be remembered that the design of this study is not truly longitudinal and that the 3, 6 and 9 week groups were assayed at different times and for these reasons comparisons should be made between the CONT, HINT and PROG groups at their respective time points. The changes in ATPase are not due to regulation changes since the Hill n and pCa50 are unchanged after training (Table 3-4 $p < .05$). The intensity of training itself did not contribute to changes early in the training program. Baldwin et al (1977) have shown that higher intensity interval training increased the levels of citrate synthase to a greater extent than a lower intensity training program in the soleus muscle of rats. Other studies have also demonstrated a greater magnitude of change with higher intensity training (Dudley et al, 1982; Harms et al 1983). Unfortunately, tissue was accidentally lost in the present study, preventing evaluation of this parameter. The PROG trained group achieved the same intensity of training as the HINT group after 9 weeks of training where changes in ATPase activity did take place. To evaluate the effects of the intensity of training with longer periods of training (ie. 8 weeks or more), a low intensity training program must be evaluated. In the early stages of training, the time-course of adaptation in the soleus and heart muscle is opposite with respect to ATPase activity. Although oxidative enzyme levels increase early in a training program these also follow a time-course progressively increasing with the training period (Dudley et al, 1982; Baldwin et al, 1977). It is possible that initially, the relative stress from the intensity of training was greater and exerted its effects on central parameters rather than on peripheral ones. Others have also reported that myosin isoenzyme shifts occur much later than changes associated with Ca^{2+} binding and sequestering and the enzyme and isoenzyme forms of energy metabolism (Baldwin et al, 1977; Green et al, 1983, 1984). The change in myofibril ATPase activity in the present study may be reflective of isoenzyme changes and are likely not comparable to oxidative enzyme changes and since the time course of adaptation of these and other parameters is not the same in skeletal muscle it is difficult to make a definitive statement regarding a relationship between central and peripheral mechanisms of adaptation.

Plantaris Muscle Adaptations

The adaptation of the myofibril ATPase activity after 9 weeks of training is in agreement with the changes noted for fast type skeletal muscle (Belcastro et al, 1980; Baldwin et al, 1975), however, these changes are not consistent with other reports (Hearn and Gollnick, 1961; Bagby et al, 1972; Syrovy et al, 1972). The discrepancies may be, due to isolation procedures and/or assay conditions in the earlier studies. The changes that occur at 9 weeks are also evident after 6 weeks but not after 3 weeks of training. The significant decrease in myofibril ATPase activity cannot be attributed to changes in the regulatory characteristics since the Hill n and pCa50 are unaffected (Table 3-5 $p<.05$). Studies that have evaluated contractile function have also reported discrepancies. Barnard et al (1970) and Fitts et al (1973), reported no changes in contractile parameters in fast muscle with endurance training. In contrast, Drachman et al (1973) observed a decreased 1/2 RT although this change was correlated to an increased Ca^{2+} uptake of the SR rather than the contractile element. In addition, Drachman et al (1973) have shown a greater resistance to fatigue as demonstrated by a better maintenance of isometric tension and maximal twitch tension after their endurance training program. The work of Green et al (1984) supports these findings since they found an increase in the relative proportion of slow isoenzymes in fast muscle after endurance training. The specific demands of exercise in the present study in both training programs may be such that a stimulus for an upward regulation of physiological function may have resulted in soleus muscle, because this muscle may not have had the necessary contractile profile to meet the demands of exercise, whereas in the case of the plantaris muscle contractile parameters were overloaded at a level that was much less than its inherent capacity. The decrease in ATPase activity in plantaris muscle could also reflect an isoenzyme shift as a result of an endurance type stimulus (Green et al, 1983, 1984; Pette, 1984). Although other parameters such as oxidative enzymes and capillarization are greatly increased early in a training program in both fast and slow muscle (Holloszy, 1977; Saltin and Rowell, 1980), these changes reflect parameters that affect energy supply, whereas, in contrast, myofibril ATPase changes and physiological function changes reflect a change in factors affecting the utilization of energy. As has previously been mentioned for soleus muscle the time course of energy utilization factors respond more slowly (Green et al, 1983, 1984; Pette, 1984) but from the results on studies of both slow and fast type muscle, it appears that muscles respond to the nature or type of stimulus placed upon it when energy utilization parameters such as myofibril ATPase activity and physiological function are being considered.

Central versus Peripheral adaptations

A possible mechanism by which the differing time-course of adaptation in myofibril ATPase activity in skeletal and cardiac muscle could be explained is provided by studies that have attempted to differentiate between central and circulatory mechanisms of adaptation (Clausen, 1976). It is possible that at the beginning of the training program the increase in cardiac myofibril ATPase activity reflected an adaptive response in the heart to a functional overload caused by the relative intensity of exercise. As previously mentioned, the increase in myofibril ATPase activity could reflect an increase in contractility.

Later in the training, the relative intensity of the exercise on the myocardium may have diminished due to peripheral adaptations that could take over a greater portion of the overload of exercise. This could be expressed as a decrease in peripheral resistance in the circulation. This could in turn negate the necessity for an increase in contractility. Evidence for such a hypothesis comes from studies that have shown that training in one limb (ie. leg) is not carried over to the contralateral untrained limb (Clausen, 1976). Also training a smaller muscle mass has a minimal effect on an untrained larger muscle mass (ie. training of the arms and measuring changes in the untrained legs) (Clausen, 1976). These two lines of evidence suggest that the periphery is partially responsible for improvements in indices of cv function and that the magnitude of central adaptation is in part due to the muscle mass used in training. The greater magnitude of change with the larger muscle mass is presumably due to a greater overload centrally. The findings of the present study suggest that the time-course of the response of the central parameters may in part be related to the time course of adaptation of skeletal muscle.

CHAPTER 5

Summary and Conclusions

Conclusions

From the results of the present study the following conclusions appear justified:

1- Cardiac muscle response to running endurance training follows a time course whereby Ca^{2+} regulation of the myofibril ATPase is accomodated in the early stages (3 and 6 weeks) of training but not after 9 weeks.

2- The temporal adaptations are not related to exercise intensity, once beyond 70-80% of Vo2max .

3-The increase in co-operativity in the heart of the trained rats suggests a possible role for the thin filament in the development of the training adaptation with run training.

4- Soleus muscle increased its myofibril ATPase activity and plantaris muscle decreased its activity in the later stages of training (6-9 weeks of training), with no apparent alterations in Ca^{2+} regulation.

5- The differing time-course of the changes in cardiac and skeletal muscles suggests a relationship between cardiac and skeletal muscle adaptation.

Limitations and recommendations for further study

1- Future studies should include contractile parameters to establish a relationship between biochemical and contractile changes as a function of training.

2- Other aspects of the regulation of the myofibril ATPase such as phosphorylation-dephosphorylation mechanisms, myosin isoenzyme changes, ATP effects and Pi effects need to be investigated to understand the precise nature of the biochemical regulation of the myosin ATPase as a function of the exercise stress.

3- The time-course of other variables affected by training need to be evaluated both in skeletal and cardiac muscle to better understand the relationship between cardiac and skeletal adaptation to exercise.

4- The findings of the present study are limited by the species used and thus the generalisability to other species is reduced.

5- The findings of the present study are limited to female rats.

Appendix A- Review of Related Literature

The purpose of the present review is to outline and present data from the major areas of research that led to the undertaking of the present dissertation. These areas will include the physiological effects of interval and continous training since these studies have focused mainly on the intensity of training as a manipulative variable for improvements with endurance training. The intensity of exercise and its effects in rats will also be reviewed. Morphological changes in the heart of humans and animals are covered since it is a parameter that has been considered in the dissertation as well. Contractile changes in the heart and skeletal muscle as a result of endurance training are also presented since these adaptations are often inferred from biochemical changes and aid in understanding the meaning of the training effect. Studies dealing with the biochemical alteration of the myosin ATPase enzyme in skeletal and cardiac muscle are reviewed since they are central to the dissertation. Finally, biochemical evidence that has led to a better understanding of the structure, function and regulation of cardiac and skeletal muscle is reviewed since this area helps to provide a more complete explanation of the underlying regulatory mechanisms associated with endurance training.

Intensity of training in humans

Much work has been devoted to endurance training and its effects, however, this area of research is beyond the scope of the present review. Excellent reviews exist outlining the adaptations that have been found to occur as a result of endurance training (Clausen, 1977: Saltin, 1980). Instead, the focus of this section is on the information accumulated over the past 20 years or so, that devotes itself to the general cardiovascular (cv) effects of intensity of training on the body.

One of the most popular methods of examining the effects of intensity as it relates to training has been to use interval versus continous training paradigms to compare largely diverging intensities of training. Unfortunately, the research done in this area suffers from many limitations. These include lack of control groups in experimental designs, lack of pre-test familiarization and lack of control of other variables (ie. frequency, duration, total work and initial fitness level) that are known to affect the magnitude of the adaptive response with endurance training (Med.Sci.Sports- Position Paper, 1978). Despite these obvious problems, some workers have adopted a philosophy that the upward manipulation of intensity is of paramount importance for adapatation and this belief may be unjustifiable (Fox and Mathews, 1975). Nevertheless, some valuable information has been derived from these studies and important inferences with regards to the design of the training programs used in the present dissertation have been made.

As previously mentioned, many studies that have attempted to evaluate intensity by means of interval training (IT) suffer from the fact that a control group was never used (Chaloupka, 1975; Fox et al, 1973; 1973 b, 1975 a, 1975 b, 1977; Harger et al, 1971; Hollering et al, 1971); Hickson et al 1971; Knuttgen et al, 1973). In general, most of these studies found only small increases in Vo2 Max (4-6 ml/kg/min) and in some cases values were not reported. Consistent decreases in submaximal exercise heart rates and blood lactate levels were also found. Unfortunately, it is difficult to evaluate the significance of these changes since no controls were used, making it impossible to make comparisons to the results of training or to other studies. In only a few cases the interval (IT) and continuous (CT) training programs were equated on total work. Knuttgen (1973) and Hickson et al (1977) demonstrated much larger increases in Vo2 max in subjects in their studies, however, no control group was used and even though this type of study has a pre-post design many limitations as to the significance of their results arise because of design problems. Knuttgen (1973) used two interval training programs one using a greater frequency (3 as opposed to 5 times a week) with a longer training interval than previous studies using interval methods. In his study the interval was three minutes in duration as opposed to only a 90 second maximum interval length in other training studies. The submaximal exercise heart rates decreased to the same extent but the Vo2 max increased to a greater extent in the program of greater frequency. Initial fitness levels were similar at the beginning of the training program. Thus, many studies have offered evidence suggesting that interval training may enhance the cardiovascular response to exercise to a modest degree and that intense training may have played a role in making that adaptation possible, however, design problems make it impossible to determine the magnitude of contribution of intensity to the training effect.

One study (Hickson et al 1977) showed large linear increases in Vo2 max with both a continuous and an interval program, however, the interval consisted of long (6 minute work bouts at 100% Vo2 max). In addition, initial fitness levels were very low at the onset of training. Other studies have actually compared IT and CT programs in the same study (Whitten and Painter, 1977; Cunningham et al, 1979; Eddy et al, 1977; Henriksson and Reitman, 1976; Roskamm, 1967). These studies used classical cardiovascular variables to evaluate the effects of the training programs and were unable to show any differences between IT and CT. Again, some studies lacked control groups and the significance of the changes can be questioned [illegible] and Painter, 1977; Eddy et al, 1977; Henriksson and Reitman, 1976) , however, studies that have used control groups (Cunningham et al, 1979; Roskamm, 1967) and have equated the training groups on total work did not show differences in Vo2 max, blood lactates, cardiac output or maximal rate of work. Indeed the only difference that was found was a greater a-v o2 difference in the IT group over the CT group (20% vs. 9%) (Cunningham et al, 1979). The intensities used in the IT and the CT programs were readjusted so that the same relative stress (ie. %Vo2 max) was maintained throughout the study, but the intensity of training was never more than 100% Vo2 max. It can be questioned whether this intensity of exercise in the IT was sufficient to have a different overload than CT on the cv system since no substantial differences were found between the two modes of training under those conditions.

Others have found that higher intensity training of a continous nature was better than lower intesity training of the same type but the training intensities were either very divergent (100% vs 60% Vo2 max) (Wenger and Macnab, 1976) or the training was not equated on total work (Atomi and Miyashita, 1980; Shepherd, 1979).

Others have examined the question of intensity by implementing very high intensity training programs and measuring the cardiovascular response of their subjects to this type of chronic overload as compared to more classical CT programs (Smith, 1978; Robinson and Sucec, 1980; Thorstensson et al, 1975; Houston and Thomson, 1977). For example, Houston and Thomson (1977) and Thorstensson et al (1975) sprint trained subjects and were unable to show a change in the classic cv responses to exercise after training. However, endurance time, maximal blood lactates, Mg^{2+} stimulated ATPase activity, creatine phosphokinase and myokinase activities were all increased with this mode of training. Smith (1978) and Robinson and Sucec (1980) used intensities of up to 125% Vo2 max under well controlled conditions (ie. control groups, same initial fitness levels, and equated workloads) and were also unable to demonstrate classical changes found with endurance training programs. Robinson and Sucec (1980) did show that this type of training resulted in improvements in the anaerobic threshold in their subjects. Smith (1978) only reported Vo2 max data.

An animal study is worth mentioning in this light. Barnard et al (1970) combined a CT and an IT program in guinea pigs on a treadmill program. The CT consisted of 2 days a week for 20 minutes and 2 days a week of IT which consisted of 30 seconds work with an equal work-rest ratio at 40 meters per minute. Changes in the total fibers staining as slow were not found until 18 weeks after the onset of training but the oxidative capacity of the muscles had still not changed. It seems that very high intensity training may have a different physiological effect than lower intensity training.

In the light of the studies reviewed some important points are evident. First, the issue of intensity as a critical and positive element in a training program has not been resolved. Second, it appears that in well controlled studies intensities of up to 100% Vo2 max done with an IT method has not provided evidence that intensity offers any substantial advantages over continuous training. Third, well controlled studies that have used high intensity training (ie. sprint training or training of up to 125% Vo2 max) do not appear to offer any advantage with respect to changing classical cardiovascular measures and evidence for different types of adapatations with high intensity IT has been put forth. Undoubtedly, the use of intensity as a component of a training program requires further investigation before the nature of its effects can be evaluated.

Intensity of exercise in rats

In animals, training studies have been designed to demonstrate a training effect but most have overlooked the relative intensity during the progression of a training program and hence researchers may not have achieved the desired skeletal muscle or cv adaptations that are typically expected with endurance training. Also, many workers have employed swimming as a model for exercise training and recent work by Flaim et al (1979) and Gleeson and Baldwin (1981) has demonstrated that swimming does not result in classic cv responses to exercise as does treadmill running in rats.

Running on the other hand, results in classical responses that can be monitored and evaluated quite easily (Shepherd and Gollnick, 1976; Patch and Brooks, 1980; Brooks and White, 1978; Taylor, Nielsen and Rook, 1970; Gleeson et al, 1983). The overload of running on treadmills and in activity wheels has been evaluated and these results indicate a linear, quantifiable relationship between speed of running and maximal oxygen consumption in untrained rats, trainability of Vo2 max and little variability in the response from one rat to another. Table I and II present the results from the studies that have examined this question.

Previous training studies have rarely used speeds of more than 25 meters·min^{-1} when examining response variables and have rarely established the relative intensity of work or readjusted this intensity periodically throughout a training program. Recently, Dudley et al, (1982) have established that male Sprague-Dawley rats can be trained to run continously for speeds up to 40 meters·min^{-1} and that 100% Vo2 max is not reached until speeds of 50 meters·min^{-1} or more. The training effects in cardiac and skeletal oxidative enzymes tended to plateau at durations of 60 minutes/day as opposed to 30 or 90 min/day. Although the oxidative enzyme changes were muscle specific these data indicate that the previous training programs of rats may not have been designed to get optimal responses from their training paradigms. In addition, serial evaluations of different training intensities has not been evaluated and may further characterize the training effect.

Table 4-1

Data from various studies on VO2max and maximum treadmill running speed of Albino rats

Author	VO2max	%grade	Speed	Weight	Sex/Train.
Pasquis et al, 1970	76.5	20%	72 m·min⁻¹	223g	F/T
Bedford	58.6	10%	26.8	461g	M/UT
et al, 1979	74.1	"	26.8	472g	M/T
"	81.1	"	26.8	207g	F/UT
"	90.6		"	254g	F/T
Patch and	71.6	15%	41.6	265g	F/UT
Brooks, 1980	81.5	"	48.9	261g	F/T

Treadmill speed is in meters·min^{-1} and Train. denotes training status

Cardiac Hypertrophy

Since the early part of this century researchers have been concerned about the possible deleterious effects of exercise on the heart. With the use of electrocardiographic and radiographic data many physicians have coined the phrase "athletic heart syndrome" for what was suspected to be pathological ventricular hypertrophy in humans engaged in athletic activity (Gott et al, 1968; Underwood et al, 1977; Frick et al, 1963; Ganse et al, 1970; Saltin and Grimsby, 1968; Astrand 1963; Bramwell, 1931). These techniques were limited for measuring hypertrophy, however, and echocardiographic techniques have permitted researchers to better understand the differences between athletes and sedentary individuals. It has also allowed for a more in depth understanding of the hypertrophic process with exercise training.

Many studies using a comparative design have shown that endurance runners, cyclists, weight lifters, wrestlers, basketball players, field hockey players, race walkers and swimmers have increased left ventricular dimensions such as volume and mass and internal dimensions when compared to age and weight matched control subjects. Left ventricle and septal wall thickness were not different from controls except in the case of wrestlers, shot-putters and weight lifters (Burke et al, 1980; Epstein et al, 1975; Krishna etal, 1980; Roeske et al, 1976; Nishimura et al, 1980; Rubal et al, 1980; Jordan et al, 1980; Gilbert et al, 1977; Nutter et al, 1975; Parker et al, 1978; Raskoff et al, 1976; Zeldis et al, 1978; Underwood and Schwade, 1977). The normal range for structural dimensions of the heart at rest is quite large and it is therefore difficult to draw conclusions as to the possible existence of a training effect or to a superior genetic endowment with studies using cross-sectional or comparative designs. Ricci et al (1982), were able to demonstrate a modest but significant increase in left ventricular mass (LVM) under resting conditions with endurance and sprint training as calculated by an increase in the internal dimension of the left ventricle. These changes were attributed to the bradycardia of training and an increased diastolic filling time rather than to a true cardiac hypertrophy. Such a change at rest may only reflect a use of a greater portion of the inherent filling capacity of the ventricle rather than a true hypertrophy of muscle fibers. A question arises with these relatively classical type of exercise training programs. Does exercise training when evaluated at rest and after a presumably steady state stage of training result in persistent structural and/or hypertrophic changes in the hearts of humans? Present evidence would suggest that if there is an effect that it is a modest one. Other training studies have raised more doubt with respect to this question since some studies have shown increases with training that was evaluated after only 3 to 12 weeks (Ehsani et al, 1978; DeMaria et al, 1978; Stein et al, 1980; Rerych et al, 1980) while others have been unable to show any echocardiographic evidence of structural or hypertrophic changes in the heart after 6 months of training (Wolfe et al, 1979). With these studies it is possible that changes must be evaluated at earlier stages of training and perhaps during exercise rather than at rest. In addition, the training progams used in these studies ought to be quantified and the relative intensity of training needs to be reestablished as the training program progresses. When examining an exercise program four variables and not three need to be controlled. These are frequency, intensity, duration and total work. Therefore, the quantifying of the magnitude of exerise based on each of these four variables may affect the nature of the adaptation. These problems as well as other design problems previously mentioned in this review must also be considered if a proper evaluation of the training stimulus is to be made possible.

This is in contrast to results in studies with dogs (Wyatt and Mitchell, 1974) and rats (Crews and Aldinger, 1974) where increases of 9% and 39% of the thickness of the left ventricular wall were found. Many reports of hypertrophy have been published in dog and rat studies. Some rat studies have reported increased heart weight to body weight ratios or have kept controls on a restricted diets when using males while others have used females presumably because they maintain their appetite and hence their body weight even with training (Bloor et al, 1970; Bhan and Scheuer, 1976; Scheuer, 1973; Malhotra et al, 1976; Carew and Covell, 1978; Barnard et al, 1980; Wyatt and Mitchell, 1978; Terjung and Spear, 1975; Oscai et al, 1975; Oscai et al, 1971; Liere and Northrup, 1957; Javeed et al, 1974; Baldwin et al, 1977). There is some question whether a change in heart weight body weight ratio (HW/BW) is a true indicator of cardiac hypertrophy and most studies with rats have been unable to show what some consider to be a true cardiac hypertrophy as a result of exercise training. Some have attempted to do this by using female rats and have failed but their training programs were evaluated after many weeks and the intensity of training was maintained at a low level. Interestingly, Baldwin et al (1977), have also shown a regression in ventricular weights with continous training evaluated after only 2, 4, 6, and 8 weeks of training. Initially though, hypertrophy, as measured by an increase in ventricular weight versus weight matched female controls was significantly increased. A high intensity interval training program also elicited this effect and it was maintained even after nine weeks of training. It was suggested that the relative intensity of training may have decreased with the continous training making the training stimulus inadequate to maintain the stress on the myocardium. Other investigators have demonstrated that rat heart can be hypertrophied if rats are subjected to an isometric training program (Muntz et al, 1981). Anversa et al (1982, 1983) have also shown that the right ventricle was hypertrophied to a greater extent in the rats used in their studies as a result of running training even if training was moderate adding a new dimension to the appreciation of cardiac function in rats with treadmill exercise.

Summary

From the literature available in both humans and animals it is apparent that there is still some doubt about the existence of a permanent hypertrophic effect of training on the heart. In addition, the work of Anversa et al (1982, 1983) has also raised more serious questions about the functional mechanisms by which the heart responds to chronic exercise.

Functional changes in the heart with training

A series of studies have examined the effects of physical training on the mechanical and contractile function of the heart. A variety of variables such as positive and negative rate of rise of pressure ($\pm dP/dt$), peaked developed tension (PDT), time to peak tension (TPT), relaxation time from PDT to 1/2 PDT also called 1/2 relaxation time (1/2 RT), peak systolic pressure (PSP), peak left ventricular systolic pressure (PVLSP), left ventricular end-diastolic pressure (LVEDP), velocity of circumferential fiber shortening (Vcf), ejection fraction (EF), stroke volume (SV), cardiac output (Q), stroke work, stroke power, isometric tension, ventricular wall stress and ventricular wall compliance have all been shown to improve after swimming and running training in rats and dogs (Crews and Aldinger, 1974; Behrson and Scheuer, 1977, 1978; Barnard et al, 1980: Nutter and Fuller, 1977; Stone, 1977; Scheuer, 1977; Dowell, 1977; Giusti et al, 1978; Hepp et al,1974; Meerson et al, 1980; Noakes et al, 1979; Penpargkul et al, 1970; Ritzer et al, 1980; Scheuer and Stezoski, 1972; Schaible and Scheuer, 1979; Riedhammer et al, 1980; Codini et al, 1977; Carew and Covell, 1978; Dowell et al, 1976, 1977;Carey et al, 1976; Tibbits et al, 1978). Other studies, however, using more moderate training programs, have been unable to show any functional changes (Williams and Potter, 1976; Cutiletta et al, 1979). These measures were taken under normal conditions as well as conditions including ischemia, hypoxia and aortic occlusion in intact anesthetized and unanesthetized animals with isolated heart and heart muscle preparations (Scheuer, 1977; Stone, 1977; Dowell, 1977; Nutter and Fuller, 1977).

Under normal conditions, regardless of the type of preparation used, one common finding seems to be an increase in $\pm dP/dt$ and a decrease in relaxation time. The finding of increased $\pm dP/dt$ and peak $\pm dP/dt$ has also been documented during maximal and submaximal exercise in dogs (Ritzer et al, 1980; Dowell et al, 1977; Barnard et al, 1980; Stone, 1977; Carey et al, 1976), however, in humans $+dP/dt$ seems to be decreased at all relative work loads at a given blood pressure (Clausen, 1976). In pacing studies with isolated heart preparations from trained rats the relationship still holds (Scheuer, 1977; Giusti et al, 1978; Behrson and Scheuer, 1977; Codini et al, 1977; Schaible and Scheuer, 1979). Researchers have used $\pm dP/dt$ as an index of contractility and extent of relaxation of the left ventricle. Some authors (Barnard et al, 1980; Behrson and Scheuer, 1978; Scheuer, 1977) have suggested that the training change in dP/dt is related to the Ca^{2+} pumping ability of the sarcoplasmic reticulum (SR) or to an in crease in sarcolemmal bound Ca^{2+} available for excitation-contraction coupling (Tibbits et al, 1981). Some authors using swimming paradigms (Scheuer, 1977; Codini et al, 1977; Giusti et al, 1978) have also demonstrated and implied a physiological parallel between biochemical (ie. increased ATPase activity) and functional changes in the heart but Dowell et al (1977) could not demonstrate this relationship. Increased ±dP/dt were also maintained in studies where anesthetized rat hearts were paced (Penpargkul and Scheuer, 1970; Codini et al, 1977) and in hearts that received isoproterenol injection (Noakes et al, 1979). Increases in + and - dP/dt were also noted during hypoxia induced in conditioned hearts of rats and dogs (Scheuer and Stezoski, 1972; Carey et al, 1976). Aortic occlusion studies have been used to examine the effects of training during the isovolumic phase of ejection of the left ventricle. During an acute bout of exercise a decrease (Codini et al, 1977) and an increase (Dowell et al, 1976) in LVEDP and peak ±dP/dt have been reported. Chronic occlusion of three days or more resulted in an increase in peak ±dP/dt and an increased LVEDP over control animals (Dowell et al, 1976). Riedhammer et al, (1980) found an increase in LVEDP after training in dogs during submaximal exercise.

In early investigations (Rushmer, 1976) it was beleived that the Starling's law of the heart was a significant factor in increasing the SV during exercise. Though training studies in humans and dogs support this view (DeMaria et al, 1978; Ehsani et al, 1978; Stein et al, 1980; Rerych et al, 1980; Ritzer et al, 1980), the previously mentioned body of literature suggests that an increase in contractility may also play a role in this adaptive process as well.

Another finding that seems consistent in many animal studies is an increase in Vcf (Behrson and Scheuer, 1977; Carew and Covell, 1978; Meerson et al, 1980; Ritzer et al, 1980; Schaible and Scheuer, 1979). Again a relationship between biochemical changes and this functional change has been implied and human studies have reported this change in one study (DeMaria et al, 1978). The models that have been employed to make these measures vary greatly and could account for many of the discrepancies in the literature. Heart muscle preparations have offered a high degree of control (Scheuer, 1977) while others concerned with physiological conditions have used isolated working heart preparations. The heart muscle prepararations offer the advantage of direct measurement of contractility and/or force production by cardiac muscle fibers, a low failure rate and reliable and comparable force-velocity data (Nutter and Fuller, 1977). The anesthetized and instrumented animals mimmick more closely stable physiologic conditions than the muscle preparations or the isolated working heart preparations. These preparations also allow for the investigation of nervous system effects (Stone, 1977; Dowell, 1977). Despite the highly complex and highly different approaches used common findings suggest each method to be potentially informative. For example, Scheuer (1977) has been able to consistently demonstrate improvements in indices of contractility (ie. increased ± dP/dt and peak ±dP/dt, Vcf, decreased 1/2 RT) by using the same preparation and the same physical training program. These improvements, however, may not be generalizable to other training methods or species. Also the use of measures such as these have been questioned as indices of contractility since they affect the functioning of the heart and are not necessarily representative of a direct change in contractility or contractile force of cardiac muscle.

ATPase activity in cardiac and skeletal muscle

Endurance training in animals causes changes in the activity of the ATPase enzyme in skeletal and cardiac muscle. The results of the studies done vary a great deal. Part of the discrepancies can be attributed to the type of preparation used, the assay conditions and also to the type and nature of the training programs used in each study.

Swimming and running have both been used as models of endurance training. Both swim training and run training studies have dealt mostly with changes in cardiac tissue. There have been very consistent changes in the hearts of swimming trained animals. On the average, increases of 17 to 30% in either myofibril, myosin or actomyosin Ca^{2+} ATPase, Ca^{2+}/Mg^{2+} ATPase, or Mg^{2+} ATPase activity have been found in the hearts of rats (Bhan et al, 1975; Bhan and Scheuer, 1972; Malhotra et al, 1976; Giusti et al, 1978; Medugorac, 1975; Penpargkul et al, 1981). Others have demonstrated up to a 55% increase in cardiac Ca^{2+} ATPase activity with the same type of preparations (Wilkerson and Evonuk, 1971; Ashok et al, 1975) but Rupp (1981) found only a 10% increase in myofibrillar Ca^{2+}/Mg^{2+} ATPase activity in the hearts of rats trained by swimming. Wilkerson and Evonuk (1971) examined the effects of swim training on skeletal as well as cardiac muscle and found a 44% increase in Ca^{2+} stimulated ATPase activity of the gastrocnemius muscle after 6 and 10 weeks of training which corresponded to the magnitude of change found in cardiac tissue in the same study. Hearn and Gollnick (1961), on the other hand, found no change in the Ca^{2+} myosin ATPase activity of the gastrocnemius muscle but did find an increase in heart muscle after 5 weeks of daily swimming. Syrovy et al (1972) examined soleus and found a 17% increase in the Ca^{2+} myosin ATPase activity but no change in the extensor digitorum longus muscle after 9 weeks of swim training.

Running programs have been less consistent in reporting increases in cardiac tissue. Watras and Gollnick (1979) have reported an 8% decrease in Ca^{2+} actomyosin ATPase activity in hearts while others have shown no change in the heart with a myofibril preparation (Baldwin et al, 1975; Carey et al, 1979; Tibbits et al, 1981) and with actomyosin (Dowell et al, 1977). The intensity of these running programs was 25 meters·min^{-1} and the duration was usually up to one hour. Others (Malhotra et al, 1981; Penpargkul et al, 1980) have trained rats at an intensity of 30 meters·min^{-1} for up to two hours and have found no changes in Ca^{2+} actomyosin ATPase activity in the hearts. A long duration (2.5 hours per session) training program at a low intensity (20 meters·min^{-1}) used by Penpargkul et al (1980) resulted in a 9% increase in Ca^{2+} actomyosin ATPase activity.

Resink et al (1981, a, b) used the same continuous training program in two different studies. This program used a speed of 25 meters·min^{-1} and the sessions were 2 hours in length. The intensity was augmented by elevating the grade to 15% rather a more popular 8% grade used in previous studies. Ca^{2+} myosin ATPase activity was increased by 14% in trained over control hearts of rats. Baldwin and Terjung (1975) were able to show up to a 13% increase in myofibril·Ca^{2+} ATPase activity in a study which used four training programs. Some animals ran at 25 meters·min^{-1} at a 10% grade for 2 to 4 hours per day. The largest increase (13%) was found with the program using a 10% grade for 2 hours per day. All four programs showed a mean increase of 8% in Ca^{2+} myofibril ATPase activity. Cardiac hypertrophy was greatest with the highest grades and then with the longest duration training programs. Baldwin et al, (1977) also used programs of different intensities in an attempt to identify the "time-course" as well as the intensity required to elicit a change in the cardiac myofibril Ca^{2+} ATPase activity of rats. One training program used an intensity of 25 meters·min^{-1} with a 25% grade with a duration of up to one hour over an eight week period. An initial increase of up to 10% in ATPase activity regressed to control values after 4 weeks of training. A second training program used an interval type training program to elicit speeds of up to 67 meters·min^{-1} during training sessions. The duration of the work bouts were up to 3 minutes. Although the total work done was considerably less in this training program than in the continous one, the intensity of the program was sufficient to cause a 15% increase in ATPase activity which persisted after 9 weeks. Fast and slow type skeletal muscle increased their citrate synthase activity in both training programs although the continous program showed increases of greater magnitude. Phosphofructokinase activity increased by 20% in the slow muscle with the continous program, but ATPase activity was not evaluated in the skeletal muscle.

Pierce et al (1979) examined the effects of only 10 training sessions on Mg^{2+} stimulated myofibrillar ATPase activity. Female rats were examined after 1, 3, 5, and 10 sessions. After 3 and 5 sessions Mg^{2+} activated ATPase activity was increased by 22% but this was down to normal values after 10 training sessions. The nature of the training program was not explained, however, but it is interesting to note that the early stage of the training program elicited a large effect as in the earlier part of the training regimen in the study of Baldwin et al (1977). Apart from these two studies all other studies were examined after 8 weeks or more of training and there were usually no changes found in these studies when ATPase activity was evaluated after these longer periods of training.

Another study by Baldwin et al (1975) attempted to look at Ca^{2+} actomyosin ATPase activity of both cardiac and skeletal muscle with the same run training program. After training for 24 weeks at 28 meters/min at a 15% grade for 2 hours per day the hearts's ATPase activity was unaffected. Similarly, fast type vastus muscle did not alter its ATPase activity. The fast oxidative glycolytic muscle decreased its activity by 20% while the slow soleus increased its activity by 20%. The ATPase activity of all the skeletal muscle types correlated well with (.933) with phosphofructokinase activity.

Bagby et al (1972) used a continuous (28.4 meters/min for one hour) and an interval type training program (18 sprints per session up to 80 meters/min with a 1:1 30 second work rest ratio). Training in this manner for 11 weeks failed to alter Ca^{2+} myosin ATPase activity in the gastrocnemius muscle. Belcastro et al (1980) trained animals at an early age (10 days after birth) and found a 19% depression in Ca^{2+} myofibril ATPase activity of the plantaris muscle in a group of rats trained to run at 40 meters/min for 2 hours a day for 6 weeks. A sprint trained group (40 bouts/day 20:30 seconds work rest ratio at 80 meters/min) did not alter its ATPase activity after the same period of time.

Finally, with respect to studies that have investigated ATPase activity Belcastro and Wenger (1982) used the same training program to evaluate effects on slow type soleus muscle. At 21 and 51, days the Mg^{2+} activated myofibril ATPase activities were 39% and 49% greater when compared to the control groups. Even though the sprint group did not change its activity, growth caused a depression in activity in the control group that did not occur in trained animals. The endurance trained group also maintained its activity and increased its activity at 51 days over its own activity at 10 days.

Factors underlying changes in ATPase activity

Several authors have attempted to determine various causes that underly ATPase changes with both swim and run training studies. Swimming training studies have shown changes related to the myosin molecule or to myosin's light chains in the heart muscle of rats. Bhan and Scheuer (1975) found an increase in HMM ATPase activity of trained animals indicating a change in the head of the myosin molecule. The change was not related to the oxidation of sulphydryl groups as shown by the use of sulphydryl modifying reagents (Bhan and Scheuer, 1975) but perhaps to a conformational change as suggested by the differential effect of ethylene glycol in the hearts of the same swim trained rats. Rupp (1981), Rupp and Jacob (1982) and Rupp et al (1984) support this contention by demonstrating that a 12 week program of swimming resulted in a shift to a greater proportion of VM1 from VM2 and VM3. The Vm1 isozyme is correlated to the speed of shortening of cardiac muscle (Rupp et al, 1983). Swimming training programs have also demonstrated an increase in the susceptibility of the light chains to phosphorylation (Penpargkul, 1977). Ca^{2+} ATPase and Ca^{2+} uptake of the sarcoplasmic reticulum (SR) have also been reported with swimming training and these changes have been offered as mechanisms by which the Vmax of the myosin ATPase could be increased (Penpargkul et al, 1980).

Running training has also suggested that light chain phosphorylation is increased with increased extracellular Ca^{2+} levels and this increase is in proportion to the increase in Ca^{2+} myosin ATPase activity in the hearts of trained animals and it has thus been implied that an enhanced capacity for transsarcolemmal Ca^{2+} flux could be responsible for a greater Ca^{2+} dependent phosphorylation of the myosin P light chains resulting ultimately in improvement of cardiac function (Resink et al, 1981, a, b). Evidence for an increase in transarcolemmal flux has been furnished by the study of Tibbits et al (1981) who suggested an increase in the Ca^{2+} content of the sarcolemma of run trained rats.

The activity of the actomyosin ATPase enzyme also corresponds to alterations in the contractility of the heart. The actomyosin ATPase activity is increased with increased contractility and diminished in states of diminished contractility (Hjalmarson et al, 1970; Goodkind et al, 1969). Because of this relationship, it has been proposed that increases in the activity of the ATPase enzyme following a training regimen be a cellular mechanism partially responsible for an improvement in contractile function of the heart. Indeed both swimming and running training in animals have provided evidence for the adaptability of the heart when it is exposed to an exercise stress. The previous section on contractile changes of the heart is also evidence for this adaptive capability of the heart with chronic exercise.

Biochemical changes in skeletal muscle have most often been related to an increased capacity for aerobic metabolism partially centered around fat oxidation (Holloszy and Booth, 1976). With regard to depression in ATPase activity in the myofibril with training some have indicated a change in myosin isozyme type as a possible mechanism by which ATPase activity could be depressed.

Evidence explaining skeletal muscle alterations that underlie ATPase changes is not as abundent, since less work has been done with this type of muscle. Conflicting evidence exists as to the effects of endurance training in skeletal muscle. Changes in skeletal muscle have been related to an increased Ca^{2+} uptake and Ca^{2+} binding ability of the SR. The slow soleus muscle has been used and a depression in Ca^{2+} ATPase and uptake of the SR has been found with swimming training (Fitts et al, 1979), but others (Bonner et al, 1976; Belcastro and Wenger, 1982) have found increases in Ca^{2+} uptake and Ca^{2+} ATPase but no change in the Ca^{2+} binding ability of the SR in soleus muscle with endurance running. The Ca^{2+} binding ability of the SR (Belcastro et al, 1980) and Ca^{2+} uptake of the SR to fast type muscle [illegible]hman et al, 1973 have also been shown to increase with endurance running in other studies. Conflicting evidence exists as to the effects of endurance training on the functioning of the SR. Muscle type (ie. slow vs. fast) and assay conditions used in different studies are undoubtedly responsible for some of these differences (Tate, 1984). Electrical stimulation studies have demonstrated the plasticity of muscle showing substantial changes in the contractile element, the SR and the intracellular content of parvalbumin (a Ca^{+2} binding protein)(Pette, 1984). (Tate, 1984).

The contractile function of skeletal muscle has also been studied and again conflicting results have been reported. Some authors (Barnard et al, 1970; Fitts et al, 1973) have also shown no changes in contractile characteristics of fast muscle with endurance training. Drachman et al (1973) on the other hand, demonstrated a decrease in the 1/2 RT in line with an increase in Ca^{2+} uptake ability of the SR. Although fast muscles do not change a great deal with respect to their characteristics a greater resistance to fatigue as demonstrated by a better maintenance of isometric tension and maximal twitch tension was demonstrated in trained animals in all of the studies. In slow muscle, up to a 15% decrease in contraction time (CT), an increase in peak +dP/dt during a twitch and during a tetanic contraction, an increase in Vmax, a decrease in 1/2 RT have all been demonstrated with endurance training (Fitts and Holloszy, 1977) An increase in maximum tetanic tension has also been demonstrated with sprint training (Staudte et al, 1973). Although these changes have not been demonstrated in conjunction with biochemical data many of these changes have been found with similar types of training programs as that used to establish the existence of biochemical adapatations with endurance training.

Stimulus for change in ATPase activity

The stimulus for adaptation of the ATPase enzyme may be different for swimming and running. Although swimming generally causes larger and more consistent changes in ATPase activity, many differences exist when compared to running. Water immersion has been considered as a factor but was refuted by Penpargkul et al (1980) who found no effect on ATPase activity of simply immersing rats in water. Also, swimming exercise causes a decrease in cathecholamine turnover rather than an increase making the fight or flight response an unlikely mechanism responsible for a greater ATPase response (Rupp et al, 1983). However, hormonal influence cannot be discounted as Pagani and Solaro (1983) were able to show myosin isoenzyme shifts in normal swim-trained rats but not in thyroidectomized rats indicating that a hormonal influence is essential for normal adaptive changes to occur.

Flaim et al (1979) have indicated that swimming exercise may not elicit a classic response in measured cv variables. After 15 minutes of exercise with tails unweighted, Q, SV, mean arterial pressure (MAP) and right and left ventricular coronary blood flow were unchanged. The Vo2 max elicited with swimming is lower than with running (McArdle, 1967; Gollnick and Shepherd, 1976). The overload of swimming exercise in Flaim's study is definitely of a lesser magnitude than swimming training where tails are usually weighted but swimming exercise in rats is definitely different than traditional exercise stimuli and this makes it difficult to use validly to evaluate true training effects.

Treadmill exercise with intensities as low as 20 meters·min^{-1}result in more classic responses of the sa[illegible]arialbles in rats (Flaim et al, 1979). Nutter et al (1981), however, have indicated th[illegible]ining at such an intensity does not result in functional adaptation of the myocardium. Furthermore, training at this low intensity does not cause as great a magnitude of adaptation in the skeletal oxidative enzyme levels. Also, Cutiletta et al (1979) found no increases in cytochrome c levels in skeletal muscle with training of up to 25 meters·min^{-1} for 8 weeks, and this raises doubt as to the potential for adaptability at these low intensities. Dudley et al (1983) have shown that the adaptive response of skeletal muscle to endurance training is related to the intensity and the duration of the exercise training sessions. This has been corroborated by Terjung (1975) who showed maximal response to training in skeletal muscle with higher intensity and duration.

Although an intensity of 25 meters·min^{-1} is sufficient to demonstrate a training effect and the Vo2 response to this exercise is equivalent to 70-80% of Vo2 max in rats (Shepherd and Gollnick, 1976; Pasquis et al, 1970; Bedford et al, 1979; Patch and Brooks, 1980; Wilson et al, 1978), others have shown that rats are capable of exercising continously for long periods of time at intensities of up to 40 meters·min^{-1} (Belcastro et al, 1980; Dudley et al, 1983) and that the training effect seems to be greater at intensities greater than 25 meters·min^{-1} (Dudley et al, 1983) depending on the muscle evaluated. In addition, some running programs using lower intensities of training (Baldwin et al, 1977; Pierce et al, 1979) have shown a greater magnitude of change in cardiac ATPase activity in the early stages of their exercise program with subsequent regression to normal values at later stages of the program and this could be related to a progressive decrease in the intensity of the overload on the myocardium. This change could also be related to peripheral changes or to an interplay between central and peripheral changes. Speculation is difficult, since no one has closely examined the time course of adaptation in the cardiac and skeletal muscle of rats.

Structure of striated muscle

Gross morphology of the myofibrils of skeletal and cardiac muscle

Both cardiac and skeletal muscle cells are striated and homologous in their structure although some structural differences exist (Adams and Schwartz, 1980). The myofibril is the basic functional unit of both types of muscle. In cardiac muscle, the fibers are interconnected end to end by tight junctions known as intercalated discs at right angles to the long axis of the myofibrils. These discs permit electrical flow with little resistance across all muscle cells upon depolarization. Skeletal muscle cells do not have any structural equivalent to these discs (Adams and Schwartz, 1980; Katz, 1977). Skeletal fibers are uniform long and parallel in arrangement, grouped in fascicles, varying in length with diameters from 10 to 100 um. Cardiac muscles, however, branch off or bifurcate forming a more complex, three dimensional network (Adams and Schwartz, 1980). The cardiac myofibrils make up 50 to 60% of the cell volume inside the sarcolemma, while the mitochondria make up 3% of the cell volume (Hirakow et al, 1980). In skeletal muscle, mitochondria are much less numerous but sarcoplasmic reticulum (SR) content is much greater.

Sarcolemma

The cell unit membrane is called the plasma membrane or plasmalemma and is 7 to 9 nanometers (nm) thick. Outside this membrane is the glycocalyx which consists of two layers; the inner or surface coat (20 nm) and the external lamina (30 nm). In the myocardium, the glycocalyx follows the plasmalemma in the T-tubules (transverse tubules) but does not do so in skeletal muscle (Langer, 1978).

The plasmalemma consists mainly of lipoproteins and is made of a lipid bilayer structure, which has high electrical resistance and areas which permit the diffusion of ions (Bennett, 1963). The glycocalyx is polyanionic, primarily because of a great amount of mucopolysaccharides, glycoproteins and sialic acid residues. This contributes to the negative charge of the polar head groups of the plasmalemma's phospholipids and creates an extracellular region with high cation binding capacity, particularly Ca^{2+} ions. The sialic acid residues which are in both coats of the glycocalyx play a major role in regulating cation permeability thru the plasmalemma (Langer, 1978; Adams and Schwartz, 1980).

Cardiac muscle has only 7% of the cisternal capacity of skeletal muscle but 400% greater surface cation-binding capacity since it has 54 times more sialic residues per unit volume per cell (Langer, 1978). Hence much recent work on regulatory processes has revealed that extracellular Ca^{2+} sources in cardiac and skeletal muscle are not the same (Dhalla et al, 1981).

Transverse tubular system

The t-tubules are invaginations into the muscle cells. In cardiac muscle, the invaginations are at the Z-line of the sarcomere and extend to the center of the myofibril and occasionally bifurcate to adjacent myofibrils. In skeletal muscle, T-tubules invaginate at the site of the H-I band and these also bifurcate. Adams and Schwartz (1980) have noted three major differences between skeletal and cardiac muscle with respect to T-tubular system. These are:

1- a larger lumen in cardiac than in skeletal muscle

2- cardiac tubules are heavily vesiculated and include the glycocalyx while skeletal muscle is not.

3- cardiac T-tubules are randomly coupled to the SR whereas in skeletal muscle these are consistently oriented to the SR.

Sarcoplasmic Reticulum

The SR is discontinous with the plasmalemma. It functions as a regulator of Ca^{2+} in the myoplasm by storing and releasing Ca^{2+} during excitation-contraction coupling (Katz, 1977). In skeletal muscle, SR is arranged in parallel and branches out in the region of the A bands but in cardiac SR the orientation is more random. The skeletal muscle SR also flows together in the A-I band region and forms the terminal cisternae which are much larger channels. Pairs of parallel channels run transversely across the myofibril in close association with the T-tubules interconnected by extensions or junctional processes of the SR membrane. The cardiac SR is dilated at its ends and these ends run very close to the T-tubules and sarcolemma and are also joined via junctional processes (Adams and Schwartz, 1980). Junctional SR (JSR) is associated with the plasmalemma or the T-tubule and free SR (FSR) is not associated with either of these.

Upon isolation, seven proteins and a lipid component are found to make up the SR. Five of the proteins are intrinsic including the Ca^{2+}ATPase (100,000 daltons (d))and a proteolipid. Ca^{2+} ATPase acts as a pump to sequester Ca^{2+} across the SR during relaxation (Adams and Schwartz, 1980; Tada et al, 1978; Van Winkel and Entman, 1979). The proteolipid appears to be involved in Ca^{2+} transport as well. Phospholamban (22,000 d) when phosphorylated enhances the Ca^{2+} ATPase activity and Ca^{2+} uptake (Tada et al, 1978). The two extrinsic proteins, calsequestrin, and a high affinity Ca^{2+} binding protein act as a Ca^{2+} sink in the SR and may also play a role in Ca^{2+} translocation (Adams and Schwartz, 1980).

Morphology of the myofibrils

The sarcomere is the basic unit of the striated muscles. A single sarcomere extends from Z-line to Z-line. The thin filament, composed of tropomyosin (Tm), F-actin and three troponins (T-I-C) are attached to the Z-line although it is unknown precisely how the attachment takes place. The M-band is the point of radial cross-linking of the thick filament myosin molecule and is in the center of the A-band (anisotropic because of the birefringent nature of the myosin molecule). The A-band is made up of myosin and interdigitating actin filaments. During contraction and relaxation, the A-band width remains the same since both filament lengths remain the same. The H-band consists of thick filaments only and the M-line of the A-band appears less dense at rest since the actin filaments do not interdigitate as far along the thick filaments. During contraction, interaction of thick and thin filaments takes place and the I-band becomes narrower since the thin filaments slide past the thick filaments.

Myosin

The myosin molecule includes two α-helical light meromyosin (LMM) rod like molecules that form a coiled-coil and a heavy meromyosin (HMM) portion which consists of two subfragments called S-1 and S-2. The S-2 subfragment is rod-shaped and is attached to LMM while the S-1 forms two globular heads at the distal end of HMM S-2 and contains the ATPase enzyme. In addition, four light chains are associated with the globular heads of the S-1 HMM subfragment (Katz, 1977; Adams and Schwartz, 1980; Mannherz and Goody, 1974, 1976). Table 4-2 presents the structural features of the myosin molecule that have been identified.

Table 4-2

Myosin Ultrastructural Components

Structure	**MW**	**Length A°**	**Reference**
Myosin filament	485,000	1650	McCubbin and Kay, 1980
LMM-skeletal	150,000	900	Katz, 1970
-cardiac	145,000	"	"
HMM-skeletal	350,000	-	"
-cardiac	345,000		Muellar et al, 1969
HMM S-1	115,000		Mannherz and Goody, 1976
HMM S-2	60,000	-	Lowey et al, 1969

Molecular weight (MW) is in daltons and length is in Angstroms

Table 4-3

Myosin light chains of cardiac and skeletal muscle

Muscle Type	Chain Name	Molecular Wt.	Reference
Fast skeletal	DTNB-LC2	18,500	Weeds and Lowey, 1971
Fast skeletal	LC1-alkali	25,000	Marrimoto and Harrington, 1974
Fast skeletal	LC3-alkali	16,000	"
Slow skeletal	LC1	27,000	Frearson and Perry, 1975
Slow skeletal	LC2	19,000	"
Slow skeletal	LC3	29,000	"
Cardiac muscle	LC1	27,000	"
Cardiac muscle	LC2	19,000	"

Molecular weight is in daltons

All components myosin but light chains are presented. HMM S-1 and S-2 are cross-bridge projections. References are in right hand column.

The remaining structural components of the myosin molecule are the light chains. The light chains of fast muscle are made up of a DTNB (5,5'-dithiobis-2-nitrobenzoic acid) sensitive (ie. DTNB treatment removes it from the myosin molecule). This chain does not cause a loss of ATPase activity and has also been called the non-essential light chain (Table 4-3). Also in fast muscle, are two alkali light chains. Light chain 1 (LC1) has 41 additional amino acid residues at the N-terminus and five amino acid substitutions (Mannherz and Goody, 1976)(Table 4-3).

In slow muscle, three light chains are found and in cardiac only two types, both of them having a 19,000 d non-essential light chain that is not sensitive to DTNB treatment. The removal of this LC2 from all muscles causes a reduction in K^+ EDTA ATPase activity and an increase Mg^{2+} ATPase activity and is therefore thought to play an integral role in actomyosin

Table 4-4

Myosin isoenzyme types in skeletal and cardiac muscle in different species

Muscle Type	Differentiation	Characteristics	Reference
Chicken Slow	LC1:LC1	Slow	Hoh et al, 1976
Chicken Slow	LC2:LC2	Slow	Hoh et al, 1978
Chicken Fast	LC1:LC1	Fast	"
Chicken Fast	LC3:LC3	Fast	Lowey et al, 1979
Chicken fast	LC1:LC3	Fast	Bechet et al, 1982
Rat atria	A1	Fast	Hoh et al, 1978
Rat atria	A2	Fast	"
Rat Ventricle	V1	Fast	"
Rat Ventricle	V2	Intermediate	"
Rat Ventricle	V3	Slow	"

Heart isozymes differentiated on the basis of heavy chain differences

interaction even though it does not directly participate in ATP hydrolysis (Malhotra et al, 1979). All three LC2 have also been shown to have an MLCK (myosin light chain kinase) dependent phosphorylatable site (serine 14), and a protein phosphatase that dephosphorylates the residue (Perrie et al, 1973; Holroyde et al, 1979a; Pires et al, 1974; Frearson and Perry, 1975). LC2 has also been called the P light chain. Its phosphate content has also been shown to turnover in beating hearts and freeze-clamping of hearts has revealed a phosphate content of 0.3 to 0.4 moles phophate per mole of LC2 (Holroyde et al, 1979; Barany and Barany, 1977; Stull and High, 1977). Ca^{2+} is also required for these reactions. LC2 also has two high affinity Ca^{2+}binding sites and these binding sites appear to be the ones involved in the phosphoryl transfer reactions.

Myosin isoenzymes are different in fast, slow and cardiac muscle as well, as can be seen in Table 4-4. Some isoenzymes such as in chicken slow and fast muscle have been shown to differ on the basis of light chain composition but rat heart isoenzymes are beleived to be classified on the basis of different myosin heavy chains (Hoh et al, 1978). Zak et al (1982), have reported a different primary structure in the heavy chains obtained from V1 and V3 isoenzymes while V2 appears to be a hybrid of V1 and V3. Thus far, only the V3 or slow isoenzyme type has been found in pig, canine and human ventricles (Clark et al, 1982).

Active Site

Although it is known that the active site of the ATPase is in the region of the HMM S1 and the light chains of the myosin molecule, the exact location of the site is not yet known. Ramirez et al (1979) have proposed a model for the activation site using the 92 amino acid peptide (p10) that had been previously isolated by Elzinga and Collins (1977). Ramirez et al (1979) felt that this peptide could be responsible for part or all of the active site because of:

1) the existence of the unusual N-methylhistidine residue at position 69. One molecule of this residue is present in all actins investigated.

2) the sulphydryl cysteine residues at position 11 and 21. These sulphydryl residues play important roles in the modification of the enzyme.

3) the DTNB light chain which also modifies the enzyme and is near this region of this peptide.

All of these structures are also near the hinge of the S2 and S1 HMM subfragments. In cardiac myosin, where N-methylhistidine is not present it has been proposed that histidine 69 could replace it. The model of Ramirez and his co-workers also included other amino acid residues which, in the proper tertiary structure, could bind the Mg·ATP. Their model could be explained by several structural associations of the nucleotide with the different residues in the p10 protein. First, the purine ring of the eight membered cyclic substrate Mg^{2+}·ATP was fit tightly into a 16 amino acid pocket by intercalating with phenylalanines 80 and 81. Second, the purine 6-amino group formed a hydrogen bond with aspartate 66 and the terminal phosphate was bound tightly to histidine 76 which donates a proton. Third, the phosphate end of ATP interacts electrostatically with the β-phosphate of lysine 78. Fourth, the Mg^{2+} is electrostatically bonded to the other two phosphate moieties and the side chains of methylhisitidine 69 and to tyrosine 72 (by a hydrogen bond). These 7 amino acids bind directly to Mg^{2+}·ATP.

The sulphydryl groups SH1 ands SH2, lysine residues in the S1 and S2 region and a reactive carboxyl group have also been implied via a possible modification of tertiary or quartenary structure of the HMM subfragments or through steric changes that alter actin-binding affinity or modify Ca^{2+}, Mg^{2+} and or K^{+}(EDTA) ATPase activities.

Myosin orientation

Myosin filaments are arranged in a head to head manner with opposite polarity in the center of the filament. The cross-bridges project helically with axial spacing of 145 angstroms (A°) (Huxley and Brown, 1967). Interaction must take place on two levels for this to occur. First, the filaments of opposite polarity interact in the center of the filament through the rod section. Second, the filaments used to extend the length of the filament must interact with each other's rod portions as well. Different coiling structures making these interactions possible have been proposed by several investigators (Huxley and Brown, 1967; Marimoto and Harrington, 1974; Squire, 1972). Hasselgrove (1980), through X-ray diffraction studies has demonstrated that each cross-bridge has two heads with opposite screw-senses (ie. both twisted in the same way but tilting in opposite directions. The tilt of the two heads in the frog sartorius studied were $\pm 30°$ to the filament axis.

Thin filament

The thin filament is made up of actin, tropomyosin (Tm) and troponin in a molar ratio of 7:1:1 (Ebashi and Endo, 1968). G-actin polymers are arranged in a double helix giving the appearance of a two coiled strand of pearls (Mannherz and Goody, 1976). The tropomyosin is in the grooves of the actin strand while the troponin complex repeats everey 385 A along the thin filament (Ebashi and Endo, 1968).

Actin

Actin makes up 60% of the thin filament protein and is homologous in cardiac and skeletal muscle. The G-actin monomer of actin weighs 41,875 d and is 55 A° in diameter. When G-actin monomer is complexed with ATP and Ca^{2+} its molecular weight is 42,300 d. G-actin is slightly assymetric and this has been attributed to a single tyrosine at position 53 in the monomer. This residue is necessary for the formation of F-actin, the polymerized form of G-actin. Actin also has a Ca^{2+} and a nucleotide binding site that are necessary for polymerization of G-actin (Barden et al, 1980; Wegner, 1982). The sites are 16 A° apart. The cations stabilize the F-actin structure by compensating for negative protein charges and reduction of electrostatic repulsion between monomers (Mannherz and Goody, 1976; Zechel, 1981). The nucleotide is not necessarily phosphorylated but ATP enhances polymerization over ADP (Katz, 1970).

Tropomyosin-(Tm)

Tm is a polar molecule 410 A° long and 20 A° wide which makes up 10-12% of the myofibrillar protein. It exists in a 1:7 stoichiometric molar ratio with actin and weighs about 68,000 d. Upon cleavage, two different chains are extracted, α and β, with the α chains predominating in a 4:1 ratio with β chains (Katz, 1970; Mannherz and Goody, 1976; Wegner, 1980). The β chain content is inversely related to the speed of contraction. For example, slow red muscle has a higher content of β chains in the slow muscle and rapidly beating hearts of smaller animals have no β chains while larger human, pig and sheep hearts have up to a 20% β

chain content. Thus, it appears that the heterogeneity of α and β chain content in Tm plays a role in ATPase function and regulation (Cummins and Perry, 1974; Leger et al, 1976).

Troponin

Troponin (Tn) is a complex of three molecules that are mainly involved in the regulation of contractile processes through their sensitivity to Ca^{2+} concentration changes in the cell. Its molecular weight (MW) is 76,000 d and the three sub-units are: Troponin-C (Tn-C, calcium binding), Troponin-I (Tn-I, troponin inhibiting), and Troponin-T (Tn-T, tropomyosin binding) (Mannherz and Goody, 1976; Perry, 1979). Each of these sub-units will briefly be considered.

Troponin-C

Tn-C has a MW of 17,840 d and except for minor differences in primary structure is homologous in different muscle types (Collins et al, 1973). The molecule is highly negatively charged at neutral pH and has four (I, II, III, IV) regions in the molecule rich in aspartic acid and glutamic acid residues which form binding loops and have been found to bind Ca^{2+} via the carboxyl side of the residues. Four moles of Ca^{2+} are bound per mole of Tn-C (McCubbin and Kay, 1980), and further investigation of this molecule has led to the discovery of two classes of binding sites in both skeletal and cardiac muscle.

In skeletal muscle, two high affinity sites for Ca^{2+} and Mg^{2+} have been found in regions III and IV, while two low affinity sites specific for Ca^{2+} have been found in regions I and II (Potter and Gergeley, 1975). In cardiac muscle, the same Ca^{2+}/Mg^{2+} sites are in regions III and IV but only the Ca^{2+} specific site in region II is active (Potter et al, 1976; Potter, 1977; Leavis and Kraft, 1978).

Ca^{2+} binding has also been shown to have positive co-operativity and Tn-I binding increases Ca^{2+} binding to all sites by one order of magnitude in skeletal muscle and ten fold in cardiac muscle. When regions III and IV are bound by Mg^{2+} or Ca^{2+}, a conformational change occurs involving secondary and tertiary structural modification, readying the molecule for actomyosin interaction. The binding of the two low affinity sites in regions I and II or only in II in the case of cardiac muscle, triggers actomyosin interaction and the activation of the ATPase enzyme. Ca^{2+} is also necessary for Tn-C interaction with Tn-I (Potter and Gergeley, 1975; Holroyde, 1980; Perry, 1979).

Troponin-I

This sub-unit has a MW of 20,864 d with 179 amino acids residues in skeletal muscle but in cardiac muscle the MW is 23,000 d in rabbit and 28,000 d in bovine cardiac muscle and this is attributable to a greater number of residues (216 because of an additional 26 at the N-terminus and other smaller additions). Only 103 0f 179 skeletal residues are homologous in cardiac and skeletal muscle (Wilkinson and Grand, 1975).Its function is generally homologous among species in at least the following three ways:

1) it inhibits Mg^{2+} stimulated actomyosin ATPase activity

2) neutralization of inhibition occurs upon interaction with Tn-C

3)it is phosphorylatable by a phosphorylase or a protein kinase

(Syska et al, 1976; Hartshorne and Muellar, 1968).

Fast muscle has a positive charge of 9 and cardiac and slow muscle have a positive charge of 14 and 18, respectively. The cardiac and slow muscle have a lower content of aspartate and glutamate residues and a higher content of lysine and arginine residues than fast muscle. A high content of proline residues accounts for the bending structure of the molecule with disparity in studies reporting 45 or 20% α and 10 or 29% β sheet (Wilkinson and Grand, 1978; Wu and Yang, 1976). Tn-I phosphorylation is not unique as other sub-units in the myofibril structure have a number of phophorylatable sites (Table 4-5) (Perry, 1979; Cole and Perry, 1975). Cardiac Tn-I is phosphorylated at its serine 20 residue at a much higher rate than skeletal Tn-I but at comparable rates at serine 146. Interaction of Tn-I with Tn-C does not prohibit phophorylation in cardiac muscle unlike skeletal muscle implying functional differences in actomyosin interaction between the two types of muscles (Cole and Perry, 1975; Moir et al, 1980).

Table 4-5

Sites of Phosphorylation of myofibrillar proteins

(Source: Perry, 1979)

Protein	Muscle	Site	Enzyme
Tn-I	Rabbit Fast	Ser-117	cAMP-dependent protein kinase
	"	Thr-11	phosphorylase kinase
"	Rabbit Cardiac	Ser-20	cAMP-dependent protein kinase
"	"	Ser-146	"
"	"	Ser-72	phosphorylase kinase
Tn-T	Rabbit Fast	Ser-1	Tn-T phosphorylase kinase
"	"	Ser-149	"
"	"	Ser-156	"
Myosin P-LC	All muscle types	Ser-14	Myosin light chain kinase

Sites are indicated by phosphorylated amino acid in primary structure

Troponin-T

Tn-T interacts with Tm, Tn-I and Tn-C establishing communication between all of these proteins in the thin filament. Tn-T positions the Tn complex every 385 A along the thin filament (Flicker et al, 1982; Mannherz and Goody, 1976). It is composed of 259 amino acids and has a MW of 30,503 d in rabbit skeletal muscle and 37,000 d and [illegible],000 d in chicken skeletal and breast muscle, respectively (Pearlstone et al, 1976). It is a highly basic protein with a +9 charge at neutral pH. The N-terminus is acidic and the carboxyl terminus is basic. A 37% α helix content has been noted with some β sheet in the carboxyl terminus. This protein, as previously described in table 4-4 binds 3 moles of phosphate per mole of Tn-T (Moir et al, 1979; Mannherz and Goody, 1976).

The Tn-T and Tn complex is thought to be an elongated molecule spanning much of the Tm length (Horwitz et al 1979; Flicker et al, 1982). The length of the whole Tn complex is 285 ±40 A° with a 160 ±35 A° tail portion. Tn-T is about 195 ±25 A° and the remaining 100 A° diameter portion is likely accounted for by Tn-C and Tn-I (Flicker, 1982). Tm probably interacts with all 3 sub-units via cysteine 190. Tn-T has two binding regions with Tn-I and Tn and the remainder of the Tn-T extends along Tm's length (Pearlstone and Smillie, 1981; Mak and Smillie, 1981). This tail portion of Tn-T along Tm suggests extensive interactions that could play a major role in controlling the switching processes that the Tn complex undergoes to allow actomyosin interaction (Flicker et al, 1982).

Function

Contraction of the myofibrils

Huxley's thin filament sliding model of contraction has been the accepted explanation for muscle contraction and has been the basis of much of the work of others since. The basis of the model suggests that myosin cross-bridges attach to the thin filaments and mechanical force is then transmitted through the heads. For actin and myosin interaction ATP hydrolysis is necessary and results in a series of conformational changes. The force generation is possible over a wide range of acto-myosin distances because of the flexibility between the S1 and S2 subfragments. The myosin head is now believed to attach at a preferred angle of 90° and generates force by moving to an angle of 45° where ATP binding promotes dissociation and a renewed cycle. The rotation through the angle or movement of the cross-bridges is postulated to occur by outward movement of the stem, a change in tilt of the S1 subfragment, a change in azimuth angle or by a combination of all three actions (Taylor, 1979).

Huxley (1969) has proposed that the tension is proportional to the extent of filament overlapping which would correspond to a greater possible number of cross-bridges interacting with actin. However, the work of Podolsky et al (1976) has demonstrated that in actively shortening muscle, force generation is primarily generated by the motion of cross-bridges rather than the absolute number attached. Matsabura et al (1980) have also shown that not all cross-bridges return to a resting state during diastole and that the force is proportional to the product of the number of heads in thin filament vicinity and the degree of thin filament activation. This failure to return to a relaxed state has been noted in skeletal muscle as well (Taylor 1979) and it has been postulated to be a potentiated state which is also considered to be an important factor to consider with respect to tension generation. Huxley and Simmons (1971) have also presented evidence for an independent elastic element which allows the cross-bridge to change lengths relative to the thin filament. This model included an attachment phase and a stretching phase (more rapid) during force generation. The elastic component allowed for a wide range of axial positions (50-100 A°) where it was possible to generate force.

Recently Eisenberg and Hill (1978) have developped a model which incorporates their biochemical model and modified the model of Huxley. What has emerged is a theoretical model of muscle contraction that enables a better understanding of the biochemical and mechanical co-ordination of muscle contraction (Stein et al, 1979). The model uses the concepts of flexible hinges, sliding filaments and an elastic component all incorporated into a biochemical scheme. The model uses an elastic cross-bridge which permits it to bind actin at a wide range of angles although with bound ADP·Pi the theoretical preferred angle is at 90°. Detachment of the cross-bridge occurs at 45° or when ATP binds myosin, again initiating a new cycle. The angle of detachment will depend on the axial position and hence the angle of attachment at the beginning of the cycle. The rate limiting step in this model is said to arise in the transition from a refractory to a non-refractory state which are unattached states. As mentioned in the biochemical breakdown of the actomyosin and myosin cycles (Stein et al, 1979; Eisenberg et al, 1980) the rate limiting transition is thought to occur because of a conformational change that must take place before myosin·ADP·Pi can re-attach to actin after ATP hydrolysis. The existence of the refractory and non-refractory states is still an issue of debate.

Another feature of this model is that ATP binding and hydrolysis need not necessarily lead to dissociation of myosin with actin. The elastic component allows variable axial placement of the myosin on the actin molecule and accordingly the free energy levels will vary according to the angles through which myosin is able to rotate. An alternative explanation is that the myosin had no elastic element and that the force producing capability of the myosin molecule depended strictly on its angle of attachment to the actin molecule. In both cases the attached myosin cross-bridges movement depends on its axial placement on actin with a consequential drop in free energy and external work being done.

ATP binding is presumed to weaken the bond with actin and return the myosin to its preferred angle of attachment (ie. 90°) for a new cycle to occur. The conformational change accompanying ADP·Pi release returns the molecule to 45°. Considering the range of the independent elastic element of the myosin molecule (if there is one) and the distance of the next site of attachment on actin this has been postulated to be at approximately 80 A°.

Marston et al (1976) suggest a lengthening of the muscle fiber in the presence of ADP. Eisenberg et al (1980) suggest that Pi release brings the molecule to 50° and the release of ADP is needed to bring it to 45°. Since the actomyosin-ADP complex is more stable, its release may be slower allowing for completion of the power stroke. The elasticity of the cross-bridges in combination with the dependence of rate constants and rapid equilibrium to axial placement of myosin on actin may explain why detachment of the AM complex is not necessary for ATP hydrolysis and force production (Eisenberg and Greene, 1980; Matsabura et al, 1980; Podolsky et al, 1976). This could also partially explain the prediction that at greater velocities the number of attached cross-bridges does not change significantly (Eisenberg et al, 1980).

Biochemical Models

Studies that have attempted to quantify different steps in the actomyosin cycle have included intrinsic fluorescence of tryptophan, absorption at 190 nm, fluorescence polarization of bound labels, stopped-flow fluorescence, light scattering, proton release or absorption coupled to a pH indicator, conductance and direct measurements of ADP and phosphate formation (Taylor, 1979). Most of the work done has the limitation of representing in vitro data and values derived from these studies are only approximations of the in vivo situation. Despite this limitation many of the in vivo states are retained and in vitro characterization of the actomyosin cycle has made it possible to hypothesize in vivo models of muscle contraction. In the following discussion abbreviations will be used to avoid repetition of frequently used terms (ie. M=myosin, A=actin, Pi=inorganic phosphate, AM=actomyosin, refractory state=(r), non-refractory state=(n)).

A number of biochemical models have been suggested to explain the contraction cycle in striated muscle (Eisenberg and Moos, 1968; Lymn and Taylor, 1971; Stein et al, 1979). Of these the model of Stein et al (1979) has become the generally accepted model. Eisenberg et al (1980) combined data from their work, the modified refractory state model (Stein et al, 1979) and a previously introduced theoretical formalism (Eisenberg and Hill, 1978) and attempted to quantify the model by suggesting equilibrium constants and free energy changes that would essentially concurr with the modified refractory state model. Eisenberg et al (1980) present an excellent schematic representation of this model and readers are referred to this diagram for reference as an aid to understanding the following discussion. The major points of this model are that all of the myosin states bound with phosphate are in rapid equilibrium with their respective actin bound states, that ATP hydrolysis can occur without dissociation of AM (ie. direct hydrolysis), that a large free energy drop occurs upon binding of ATP to AM and finally that a slow rate limiting transition occurs after the initial Pi burst.

The kinetic steps of the AM cycle will be reviewed in detail. Some of the values for equilibrium constants and free energy changes that occur in the model are assumed. Where asssumptions are made [illegible]ill be noted.

<u>Myosin cycle</u>

1- The binding constant of ATP to M (K1) is about 10^{10} or 10^{11} M^{-1} A large free energy drop occurs at this point. The binding of ATP is presumed to require a two-step reaction with a conformational change allowing M·ATP binding. The second order rate constant of ATP binding is about 10^{6} M^{-1} sec^{-1}. The forward rate is very fast and the reverse rate is quite slow and therefore M·ATP binding is essentially irreversible.

2- The hydrolysis of ATP or the Pi burst has an equilibrium constant (K5) of 10^{1} M^{-1} and therefore almost no free energy change occurs at this point. If you increase the pH or the ionic strength the forward rate constant increases from 20 to 200 sec^{-1}. This step is 200 times faster then the Vmax. Both K1 and K5 are derived from experimental data values and are assumed to be the same in vivo as well as in vitro (Eisenberg et al, 1980).

3- The existence of a transition from a refractory to a non-refractory state distinct from Pi release is still being debated but the equilibrium constant (K7) of this postulated step was assumed to be 3.3 x 10^{-2} M both in vitro and in vivo. This value shifts the equilibrium toward the refractory state, thereby guaranteeing a rate-limiting transition before force production (ie. AM interaction) during an isotonic contraction.

4- The release of Pi is likely a two-step reaction which causes a conformational change in the myosin molecule and has a constant (K9) of 7.06 M. This reaction is very slow in the absence of actin and is rate limiting above 5°C in the myosin cycle. This step may be reversible. When bound to actin a large effective free energy change is evident as described in the AM cycle.

5- The final step in the cycle is the release of ADP and this step has a constant (K11) of about 10^{5} M^{-1}. It is rate limiting at temperatures below 5°C.

<u>Actomyosin cycle</u>

The actomyosin cycle has two components: associated and dissociated states with myosin and the transition of the AMT.

1-The binding of S1 to A (K2) has a binding constant of 10^{7} or 10^{8} M^{-1}, is strengthened with weakened ionic strength is weakened 30 fold by ADP and 3000 times by ATP or ADP·Pi. Because of this M·ADP and M states bind ATP more strongly compared to M·ATP, M·ADP·Pi(r), M·ADP·Pi(n). The second order rate constant for this reaction is 5 x 10^{6} M^{-1} and this rate is diffusion limited only. With 50 um actin K1, K5, K4 and K6 are in equilibrium and so their second order rate constants are probably similar. Stein et al (1979) suggest that if equilibrium exists the differences in the binding constants would be dependent on dissociation rates rather than association. They also suggest that M·ATP and M·ADP·Pi(r) would rapidly dissociate at a rate of 10^3 sec^{-1}.

2- The association of AM with ATP (K4) is very rapid (10^3 sec^{-1}) competing with rapid dissociation of AM by ATP (K3). The equilibrium constant (K4) is 2 x 10^2 M^{-1} and is presumed to be the same in vivo. Like all the reactions in the AM cycle this one is also presumed to be a two-step reaction. The high binding constant of product release (K9) is quite high (7.06 M) and with in vivo ATP levels of 3 mM or more a free energy drop (9.5 kcal) accompanies ATP bi[illegible] forward reaction irreversible.

3- The hydrolysis of [illegible] is controversial and is thought to occur only at high actin concentrations. Eisenb[illegible] (1980) have postulated an equilibrium constant for K6K8 and [illegible] approximate a rate constant of 5.48 x 10^2 sec^{-1}. Evidence suggests this step does occur [illegible] ATPase is not inhibited at high actin concentrations. If it were true, M·ATP and [illegible]ADP·Pi states would be expected to occur but do not (Stein et al, 1979). The initial Pi burst is thought to be even higher than on myosin alone (Stein et al, 1979).

4- There is evidence indicating that after ATP hydrolysis, a slow step occurs which limits the ATPase rate. [illegible] et al (1979) and Eisenberg et al (1980) favor the theory of a transition from a refractory to a non-refractory state (M·ADP·Pi(r) to A[illegible]DP·Pi(n)) (K8) which occurs before Pi release and has a rate that is independent of actin. It has been postulated that this occurs in the M cycle since ATPase rate dependence on actin (Kapp) is 4 times greater than S1 binding to actin (K13) (Stein et al, 1979). This means that K13 would not be favored but rather K14 would be favored and AM would reassociate at this point. It is not known how the refractory to non-refractory state transition comes about although oxygen exchange (O^{18}) expirements (Sleep et al, 1980) also indicate that this occurs predominantly in the dissociated states M·ADP·Pi(r) and M·ADP·Pi(n) since K7 is compatible with oxygen exchange. Thus, water addition and oxygen exchange are thought to limit the rate of transition from the refractory to non-refractory states. When A attaches Pi release is very fast (since oxygen exchange decreases).

5- Eisenberg et al (1980) have postulated an equilibrium constant (K10) of $4.29 \times 10^{4}\ M^{-1}$ for the release of Pi. The forward rate of release of Pi is very rapid as is ADP release (1000 sec^{-1}). ADP release has an equilibrium constant of $1.0 \times 10^{-4}\ M^{-1}$. These reactions are probably irreversible and a large free energy drop probably occurs with Pi release.

Some assumptions have been made by Eisenberg et al (1980) to simplify the understanding of the cross-bridge cycle. First, K13 is thought not to exist so that the refractory state cannot bind actin. Second, many of the equilibrium constants are fitted assumptions based on the model of Stein et al (1979) and other experimentally derived values in other steps. Third, they assume that ATP hydrolysis cannot occur without AM dissociation. Stein et al (1979) have put forth evidence to the contrary. Fourth, they assume that myosin heads interact with only one actin molecule but recent evidence suggests that each myosin molecule may bind several actin molecules simultaneously. This increase in complexity would in turn change the quantitative assumptions of the equilibrium constants and forward rate constants associated with the cross-bridge cycle. Also, many of the reactions in the cycle are thought to involve two steps which in itself increases the complexity of the model.

In spite of these limitations the work done in Maryland by Eisenberg and his co-workers is currently the most complete explanation of the cross-bridge cycle. The modified refractory state model put forth by Stein et al (1979) essentially uses a four state model [illegible] explain the mechanism of muscle contraction.

Tn-Tm regulation of cross-bridge cycling

Two theories have developped regarding the relaxation in the cross-bridge cycle. The first is a steric hindrance model (Huxley, 1972). This model is based on X-ray diffraction and electron microscope studies and suggests that the Tm molecule moves away from the center of the actin groove where it is placed during contraction by Tn (bound by Ca^{2+}). When Ca^{2+} is removed Tn maintains Tm in its relaxed position away from the center. Recently, biochemical evidence has cast doubt on the steric hindrance model and Taylor and Amos (1981) have shown that Tm is located on the oppposite side of the actin groove to block actomyosin interaction. They propose a new shape for S1 and a three-dimensional structure which has Tm and the S1 subfragment come into close contact in the long pitch of the actin-helical groove during relaxation. During contraction however, the Tm is well away from the S1 and as mentioned on the opposite side. The trapping of Tm in the long pitch of the groove could explain cross-bridge binding without Ca^{2+} as in rigor (Taylor and Amos, 1981).

A second theory results from an accumulation of biochemical evidence that compromises the first theory. Eaton et al (1975) have pointed out that Tm binding to actin is strengthened in the absence of ATP. Tm alone only partially inhibits the ATPase activity. A series of papers (Greene and Eisenberg, 1980; Adelstein and Eisenberg, 1980; Johnson et al, 1979) have disputed the existence of a steric hindrance model as well. This model does not account for attached and detached states in the AM cycle and the possible equilibrium between these states (ie, AM cycle is probably not an all or none phenomenon). Ca^{2+} binding to Tn will influence the rates and extent of contraction and relaxation and also disputes the existence of a simple steric hindrance model. The model also does not predict which of the myosin states is prevented from binding actin when Ca^{2+} is not present.

Work from the lab of Eisenberg (Chavlovich et al, 1981; Chavlovich and Eisenberg, 1982; Greene and Eisenberg, 1980) has resulted in more biochemical evidence for a new theory of Tm-Tn regulation of the actomyosin ATPase activity.

Eisenberg and Greene (1980) conducted some studies with regulated actin (includes Tn-Tm) and unregulated (no complex) actin. In the presence of low levels of S1-ADP without Ca^{2+} binding regulated actin bound weakly compared to unregulated actin. At high levels of S1-ADP both bind strongly and regulated actin was found to bind in a co-operative manner similar to the co-operative binding model of Hill. Therefore, an allosteric weakly bound state was thought to occur in the absence and presence of Ca^{2+}. A lower concentration of strong binding sites for S1-ADP on actin (perhaps one actin for every seven G-actin monomers) has been suggested to occur under these conditions (Trybus and Taylor, 1980). The interaction of Tm molecules in an end to end manner has also been put forth as an explanation for the co-operative change in states from activation to inactivation (Chavlovich et al, 1981). The Tn-Tm complex could thus alter the entire free energy profile by weakening the A·M·ADP state proposed to be the 45° state in the cross-bridge cycle. But the fact that the binding is lower in the presence of low S1-ADP in regulated actin still does not support the steric hindrance model, since at rest the muscle exists with bound ATP or ADP·Pi. More work (Chavlovich et al, 1981; Chavlocich and Eisenberg, 1982) with regulated and unregulated actin has shown that the maximum ATPase rate (Vmax) is decreased by 96% in the absence of Ca^{2+} but that the association constant was the same regardless of Ca^{2+} levels. Therefore, the extent of activation is presumably controlled co-operatively by Ca^{2+} bound to the Tn-Tm complex without affecting the affinity of actin for S1. Thes investigators have proposed that a conformational change or non-competitive binding of the Tm-Tn complex could account for these results and that in the absence of Ca^{2+} the Tn-Tm complex blocks a step in the ATPase cycle, perhaps the release of Pi from the A·M·ADP·Pi complex.

Regulation

Calcium

Since the early 1960's a considerable body of evidence has accumulated testifying to the importance of the calcium ion as a regulator of a number of cellular processes including muscular contraction (Ebashi, 1980). In fact calcium is recognized as a nearly universal messenger in the regulation of function of all animal cells. It is known that in excess calcium acts as a toxic agent leading to dysfunction and cellular death. Fluctuations of calcium in and out of the cytosol are transient even in cells that have a sustained response (Rasmussen and Barrett, 1984). In this discussion aspects of Ca^{2+} metabolism that relate to muscular contraction are reviewed. These include sources of Ca^{2+} in cardiac and skeletal muscle, the Ca^{2+} requirements for contraction to take place, the co-operative aspects of Ca^{2+} regulation of the myofibril ATPase activity and the physiological and mechanical correlates of ATPase activity especially in heart muscle.

Calcium sources

In skeletal muscle, it has been fairly well established that the extracellular calcium is not essential for contraction to take place (Frank, 1982). In cardiac muscle, however, the removal of extracellular calcium leads to a rapid decline in force production indicating a need for the extracellular compartment (Rich and Langer, 1975). These findings have led to studies from Langer's laboratory to investigate the relationship between extracellular calcium and cardiac function (Langer, 1978; Phillipson et al, 1980; Bers, Phillipson and Langer, 1981). In these studies it was shown that isolated sarcolemmal vesicles had specific phospholipid and sialic acid binding sites for Ca^{2+} and that the degree of contractility was directly dependent on the extent of Ca^{2+} binding to these sites. Abundant low affinity receptors were suggested to be involved in Ca^{2+} flux in and out of cells. Binding of Ca^{2+} was also thought to govern the rate of decline and rate of increase in tension development in the myofibrils. The direct relationship between sarcolemmal Ca^{2+} binding and contractility has also been demonstrated by Dhalla et al (1982).

In both types of muscle the mechanism by which the extracellular calcium could control contractility or tension development has also been investigated. Two fields of thought have emerged. One theory is that the extracellular calcium, by binding to the membrane with a subsequent increased influx from extracellular sources, could account for all the calcium necessary for cardiac contraction. This would not be be the case in skeletal muscle. This increase in calcium could be brought about by a Na^{+}/Ca^{2+} exchange (electroneutral exchange) and/or by a Ca^{2+} current during the plateau phase of depolarization of cardiac muscle (Langer, 1978; Langer, 1980; Fabiato and Fabiato, 1979; Dhalla et al, 1982; Drummond, 1979). Whether the net influx of Ca^{2+} from these sources is sufficient to raise myoplasmic Ca^{2+} to the levels necessary for contraction is still a question of debate (Fabiato and Fabiato, 1979) and an alternate theory has been suggested that would agree with the involvement of extracellular calcium. This theory is that of a calcium induced calcium release, whereby extracellular calcium influx causes a larger influx of calcium from the SR to the myoplasm causing an elevation in calcium levels sufficient for contraction to take place. This theory has been hypothesized to be the mechanism of activation in both skeletal and cardiac muscle (Frank, 1982; Fabiato and Fabiato, 1979).

Calcium requirements

Whatever the mechanism of Ca^{2+} influx into the cell, the calcium ion is necessary for direct activation of the myofibril and the degree of activation is directly dependent on the calcium concentratrion in the cell (Solaro et al, 1974).

In cardiac muscle, the amount of calcium necessary for tension production was deduced by monitoring Ca^{2+} binding, the biochemical ATPase activity and the mechanical (tension development) correlates of myofibril activation. Binding, tension and activity increased in parallel with low values a[illegible]ree Ca^{2+} until 10^{-6} M free Ca^{2+} where these all increased sharply up to 10^{-5} M. Although the sigmoidal relationship of tension and activity were not directly superimposed they displayed similar activation curves over the free Ca^{2+} concentrations between 10^{-8} and 10^{-5} M free Ca^{2+} (Solaro et al, 1974). Solaro et al (1974) had also demonstrated further binding without an increase in activity or tension suggesting that complete saturation of binding sites was not necessary as had already been demonstrated in skeletal muscle (Bremel and Weber, 1972).

In vivo studies have also substantiated the in vitro data with direct measurements of Ca^{2+} levels at rest and during contraction with the Ca^{2+} sensitive fluorescent substance known as aequorin and by calcium sensitive microelectrodes (Allen and Blinks, 1978; Marban et al, 198[illegible]). These studies support the contention of a beat to beat regulation of myoplasmic Ca^{2+} and also support the work of Solaro et al (1974) and Bremel and Weber (1972) by showing that activation was co-operative in nature and that intracellular calcium reaches 10^{-5} M for significant tension generation.

Co-operative activation of the myofibrils by calcium

Calcium activates the myofibril by binding TnC as described earlier. In skeletal muscle, there are two low affinity sites that are bound and in cardiac there is apparently only one active low affinity site. Both cardiac and skeletal muscle have high affinity sites but these are not beleived to be involved in the activation process. The reason for this is that the high affinity sites also bind Mg^{2+} and the presence of high levels of Mg^{2+} coupled with the dissociation constant of Ca^{2+} for this site render these sites unavailable for Ca^{2+} and/or too slow to be involved in the activation process (Johnson et al, 1979). Different approaches (Holroyde et al, 1980; Robertson et al, 1981) have shown that activation of the myofibril ATPase occured at Ca^{2+} concentrations when binding of the low affinity sites took place whereas all the high affinity sites were already bound. Also the on and off rates on the four binding sites was only analogous to the contraction-relaxation cycle in the case of the low affinity sites. Kohama (1979) has challenged this showing that activation can occur even if Ca^{2+} binding is restricted to the high affinity sites but whether this situation is possible in vivo as well as in vitro is questionable.

Rupp (1980a; 1980b) has described a method by which this co-operative effect can be evaluated both in cardiac and skeletal muscle. With the use of the classical Hill equation the sigmoidal curve of myofibril ATPase versus free Ca^{2+} can be evaluated. By using the Hill equation the slope or Hill n and the pCa50 (ie. Ca^{2+} required for 50% activation) can be derived. The consequence of the Hill n is that an indication of co-operativity of Ca^{2+} binding can be seen. If the slope (Hill n) is 1.0 then the Ca^{2+} binding is independent but if it is greater than 1.0, as is usually the case in myofibril activation under normal circumstances then Ca^{2+} binding to one site enhances binding to the second site and likely to other sites on adjacent actin molecules. The more co-operative the binding the greater the slope (Hill n) of the curve and this will mean a smaller range of Ca^{2+} needed for full [illegible] the time course (ie. higher ATPase at a particular Ca^{2+} level) and the extent of activation (greater maximal ATPase activity).

The measurement of the pCa50 on the other hand would be analogous to the Km of the myosin ATPase although not a true one since Ca^{2+} is not the true substrate of the enzyme. A higher pCa50 would translate into a greater level of Ca [illegible] comparable binding at a lower pCa50 (ie. Ca^{2+} sensitivity is decreased with a higher p[illegible] vice versa). This information is important in describing the actin mediated regulation of the contraction cycle. The importance of the actin molecule (including the Tn-Tm complex) has been demonstrated by a number of previously mentioned studies (Greene and Eisenberg, 1980; Dancker, 1980; Chong et al, 1983; Chavlovich et al, 1981; Chavlovich and Eisenberg, 1982).

In cardiac muscle, the sigmoidal curve can be described as being analogous to diastole with low free Ca^{2+} ranges and as systole at activating levels of free Ca^{2+}. In skeletal muscle, the same principle applies with relaxation at low levels of free Ca^{2+} and activation at high levels of free Ca^{2+}.

Physiological consequences of co-operativity

Presumably if Ca^{2+} is the activator of myofibril ATPase and if ATPase activity is necessary for contraction to occur, then there should be a relationship between Ca^{2+} binding and physiological parameters. Indeed a good relationship between tension and ATPase over the range of Ca^{2+} activating levels is well known (Winegrad, 1971; Solaro et al, 1974; Bremel and Weber, 1972; Brenner and Jacob, 1972; Herzig and Ruegg, 1980). Although Ca^{2+} also regulates tension development, immediate stiffness, Vmax (maximum unloaded shortening velocity) and length-tension relationships, the relationship between these is also dependent on sarcomere length and internal afterload induced by length changes (Brenner and Jacob, 1980).

Herzig and Ruegg (1980) have substantiated that immediate stiffness and myocardial tension increases are paralleled by increases in Ca^{2+} and ATPase activity suggesting a participation of a greater number of cross-bridges through Ca^{2+} binding and activation. However, the velocity of tension development was related to Ca^{2+} only at low levels but at higher levels the relationship diminished suggesting that the participation of other factors besides myoplasmic Ca^{2+} would contribute to the velocity of contraction in heart muscle. Herzig and Ruegg (1980) have also demonstrated that Vmax required a greater pCa50 than does tension itself. Thus, other factors must play a role in developping maximal velocity in muscle since Vmax can be altered independently of tension and stiffness.

A number of researchers have looked at sarcomere length to investigate its effects on Vmax both in cardiac and skeletal muscle (Brenner, 1980; Brenner and Jacob, 1980; Allen and Blinks, 1978). By increasing sarcomere length, quick-release expirements demonstrate a greater velocity of shortening. Although this velocity is independent of Ca^{2+} concentration, the pCa50 does affect the slope of the loss of velocity with decreasing sarcomere lengths demonstrating a calcium dependence of the velocity of shortening at a particular sarcomere length.

In cardiac muscle, the length-tension relationship seems more directly dependent on the pCa. Frog cardiac muscle increased its tension with an increase in sarcomere length and an increase in Ca^{2+} sensitivity (Allen and Blinks, 1978; Fabiato and Fabiato, 1978b). Increasing the sarcomere length beyond its optimal level had an opposite effect, however, with a 20% reduction in tension in frog heart (Fabiato and Fabiato, 1978). Thus, simply stated stretch induced increase in Ca^{2+} sensitivity can potentially result in a greater number of active cross-bridges. Ca^{2+} release from the SR has also been reported to be inhibited by a decrease in saromere length (Fabiato and Fabiato, 1975b). Therefore, stretching the sarcomere up the ascending limb of the sarcomere length-tension curve would result in optimized Ca^{2+} regulation and an improvement in cardiac function (Katz, 1980). This as Katz (1980) has explained in greater detail would provide a mechano-chemical basis for the Starling mechanism.

Other factors modulate contractility besides Ca^{2+} activation. Some of tese including regulation by phophorylation-dephosphorylation reactions, Mg^{2+}, MgATP, pH and Pi will be briefly examined here.

Phosphorylation

Many tissues are known to be regulated by phosphorylation-dephosphorylation reactions and fragments of myofibrillar protein known to be phosphorylated are TnI, TnC, Tm and myosin light chains. However, only TnI and the myosin light chain will be considered since most of the phosphate incorporated in myofibrils occurs in these two subfragments. Phosphorylation reactions involving these sub-fragments usually includes a second messenger system beginning at the membrane and these are mediated by cAMP or the Ca^{2+} ion. Both of these cAMP messengers are bound intracellularly by a protein kinase or calmodulin type proteins in the case of Ca^{2+} and exert their effects directly or indirectly. cAMP phosphorylates enzymes in the sarcolemma, SR and myofibril and Ca^{2+}-calmodulin catalyze phosphorylation reactions via a Ca^{2+}-calmodulin dependent protein kinase. Usually, the phosphate group binds a serine group and adds two negative charges to the protein and this may be the chemical basis of the physiological reactions mediated by phosphorylation. Dephosphorylation occurs via specific protein phosphatases (Barany and Barany, 1981; Perry, 1979).

Troponin I

Some of the actions of TnI phosphorylation have been characterized and the consequences of TnI phophorylation are briefly discussed here. TnI is phosphorylated at serine 20 of the molecule in cardiac muscle and at threonine 11 and serine 117 in skeletal muscle. Other sites exist on the molecule but these account for only 2% of phosphate incorporation in the molecule. This phosphate incorporation is evident in both skeletal and cardiac muscle. (Perry, 1979; Solaro et al, 1976; Barany and Barany, 1981). But phosphorylation is not induced by contraction of skeletal muscle or isoproterenol treatment suggesting that kinases do not actively phosphorylate skeletal muscle. (Barany and Barany, 1981). In cardiac muscle phosphorylation increases with isoproterenol, epinephrine or adrenaline treatment. The amount of phosphate incorporated varies from study to study but all show a substantial increase from resting levels (Robertson et al, 1982; Moir et al, 1980; England, 1975).

The increase in phosphate incorporation observed in TnI has also been paralleled to force development in response to adrenaline (Solaro et al, 1976) but the peak of force and the peak of incorporation of phosphate are not simultaneous with the decay of force (England, 1976; Westwood and Perry, 1981).

Phosphate incorporation also decreases the Ca^{2+} sensitivity and causes a rightward shift of the pCa-ATPase relationship (Ray and England, 1976; Wyborny and Reddy, 1978; Holroyde et al, 1979c; Holroyde et al, 1980; Resink et al, 1979; Yamamoto and Ohtsuki, 1982). Others (Bailin, 1979; Yamomoto and Ohtsuki, 1982) have also shown that an increase in Ca^{2+} concentration is also required for basal and maximal ATPase activity and that at normal levels of Ca^{2+} a reduction in ATPase activity is caused by TnI phosphorylation (Yamomoto and Ohtsuki, 1982; Resink et al, 1979; Bailin, 1979; Wyborny and Reddy, 1978). Resink and Gevers (1982) showed that phosphorylation decreased Vmax and dephosphorylation caused increases in Vmax. In addition to these findings, a decrease in $Ca2^{2+}$ binding accompanying a decrease in Ca^{2+} sensitivity and a decreased ATPase activity has been noted. Thus, Ca^{2+} removal or a reduced affinity for Ca^{2+} at the TnC binding sites is caused by TnI phosphorylation (Holroyde et al, 1979c; Robertson et al, 1982).

The previously mentioned authors and others (Katz, 1980; Kranias and Solaro, 1983) have come to a consensus regarding the physiological effect of TnI phosphorylation in cardiac muscle. Since Ca^{2+} sensitivity, myofibril ATPase and Vmax are all decreased by phosphorylation, researchers have suggested that cardiac relaxation rate is increased in a catecholamine stimulated inotropy. This appears to act synergistically with cAMP dependent phosphorylation of phospholamban in the SR which increases Ca^{2+} uptake from the myoplasm. Therefore increased Ca^{2+} dissociation from Tn, changes in the Ca^{2+} flux and binding at the SL and increased Ca^{2+} uptake by the SR speed up relaxation (Kranias and Solaro, 1983; Katz, 1980). This could also be facilitated by a reduced actin-actin transduction leading to a decreased AM interaction and thus a reduced Vmax over the range of activating Ca^{2+} concentrations (Resink and Gevers, 1982). It has also been suggested that TnI phosphorylation may represent a negative feedback mechanism in response to the β-adrenergic stimulated positive intropic response reducing or smoothing out the positive inotropy by decreasing the myofibril ATPase activity (Perry, 1979; Solaro et al. 1976; Moir etal, 1980). Dephosphorylation of Tn by specific phosphatases would presumalbly renew the cycle and this would allow for a beat to beat regulation of cardiac contraction.

Myosin light chain

Both cardiac and skeletal muscle undergo phosphorylation-dephosphorylation reactions of one or more myosin-P light chains. Specific kinases and phosphatases catalyze these reactions (Frearson and Perry, 1975; Barany and Barany, 1981). Some have theorized that phosphorylation of the P-light chain could play a role in regulating myosin and thus contractility, however, evidence supporting this concept is still obscure (Stull et al, 1982; Barany and Barany, 1981). The P-light chain also serves as the Ca^{2+} binding site of the thick filament (Holroyde et al, 1979c) and myosin undergoes a conformational change upon binding at activating levels (Marimoto and Harrington, 1974).

The kinase of the myosin P-light chain is activated by a Ca^{2+}-calmodulin complex (4 Ca^{2+} bound/mole of calmodulin) and since calmodulin is ubiquitous the extent of phosphorylation is dependent upon Ca^{2+} fluxes, myosin light chain kinase (MLCK) levels and activity and phosphatase activity (Stull et al, 1982). Kardami and Gratzer (1982) have shown that phosphorylation may reduce the affinity of AM interaction via altered Ca^{2+} binding properties of the myosin light chain.

It has been determined that light chains are partially phosphorylated even at basal levels of muscle activity, but the exact content has been reported as between 0.3 and 0.5 mole of Pi/mole of light chain (Jeacoke and England, 1980; Barany et al, 1983; Westwood and Perry, 1981).

P-light chain phosphorylation during diastole has been correlated with tension development in hearts and thus phosphorylation was presumed to predetermine the magnitude of tension development (Kopp and Barany, 1979) but many studies have not been able to show this relationship in skeletal muscle (Kushmerick and Crow, 1983; Crow and Kushmerick, 1982; Kerrick et al, 1980). However, researchers are in disagreement on this point since phophorylation has been shown to increase with tension and tetanic stimulation in skeletal muscle in other studies (Stull et al, 1980; Hager et al, 1982; Barany et al, 1983; Barany et al, 1980).

Myosin ATPase activity has been shown to increase in rat hearts with phosphorylation (Resink and Gevers, 1982; Holroyde et al, 1979a) but another study (Reddy and Wyborny, 1979) did notr show a significant change. The actin activation of myosin Mg^{2+} ATPase was increased in rat hearts, which may be more important (Reddy and Wyborny, 1979) since phosphorylation is thought to mediate AM interaction (Perry, 1981). In skeletal muscle, many of these effects on actomyosin ATPase have also been reported (Pemrick, 1980). Therefore, actin activation enhancement in both these types of muscle support the speculation that phosphorylation may modulate AM interaction (Perry, 1981).

With peak inotropic response to β-adrenergic stimulation in hearts, many workers did not observe any phosphorylation (Holroyde et al, 1979a; Jeacoke and England, 1980;High and Stull, 1980; Westwood and Perry, 1981) but others have shown that phosphorylation of the P-light chain increased and decreased with positive and negative inotropy (Kopp and Barany, 1979). Resink and Gevers (1982) have shown that withdrawal of β-adrenergic stimulation that cAMP levels declined along with heart rate and P-light phosphorylation. Others (Resink et al, 1981a; Resink et al, 1981b) have also shown that running training results in enhanced light chain phosphate content in trained animals in the presence of elevated Ca^{2+} levels in perfused hearts. This increase in phosphorylation was in parallel with the Vmax of myosin ATPase activity. Therefore, some authors contend that phosphorylation parallels increases in contractility in the myocardium. Others (Alexis and Gratzer,1978; Kardami and Gratzer, 1982) have investigated the effect of phosphorylation on the Ca^{2+} binding site of myosin. Phosphorylation caused a decrease in Ca^{2+} sensitivity and Ca^{2+} binding to the light chain. However, disagreement exists on this point as well, as Holroyde et al (1979b) were not able to find this effect. They suggest that Ca^{2+} binding to myosin may represent a distinct regulatory mechanism.

Other investigators have noted that SL, SR and myofibril phosphorylation (TnI and P-light chain included) represent a part of a coordinated regulatory mechanism as previously mentioned (Perry, 1979; Katz, 1980; Barany et al, 1983). Substantiation of this theory requires further investigation because of the great discrepancy in the findings to date.

Magnesium

The measurement of free Mg^{2+} in all muscles poses great difficulties but ^{31}P NMR spectroscopy has provided a value of 0.6 mM as a physiological level (Gupta and Moore, 1980). Polemini and Page (1973) has stated the physiological range to be between 0.1 and 1mM. Several authors have studied varying intracellular Mg^{2+} concentrations reporting on its effects on contractile and Ca^{2+} activation parameters in heart and skeletal muscle of different species (Fabiato and Fabiato, 1975; Kerrick and Donaldson, 1972; Solaro and Shiner, 1976; Donaldson et al, 1978). Therefore, this compunds the problems since magnesium levels may also be tissue and species specific.

In skinned rat cardiac cells maximum tension was increased at high Mg^{2+} (10 mM) but not from 1 to 5 mM Mg^{2+} (Donaldson et al, 1978). The significance of an increased tension at high (10mM) Mg^{2+} is questionable since physiological levels may not exceed 1mM. The major effect in cardiac muscle appears to be a rightward shift in the pCa-%tension curve with increasing Mg^{2+}, which indicates that the muscle is less sensitive to Ca^{2+} activation. However, despite this shift in the curve there is no change in the slope (Hill n) of the curve indicating that Mg^{2+} is not directly affecting Ca^{2+} binding to TnC (Donaldson et al, 1978) but rather reducing the sensitivity of the ATPase to Ca^{2+}. When Mg^{2+} levels are lowered there is an increase in the Hill n and a decrease in maximum tension. Fabiato and Fabiato (1975a) have also substantiated that increased Mg^{2+} shifts the pCa-%tension relationship to the right. In skeletal muscle, increasing Mg^{2+} resulted in decreases of submaximal tension generation (Kerrick and Donaldson, 1972) and Hill n in skeletal muscle of frog.

Solaro and Shiner (1976) have shown that biochemical alterations substantiate the previous findings. In skeletal muscle, an increase in Mg^{2+} levels shifts the pCa-ATPase relationship to the right and increases the slope or Hill n of the curve. Mg^{2+} also causes a decrease in myofibril ATPase at all levels over the entire range of Ca^{2+} levels. The Mg^{2+} levels varied between .04 mM and 10 mM. However, in cardiac muscle the biochemical alterations were different. When Mg^{2+} is increased from .04 to 1 mM there is an increase in ATPase rather than a decrease and the entire pCa-ATPase curve is shifted leftward. Furthermore, at 10 mM Mg^{2+} and greater than pCa5 the ATPase is greater than with .04 mM Mg^{2+}. This result is in opposition to pCa-%tension results and opposite to skeletal results. But Donaldson et al (1978) also found an increase maximum tension at high Mg^{2+} levels. Again the physiological significance is questionable but in pathological states or conditions promoting ionic imbalance these findings may be of importance (Rupp, 1980).

The mechanism by which Mg^{2+} has its effects on the myofibrils is beleived to be related to the Ca^{2+} and Mg^{2+} binding sites of the myofibrils (Solaro and Shiner, 1976). Solaro and Shiner (1976) could explain their results via a decreased calcium binding to troponin in skeletal and an increase in cardiac muscle with high Mg^{2+} levels. Ca^{2+} binding to myosin was depressed under these conditions which as with troponin was opposite low Mg^{2+} conditions. The depressed ATPase activity in cardiac muscle at low Mg^{2+} corresponds to Bremel and Weber's (1975) similar finding at um Mg^{2+} levels with PCa increasing from 7 to 5. Therefore, Ca^{2+} requirements at different Mg^{2+} levels is quite different and the relationship between binding and ATPase is very steep.

A role for Mg^{2+} has been suggested by Potter et al (1981) who found that at low and high Mg^{2+}, Mg^{2+} non-competitively inhibited Ca^{2+} binding to TnC but this effect was not found at physiological Mg^{2+} levels (1-2 mM). This does not explain Solaro and Shiner's (1976) finding of increased binding at high Mg^{2+} levels in cardiac muscle. The role magnesium plays in the muscle is still largely unexplained mostly because of difficulties in determining intracellular concentrations and fluxes in and out of muscle cells. Theories include an interaction of Ca^{2+}

and Mg^{2+} on binding sites of both TnC and myosin but are only tentative explanations for an unsolved question.

Inorganic Phosphate-Pi

Inorganic phophate (Pi) is a proton donating anion and in the presence of a 10 mM concentration has been shown to stimulate mitochondrial Ca^{2+} uptake at a pH below7.2. At a pH higher than 7.2 this anion stimulates Na^{+}/Ca^{2+} exchange and inhibits the Na^{+}/K^{+} pump (Ponce-Hornos et al, 1982). It was suggested from these studies that this sarcolemmal Ca^{2+} uptake could have an effect on the role of extracellular Ca^{2+} as an activator of myofibrils and a trigger for the release of SR Ca^{2+}. Pi is also important in regulating at the SR and myofibrils. Herzig and Ruegg (1980) have shown a 50% decrease in isometric force and stiffness (20 mM Pi)and a decrease in Vmax (10 mM Pi) with Pi addition. Herzig et al (1981) have also shown that vanadate (Vi), an analog of Pi, in uM concentratrions can decrease maximum isometric tension development, but increased ATPase activity at any activation level above basal levels. Since ATP turnover was increased while tension was decreased it was suggested that the energy cost of tension maintenance was increased. This was presumably brought about by an alteration of AM interaction whereby Pi and/or Vi increased the rate of turnover and thus decreased the amount of time that the cross-bridges spent in active force generation. Herzig et al (1981b) failed to show a change in Ca^{2+} binding or Ca^{2+} sensitivity due to Pi and Vi even though tension and stiffness were decreased.

Vi has also been shown to depress Ca^{2+} uptake and myofibril ATPase activity (Solaro et al, 1980b). These authors have also shown a positive inotropic response (increased LVP development and force development) in isolated perfused hearts and decreased TnI phosphorylation which could also increase positive inotropy. A decrease in actin cofactor activity was suggested to play a role in reducing the myofibril ATPase. This conflicts with Herzig et al (1981) who found an increase in ATPase activity but this could be due to the fact that Herzig et al (1981) used an indirect method to assay ATPase as opposed to a direct biochemical method by Solaro et al (1980b).

MgATP

MgATP^{2+} serves both as a substrate for actomyosin ATPase and as a plasticizing agent. As a substrate it provides the free energy for contraction and as it binds it weakens the AM interaction thereby maintaining muscle extensibility (Rupp, 1980; Marston et al, 1979). Physiological levels are beleived to be between 3 to 6 mM in skeletal muscle, complexed with Mg^{2+}(Burt et al, 1976).

Varying ATP concentrations from uM to mM levels yields a bell-shaped tension-MgATP curve both in striated muscle and skeletal cells of frogs (Fabiato and Fabiato, 1975b). At low MgATP (without Ca^{2+}) 15-20% of maximal tension is generated (pMgATP 7). With pMgATP of 5.5, 50% of maximal tension is generated and only 5-10% is generated with pMgATP of 4.0 or more (Fabiato and Fabiato, 1975). Reuben et al (1971) have suggested that this was the expression of substrate inhibition. Bremel and Weber (1972) suggest that tension at low ATP levels is due to a threshold-dependent cross-bridge formation of rigor complexes that are a result of co-operativity of the myosin head with the actin filament ("on-state"). Substrate saturation and ATP hydrolysis continue until the high numberof dissociated or "off-state" cross-bridges brought about by high MgATP is no longer compensated for by substrate binding. Therefore, hydrolysis decreases and reaches a minimum with an increase in "off-state" AM sites. It is questionable whether high and low MgATP levels is representative of a physiological phenomenon since ATP levels are beleived to be well maintained (Godt, 1974), however, maximal isometric tension, the pCa-%maximum tension, pCa-ATPase and the slope or co-operative nature of the pCa-tension curve are all affected by varying ATP levels (30um to 30 mM) in both skeletal and cardiac muscle (Best et al, 1977; Fabiato and Fabiato, 1975; Rupp, 1980; Godt, 1974; Kerrick and Donaldson, 1975; Ferenczi et al, 1979; Bremel and Weber, 1972).

In cardiac muscle an increase of MgATP from 30 uM to 2mM resulted in a 25% reduction in tension (Best et al, 1977). Fabiato and Fabiato (1975b) similarly found a decrease in tension with increasing levels of ATP. These results have also been reported in skeletal muscle (Orentlicher et al, 1977). Ferenczi et al (1979) also demonstrated a hyperbolic relationship between maximal shortening velocity and MgATP varying between 10 um and 10 mM. In contrast, Godt (1974) and Brandt et al (1972) have shown that maximal tension was independent of MgATP.

Increasing MgATP shifts the pCa-tension curve to the right (ie. increases Ca^{2+} requirements) and vice versa on cardiac (Fabiato and Fabiato, 1975b; Best et al, 1977; Rupp, 1980) and skeletal muscle cells (Fabiato and Fabiato, 1975b; Orentlicher et al, 1977; Godt, 1974; Kerrick and Donaldson, 1975). Myofibril ATPase behaviour is similar when varying ATP from 20 um to 2 mM (Weber, 1969). Portzhel et al (1969), however, observed this effect in crab myofibrils but not in rabbit myofibrils.

The behaviour of the co-operative nature (Hill n) of the pCa-tension curve is more contradictory. In cardiac muscle, Best et al (1977) reported an increased Hill n with an increased MgATP. Reducing ATP from 4 mM to 100 uM resulted in a reduced Hill n from 1.9 to 1.2 without affecting Ca^{2+} sensitivity. Tension also increased at low to midrange Ca^{2+} values (pCa 6.1) under these conditions but decreased tension was noted at higher Ca^{2+} levels (pCa 4.7). Rupp (1980) found an increased Ca^{2+} sensitivity with ATP levels between 100 um and 30 um. In skeletal muscle, some reports agree with findings in cardiac muscle (Godt, 1974; Orentlicher et al, 1977) while others could show no effect (Brandt et al, 1972).

Two explanations have emerged to explain MgATP's effects on Ca^{2+} activation parameters. Weber and Murray (1973) contend that low MgATP reduces troponin's inhibitory effect and rigor complexes are formed. This could also affect Ca^{2+} binding. The high affinity sites become more like the low affinity sites when the extent of rigor complex formation increases. But this, aside from shifting the pCa-tension curve to the left should increase co-operativity but such is not the case in two studies in cardiac cells (Rupp, 1980; Best et al, 1977). In contrast, Orentlicher et al (1977) propose the existence of three states between the enzyme-substrate complex; ES2, ES and E. ES2 is the double nucleotide bound state (off-state). ES is a single nucleotide bound state (on-state) and E is a nucleotide free state (potentiated). As substrate concentrationincreases, Ca^{2+} activated tension goes from an active potentiated state at low levels to a competely relaxed state (inhibited ES2) at higher levels.

PH effects

Decreasing the cellular pH either by a respiratory or metabolic acidosis is known to affect both cardiac and skeletal muscle, the main effect usually being a negative inotropic effect on many metabolic processes and tension generation (Boos and Boron, 1981). Respiratory acidosis (eg. increasing pCO_2) has a more profound effect on force production and provides evidence that H^+ acts primarily intracellularly (Poole-Wilson and Langer, 1975; Roos and Boron, 1981).

Normal intracellular pH measured by ^{31}P NMR spectroscopy is between 7.0 and 7.2 (Jacobus et al, 1982; Salhany et al, 1979). The H^+ production of the cell and hence pH state in the cytoplasm arises from a multiplicity of metabolic processes inside the cell and ion exchange processes across the cell membrane (Gevers, 1977). Salhany et al (1979) have questioned the premise of lactate production as the primary determinant of cell pH and in heart it has been suggested that ATP hydrolysis itself may be the major source of proton generation (Gevers, 1977). Indeed processes related to ATP hydrolysis are affected by excess H^+ production. The effect intracellular acidosis has on tension generation has been examined both in cardiac and skeletal muscle preparations. A range of pH and Ca^{2+} concentrations have been used and in both muscle types a depression in maximal tension was noted at pH 6.5 (Donaldson and Hermansen, 1978; Donaldson et al, 1981) or 6.2 (Fabiato and Fabiato, 1978) at high ca^{2+} concentrations. The pCa-%tension curve is shifted to the right with a resultant increase in pCa50 with decreasing pH. This effect on submaximal tension was more pronounced in cardiac muscle (ie. increased Ca^{2+} requirements at pCa50 by a factor of 5.5 in cardiac and by 3.5 in skeletal muscle) (Donaldson et al, 1981; Fabiato and Fabiato, 1978). The pH effects are reversible but cannot be overcome by increasing Ca^{2+} suggesting that the mechanism of impairment involves much more than a simple competition between H^+ and Ca^{2+} for myofibril sites (Fabiato and Fabiato, 1978). Absolute force generation was also shown to be impaired in cardiac and fast fibers at all levels of Ca^{2+} (pCa 7.75 to 4.0) but not in slow soleus (Donaldson and Hermansen, 1978) but the slope or the co-operativity was not shown to be affected by others (Rupp, 1980; Donaldson et al, 1981). Perfused hearts have also shown a 50% depression in LVP when pH was decreased by 0.22 but it was only necessary to decrease it by 0.09 under conditions of ischemia for the same effect suggesting a different mechanism for the reduction of contractility (Jacobus et al, 1982).

Myofibril ATPase activity of cardiac and skeletal muscle was also altered with pH changes. Basical activity of cardiac ATPase activity was shown to be unaffected by a pH range of 6.4 to 7.2 in one study (Kentish and Nayler, 1979) but depressed at pH 6.4 in another (Okabe and Hess, 1981). Fast muscle ATPase was slightly increased by a mild acidosis (Kentish and Nayler, 1979). Maximal cardiac ATPase was decreased by 20% at pH 6.5 from 7.0 but skeletal muscle was unaffected (Kentish and Nayler, 1979). The pCa-ATPase relationship behaves in the same manner as the pCa-%tension relation with a rightward shift at low pH. Ca^{2+} requirements for a pCa50 increased 4 fold and requirements for a full ATPase increased by 5 fold as pH was reduced to 6.4 (Kentish and Nayler, 1979). This is in general agreement with the tension generation data. Okabe and Hess (1981) found an increased sensitivity to pH with an increase in Ca^{2+} concentrations. At a pCa of 7.0 and pH 6.8 ATPase is not affected but is depressed at a pCa of 6.65 (pCa increased from 6.6 to 6.3) and at pH 6.4 the basal, pCa50 and maximal ATPase were all depressed.

Combining acidosis with different Mg^{2+} and Mg ATP^{2-} levels has also been examined. With high Mg^{2+} (10mM) the sensitivity of tension is unaffected at pH 6.5. Thus, the H^{+} effect on Ca^{2+} is Mg^{2+} dependent (Donaldson and Hermansen, 1978). $MgATP^{2-}$ reduction did not alter changes that occur with acidosis, but enhanced responses at normal pH to very low ATP was reversed at pH 6.5 suggesting a possible H^{+} $MgATP^{2-}$ interaction with acidosis (Donaldson et al, 1981; Orentlicher et al, 1977).

Competition for binding sites between H^{+} and Ca^{2+} has been suggested as a mechanism causing negative inotropy but this is contoversial and Fuchs (1979) was not able to show any change in Ca^{2+} binding at a pH range of 6.2 to 7.4. In contrast, Robertson et al (1978 a,b) have shown that the Ca^{2+} specific sites of TnC were pH sensitive. Mattiazzia et al (1979), showing a reduced tension development and rate of rise of tension suggested a non-competitive interaction between the two cations. H^+ may bind to a multiplicity of other sites in the contractile proteins and in this regard Reisler and Liu (1982) have demonstrated that the S2 region is very sensitive to small alterations in charge balance.

An impairment of SR transport has also been implicated. In skeletal muscle, the pH optimum for Ca^{2+} loading depended on the Ca^{2+} concentration (ie. low Ca^{2+} had higher pH optimums of 7.0 to 7.4). Acidosis increase loading at high Ca^{2+} levels (pCa 7.0- pH 7.0 to 6.6 and pCa 6.0- pH 6.6 to 6.2). In cardiac muscle the Ca^{2+} dependency of pH optimum was not as clear. Acidosis depresses Ca^{2+} induced Ca^{2+} release and greater Ca^{2+} is required to achieve a caffeine induced contracture. Thus, these authors theorize a graded release of Ca^{2+} from cardiac SR (Fabiato and Fabiato, 1978, 1979). Okabe and Hess (1981) have also noted a depression in rate and extent of Ca^{2+} uptake with acidosis. This depression in SR function is probably a contributor to the impairment of cardiac and skeletal function observed in other studies. But, in contrast to cardiac muscle, in skeletal muscle the acidotic effects may in part compensate for the effects on the myofibrils.

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Appendix B-Training Programs

Programs

A - Progressive increase in intensity

B - High Intensity

Time-course of training

Train 9 weeks - 0 3 6 9x

Train 6 weeks - 0 3 6x

Train 3 weeks - 0 3x

x - denotes sacrifice time.

PROGRAM A

Day	Speed	Duration
	Week One	
Day one.	25 m/min	15 min
Day two	25 m/min	20 min
Day three	25 m/min	30 min
Day four	25 m/min	30 min
Day five	25 m/min	35 min
	Week Two	
Day one	25 m/min	35 min
Day two	25 m/min	40 min
Day three	25 m/min	40 min
Day four	25 m/min	45 min
Day five	25 m/min	45 min
	Week three	
Day one	25 m/min	50 min
Day two	25 m/min	50 min

Day three	25 m/min	55 min
Day four	25 m/min	55 min
Day five	25 m/min	60 min
	Week four	
Day one	25 m/min	60 min
Day two	30 m/min	30 min
Day three	30 m/min	30 min
Day four	30 m/min	35 min
Day five	30 m/min	35 min
	Week five	
Day one	30 m/min	40 min
Day two	30 m/min	40 min
Day three	30 m/min	45 min
Day four	30 m/min	45 min
Day five	30 m/min	50 min
	Week six	
Day one	30 m/min	50 min
Day two	30 m/min	55 min
Day three	30 m/min	55 min
Day four	30 m/min	60 min
Day five	30 m/min	60 min
	Week seven	
Day one	35 m/min	30 min
Day two	35 m/min	30 min
Day three	35 m/min	35 min
Day four	35 m/min	35 min
Day five	35 m/min	40 min

Week eight

Day one	35 m/min	40 min
Day two	35 m/min	45 min
Day three	35 m/min	45 min
Day four	35 m/min	50 min
Day five	35 m/min	50 min

Week nine

Day one	35 m/min	50 min
Day two	35 m/min	55 min
Day three	35 m/min	55 min
Day four	35 m/min	60 min
Day five	35 m/min	60 min

Training Program B

Week one

Day one	35 m/min	10 min
Day two	35 m/min	15 min
Day three	35 m/min	21 min
Day four	35 m/min	21 min
Day five	35 m/min	25 min

Week two

Day one	35 m/min	25 min
Day two	35 m/min	25 min
Day three	35 m/min	25 min
Day four	35 m/min	30 min
Day five	35 m/min	30 min

Week three

Day one	35 m/min	30 min
Day two	35 m/min	30 min
Day three	35 m/min	30 min
Day four	35 m/min	30 min
Day five	35 m/min	32 min
	Week four	
Day one	35 m/min	32 min
Day two	35 m/min	34 min
Day three	35 m/min	34 min
Day four	35 m/min	35 min
Day five	35 m/min	35 min
	Week five	
Day one	35 m/min	36 min
Day two	35 m/min	36 min
Day three	35 m/min	38 min
Day four	35 m/min	38 min
Day five	35 m/min	40 min
	Week six	
Day one	35 m/min	40 min
Day two	35 m/min	40 min
Day three	35 m/min	40 min
Day four	35 m/min	42 min
Day five	35 m/min	43 min
	Week seven	
Day one	35 m/min	43 min
Day two	35 m/min	43 min
Day three	35 m/min	45 min

Day four	35 m/min	45 min
Day five	35 m/min	45 min
	Week eight	
Day one	35 m/min	45 min
Day two	35 m/min	50 min
Day three	35 m/min	50 min
Day four	35 m/min	50 min
Day five	35 m/min	55 min
	Week nine	
Day one	35 m/min	55 min
Day two	35 m/min	55 min
Day three	35 m/min	55 min
Day four	35 m/min	55 min
Day five	35 m/min	60 min

Total work in meters for program A:

Week 1- 3250 meters

Week 2- 5125 meters

Week 3- 6750 meters

Week 4- 5400 meters

Week 5- 6600 meters

Week 6- 8400 meters

Week 7- 5950 meters

Week 8- 8050 meters

Week 9- 9800 meters

Total work done= 59,325 meters for Program A after 9 weeks. Program A averaged 6591 meters per training week and 1318 meters per training session.

Program B was similar with a total of 59,255 meters of work done for the training program.

Appendix C- Biochemical assays

A.Isolation of myofibrils from muscle (Perry and Corsi, 1951).Reagents and Chemicals

1. Buffer 1: 39 mM Na-borate; 25 mM KCl; 5 mM EDTA; pH 7.1

2. Buffer 2: 39 mM Na-borate; 50 mM Tris; pH 7.1

3. Wash Solution: 50 mM Tris; 5 mM $MgCl_2$; 100 mM KCl; 0.5% Triton X-100, pH 7.4

Suspension Medium: 150 mM KCl; 50 mM Tris; pH 7.4

Procedure

1. Homogenize tissue in 20 volumes of cold buffer 1 for 20 seconds at a setting of 7 with a Polytron Tissue Homogenizer (Pt-10).

2. Centrifuge the homogenate at 1000xg for 10 minutes using a Sorvall RC2-B Centrifuge.

3. Decant supernatant and discard. Resuspend the pellet in 20 volumes of Buffer 1, centrifuge at 2200 for 10 minutes and discard supernatant.

4. Resuspend pellet in 20 volumes of Buffer 2, centrifuge at 1000xg for 10 minutes and discard supernatant.

5. Repeat step 4.

6. Resuspend pellet in 20 volumes of Wash Solutionand centrifuge at 1000xg for 10 minutes and discard supernatant.

7.Repeat step 6.

8. Resuspend pellet in 20 volumes of Suspension Medium.

9. Take 0.1 mls. for protein determination (Lowry et.al, 1951.)

B. Protein Determination

Reagents and Chemicals

1. 0.5% cupric sulphate ($CuSO_4.5H_2O$)

2. 1.0% sodium potassium tartrate ($NaKC_4H_4O_6$)

3. 2.0% sodium carbonate(Na_2Co_3) pH to 12.5 with 10N NaOH. 4. Lowry C Solution: 61.7% $NaCo_3$, 35.7% distilled H_2O, 1.3% $CuSo_4$, 1.3% $NaKc_4H_4O_6$= total solution

5. Folin Reagent: 1 to 1 (v/v) distilled water.

6. 0.3N KOH

STANDARD CURVE

Stock (ml)	Buffer (ml)	Conc (mg/ml)	Conc.(ug/ml)
0.0	0.5	0.0	0.0
0.1	0.4	1.0	6.0
0.2	0.3	2.0	12.0
0.3	0.2	3.0	19.0
0.4	0.1	4.0	25.0
0.5	0.0	5.0	30.0

(Protein Stock was 5 mg/ml Bovine Serum Albumin in distilled water)

Procedure

a) Solubilizing Protein

1. Take 0.1 ml. fo homogenate or standard solution

2. Add 0.2 ml. of 0.3N KOH

3. Incubate in water bath at 37 C. for 30 minutes.

Reaction Mixture

1. Take 0.1 ml (twice) of soluble protein solution from above.

2. Add 5.0 ml of freshly prepared Lowry C Solution to duplicate tubes.

3. Add 0.3 ml of folin reagent to each tube while vortexing. Insure each tube is mixed for the same length of time.

4. Let reaction mixture stand for at least 0.5 hours.

c) Spechtrophotometric Analysis

1. Set spectrophotometer wavelength at 750 nm (Pye-Unicam SP8-100).

2. Use protein blank from the standard curve as the reference.

3. Vortex each sample before the analysis of each sample and record optical density.

C.Incubation for myofibril ATPase activity (Goodno et al, 1978.)

Reagents and Chemicals

1. Incubation Medium: 100 mM KCl, 4 mM Tris, 4 mM $MgCl_2$, pH 7.0.

2. $CaCl_2$ - 5 Conc.: 0.01, 0.1, 1.0, 10.0, 100.0 with 5 mM EGTA, pH to 7.0 at 30 C.

Trichloroacetic acid (TCA), 20%, made fresh weekly.

4. Mg.ATP - 50 mM

5. KOH - 1.0N

Suspension Medium (Same as in isolation Procedure)

Procedure

a) Reaction Mixture 1. take 1 ml of incubation buffer into both A and X tubes.

2. Add Ca^{2+} - 0.1 mls.

3. Add 1 ml. of 20% TCA to each X tube

4. Add 0.5 mls. of protein (after adjusting to 2 mg/ml.).

5. Taking into account the later addition of 0.2 ml of Mg.ATP solution, make up each tube to a total of 2 mls. volume with the addition of 0.2 mls. of suspension medium.

b) Incubation Procedure

1. Vortex each tube and incubate at 30 C for 5-10 minutes.

2. At 30 second intervals add 0.2 mls. of Mg.ATP solution to every tube and allow an incubation time of exactly 5 minutes. Vortex constantly.

3. Add 1.0 ml. of TCA to the A tubes at the same 15 second intervals. Vortex.

c) Centrifuge every tube for 10 minutes at 1000xg and save supernatant for phosphate analysis.

N.B. See Free Ca^{2+} calculations - Katz et al, 1970.

D.Phosphate Assay (Taussky and Shorr, 1953)

Reagents and Chemicals

1. Sulphuric Acid - 15N: 28 mls. od H_2SO_4 (98%) and make up to 400 mls. with distiled water.

2. Ammonium-Molybdate-Ferrous-Sulphate Solution: add 0.5 gms. of ammonium-molybdate to 5.0 mls. of 10N H_2SO_4. Dissolve completely and make volume up to 8.0 mls. Add 2.5 gms. of ferrous sulphate and make volume up to 50.0 mls. (make fresh daily).

Standard Curve

Stock (mls.)	Water (mls.)	Final Conc. (umoles)
1.00	0.00	1.00
0.50	0.50	0.50
0.10	0.90	0.10
0.05	0.95	0.05
0.01	0.99	0.01
0.00	1.00	0.00

Procedure

a) Reaction Mixture

1. To each standard, add 1.0 ml. of 20% TCA and divide mixture in half (duplicate).
2. Take 1.0 ml. in duplicate tubes from supernatant of the ATPase reaction.
3. Add 1.0 ml. of ammonium-molybdate solution to every tube and vortex.
4. Let stand for 5.0 minutes.

b) Spectrophotometric Analysis

1. Set spectrophotometer wavelength at 700 nm (Pye-Unicam SP8-100).
2. Use distilled water as the reference.
3. Analyze each sample and recordoptical density.

Calculations

(Sample (mM)-Blank (mM)/1000/5min./2mls.x 7.4 (dilution factor)

Answer = X umoles Pi·mg^{-1}min^{-1}

Appendix D- Calculations from Hill Equation

1. Hill plots are used to determine n (interaction coefficient or slope)

The equation to determine these plots reads as follows:

$$LOG\ V/Vmax-V$$

2. Once the Hill n has been calculated the activation energy which was allowed to vary in this experiment was calculated for each Ca^{2+} concentration used as follows:

$$Vmax/1+Q/Ca^{2+}\ conc.x\ n$$

therefore, Ca^{2+} conc with n as its exponent

results in the Ca^{2+} to the nth x (Vmax/v -1) = Q

n = Hill value

Ca^{2+} is expressed in moles

Vmax is previously determined from the experiment

v is equal to a velocity (ATPase activity) at a specific Ca^{2+} concentration.

<u>EXAMPLE</u>

Ca^{2+} conc. = pCa 7

Vmax = 0.129 umol $Pi\cdot mg^{-1}min.^{-1}$

v = 0.051 umol $Pi\cdot mg^{-1}min^{-1}$

n = 1.2 from Hill plot

$10^{-7\ 12}$ (.129/.051-1) = Q

10^{-84}(1.529) = $6.1x10^{-6}$

Q varies for each Ca^{2+} and also absolute ATPase activities.

3. Determine Hill Equation:

$V = Vmax/1+Q/Ca^{2+}$ to the nth.

4. Use Interpolation Program with Cubic Spline to plot sigmoidal curve of ATPase activity vs. Ca^{2+} concentration

Appendix E- Raw Data

Raw data for anthropometric measures

Left column contains ID.

ID	BW	HW	TV	RV	LV	H/B	V/B	V/H	R/H	L/R
00121	242	764	654	145	473	316	270	619	190	326
00221	242	773	668	151	478	319	276	618	195	316
00321	217	677	621	126	438	312	286	647	186	348
00421	222	734	656	120	481	331	295	655	164	401
00521	208	628	568	111	424	302	273	675	195	382
00621	247	807	743	179	519	330	301	643	241	290
00721	220	656	563	101	438	298	256	668	154	434
00821	232	677	600	105	456	305	259	674	155	434
00921	241	735	652	133	484	304	271	659	181	364
01021	234	711	630	113	475	331	269	668	156	420
01121	223	739	653	141	466	309	293	631	191	330
01221	238	736	660	146	457	292	277	621	198	313
01322	272	958	840	207	537	352	309	561	216	259
01422	253	785	672	136	472	310	266	601	173	347
01522	252	774	685	145	492	307	272	636	187	339
01622	250	799	696	140	510	320	278	638	175	364
01722	256	910	785	199	522	355	307	574	219	262
01822	277	1015	907	203	626	366	293	619	185	375
01922	251	833	718	154	509	332	286	611	173	331
02022	263	943	831	163	611	359	316	648	173	380
02122	252	828	728	143	544	329	289	657	213	306
02222	265	860	784	183	560	325	296	651	217	283
02322	282	890	790	193	547	316	280	615	194	323

02423	288	910	823	193	570	314	286	626	212	310
02523	314	987	891	183	587	222	196	595	185	321
02623	385	856	753	196	490	320	281	572	229	250
02723	262	839	735	154	536	306	278	639	184	348
02823	289	874	803	152	547	286	273	616	179	341
02923	277	793	700	142	547	335	253	616	189	244
03023	269	900	800	170	580	316	297	644	239	308
03123	273	862	765	206	502	308	280	582	212	369
03223	260	802	728	170	523	349	280	652	176	310
03323	263	917	810	161	594	306	308	615	201	
03411	213	557	512	127	356	261	240	639	228	283
03511	220	607	554	118	409	276	252	674	194	347
03611	208	559	509	097	378	269	245	676	174	390
03711	200	577	522	111	380	289	261	659	192	342
03811	206	589	522	118	373	286	253	633	200	316
03911	226	661	592	115	447	292	262	676	174	389
04011	220	612	547	114	400	278	249	654	186	351
04111	222	660	596	141	431	298	268	653	214	306
04211	211	621	571	109	431	294	271	694	176	395
04311	215	632	569	121	404	294	265	639	192	334
04411	219	600	543	109	409	274	248	682	182	375
04512	239	703	640	150	435	274	266	635	211	307
04612	247	678	611	127	449	267	247	662	187	354
04712	244	651	600	132	433	267	246	665	203	328
04812	230	694	640	137	458	302	278	660	197	334
04912	256	832	744	181	529	325	291	636	218	292
05012	227	641	573	114	432	282	252	674	178	379

05112 222 586 516 105 375 264 232 640 179 357

05212 259 811 756 205 482 313 292 594 253 235

05312 224 675 614 147 439 301 274 650 218 299

05412 238 743 692 164 467 312 291 629 221 285

05512 257 715 657 183 383 278 256 536 256 209

05613 253 913 807 213 530 287 252 581 233 249

05713 247 693 605 114 444 281 245 641 165 389

05813 265 722 648 199 402 272 245 557 276 202

05913 254 728 642 124 488 287 253 670 170 394

06013 275 850 713 147 477 310 259 561 173 324

06113 224 665 586 124 420 297 262 632 187 339

06213 247 704 641 133 481 285 260 683 189 362

06313 260 743 667 155 466 286 257 627 209 301

06413 255 725 632 134 465 284 248 641 185 347

06513 254 723 615 143 472 285 242 653 198 330

06631 237 816 733 138 543 344 309 665 169 393

06731 224 688 640 140 480 310 286 697 204 343

06831 232 745 661 126 537 321 285 721 169 426

06931 216 720 653 126 476 333 284 661 175 378

07031 220 709 622 119 475 322 283 670 168 399

07131 243 746 657 118 510 307 270 684 158 432

07231 228 716 624 111 493 314 274 689 155 444

07331 224 722 637 121 489 322 284 677 168 404

07432 253 837 730 147 543 331 289 649 176 369

07532 253 868 768 178 565 343 304 651 205 317

07632 251 806 710 157 514 321 284 638 195 327

07732 243 810 712 158 539 333 293 665 193 341

07832 216 662 594 128 432 306 274 653 171 338

07932 272 832 698 142 580 326 288 697 181 409

08032 227 740 680 134 517 308 300 699 236 386

08132 255 785 695 185 583 306 273 743 195 315

08233 251 805 716 122 568 321 285 706 152 466

08333 250 711 646 146 461 332 293 648 205 316

08433 244 801 728 167 527 328 298 658 209 316

08533 291 787 712 147 543 270 245 690 187 369

08633 238 935 790 179 605 393 332 647 191 338

08733 251 811 699 154 497 332 278 674 185 323

08833 235 784 695 138 525 334 296 670 176 380

08933 243 816 736 157 553 336 303 678 192 352

09033 247 850 751 145 587 344 304 691 171 405

Myofibril ATPase raw data for heart

ID E .01 .1 1.0 10 100E4 E10 M4 M10 N Km

09111 027 030 033 094 141 143 070 024 136 120 1556 631

09211 020 029 035 176 205 207 038 024 193 145 1500 653

09311 051 053 059 108 155 166 043 011 163 128 1207 664

09411 032 038 028 130 203 203 022 019 192 156 1553 645

09511 044 064 036 089 141 153 035 019 143 113 1363 644

09611 028 041 030 067 119 137 028 043 109 091 1256 648

09711 024 035 049 122 137 130 036 031 134 104 1191 678

09811 016 006 017 096 114 114 022 020 106 079 1409 648

09911 000 027 034 101 108 117 02[illegible] 024 111 084 1188 678

10021 044 066 067 162 184 178 040 037 183 122 1255 676

10121 026 061 071 133 152 141 028 043 156 120 1122 688

10221 002 021 040 109 128 133 033 041 127 095 1227 668

10321	025	039	039	126	145	170	030	026	154	121	1301	665
10421	044	053	077	147	162	160	039	035	163	119	1123	693
10521	035	049	064	150	164	149	035	038	164	135	1206	683
10621	035	038	052	116	131	123	023	032	156	117	1152	681
10721	046	046	059	139	171	163	036	038	161	127	1258	668
10821	010	024	045	103	108	108	038	039	106	088	1093	695
10921	053	068	077	203	218	222	011	019	173	147	1193	682
11031	026	031	039	158	243	234	022	027	188	157	1551	642
11131	021	031	033	123	135	135	011	013	119	096	1312	666
11231	018	040	043	130	162	141	031	030	134	108	1328	688
11331	[illegible]	026	025	108	130	128	022	023	127	103	1057	688
11412	[illegible]	[illegible]	024	081	094	089	005	006	084	072	1222	664
115[illegible]	[illegible]	[illegible]	020	072	086	080	017	021	092	077	1229	657
11612	019	022	025	075	094	088	011	012	087	082	1210	658
11712	016	017	023	060	065	078	003	013	077	057	1042	681
11812	025	031	037	111	158	163	021	030	143	126	1358	687
11912	036	036	036	091	137	129	071	024	117	108	1307	688
12012	023	033	039	103	155	163	029	033	151	138	1324	688
12112	015	037	043	087	177	123	031	029	132	117	1372	686
12212	019	055	074	189	166	156	031	033	135	114	1383	686
12312	024	024	032	106	153	145	039	048	167	136	1354	687
12412	018	026	027	032	131	110	031	035	129	104	1396	686
12512	017	027	026	114	140	116	025	028	142	128	1291	682
12622	018	029	032	099	135	124	018	021	121	098	1321	652
12722	013	027	027	110	149	157	019	023	148	132	1416	646
12822	021	024	021	079	118	125	021	021	124	109	1370	641
12922	020	040	042	147	210	210	011	010	095	060	1464	644

13022	025	020	023	085	117	117	011	017	107	093	1342	646
13122	011	042	042	148	229	229	019	022	212	169	1517	643
13222	003	021	027	128	129	129	018	022	145	109	1346	683
13322	026	038	036	232	213	213	034	041	210	183	1397	651
13432	020	041	050	176	206	190	020	020	179	134	1405	661
13532	026	028	027	152	187	184	027	039	233	190	1524	646
13632	023	025	029	097	138	139	019	019	174	092	1359	643
13732	011	023	026	122	178	180	022	020	180	132	1510	640
13832	003	021	026	127	182	190	019	022	180	132	1521	640
13932	012	014	023	135	208	227	026	026	183	140	1613	639
14032	012	026	025	090	122	123	034	027	170	087	1340	648
14132	028	034	039	165	219	212	037	042	210	161	1503	647
14213	001	004	018	100	165	103	023	013	157	139	1566	631
14313	029	031	067	155	171	160	023	020	147	121	1214	682
14413	019	039	031	056	150	159	030	030	134	116	1382	636
14513	023	029	026	081	113	115	022	016	118	105	1291	649
14613	008	020	028	080	088	084	042	026	095	076	1142	675
14713	013	033	037	098	152	120	028	037	146	133	1339	648
14813	008	026	040	011	122	116	009	015	132	112	1202	646
14913	074	028	038	088	122	128	031	042	131	104	1218	658
15013	018	055	025	070	118	160	019	040	155	105	1324	642
15113	023	033	055	139	165	102	020	022	141	109	1261	669
15213	023	023	028	085	103	183	018	020	101	090	1297	654
15323	031	036	029	099	186	156	008	008	159	131	1503	635
15423	023	019	023	101	142	168	000	005	155	142	1435	649
15523	015	003	010	058	128	061	009	020	135	115	1104	658
15623	027	074	084	136	168	158	069	070	151	143	1115	685

15723 082 082 085 136 163 163 101 095 168 144 1090 690

15823 024 029 024 083 146 153 003 004 143 129 1435 636

15923 019 016 027 105 159 154 026 020 157 128 1447 641

16023 023 025 023 078 125 123 022 023 112 131 1374 640

16123 016 023 023 086 127 113 008 006 114 096 1466 628

16223 014 029 029 088 119 117 012 031 117 103 1286 652

16333 017 027 025 082 120 122 027 029 125 101 1286 645

16433 017 022 013 057 097 102 015 017 120 094 1401 631

16533 022 024 018 068 133 140 019 024 139 102 1463 630

16633 026 028 030 145 179 122 016 014 167 129 1476 649

16733 029 033 042 104 150 149 028 031 139 104 1295 654

16833 029 034 032 093 154 160 025 032 156 123 1386 643

16933 013 009 009 055 098 088 019 015 088 073 1496 624

17033 042 048 068 101 108 091 044 021 093 060 1400 639

Raw data for myofibril ATPase in Plantaris

17111 049 066 090 375 473 487 063 034 394 216 165 653

17211 055 086 095 364 419 400 025 058 400 262 158 661

17311 043 050 054 423 587 585 045 026 559 418 188 639

17411 059 064 072 557 618 614 049 035 620 393 184 652

17511 060 063 074 436 520 520 057 036 499 267 175 650

17611 040 041 048 354 452 460 034 017 461 286 179 642

17711 051 062 072 418 511 511 058 036 507 329 175 649

17821 050 057 118 391 450 438 089 045 465 307 155 665

17921 051 068 078 361 460 450 060 028 462 347 168 650

18021 079 074 083 295 429 440 069 038 407 245 163 648

18121 052 060 082 413 466 491 059 031 487 325 167 657

18221 033 033 082 499 603 465 037 020 607 467 179 649

18321	056	059	064	402	442	613	085	017	427	328	171	656
18421	038	046	053	446	535	548	039	021	524	367	184	645
18531	069	078	121	408	541	523	073	038	534	302	164	655
18631	049	077	114	381	439	407	029	059	426	246	155	665
18731	122	164	172	513	574	559	122	068	567	424	156	671
18831	139	185	198	445	440	452	093	046	419	335	098	658
18931	083	102	137	410	455	464	089	041	447	287	151	671
19031	039	072	089	347	478	470	078	041	505	331	166	649
19131	032	038	046	342	498	527	039	026	491	274	185	637
19212	073	088	095	571	612	648	072	043	620	408	176	660
19312	035	051	078	370	444	435	074	032	307	390	166	654
19412	060	071	073	376	567	573	062	030	533	258	179	642
19512	076	072	078	320	591	638	085	053	684	530	180	638
19612	080	096	099	409	476	518	083	034	496	377	163	659
19712	078	070	086	334	457	466	081	048	527	365	165	650
19812	052	047	053	385	466	486	052	023	535	318	178	645
19912	069	082	089	494	543	581	075	035	535	380	172	658
20012	091	106	112	337	564	573	101	084	576	398	168	646
20122	156	168	178	457	481	524	174	088	518	319	146	685
20222	050	050	064	408	552	511	047	078	537	403	181	642
20322	134	138	174	384	437	429	172	083	452	335	141	678
20422	050	051	067	320	495	470	063	038	490	396	175	641
20522	063	056	071	330	425	406	080	037	455	313	166	649
20622	054	062	079	391	464	427	067	032	408	302	167	655
20722	032	064	092	423	516	523	058	031	467	381	169	653
20822	030	040	182	320	402	394	033	007	397	203	134	677
20932	033	028	027	295	414	412	021	008	448	256	189	633

21032 145 158 143 351 431 468 124 064 435 259 152 663

21132 075 098 123 505 612 612 093 058 585 469 170 656

21232 048 047 048 283 404 439 053 019 405 277 174 640

21332 045 050 058 353 459 438 047 021 476 329 175 645

21432 037 054 054 218 428 445 046 029 429 253 174 635

21532 038 044 050 325 471 484 038 015 455 319 180 639

21632 058 058 061 323 478 476 046 027 467 306 176 641

217[illegible] 058 062 077 526 624 648 056 032 626 420 183 649

21813 039 050 055 399 536 543 043 027 535 357 184 641

21913 047 046 058 620 660 673 040 025 579 517 192 652

22013 033 058 069 556 582 586 070 028 516 378 182 659

22113 069 075 090 548 633 671 067 029 611 443 179 653

22213 024 031 043 511 527 534 025 017 528 478 189 656

22313 029 030 042 511 509 520 022 019 524 411 164 679

22413 024 037 048 497 493 518 022 012 513 388 148 683

22523 042 049 055 527 557 571 043 026 553 385 186 654

22623 027 036 082 506 511 530 029 014 510 342 172 676

22723 021 023 032 524 512 524 031 010 534 402 140 675

22823 066 071 097 437 486 502 076 034 428 193 165 661

22923 037 052 062 451 560 563 044 025 552 337 183 645

23023 028 037 054 347 448 447 036 014 434 274 176 644

23123 037 046 059 285 447 456 027 011 409 267 174 640

23223 083 046 083 339 429 439 069 046 429 302 163 653

Raw data for Myofibril ATPase of Soleus

23311 013 013 011 115 191 187 009 003 189 149 175 624

23411 016 018 023 104 168 177 016 018 174 145 152 636

23511 010 013 013 115 142 145 011 010 145 105 158 637

23611	016	014	017	098	141	149	014	014	112	106	151	636
23711	024	023	021	112	156	159	021	014	162	119	150	639
23811	015	010	020	109	138	131	018	014	161	125	146	644
23911	015	015	018	109	154	149	016	010	174	137	154	637
24021	023	022	024	134	196	209	020	016	205	170	157	637
24121	014	015	019	147	195	191	018	011	199	158	162	637
24221	018	028	018	120	164	171	025	020	169	139	156	637
24321	023	019	019	105	152	166	014	009	156	120	151	637
24421	002	010	010	073	144	146	014	009	140	105	165	621
24521	013	017	023	113	158	170	016	014	172	122	149	641
24621	015	009	018	129	181	179	020	016	177	133	161	635
24721	010	017	015	111	169	176	009	005	174	148	162	631
24831	012	012	015	145	190	189	014	014	191	148	168	634
24931	008	011	010	112	175	175	013	009	186	136	173	624
25031	016	014	016	082	148	159	010	008	151	116	155	629
25131	009	010	012	080	121	126	009	008	121	092	152	631
25231	020	018	019	090	160	[illegible]	014	014	168	129	154	631
25331	016	016	019	079	156	156	021	015	163	128	153	629
25431	024	029	025	123	156	138	021	021	150	124	146	646
25531	012	015	015	113	145	142	015	007	139	135	155	638
25612	010	012	019	119	151	147	018	017	143	118	151	642
25712	030	025	050	140	140	145	021	016	129	107	120	615
25812	023	022	024	122	190	199	017	013	187	144	152	639
25912	028	016	034	098	166	178	040	018	199	175	141	642
26012	019	029	025	146	175	143	021	007	157	128	151	647
26112	011	014	017	101	171	175	014	014	186	126	160	630
26212	021	023	035	163	224	224	014	010	222	176	168	636

26312	024	023	026	180	220	231	032	025	180	169	152	649
26412	023	023	017	175	215	216	023	014	214	175	160	644
26522	013	010	013	102	163	159	010	009	154	139	164	628
26622	022	021	023	135	164	166	017	016	168	132	150	646
26722	011	016	008	124	188	196	012	006	217	171	135	652
26822	014	014	014	060	138	153	014	012	147	111	159	637
26922	017	013	021	125	203	196	022	023	202	176	163	632
27022	017	014	011	145	166	146	006	006	142	147	169	637
27122	003	009	011	140	208	216	012	008	203	164	179	625
27222	019	020	021	134	175	185	014	014	180	136	155	640
27332	015	013	016	104	168	175	015	019	181	147	160	630
27432	010	016	021	113	171	182	014	013	183	142	154	636
27532	019	016	020	104	184	186	016	013	165	147	159	630
27632	034	030	027	103	161	157	028	029	154	141	145	640
27732	016	017	018	099	181	192	016	010	185	142	161	629
27832	018	016	021	099	162	165	010	007	165	130	152	634
27932	019	017	018	078	161	163	023	017	169	127	156	627
28032	010	022	029	141	201	206	016	014	201	157	154	641
28113	018	020	027	115	148	148	020	015	104	123	141	648
28213	067	087	108	164	191	194	070	046	190	153	119	691
28313	041	044	059	144	177	167	038	027	169	139	134	662
28413	019	016	024	089	122	122	016	015	124	102	109	682
28513	025	026	038	119	142	138	027	025	151	130	127	662
28613	008	012	024	129	153	154	013	011	152	124	146	649
28713	010	014	020	135	170	171	017	015	176	139	155	641
28813	024	028	038	167	188	193	021	020	212	169	143	659

Initial Control Data on hearts

.01	.063	.065	.095	.083	.095	.096	.088
.10	.090	.096	.101	.083	.101	.095	.090
1.0	.143	.163	.146	.120	.146	.163	.143
10.0	.181	.211	.168	.145	.168	.211	.181
100	.181	.211	.171	.145	.171	.211	.181
X	.063	.065	.073	.060	.073	.065	.063

Appendix F- Tables of Mg and EGTA conditions

Varying mm Mg^{2+} levels in cardiac muscle

Training	0.04 mm	S.E.M.	1.0 mm	S.E.M.	10.0 mm	S.E.M.
Three Weeks	C .143	.012	.147	.011	.113	.009
Training	P .154	.011	.156	.010	.119	.006
	H .142	.018	.168	.030	.116	.016
Six Weeks	C .121	.009	.130	.011	.105	.008
Training	P .145a	.017	.163a	.018	.119a	.015
	H .189a	.008	.180a	.013	.134a	.014
Nine Weeks	C .132	.006	.134	.009	.110	.005
Training	P .141	.007	.146	.007	.126a	.006
	H .128	.010	.130	.010	.098	.007

(C-control P-progressive H-high intensity and a denotes significance)

ATPase activity at 10 um Ca^{2+} with

varying mm Mg^{2+} levels in soleus muscle

Training	0.04 mm	S.E.M.	1.0 mm	S.E.M.	10.0 mm	S.E.M.
Three Weeks	C .159	.010	.156	.008	.126	.007
Training	P .174	.008	.170	.007	.137	.008
	H .159	.009	.156	.008	.126	.006
Six Weeks	C .179	.011	.179	.011	.146	.010
Training	P .177	.011	.175	.009	.147	.008
	H .175	.006	.173	.005	.141	.004
Nine Weeks	C .166	.010	.161	.009	.135	.007
Training	P .190	.015	.185	.014	.150	.012
	H .195	.013	.188	.012	.152	.010

(C-control P-progressive H-high intensity and a denotes significance)

ATPase activity at 10 um Ca^{2+} with

varying mm Mg^{2+} levels in plantaris muscle

Training		0.04 mm	S.E.M.	1.0 mm	S.E.M.	10.0 mm	S.E.M.
Three Weeks	C	.507	.033	.511	.035	.329	.032
Training	P	.455	.088	.484	.063	.325	.081
	H	.484	.021	.489	.020	.314	.022
Six Weeks	C	.535	.042	.524	.023	.380	.029
Training	P	.465	.019	.471	.019	.331	.027
	H	.462	.021	.462	.025	.308	.027
Nine Weeks	C	.554	.017	.571	.024	.424	.020
Training	P	.481	.023	.494	.019	.313	.026
	H	0	0	0	0	0	0

(C-control P-progressive H-high intensity and a denotes significance)

ATPase activity with 5 mm EGTA and

varying mm Mg^{2+} levels in cardiac muscle

Training		0.04 mm	S.E.M.	1.0 mm	S.E.M.	10.0 mm	S.E.M.
Three Weeks	C	.035	.012	.027	.005	.024	.003
Training	P	.035	.005	.032	.006	.035	.002
	H	.022	.005	.022	.002	.023	.004
Six Weeks	C	.026	.006	.019	.006	.026	.004
Training	P	.019	.003	.017	.003	.022	.003
	H	.025	.003	.017	.003	.027	.003
Nine Weeks	C	.024	.003	.022	.006	.025	.008
Training	P	.026	.011	.027	.007	.028	.010
	H	.024	.003	.024	.009	.023	.003

(C-control P-progressive H-high intensity and a denotes significance)

ATPase activity with 5 mm EGTA and

varying mm Mg^{2+} levels in soleus muscle

Training	0.04 mm	S.E.M.	1.0 mm	S.E.M.	10[illegible] mm	S.E.M.
Three Weeks	C .015	.004	.016	.004	.012	.004
Training	P .017	.002	.015	.003	.013	.002
	H .014	.005	.015	.006	.013	.005
Six Weeks	C .022	.009	.021	.007	.015	.005
Training	P .013	.005	.014	.006	.012	.006
	H .017	.006	.018	.008	.015	.007
Nine Weeks	C .028	.019	.026	.019	.028	.008
Training	P .020	.011	.020	.007	.014	.010
	H .016	.003	.018	.009	.013	.003

(C-control P-progressive H-high intensity and a denotes significance)

ATPase activity with 5 mm EGTA and

varying mm Mg^{2+} levels in plantaris muscle

Training		0.04 mm	S.E.M.	1.0 mm	S.E.M.	10.0 mm	S.E.M.
Three Weeks	C	.058	.010	.051	.004	.036	.005
Training	P	.063	.018	.051	.006	.031	.012
	H	.075	.032	.076	.041	.046	.005
Six Weeks	C	.076	.005	.068	.006	.043	.006
Training	P	.087	.021	.071	.018	.049	.011
	H	.058	.013	.060	.014	.030	.008
Nine Weeks	C	.043	.019	.040	.006	.024	.007

Appendix G- Statistical Summary Tables

Anthropometric data summary tables for 1-way ANOVA

Three Week Groups

Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	1617.1	808.6	8.0	.0017
Within Groups	28	2815.7	100.6		
Total	30	4432.8			

Heart Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	99847.6	49923.8	26.5	.0000
Within Groups	28	52719.4	1882.8		
Total	30	152566.9			

Ventricular Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	66302.9	33151.4	21.2	.0000
Within Groups	28	43859.5	1566.4		
Total	30	110162.4			

Right Ventricular Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	1220.5	610.3	2.2	.1254
Within Groups	28	7634.3	272.7		
Total	30	8854.8			

Left Ventricular Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	48891.2	24445.6	34.1	.0000
Within Groups	28	20060.7	716.5		
Total	30	68951.9			

Heart weight/Body weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	2.54	1.27	27.9	.0000
Within Groups	87	2815.7	100.6		
Total	30	4432.8			

Vent Weight/Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.0058	.0029	6.88	.0037
Within Groups	28	.012	.0040		
Total	30	.0178			

Right Vent/Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.0021	.0010	2.63	.0897
Within Groups	28	.0111	.0039		
Total	30	.0132			

Left Vent/Heart

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.0058	.0029	6.88	.0037
Within Groups	28	.0012	.0004		
Total	30	.018			

Left Vent/Right Vent

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	1.41	.707	4.05	.0286
Within Groups	28	4.89	.175		
Total	30	6.31			

Six Week Groups

Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	2522	1261	6.44	.0052
Within Groups	27	5283	195.7		
Total	29	7805			

Heart Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	158420	79210	15.1	.0000
Within Groups	27	142100	5263		
Total	29	300520			

Ventricular Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	88362	44181	9.73	.0007
Within Groups	27	122540	4538		
Total	29	210900			

Right Vent Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	2429	1214	1.63	.2153
Within Groups	27	20161	747		
Total	29	22590			

Left Vent Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	60605	30302	14.4	.0001
Within Groups	27	56963	2110		
Total	29	117570			

Heart Weight/Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	1.13	.567	14.7	.0000
Within Groups	27	1.04	.039		
Total	29	2.18			

Vent Weight/Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.384	.192	6.69	.0044
Within Groups	27	.774	.029		
Total	29	1.16			

Left Vent/Heart Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.015	.073	5.74	.0084
Within Groups	27	.0342	.013		
Total	29	.049			

Right Vent/Heart Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.0021	.0011	2.11	.1412
Within Groups	27	.0137	.0051		
Total	29	.016			

Left Vent/Right Vent

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.8600	.4298	2.19	.1316
Within Groups	27	5.30	.197		
Total	29	6.16			

<u>Nine Week Groups</u>

Nine Week Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	8645	4323	6.76	.0043
Within Groups	26	16618	639		
Total	28	25264			

Heart Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	81158	40579	9.58	.0008
Within Groups	26	110110	4235		
Total	28	191260			

Vent Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	78382	39191	13.2	.0001
Within Groups	26	77286	2973		
Total	28	155670			

Right Vent Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	3537	1768	2.90	.073
Within Groups	26	15839	609.2		
Total	28	19375			

Left Vent Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	41966	20983	14.02	.0001
Within Groups	26	38907	1496		
Total	28	80873			

Heart Weight/ Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.9560	.4778	6.44	.0053
Within Groups	26	1.93	.074		
Total	28	2.884			

Vent Weight/Body Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.7793	.3870	7.56	.0026
Within Groups	26	1.33	.052		
Total	28	2.105			

Left Vent/Heart Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.0181	.095	8.75	.0012
Within Groups	26	.027	.0103		
Total	28	.0450			

Right Vent/Heart Weight

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	.013	.0064	.977	.3900
Within Groups	26	.017	.0065		
Total	28	.0183			

Left Vent/Right Vent

Source	D.F.	Sum Squares	Mean Squares	F-ratio	F-prob
Between Groups	2	4.13	2.07	2.46	.1048
Within Groups	26	21.8	.8385		
Total	28	25.93			

<u>Statistical analysis on heart ATPase and regulation</u>

Three way anova on heart

ATPase with differing pCa

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
A	0.014	2	0.007	2.91	0.061
B	0.025	2	0.013	5.13	0.008
AB	0.011	4	0.003	1.14	0.343
S-within	0.174	71	0.002		
C	0.975	4	0.244	628.3	0.001
AC	0.009	8	0.001	2.88	0.004
BC	0.012	8	0.001	3.86	0.001
ABC	0.012	16	0.001	1.97	0.015
CS-within	0.110	284	.000		

A-group, B-time, C-ATPase

Three way anova on heart ATPase with differing Magnesium levels and no Calcium

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
A	0.000	2	0.000	0.494	0.612
B	0.001	2	0.001	1.40	0.254
AB	0.001	4	0.000	0.773	0.547
S-within	0.032	71	0.000		
C	0.000	2	0.000	2.09	0.128
AC	0.000	4	0.000	0.828	0.509
BC	0.000	4	0.000	1.11	0.355
ABC	0.000	8	0.000	0.523	0.837
CS-within	0.014	142	.000		

A-group, B-time, C-ATPase

Three way anova on heart ATPase

with differing Magnesium levels

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
A	0.012	2	0.006	2.67	0.076
B	0.010	2	0.005	2.15	0.054
AB	0.022	4	0.006	2.45	0.054
S-within	0.162	71	0.002		
C	0.050	2	0.025	140.6	0.001
AC	0.002	4	0.001	2.86	0.026
BC	0.002	4	0.000	2.62	0.038
ABC	0.004	8	0.000	2.74	0.008
CS-within	0.025	142	.000		

A-group, B-time, C-ATPase

Two way anova on Hill n values in heart

Source	Sum Squares	Mean Squares	D.F.	F-ratio	F-prob
Mean	128.2	128.2	1	9179.9	0.000
Time	0.06	0.03	2	2.48	0.091
Treat	0.20	0.10	2	7.37	0.001
Time/Treat	0.11	0.02	4	1.99	0.105
Res	0.93	0.01	67	0.000	0.000

Two way anova on pCa50 values in heart

Source	SumSquares	Mean Squares	D.F.	F-ratio	F-prob
Mean	3247	3247	1	151194	0.000
Time	0.28	0.14	2	6.72	0.002
Treat	0.24	0.12	2	5.81	0.0046
Time/Treat	0.21	0.05	4	2.52	0.049
Res	1.46	0.02	68	0.000	0.000

Statistical analysis on soleus ATPase and regulation

Three way anova on soleus

ATPase with differing pCa

Source	**Sum Squares**	**D.F.**	**Mean Squares**	**F-ratio**	
A	0.001	2	0.001	0.450	0.639
B	0.018	2	0.009	6.61	0.002
AB	0.006	4	0.001	1.09	0.371
S-within	0.087	65	0.001		
C	1.73	4	0.433	1766	0.001
AC	0.006	8	0.001	3.00	0.003
BC	0.005	8	0.001	2.56	0.011
ABC	0.008	16	0.000	1.96	0.016
CS-within	0.064	260	0.000		

A-group, B-time, C-ATPase

Three way anova on soleus ATPase with

differing Magnesium and no Calcium

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
A	0.001	2	0.000	2.17	0.123
B	0.001	2	0.001	2.85	0.065
AB	0.001	4	0.000	1.017	0.406
S-within	0.013	65	0.000		
C	0.001	2	0.000	25.3	0.001
AC	0.000	4	0.000	0.837	0.504
BC	0.000	4	0.000	0.515	0.725
ABC	0.000	8	0.000	0.614	0.765
CS-within	0.002	130	.000		

A-group, B-time, C-ATPase

Three way anova on soleus ATPase

with differing Magnesium levels

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
A	0.004	2	0.002	1.13	0.331
B	0.013	2	0.006	3.25	0.046
AB	0.009	4	0.002	1.16	0.339
S-within	0.130	65	0.002		
C	0.054	2	0.027	311.5	0.001
AC	0.000	4	0.000	0.817	0.516
BC	0.000	4	0.000	0.444	0.777
ABC	0.001	8	0.000	1.06	0.397
CS-within	0.011	130	.000		

A-group, B-time, C-ATPase

Two way anova on Hill n of soleus muscle

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
Main effects	0.003	4	0.001	5.17	0.001
Group	0.002	2	0.001	6.63	0.002
Time	0.001	2	0.000	3.88	0.03
Grp/Time	0.001	4	0.000	2.44	0.055
Explained	0.004	8	0.000	3.81	0.001
Res	0.008	65	0.000		
Total	0.012	73	0.000		

Two way anova on pCa50 of soleus muscle

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
Main effects	0.429	4	0.107	9.82	0.000
Group	0.140	2	0.070	6.41	0.003
Time	0.296	2	0.148	13.58	0.000
Grp/Time	0.107	4	0.027	2.45	0.055
Explained	0.536	8	0.067	6.14	0.000
Res	0.710	65	0.011		
Total	1.25	73	0.017		

Statistics on plantaris ATPase and regulation.

Three way anova on plantaris

ATPase with differing pCa

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
Mean	21.59	1	21.59	2760	0.000
Group	0.019	2	0.009	1.20	0.311
Time	0.006	1	0.006	.83	0.368
GT	0.028	2	0.014	1.81	0.176
S-within	0.313	40	0.008		
Cal	8.05	4	2.01	1066	0.000
CG	0.044	8	0.006	2.93	0.0044
CT	0.012	4	0.002	1.52	.1983
CGT	0.015	8	0.0019	0.98	0.4537
CS-within	0.302	160	.002		

G-group, T-time, C-calcium

Three way anova on plantaris ATPase with differing Magnesium levels and no Calcium

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
Mean	0.434	1	0.434	227.2	0.000
Group	0.001tab 3>2		0.000	0.17	0.840
Time	0.002	1	0.002	1.10	0.299
GT	0.009	2	0.005	2.53	0.092
S-within	0.076	40	0.002		
EGTA	0.022	2	0.011	51.1	0.000
EG	0.001	4	0.0003	1.42	0.2401
ET	0.0004	2	0.0002	0.84	0.4349
EGT	0.0005	4	0.0001	0.59	0.6716
CS-within	0.018	80	.0002		

G-group, T-time, E-EGTA

Three way anova on plantaris ATPase

with differing Magnesium

Source	Sum Squares	D.F.	Mean Squares	F-ratio	
Mean	26.0	1	26.0	2280	0.000
Group	0.038	2	0.019	1.66	0.2033
Time	0.0005	1	0.0005	0.04	0.839
GT	0.026	2	0.013	1.14	0.3308
S-within	0.456	40	0.011		
Mag	0.755	2	0.378	334	0.000
MG	0.005	4	0.0013	1.15	0.3380
MT	0.004	2	0.002	1.90	.1566
MGT	0.003	4	0.0008	0.68	0.6103
CS-within	0.090	80	.001		

G-group, T-time, M-magnesium

Two way anova on 9 week plantaris calcium

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
Mean	8.83	1	8.83	1186	0.000
Group	0.05	1	0.05	6.81	0.021
Error	0.104	14	0.007		
Calcium	4.15	4	1.04	658.2	0.000
CG	0.04	4	0.009	5.66	0.0007
Error	0.088	56	0.002		

Two way anova on 9 week plantaris EGTA

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
Mean	0.06	1	0.06	90.4	0.000
Group	0.00001	1	0.00001	0.01	0.906
Error	0.0097	14	0.0007		
EGTA	0.0041	2	0.002	30.9	0.000
EG	0.000003	4	0.00001	0.22	0.806
Error	0.0019	28	0.00007		

Two way anova on 9 week plantaris magnesium

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
Mean	10.7	1	10.7	1383	0.000
Group	0.09	1	0.09	11.7	0.004
Error	0.108	14	0.0078		
Mag	0.026	2	0.13	121.1	0.000
MG	0.004	2	0.002	1.64	0.2112
Error	0.030	28	0.001		

Two way anova on Hill n of plantaris muscle

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
Main effects	0.001	3	0.000	0.807	0.496
Group	0.001	2	0.000	1.28	0.307
Time	0.000	1	0.000	0.009	0.923
Grp/Time	0.001	2	0.000	1.23	0.301
Explained	0.001	5	0.000	0.975	0.441
Res	0.014	56	0.000		
Total	0.015	61	0.000		

Two way anova on pCa50 of plantaris muscle

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
Main effects	0.046	3	0.15	1.03	0.387
Group	0.036	2	0.018	1.22	0.303
Time	0.009	1	0.009	0.58	0.451
Grp/Time	0.067	2	0.034	2.27	0.113
Explained	0.113	5	0.023	1.52	0.197
Res	0.830	56	0.015		
Total	0.943	61	0.015		