1990

An examination of the force-interval relationship in chronic diabetic rat papillary muscle.

Tina Ann. McComb
University of Windsor

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

AN EXAMINATION OF THE FORCE-INTERVAL RELATIONSHIP IN CHRONIC DIABETIC RAT PAPILLARY MUSCLE

by

Tina Ann McComb

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

Windsor, Ontario, Canada

1990

ISBN 0-315-61868-X

ACP-1890

ABSTRACT

The purpose of the present study was to fully characterize the force-interval relationship of the right ventricular papillary muscle from chronic diabetic rats, and, to more closely examine the alterations in the beta phase reported previously. To examine the beta phase alterations two manipulations were utilized during a 121 second rest period; 1. the transmembrane Na^+ gradient was reduced by 40% to promote calcium influx during rest and therefore investigate the role of the Na^+/Ca^{2+} exchanger in force development, and 2. extracellular Ca^{2+} was reduced from 1.0 mM to 0.3 mM during rest to limit calcium availability and determine the role of calcium load on force development.

The condition of chronic diabetes resulted in several alterations in the force-interval relationship, primarily in the beta phase of force development. Alterations in the percent contribution of beta to the total amplitude and the rate and time constants of the beta phase were identified and suggest alterations in the capacity of the diabetic tissue to transport calcium through the exchange compartment of the sarcolemma to the sarcoplasmic reticulum release compartment.

Reduction of the transmembrane Na^+ gradient by 40% resulted in an improvement in force development of the

chronic diabetic suggesting a limitation of the chronic diabetic tissue to allow calcium influx through Na^{+}/Ca^{2+} exchange during rest that may be associated with the depression in the beta phase reported.

A decrease in extracellular calcium during rest resulted in a reduction in force development ($p<0.05$) in the diabetic suggesting that calcium availability is reduced and that calcium overload is not a factor in producing the depression in the beta phase reported.

DEDICATION

I wish to dedicate this thesis to the memory of my late grandfather, William Potter. There have been so many special times in my life that I wish I could have shared with him, this is but one.

ACKNOWLEDGEMENTS

The completion of any major project is rarely achieved without the support and expertise of a number of significant contributors. It is with this in mind that I would like to express my sincere gratitude to those who have helped me realize my goal.

Dr. Kenji Kenno, to whom I owe a great deal of thanks, without his sincere interest in his students I may not have pursued graduate studies. As an advisor and a friend he has helped me to develop both academic and personal skills by providing the guidance, opportunity and interest so important to the completion of such a project.

To Dr. Paul Taylor who provided valuable assistance throughout the course of my studies. His kindness, expertise and undying enthusiasm will always be remembered.

To Dr. Ray Hermiston, who has been an important contributor to my education as a thesis committee member, teacher, advisor and friend.

To my lab partners and friends, Jim Gamble and Mark Kontulainen and to my close friend Ruth Bell, whose assistance, understanding, and encouragement have made the road to completion both shorter and more enjoyable.

To my parents, Dave and Nancy McComb, without the importance of education instilled in me at a young age, the opportunity and encouragement to succeed and the faith shown in my efforts, this would never have been possible.

To my sister, Janine, whose support, friendship and positive outlook have been a never ending source of inspiration. Every graduate student could use a sister like her.

To my brother, Dave, who in his own way has been a source of motivation.

To grandma Potter and grandma and grandpa McComb who have given nothing but praise and encouragement, I give a special thanks.

Finally, to Michel who has been there for me through it all. He has been a teacher, a supporter, a critic, an editor, a listener, a counsellor, coach and cheerleader, he was and is everything to me.

(And of course, to Nimika)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION

The condition of diabetes mellitus is associated with an increased incidence of cardiac dysfunction and a higher than average mortality rate as a result of heart failure (Pierce et al., 1988). Clinical reports of cardiac function in diabetic patients have shown abnormalities in myocardial shortening, left ventricular ejection and the temporal components related to both contraction and relaxation (Pierce et al., 1988).

Experimentally, cardiac function has been examined in diabetic animal models where diabetes is induced by the injection of either streptozotocin or alloxan, two chemicals which function to destroy the insulin secreting beta cells of the pancreas.

Laboratory investigations of whole heart preparations from chronic diabetic animals have shown decreases in: cardiac output; coronary flow; stroke volume; aortic flow; left ventricular pressure development; and maximum positive and negative rates of pressure development (Penpargkul et al., 1980, Vadlamudi et al., 1982).

Studies of isolated papillary muscle preparations have also reported alterations in cardiac muscle performance with chronic diabetes including: a decreased rate of force development and relaxation and, an increased time to peak shortening, time for 50% relaxation and time

for relaxation (Fein et al., 1980).

In an attempt to identify the mechanisms underlying these alterations, investigators have examined the subcellular systems primarily responsible for force development; the sarcolemma, the sarcoplasmic reticulum and the contractile proteins.

Biochemical investigations of fragmented sarcolemma from chronic diabetic rat hearts have identified lesions in the Ca^{2+} ATPase pump (Heyliger et al., 1987), the Na^{+}/Ca^{2+} exchanger (Makino et al., 1987), and the Na^{+}/K^{+} ATPase pump (Pierce and Dhalla,1983, Ku and Sellers, 1982). The depressions in the Ca^{2+} ATPase pump and the Na^{+}/Ca^{2+} exchanger, both of which function to lower intracellular levels of free calcium following contraction, may contribute to the mechanical alterations in relaxation that have been reported with chronic diabetes. The lesion in the Na^{+}/K^{+} ATPase pump reported with chronic diabetes may indirectly influence the process of relaxation by creating a reduction in the transmembrane Na^{+} gradient thus altering the functional capacity of the Na^{+}/Ca^{2+} exchanger.

Lopaschuk et al., (1983) and Ganguly et al., (1983) have identified a depression in both the calcium uptake ability and the ATPase activity of the ATP dependent sarcoplasmic reticulum (SR) Ca^{2+} pump in SR microsomes

isolated from chronic diabetic rat hearts. Due to the role of this pump in calcium sequestration during the process of relaxation, these studies suggest that the depression seen in the function of the SR Ca^{2+} ATPase pump with chronic diabetes, may also contribute to the reported mechanical alterations in relaxation.

Finally, a physiological examination of the contractile proteins has revealed a decrease in the ATPase activity associated with the myosin head and a shift in the distribution of myosin isozymes with chronic diabetes. In normal rat cardiac muscle, the V_1 myosin form possessing high ATPase activity is predominant with the low ATPase activity, V_3 form, accounting for only a small fraction of the total (Dillman, 1980). With chronic diabetes however, a reversal of the normal state is seen with the V_3 form becoming predominant and the V_1 form contributing only a small percentage to the total (Dillman, 1980, Garber and Neely, 1983). This shift in predominance to a slower ATPase activity associated with the myosin head may in part account for the decrease in rate and the increase in time of contraction of chronic diabetic cardiac muscle.

Biochemical and physiological studies provide evidence to suggest that isolated mechanisms are responsible for the mechanical alterations reported with

chronic diabetes. These studies do not, however, attempt to explain the complex interactions of the various subcellular systems associated with force development. Such an approach becomes necessary in applying these findings to a working, physiological system. In an attempt to examine the interactions of these systems, investigators have made use of the force-interval relationship and associated calcium compartment modelling to gain insight into the intracellular handling of free calcium in and between the subcellular systems involved in excitation-contraction coupling.

The force-interval relationship in cardiac muscle suggests that with the regulatory factors of performance held constant ie. preload, afterload and contractile state, if a muscle of fixed length is stimulated to contract at various rates and rhythms, the developed force is not constant but reflects a sensitivity of the muscle to the interval between contractions (Seed and Walker, 1988). This sensitivity is thought to represent an intrinsic property of the muscle and reflects the time dependent handling of intracellular free calcium.

The processes of calcium handling that occur between contractions have most commonly been described with the use of a calcium compartment model. The most recent model of calcium handling, developed by Schouten (1985, 1987)

for rat trabeculae muscle, is comprised of three compartments; 1. a release compartment that releases the activator calcium required for myofilament activation; 2. an uptake compartment that sequesters calcium, aiding in relaxation, both of these compartments are presumably located in the sarcoplasmic reticulum; and 3. an exchange compartment located in the sarcolemma that is related to the slow calcium movements between the extracellular space and the release compartment, Fig.1.

The exchange mechanism is believed to be composed of the Na^+/Ca^{2+} exchanger and the Na^+/K^+ ATPase pump (Schouten, 1985). The movements of calcium in and between these compartments is thought to occur in a time dependent manner and directly influences force development.

A typical force-interval curve is depicted in Fig.2. This curve reveals two force generating phases termed alpha and beta respectively, and a third phase of force loss termed gamma (Schouten,1985).

The initial phase, alpha, seen at short time intervals of 1-10 seconds, is thought to represent the time dependent transfer of calcium from the uptake to the release compartment of the sarcoplasmic reticulum.

The second phase, beta, expressed at longer time intervals of 10-100 seconds, is thought to represent the movement of extracellular calcium through a sarcolemma

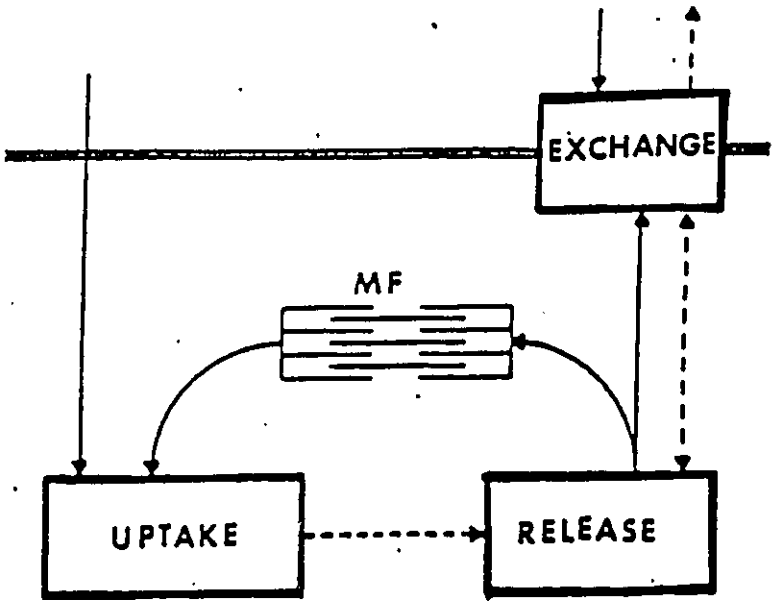

Figure 1: The calcium compartment model (modified from Schouten, 1985)

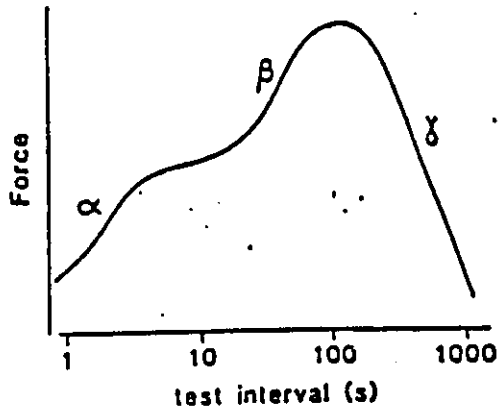

Figure 2: Typical force-interval curve (Schouten, 1985)

exchange compartment and subsequent accumulation within the SR release compartment.

Finally, the third phase, gamma, at time intervals greater than approximately 100 seconds, is thought to represent loss of calcium from the release compartment of the SR and subsequent removal from the cell through the exchange compartment.

A preliminary investigation of the force generating phases of the force-interval relationship in chronic diabetic rat papillary muscle has identified the following relationship, Fig. 3.

Analysis of the force-interval curves of both the control and the chronic diabetic rat papillary muscles reveals two distinct phases of force recovery, similar to that previously reported in the literature for control (Schouten, 1985, Johannsson and Asgrimsson, 1989, Taylor et al., 1989) and hypertrophied hearts (Taylor et al., 1989).

Results of this study have not identified an alteration in the alpha phase of the force-interval curve with chronic diabetes, suggesting that the time-dependent uptake and transfer of calcium to the release compartment is not compromised.

A significant depression in the beta phase of the force-interval curve was however reported at all time

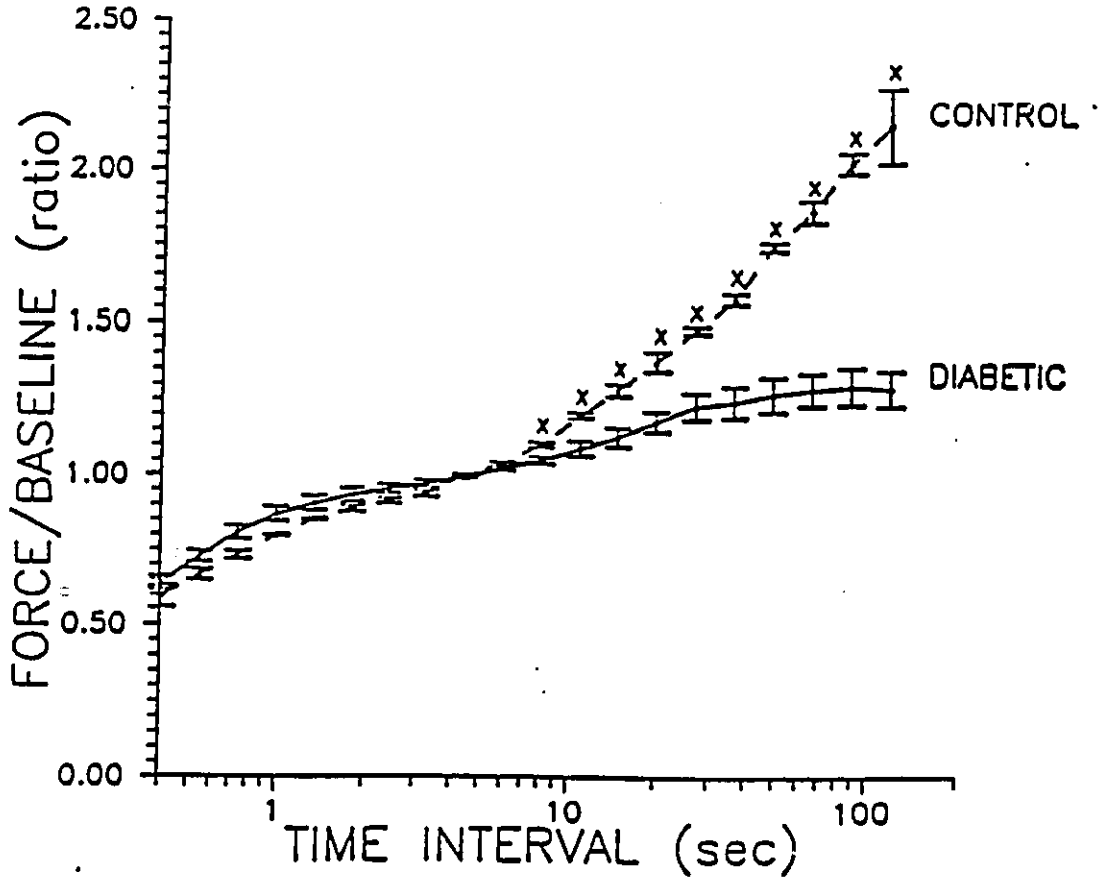

Figure 3: Force-interval curve in control (broken) and chronic diabetic (solid) papillary muscle, 1.0 mM Ca^{2+}. Values represent mean ± S.E. of 5 animals, x $p < 0.05$ (McComb, 1990).

intervals of 8.13 seconds and greater ($p<0.05$). Based on the calcium compartment modelling of Schouten (1985), an alteration in the beta phase of force development would suggest that the movement of calcium through the exchange compartment and/or the accumulation within the release compartment is altered with chronic diabetes.

Biochemical examinations of chronic diabetic rat hearts have revealed lesions in both of the components thought to comprise the exchange compartment, ie. the Na^+/K^+ ATPase pump (Pierce and Dhalla, 1983, Ku and Sellers, 1982) and the Na^+/Ca^{2+} exchanger (Makino et al., 1987).

The depression in the Na^+/K^+ ATPase pump activity reported with chronic diabetes (Ku and Sellers, 1982, Pierce and Dhalla, 1983) may exert a profound effect on intracellular cation homeostasis. A reduction in the function of this pump, normally acting to extrude intracellular sodium while facilitating the entry of extracellular potassium, would allow an accumulation of sodium within the cell and thereby reduce the transmembrane gradient for sodium.

A reduction in the membrane gradient for sodium would have a significant influence on Na^+/Ca^{2+} exchange activity as the Na^+/Ca^{2+} exchanger is driven by the electromotive force for Na^+ moving down its concentration gradient. The

consequences of this Na^+ gradient reduction in relation to Na^+/Ca^{2+} exchange activity are twofold.

Firstly, a reduction in the sodium gradient has been reported to shift the bidirectional Na^+/Ca^{2+} exchanger to significantly favour calcium entry during the action potential (Bers, 1987). Several investigators have provided evidence for enhanced calcium influx under these conditions and have suggested that functioning of the exchanger in this manner contributes to the positive inotropic effect of sodium gradient reduction (Sonn and Lee, 1988, ter Keurs et al., 1987, Bers, 1987, Sutko et al., 1986, Im and Lee, 1984). Few studies have focused on the possible contribution of the Na^+/Ca^{2+} exchanger to calcium entry during rest, however, Walford et al. (1984) have reported Ca^{2+} influx, measured as an increase in resting force and scattered light fluctuations, in unstimulated rat ventricular muscle under conditions of Na^+ gradient reduction. Shattock and Bers (1989) have provided additional evidence, with use of extracellular Ca^{2+} selective microelectrodes, that cessation of stimulation in rat ventricular muscle results in loss of calcium from the extracellular space indicating calcium influx via Na^+/Ca^{2+} exchange, that may be enhanced with Na^+ gradient reduction. Bers et al., (1989) recently reported that this calcium entry during diastolic

intervals contributes to the rest-induced gain in SR calcium seen with rapid cooling contractures, and accounts for the phenomenon of rest potentiation reported by several investigators (Bers, 1989, Taylor et al., 1989, Schouten, 1985, Ragnarsdottir et al., 1982).

Secondly, a reduction in the electromotive force for Na^+ entering the cell will reduce the calcium extrusion potential of the Na^+/Ca^{2+} exchanger. Additionally, calcium extrusion via Na^+/Ca^{2+} exchange has been reported to be reduced with chronic diabetes as a result of alterations in the phospholipid microenvironment surrounding the exchanger (Makino et al., 1987).

Both of the above conditions, ie. enhanced calcium entry and depressed calcium removal may contribute to an accumulation of intracellular calcium. Several investigators have suggested that the depression in cardiac performance with chronic diabetes was associated with a condition of intracellular calcium overload (Dhalla et al., 1985, Makino et al., 1987, Ganguly et al., 1987, Afzal et al., 1988, Tani and Neely, 1988). The accumulation of intracellular calcium as a result of the above two processes associated with the Na^+/Ca^{2+} exchanger, in addition to the depressed sequestration of calcium by the sarcoplasmic reticulum Ca^{2+} ATPase pump (Lopaschuk et al., 1983) and depressed extrusion of

calcium by the sarcolemmal Ca^{2+} ATPase pump (Heyliger et al., 1987), lead to a condition of calcium overload which may have a profound effect on performance.

Of particular interest in the present investigation is the extent to which these processes may compromise force development during the beta phase of the force-interval curve. According to the calcium compartment modelling of Schouten (1985) calcium entry from the extracellular space through the exchange compartment contributes to the releasable calcium store within the SR and subsequently influences force development. As a result of the reduced sodium gradient and associated influences on the Na^{+}/Ca^{2+} exchanger as outlined above with chronic diabetes, calcium entry from the extracellular space during the beta phase of the force-interval relationship may contribute to the condition of calcium overload already presumed present. Consequently, the depression reported in the beta phase of the force-interval relationship with chronic diabetes may be the result of a condition of calcium overload further precipitated by the entry of calcium through the exchange compartment of the sarcolemma at longer rest intervals.

Ideally, in order to investigate the nature of the involvement of the Na^{+}/Ca^{2+} exchanger in contributing to the depression in the beta phase of the force-interval relation with chronic diabetes, the effect of Na^{+}/Ca^{2+}

exchange stimulation to promote calcium removal and minimize the condition of calcium overload would be desired. However, at present there does not appear to be any satisfactory means by which to stimulate the exchanger in an isolated muscle preparation. An alteration in the ion composition of the buffer to favour Na^+/Ca^{2+} exchange, ie. increased extracellular sodium, does not appear to stimulate Na^+/Ca^{2+} exchange and promote calcium extrusion (Sonn and Lee, 1988). Additionally, there does not appear to be a chemical agent specific enough to stimulate Na^+/Ca^{2+} exchange activity promoting calcium extrusion.

As stimulation of the Na^+/Ca^{2+} exchanger does not appear to be a feasible means of examining the beta phase at the present time, a further reduction in the Na^+ gradient, favouring calcium entry via Na^+/Ca^{2+} exchange, may provide an indication of the contribution of this mechanism to the depression previously reported in the beta phase of the force-interval curve with chronic diabetes.

Additionally, limiting extracellular calcium and therefore the calcium available for entry through the exchange compartment during the beta phase, may provide insight into the contribution of intracellular calcium overload to the depression previously reported during long periods of rest in the chronic diabetic rat.

Therefore, due to the biochemical and physiological evidence from chronic diabetic rat hearts supporting lesions in calcium regulating proteins, as well as results of the preliminary investigation reporting a depression in the beta phase of the force-interval curve with chronic diabetes, the intent of this investigation was to : 1. fully characterize the force-interval curve of the chronic diabetic rat heart and 2. examine calcium movement through the Na^+/Ca^{2+} exchanger as a mechanism contributing to the depression in the beta phase of the force-interval curve reported with chronic diabetes.

The specific objectives of this investigation were to:

1. fully characterize the force-interval curves of control and chronic diabetic papillary muscles in 1.0 mM Ca^{2+} with use of mathematical modelling;

2. examine the role of the Na^+/Ca^{2+} exchanger, through Na^+ gradient reduction, in contributing to the depression in the beta phase of force development with chronic diabetes;

3. examine, through extracellular calcium reduction, the possible role of intracellular calcium overload in contributing to the depression in the beta phase of force development with chronic diabetes.

METHODS

Diabetic model

Male Wistar rats (175-200g) (Charles River, Que.), were made diabetic with a single tail vein injection of streptozotocin (65 mg/kg, Sigma) dissolved in freshly prepared 0.05 M citrate buffer, pH 4.5. Control and diabetic animals were housed individually in a temperature, 23 degrees Celsius, and light controlled room, on a 12 hour light/dark cycle, according to the standards of the Canadian Council for Animal Care. Animals were provided with Purina rat chow and tap water ad libitum. All animals were sacrificed between 30 and 45 days post injection. Diabetes was assessed periodically with Ketodiastix (Ames) testing of urine glucose. Quantitatively, diabetes was assessed with Analox LM3 glucose analysis of blood samples taken from the inferior vena cava at the time of sacrifice. Animals were considered diabetic when blood glucose values were >300mg/100ml. As well, a qualitative examination gave initial indications of diabetic state, ie. polydipsia, polyphagia, polyuria and loss or maintenance of body weight.

Animals were randomly assigned to one of two groups 1. control, 2. chronic diabetic; and one of three experimental conditions, 1. 1.0mM Ca^{2+}, 2. 1.0mM Ca^{2+}/40%

Na^+ reduction and 3. 0.3 mM Ca^{2+}.

Muscle Preparation and Mounting

To prevent blood clotting, ether anaesthetized rats were injected with 107 units of heparin (Sigma) per kilogram of body weight into the inferior vena cava. Hearts were then rapidly excised and the aorta was cannulated horizontally in a dissecting bath. Hearts were then perfused via the Langendorff technique at a flow rate of 10-15ml/min for a period of approximately five minutes to wash out all residual blood. The perfusate utilized for this procedure was a modified Krebs-Hensleit bicarbonate buffer (KHB) with the following salt concentrations (mM): NaCl 117.1, KCl 5.0, $MgCl_2.6H_2O$ 1.2, Na_2SO_4 1.2, $NaH_2PO_4.2H_2O$ 2.0, $NaHCO_3$ 27.0, Dextrose 10.0 and $CaCl_2$ 1.0 mM. The perfusate was equilibrated with 95% O_2 and 5% CO_2 at a temperature of 26 degrees Celsius, pH 7.4. Following wash out, to facilitate dissection, the contractions of the heart were stopped by raising the K^+ concentration to 15 mM. Thin right ventricular papillary muscles (<0.5 mm^2) were isolated and removed under a dissecting microscope according to a modified isolation procedure for right ventricular trabeculae as described by ter Keurs et al. (1980). The papillary muscle was then mounted horizontally in a muscle bath, perfused with a

KHB, composition as above. Temperature was monitored throughout the experiment by means of a thermistor located within the muscle bath. The ventricular end of the muscle was restrained in a cradle apparatus on a micromanipulator for length adjustment, and the valvular, tendinous end was placed over a tungsten wire hook suspended on a strain-gauge force transducer (Grass FT03) Figure 4.

After mounting, the muscle length was slowly adjusted to Lmax, ie. the length at which maximum force production is achieved (Jewell,1977). The muscle was electrically paced by passing current from a stimulator through a pair of platinum wire electrodes placed near the ventricular end of the tissue. The frequency of stimulation was set at 1.0 Hz with a stimulus pulse of 4 msec in duration. Voltage of stimulation was gradually increased from zero until threshhold was determined at which point the voltage was increased an additional 50% above the threshhold. Stimulation was regulated via an on-line computer system. The preparation was allowed to equilibrate under these conditions for at least one hour. A viable preparation was characterized as having stable tension development and absence of spontaneous contractions. On average, 70% of all preparations, excluding those hearts without suitable papillary muscles, were viable preparations. After this equilibration time, Lmax was checked and

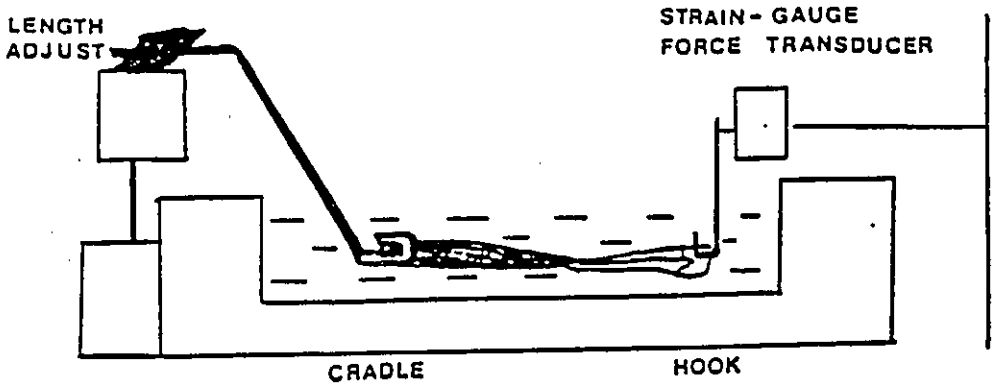

Figure 4: Experimental muscle bath

readjusted if necessary. Following equilibration, a series of ten to fifteen function curves was collected to evaluate the basic twitch parameters of the muscle under the conditions of the experiment.

Force-Interval Data Collection

Muscles were paced at a control frequency of 1.0 Hz during equilibration to increase the stability of the preparation and were paced at 0.2 Hz throughout the experimental period. Interpolated into the control pacing was a series of test intervals which included; a predetermined period of rest (absence of electrical stimulation) followed by the resumption of control pacing (Figure 5).

The beat immediately following the test interval, the test contraction, was utilized in the construction of the force-interval curve. The test intervals consisted of the following timing delays: 0.40, 0.54, 0.73, 0.99, 1.34, 1.81, 2.44, 3.30, 4.46, 6.02, 8.13, 10.97, 14.81, 20.00, 27.00, 36.45, 49.21, 66.44, 89.70, 121.00, and 130.00 seconds. These intervals were chosen, based on a logarithmic scale, to include a sufficient number of data points to represent the entire ascending limb of the force-interval curve. The test contraction, as well as several beats following, were stored on floppy disk for

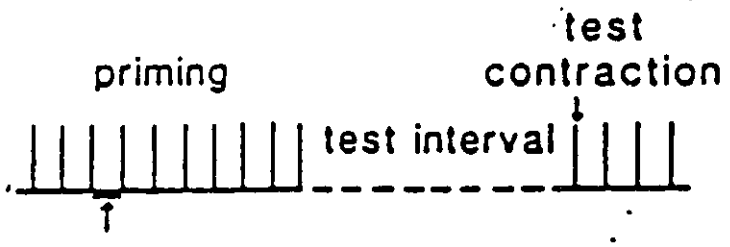

Figure 5: Pacing protocol (Schouten, 1985)

further analysis of the force curves.

Mechanical Analysis

Force signals produced by contraction of the muscle were transmitted from the force transducer to a Grass preamplifier (model #7P122D) with Grass regulated power supply (Model RPS 107E) where they were amplified according to a preset sensitivity. The force signals were converted to digital form by a 12 bit A/D converter. The signal was displayed for visual confirmation of the contraction pattern on a BK Precision 20 MHz Oscilloscope (Model 2120A). Data were collected at a sampling rate of 200 Hz to 1kHz and each force curve contained 256 data points. Data were stored on floppy disk and force curves were analyzed for the basic twitch parameters.

This analysis included evaluation of the peak force, time to peak force and time for relaxation and the rates of force development (+dF/dt) and force loss (-dF/dt). Force values were normalized and expressed as milliNewtons (mN) force per cross sectional area (mm^2). Length, width and depth of the muscle were measured with use of a reticle in the eye piece of a dissecting microscope.

In the construction of a force-interval curve, developed force of the test contraction was expressed as F/B, where F is the force of the first beat following the

test interval and B is the baseline force of the beat immediately preceding the test interval. The data were expressed in this manner to account for any alterations in resting tension that may have occurred over the course of the experiment. Further analysis of the dynamic interactions made use of a mathematically developed curve fitting program which represents the developed forces achieved for the various test intervals. This mathematical model allowed the examination of the specific exponential characteristics of the force-interval curve ie. the biphasic response representing the two processes alpha and beta. The mathematical model employs a least squares analysis with a steepest descent method to arrive at the specific parameter of fit. The model uses a linear combination of terms in the general form, $A_i * (1-\exp(-a_i t))$ where A_i is the amplitude or force parameter, a_i is the inverse time constant of the particular process, and t is the length of the test interval. Thus in analysis of the force-interval relationship, two terms were utilized to evaluate the two simultaneously occurring phases and were represented as following:

$$f(t) = F_{end} * (1-M* \exp(-at)) - (1-M)*\exp(-bt))$$

where $F_{end} = A_1 + A_2$ (phase 1-- alpha + phase 2--beta), and $M = A_1/(A_1 + A_2)$ or more simply, the contribution of

the alpha phase to the total curve (Taylor et al., 1989).

Beta Phase Data Collection

To examine the beta phase of the force-interval relation specifically, a single time point was utilized to represent the processes occurring during the beta phase. A period of rest of 121 seconds was chosen as it represents maximum force development during the beta phase.

To examine the involvement of the Na^+/Ca^{2+} exchanger, a component of the exchange compartment (Schouten, 1985), the Na^+ gradient was reduced during the 121 second rest interval to facilitate calcium entry during this period. Sodium gradient reduction was achieved, with little or no change in the resting membrane potential (Sheu and Fozzard, 1982), by replacing the buffer surrounding the muscle with a modified KHB, NaCl reduced by 40% to 70.26 mM. In order to maintain osmolarity between the control and NaCl reduced buffers, LiCl, 46.84 mM, was used as a replacement for the NaCl omitted. The control buffer was resumed following the rest interval.

In a second set of experiments to evaluate the role of intracellular Ca^{2+} overload in the beta phase, during the test interval of 121 seconds, the control buffer was replaced with a KHB of low Ca^{2+}, 0.3 mM to minimize calcium entry through the exchange compartment, and

therefore minimize calcium overload.

Design and Analysis

The independent variables in this study were:

1. group
2. experimental condition

The dependent variable in this study was:

1. force development in papillary muscle of control and chronic diabetic rats at control and rest intervals.

Independent T-tests were used where appropriate and analysis of variance of a 2 X 2 design was utilized for both beta phase experiments to determine if significant differences existed. A Tukey's HSD post hoc test was utilized to identify the location of any statistically significant differences.

RESULTS

Animal Characteristics

The condition of chronic diabetes was confirmed in this study by several parameters. The experimental animals exhibited polydipsia, polyuria, polyphagia and diarrhea, all positive indicators of the condition of diabetes mellitus. Additionally, frequent testing of urine glucose provided support that chronic diabetes had been achieved.

Quantitative measures of body weight, blood glucose, right and left ventricular weights and heart weight/body weight ratios for the experimental groups, both control and chronic diabetic (Appendix A), are reported in Table 1 and provide evidence that the condition of chronic diabetes was achieved.

Twitch Characteristics

Analysis of the basic twitch parameters of right ventricular papillary muscles of both control and chronic diabetic groups (Appendix B) reveals a number of significant differences between the two groups, Table 2. The differences reported with this study are consistent with those reported in the literature for chronic diabetic papillary muscle (Fein et al., 1980) and further verify that the condition of chronic diabetes had been achieved.

Table 1

Characteristics of control and chronic diabetic rats

Parameter	Control	Diabetic
Body Wt. (gm)	393±10	241±21*
Blood Glucose (mg/dL)	154±8	521.7±23*
RV Wt. (gm)	0.12±.004	0.08±.009*
LV Wt. (gm)	0.84±.03	0.63±.05*
Ht. Wt./Body Wt. Ratio	2.40±.07	2.97±.09*

Values are represented as means ± S.E. of 5 animals,
* $p < .05$

Table 2

Twitch characteristics of control and chronic diabetic rat papillary muscle

PARAMETER	CONTROL	DIABETIC
Force (mN/mm^2)	14.02±3.6	6.05±0.65*
+ dF/dt ($mN/sec/mm^2$)	230.51±53.8	82.66±16.1*
- dF/dt ($mN/sec/mm^2$)	194.25±48.8	55.34±9.04*
Time to Peak Force (msec)	96.07±3.17	142.51±5.34*
Time for Relaxation (msec)	158.87±10.6	268.11±15.2*

Values are represented as means ± S.E. of 5 animals, * $p < 0.05$.

Force-Interval Data

The force-interval curves, fitted from raw data (Appendix C), of control and chronic diabetic rat papillary muscles are seen in Figure 6 and as previously reported (McComb, 1990), show two phases of force generation, termed alpha and beta.

To further characterize the force-interval curves, the percentage contribution, maximum rate and time constant of each phase was mathematically calculated and are presented in Table 3.

The condition of chronic diabetes resulted in an increase in the alpha phase contribution to the total amplitude of 29.6% with a concomitant decrease in the beta phase contribution. These alterations are clearly illustrated in Figure 7 where the total curve and each of the isolated phases, alpha and beta, are depicted on a single graph for each experimental group.

The maximum rates of both the alpha and beta phases were slower following chronic diabetes when compared to the rates calculated for the control papillary muscles.

There appeared to be no change in the time constant (time to reach 66% of the amplitude) of the alpha phase of the chronic diabetic group, however, the beta phase time constant of the diabetic group was faster owing to the greatly reduced amplitude of this phase.

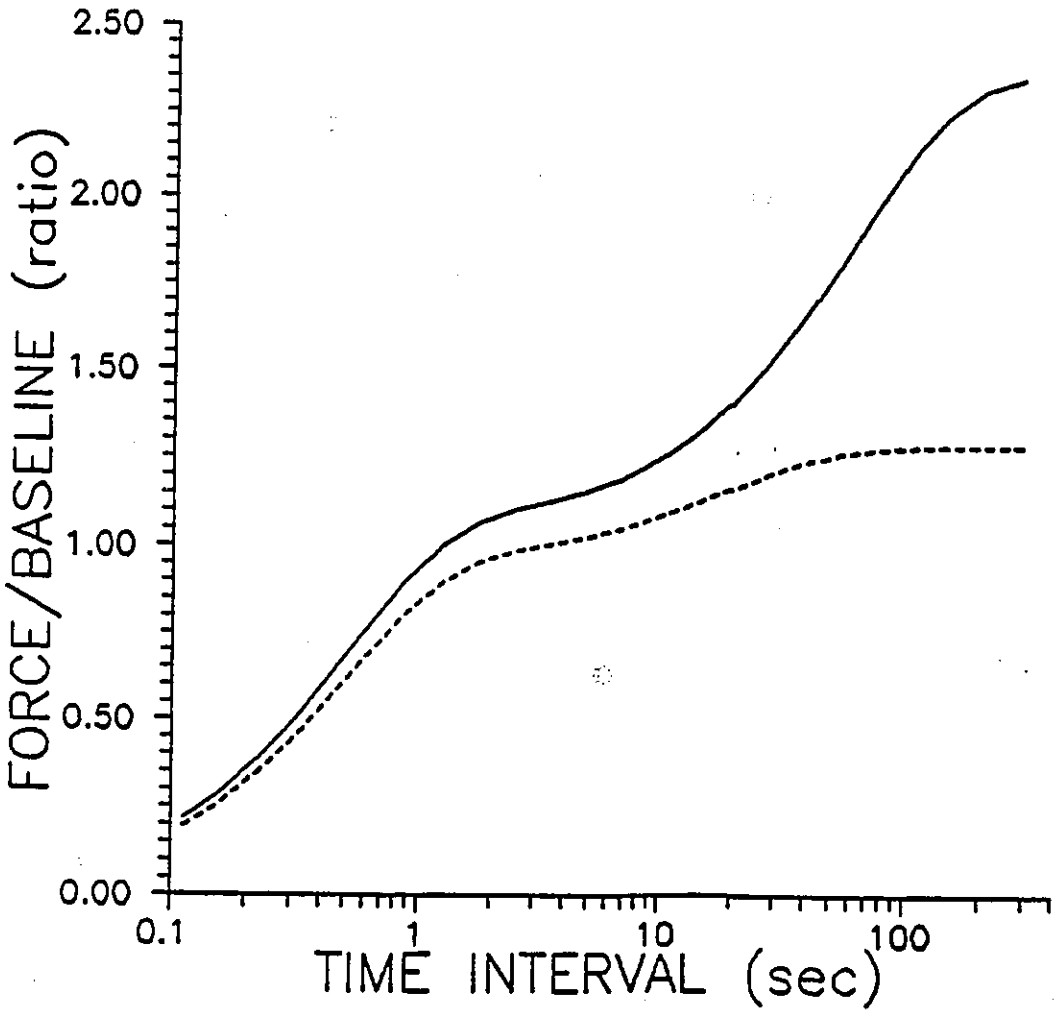

Figure 6: Force-interval curves of control (solid) and chronic diabetic (broken) papillary muscles, fitted data, n = 5.

Table 3

Mathematical analysis of the force-interval curves of control and chronic diabetic papillary muscles

PARAMETER		CONTROL	DIABETIC
Percent Contribution (%)	alpha	44.98	74.59
	beta	55.02	25.41
Maximum Rate (units/msec)	alpha	1750.00	1572.40
	beta	17.90	12.27
Time Constant (sec)	alpha	0.50	0.49
	beta	56.13	19.32

Data fitted from raw data of 5 animals/group.

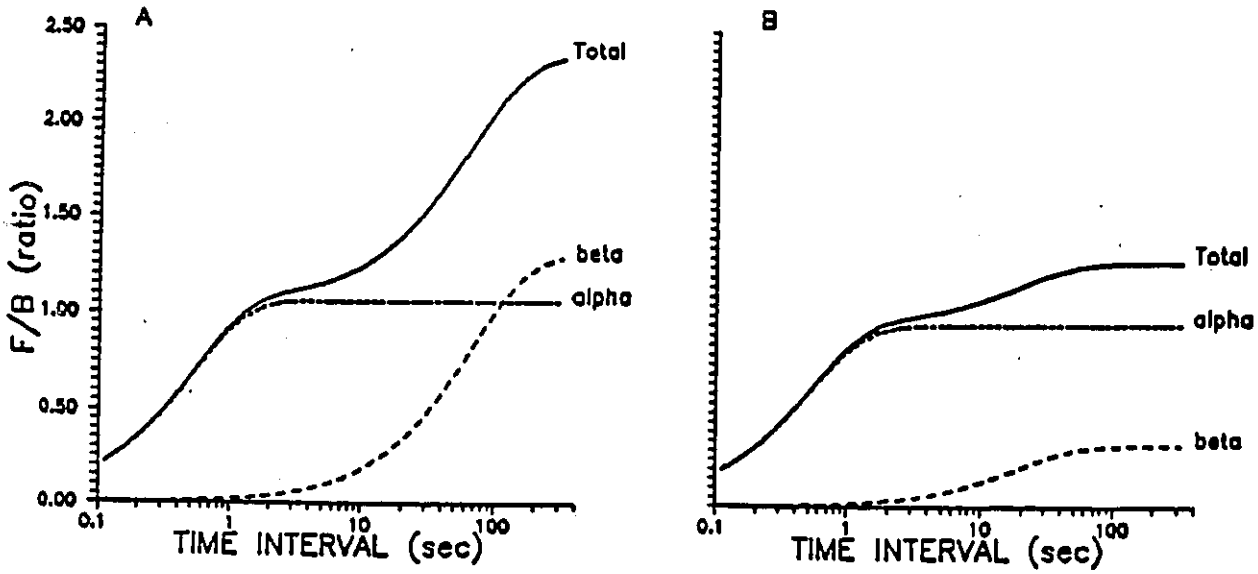

Figure 7: Force-interval curves of fitted data from control (A) and chronic diabetic (B) papillary muscles with isolation of the alpha and beta phases, n = 5.

Decay of Potentiation

Decay of potentiation was examined in an attempt to estimate the fraction of recirculating calcium as well as to gain further insight into the processes regulating calcium movement in the chronic diabetic. The control group required greater than 5 beats following the first potentiated beat to return to steady state levels, where the diabetic group had reached steady state in as few as 3 beats (Appendix G). Schouten (1985) has suggested that the accuracy of determination of the recirculation fraction is dependent upon a high degree of force development and complete decay of potentiation in 5-10 beats. The recirculation fraction in control muscles was estimated to be 60%, similar to that reported in the literature (Ragnarsdottir et al., 1982, Schouten, 1985). I was however, unable to make an accurate assessment of the recirculation fraction in the chronic diabetic group due to the limited number of beats required to return to steady state and the low degree of force development following the rest period.

Beta Phase Analysis

1. 40% Na^+ Reduction

In an attempt to examine the role of the Na^+/Ca^{2+} exchanger in the dramatic shift in the beta phase of

force generation with chronic diabetes the extracellular sodium concentration was decreased from 117.1 mM to 70.9 mM during the rest interval of 121 seconds.

The control papillary muscle showed a 129% increase ($p<.05$) (Appendices H and I) in force development when the extracellular buffer was replaced during the 121 second rest interval. An improvement in force generation was also seen in the diabetic group, however, statistical significance was not reached and the degree of change was much less pronounced with only a 47% increase in force development (Figure 8).

2. 0.3 mM Ca^{2+}

A second set of experiments to examine the role that extracellular calcium might play in the shift of the beta phase of the force-interval relationship, the calcium concentration of the KHB was reduced from 1.0 mM to 0.3 mM Ca^{2+} during the rest period of 121 seconds. The results indicated a 95% decrease ($p<.05$)(Appendices H and I) in force development of the control papillary muscle group but only a 44% decrease ($p<.05$)(Appendices H and I) with chronic diabetic preparations (Figure 9).

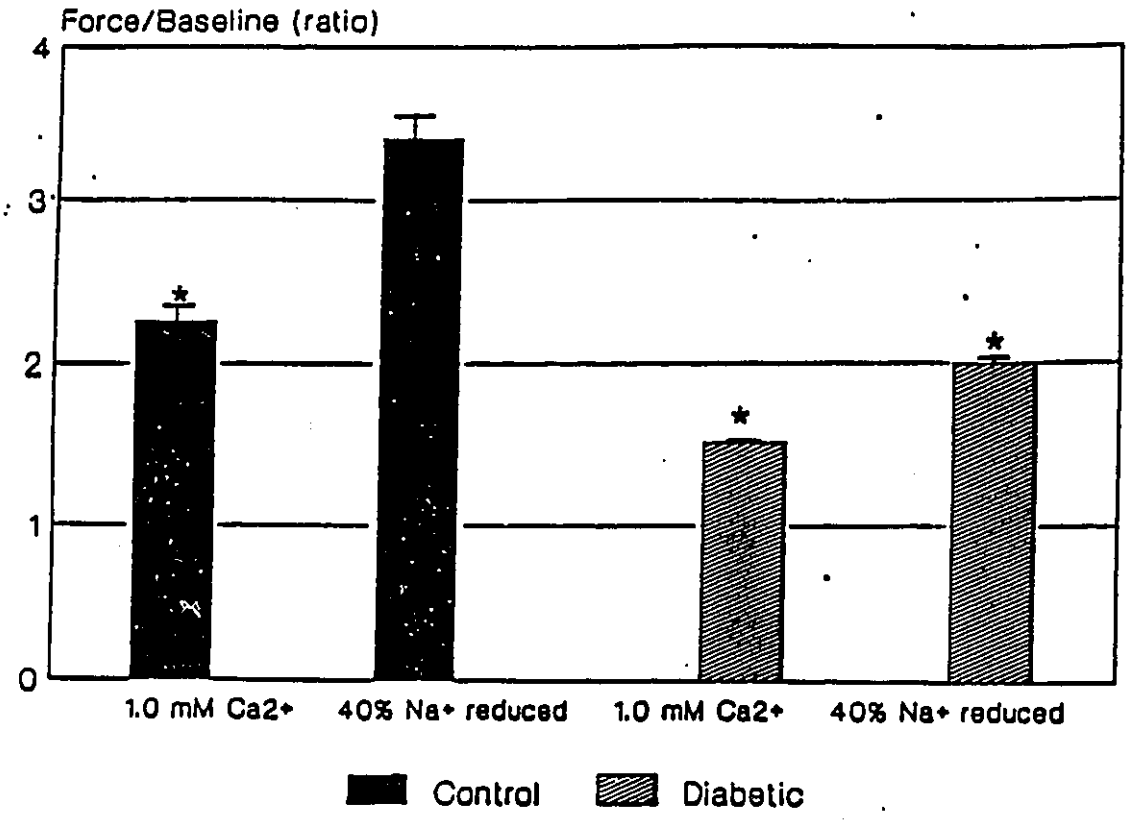

Figure 8: Influence of a 40% Na^+ reduction on the beta phase of the force-interval curve of control and chronic diabetic papillary muscles, * $p < 0.05$, significantly different than 40% reduction in control, test interval = 121 seconds, n = 5

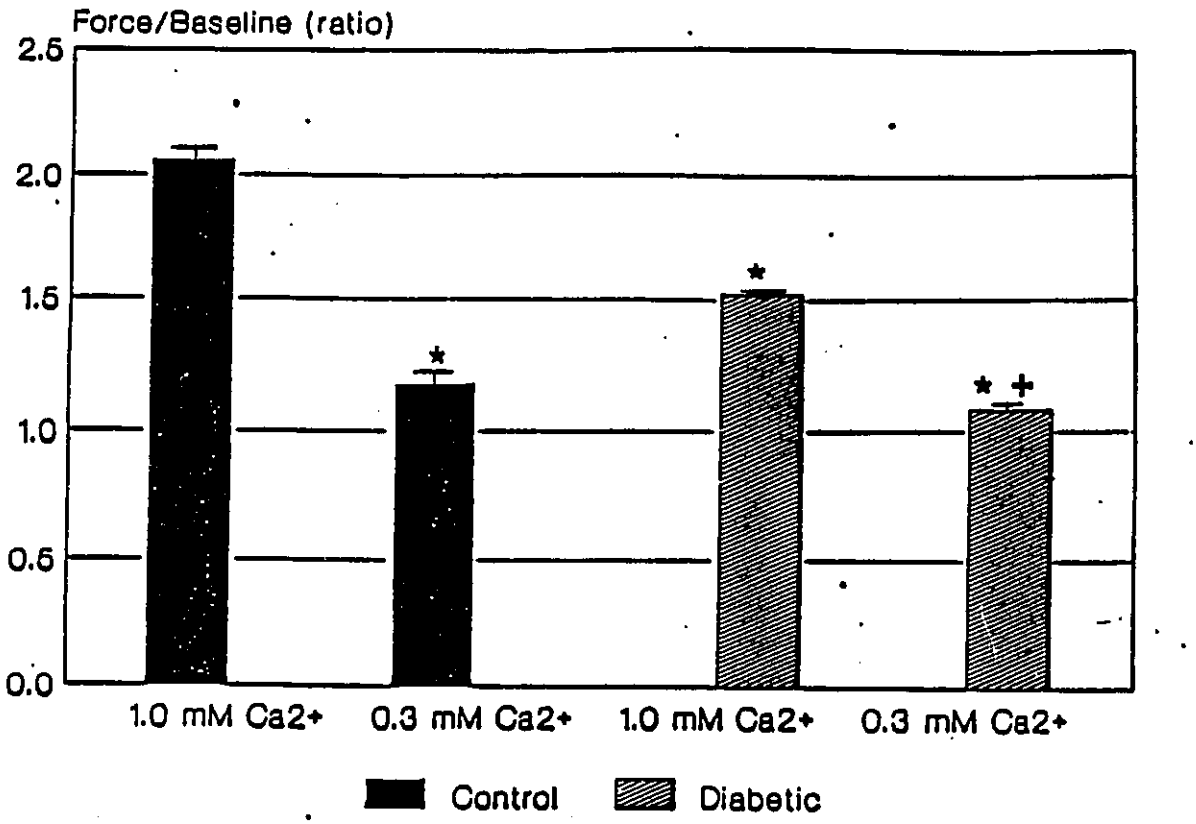

Figure 9: Influence of a 0.3 mM Ca^{2+} on the beta phase of the force-interval curve of control and chronic diabetic papillary muscles, * $p < 0.05$, significantly different than control in 1.0 mM Ca^{2+}, + $p < 0.05$, significantly different than diabetic in 1.0 mM Ca^{2+}, test interval = 121 seconds, n = 5.

DISCUSSION

Investigations of cardiac performance in chronic diabetic rat hearts have identified a number of alterations in the parameters of contraction and relaxation (Fein et al., 1980, McComb, 1990) that have been confirmed in the present study. These alterations in contractile function have been related to the reported functional changes in the sarcolemma (Pierce and Dhalla, 1983, Heyliger et al., 1987, Makino et al., 1987), sarcoplasmic reticulum (Lopaschuk et al., 1983, Ganguly et al., 1983) and contractile proteins (Dillman, 1980, Garber and Neely, 1983) seen with chronic diabetes.

Alterations in these biochemical and physiological components have been examined independently and are therefore unable to account for the complex interactions of the various subcellular systems during the processes of excitation-contraction coupling. In an attempt to examine these complex processes, investigators have made use of the force-interval relationship and associated calcium compartment modelling to examine the dynamic interactions of the subcellular systems involved in a working physiological preparation.

The force-interval relationship in cardiac muscle suggests that the excitation-contraction coupling processes of the muscle are sensitive to the time interval

between contractions and reflect the intracellular handling of free calcium during this period.

An examination of the force-interval relationship in both control and chronic diabetic rat hearts has revealed a biphasic response to varying periods of rest Fig. 6, similar to that reported in the literature for control (Schouten, 1985, Johannsson and Asgrimsson, 1989, Taylor et al., 1989) hypothyroid (Pogessi et al., 1987) and hypertrophied hearts (Taylor et al., 1989).

Mathematical analysis of these curves allows for extensive characterization of the two phases of force generation seen in the force-interval curve. This analysis provides information about the percentage contribution of each phase to the total amplitude of the curve; the maximum rate of each of the phases; and the time constants (time to reach 66%) of the individual phases.

The percent contributions of the alpha and beta phases represent the capacity of each process to transfer Ca^{2+} to the release sites and therefore contribute to force generation. With chronic diabetes, the capacity of the alpha phase to transport calcium between the uptake and release sites increases by 29.6% over the control group. The increase in the alpha phase contribution may however, only be a compensation for the concomitant

decrease seen in the capacity of the beta process to transfer calcium from the exchange compartment to the release sites. This compensation is reflected in the alpha phase Force/Baseline ratios of the control and chronic diabetic papillary muscles, which illustrate that the actual force contribution of the alpha process to the total amplitude of the curve does not change, Figure 7. Furthermore, given that the maximum rate of the alpha phase was reduced only 10%, despite biochemical reports of a depression in the SR Ca^{2+} ATPase pump following chronic diabetes (Lopaschuk et al., 1983), and that the time constant for the alpha phase was not altered, it therefore appears that the alpha process of the force-interval relationship in the chronic diabetic is not appreciably different from that of the control.

The most significant factor responsible for the alteration in the total amplitude of the force-interval curve with chronic diabetes, therefore seems to be the reduction in the beta phase contribution, which represents the movement of extracellular calcium through the exchange compartment and subsequent accumulation within the release compartment.

Specifically, alterations in the exchange compartment with chronic diabetes may limit calcium movement and therefore account for the reduction in the capacity of the

beta phase to contribute to force generation. Indeed, a decrease in the activity of both of the components thought to comprise the exchange compartment, ie. the Na^+/K^+ ATPase pump and the Na^+/Ca^{2+} exchanger, has been reported in biochemical studies of chronic diabetic rats (Pierce and Dhalla, 1983, Ku and Sellers, 1982, Makino et al., 1987).

Examination of the maximum rate of the beta phase revealed a 31% decrease with chronic diabetes. This reduction may reflect alterations in Na^+/Ca^{2+} exchange activity and indicate a reduced movement of calcium through the exchange compartment and accumulation within the release compartment.

Although the maximum rate of the beta phase is reduced with chronic diabetes, analysis of the beta time constant indicates a 71% decrease in the time to reach 66% of maximum force. Since the time constant is dependent upon the maximum amplitude of the curve, as well, as the contribution of the individual phase, this apparent reduction in time reflects the depression seen in both of the parameters noted rather than an accelerated process.

In summary, mathematical analysis of the force-interval curves of control and chronic diabetic rat papillary muscle reveals distinct alterations primarily in the beta phase of force development although minimal

alterations in the alpha phase do appear to exist. This alteration in the beta phase seems to reflect a decrease in both the rate and capacity of the chronic diabetic heart to transport calcium from the sarcolemmal exchange compartment to the sarcoplasmic reticulum release compartment.

Beta Phase Analysis

Given the current modelling of the force-interval relationship (Schouten, 1985, 1987), the depression in the beta phase would suggest an alteration in the ability of the chronic diabetic heart to transport calcium between the exchange and release compartments during the beta time intervals.

It was hypothesized, based on the literature (Afzal et al., 1988, Ganguly et al., 1987, Dhalla et al., 1985), that the depression in the beta phase with chronic diabetes may be associated with intracellular calcium overload. Given that the beta phase is highly dependent upon Na^+/Ca^{2+} exchange activity, specifically a reversal of the Na^+/Ca^{2+} exchanger, it would have been ideal to stimulate efflux of calcium by the Na^+/Ca^{2+} exchanger and therefore lower intracellular calcium levels. However, since no effective and specific means of stimulating the Na^+/Ca^{2+} was available, calcium entry was enhanced by

reducing extracellular Na^+ during the rest interval. The basic premise was, if calcium movement through Na^+/Ca^{2+} exchange in the chronic diabetic was involved in intracellular calcium overload, then an extracellular sodium reduction would further promote calcium entry and/or inhibit Na^+/Ca^{2+} exchange activity resulting in an even further reduction in force development.

Experimentally, several investigators have utilized sodium gradient reduction and have reported a positive inotropic response thought to be associated with enhanced calcium entry via Na^+/Ca^{2+} exchange activity (Sonn and Lee, 1988, Bers, 1987).

My results indicated, as expected, a statistically significant increase in force production in the control muscle. However, with chronic diabetes, although not statistically significant, a physiologically significant increase in force production was also reported, contrary to my original hypothesis.

Therefore, since this severe reduction of extracellular Na^+ (40%) did not further depress force production in the diabetic papillary muscle but slightly improved it, it suggests that intracellular calcium overload is NOT responsible for the depression in the beta phase seen with chronic diabetes. It is interesting to note that in one study examining the $^{45}Ca^{2+}$ content of

isolated cardiac myocytes from chronic diabetic rats a decreased level of intracellular calcium was reported (Horackova and Murphy, 1988).

It therefore appears that a depression in calcium entry via the Na^+/Ca^{2+} exchanger and subsequent accumulation within the SR may account for the beta phase depression reported in this study.

Makino et al., (1987) have reported an attenuation of Na^+ dependent Ca^{2+} uptake in isolated sarcolemmal preparations from chronic diabetic rats. The alteration in Na^+/Ca^{2+} exchange activity that was reported may be related to changes in the phospholipid environment surrounding the exchanger. Interestingly, Pierce et al., (1983) have also reported a significant alteration in the phospholipid composition of diabetic cardiac tissue which may be related to alterations in membrane protein function due to the intimate association between protein regulation and membrane composition.

Although function of the exchanger in the reverse mode, which facilitates calcium entry during prolonged periods of rest, has not been examined in the chronic diabetic, it is possible that an impairment of the exchanger functioning in the reverse mode exists, particularily if the phospholipid environment has an influence on its action. In this case, an alteration in

the reverse mode functioning of the Na^+/Ca^{2+} exchanger would limit Ca^{2+} entry and therefore force development as seen in the beta phase of the force-interval curve.

Therefore, the reduction in the transmembrane gradient for Na^+ may have provided a more favourable environment for the entry of calcium in the diabetic, therefore improving force development.

Although this hypothesis seems possible, it does not support the ultrastructural and biochemical studies suggesting the existence of high intracellular levels of calcium which may influence force development.

An alternative explanation for the depression in the beta phase may involve the examination of the "normal" functioning of the Na^+/Ca^{2+} exchanger during periods of quiescence.

In control rat ventricular muscle, with use of extracellular calcium selective microelectrodes, Shattock and Bers (1989) have reported a loss of extracellular calcium during rest, indicating calcium entry into the cell via Na^+/Ca^{2+} exchange. Calcium entry via the Na^+/Ca^{2+} exchanger is thermodynamically favoured when the resting membrane potential (E_m) exceeds the reversal potential of the exchanger (E_{Na-Ca}). In rat ventricular muscle, following stimulation, an elevation of intracellular Na^+ activity (a^i_{Na}) reduces E_{Na-Ca} to a

point more negative than E_m therefore favouring calcium entry and promoting the gain in SR calcium recently reported with rest (Bers, 1989).

At present, no examination of the Na^+/Ca^{2+} exchanger during rest has been conducted in the chronic diabetic rat. However, the proposed functioning of the exchanger as an influxer during rest may not be appropriate to the conditions of the diabetic tissue. Several investigators have suggested that elevated levels of both Na^+ and Ca^{2+} exist in chronic diabetic rat ventricular muscle (Dhalla et al., 1985, Ganguly et al., 1987, Afzal et al., 1988). The elevation of these ions, particularily calcium may increase E_{Na-Ca} to a more positive potential favouring calcium efflux (Shattock and Bers, 1989, Lagnado and McNaughton, 1990) and/or creating a point of equilibrium rather than favouring calcium influx during rest.

Therefore, with a 40% sodium gradient reduction, one of two processes may explain the increase in force development seen in the chronic diabetic papillary muscle. Firstly, the reduction in the Na^+ gradient may simply limit calcium efflux allowing more calcium to become available for accumulation within the release compartment of the SR.

Secondly, due to the severe reduction of extracellular Na^+, the E_{Na-Ca} may be sufficiently reduced

to a potential more negative than E_m to promote calcium entry during prolonged rest therefore contributing to the releasable store and improving force development.

In any case, the alteration in the Na^+/Ca^{2+} exchanger and its influence on the force-interval relation is of an extremely complex nature. Further investigations into the specific functions of the Na^+/Ca^{2+} exchanger in the state of chronic diabetes must be conducted before any precise conclusions can be reached regarding the exact role of Na^+/Ca^{2+} exchange in the beta phase depression. The Na^+/Ca^{2+} exchanger does however appear to be intimately involved in the depression reported in the beta phase of the force-interval relation with chronic diabetes.

A second experiment designed to further examine the hypothesized condition of calcium overload in the chronic diabetic supported the findings of the first experiment utilizing Na^+ gradient reduction. By reducing extracellular calcium and therefore limiting its entry during the beta phase, an attenutation of force development was seen in both control and chronic diabetic groups. If a condition of calcium overload had been present, it would be expected that limiting the availability of calcium during the rest interval, should ameliorate tension development in the chronic diabetic. Since force generation was not improved with lowering

extracellular calcium, again, it does not seem likely that a condition of calcium overload existed or was responsible for the depression in the beta phase reported with chronic diabetes.

The reduction in force development with decreasing extracellular calcium appears to be simply the result of a lack of calcium availability. It is interesting to note however, that there was a more significant reduction in the control group as opposed to the diabetic group when calcium was reduced during the rest interval.

The sensitivity of chronic diabetic cardiac muscle to its calcium environment has not been clearly elucidated. Bielefeld et al., (1983) have reported an increased sensitivity of isolated perfused hearts from chronic diabetic rats to decreasing levels of extracellular calcium, resulting in a dramatic decrease in pressure development. In contrast however, Tani and Neely (1988) reported a similar reduction in left ventricular pressure development to increasing levels of extracellular calcium. Recently Ramanadham et al. (1990) examined the calcium response of isolated right ventricular strips from chronic diabetic rats and reported an improvement in force development with both increasing and decreasing extracellular calcium levels, indicating a "supersensitivity" of the chronic diabetic to its calcium

load.

The results of the present experiment are in accord with the reports of Ramanadham et al. (1990) suggesting a supersensitivity of the diabetic papillary muscle to extracellular calcium, as a reduction of extracellular Ca^{2+} from 1.0 mM to 0.3 mM during rest indicated a less pronounced influence on force development of the diabetic group.

Clearly, the response of diabetic cardiac tissue to its calcium environment is highly equivocal. Further research must be conducted to arrive at an understanding of the sensitivity of chronic diabetic cardiac muscle to its extracellular calcium load.

While biochemical evidence related to intracellular calcium movement provides an explanation for some of the alterations in the force-interval relationship with chronic diabetes, the contributions of other associated factors, namely sarcomere length, myosin isozyme distribution and hypothyroidism, must not be overlooked.

The dependence of force production on optimal muscle/sarcomere length has been well documented (Schouten, 1985, Kentish, 1986, Jewell, 1977). The significant influence of muscle length on the force-interval relationship has been recently reported in our lab by Gamble (1990) where the degree of force development

following rest is significantly augmented at muscle lengths shorter than optimum. Unpublished observations by Taylor et al., (personal communication) have substantiated these findings when sarcomere distances were measured with video imaging techniques. While the present protocol establishes Lmax, the length at which maximal force development is achieved, it does not measure or set sarcomere distances. In addition, the optimal sarcomere distance for the diabetic model has not been determined experimentally but ultrastructural evidence suggests that the presence of contracted sarcomeres is not uncommon (Afzal et al., 1988). Further studies examining the interaction between muscle length and chronic diabetes must be conducted to identify the contribution of this factor to the results of the present study.

Chronic diabetes results in an alteration in the distribution of the three isoforms of myosin. A shift in the predominance of the myosin isoform from the V_1 form with fast ATPase activity to the V_3 form with a slower ATPase activity has been reported (Dillman, 1980, Garber et al., 1983). This shift in isozyme distribution has been associated with a decrease in the contractile function of the chronic diabetic heart. Studies which attempt to normalize myosin isozyme distribution have not examined cardiac function (Dillman et al., 1984, Garber et

al., 1983) and therefore conclusive evidence cannot be provided regarding its relationship to depressed mechanical performance.

The role of altered myosin isozymes in the changes in the force-interval relationship with chronic diabetes cannot be excluded, however, they may not play a significant role for the following reasons: 1. any alterations in force development as a result of myosin isozyme distribution should be constant therefore influencing both phases of force development, but, the alpha phase changes very little with chronic diabetes; and 2. since force development is expressed as a ratio, F/B, the isozyme distribution is consistent over both beats where only the improvement in developed force becomes an important factor.

In any case, until the contribution of myosin isozyme distribution to the force-interval relationship has been elucidated, the influence of this factor in producing alterations in the force-interval curve cannot be eliminated.

Finally, the condition of chronic diabetes mellitus is accompanied by a secondary condition of hypothyroidism which alone has been reported to influence force development (Poggesi et al., 1987) and results in contractile characteristics similar to the chronic

diabetic. Studies attempting to improve cardiac function in chronic diabetics by normalizing thyroid hormone levels have been largely unsuccessful (Tahiliani and McNeill, 1984, Garber et al., 1983) suggesting that further alterations are responsible for cardiac dysfunction in chronic diabetes. Additionally, an examination of the force-interval relationship in hypothyroid animals has shown no change in the late phase or beta phase of the force-interval relationship when compared to control (Poggesi et al., 1987), where chronic diabetes results in a significant alteration in the same phase (McComb, 1990). It therefore does not appear that the results of the present study are influenced by the hypothyroid condition, however, this possiblity cannot be completely eliminated at present.

CONCLUSIONS

The condition of chronic diabetes results in several alterations in the force-interval relationship, primarily in the beta phase of force development. Alterations in the percent contribution of beta to the total amplitude and the rate and time constants of the beta phase were identified and suggest alterations in the capacity of the diabetic tissue to transport calcium through the exchange compartment to the release compartment.

The Na^+/Ca^{2+} exchanger, a component of the exchange compartment, appears to be partly responsible for the depression in the beta phase reported. Sodium gradient reduction, a method by which calcium entry via Na^+/Ca^{2+} exchange is enhanced, results in an improvement in force development in the chronic diabetic muscle. This improvement in force development suggests that under "normal" conditions calcium entry is somehow limited in the chronic diabetic therefore resulting in a decreased force development.

A condition of calcium overload was eliminated as a possible contributor to the depression in beta phase as an improvement in force generation with Na^+ reduction, and a decrease in force development with extracellular Ca^{2+} reduction were identified in the chronic diabetic model.

REFERENCES

Afzal, N., Ganguly, P., Dhalla, K., Pierce, G., Singal, P., and N. Dhalla, (1988). Beneficial effects of verapamil in diabetic cardiomyopathy. Diabetes, 37 (7), 936-942.

Bers, D. M., (1989). SR Ca loading in cardiac muscle preparations based on rapid-cooling contractures. Am. J. Physiol., 256, C109-C120.

Bers, D.M., (1987). Mechanisms contributing to the cardiac inotropic effect of Na pump inhibition and reduction of extracellular Na. J. Gen. Physiol., 90, 479-504.

Bielefeld, D., Pace, C., and B. Boshell, (1983). Altered sensitivity of chronic diabetic rat heart to calcium. Am. J. Physiol., 245, E560-E567.

Dhalla, N., Pierce, G., Innes, I., and R. Beamish, (1985). Pathogenesis of cardiac dysfunction in diabetes mellitus. Can. J. Cardiol., 1 (4), 263-281.

Dillman, W., (1984). Fructose feeding increases Ca^{++}-activated myosin ATPase activity and changes myosin isoenzyme distribution in the diabetic rat heart. Endocrinology, 114 (5), 1678-1685.

Dillman, W., (1980). Diabetes mellitus induces changes in cardiac myosin of the rat. Diabetes, 29, 579-582.

Fein, F., Kornstein, L., Strobeck, J., Capasso, J. and E. Sonneblick, (1980). Altered myocardial mechanics in diabetic rats. Circ. Res., 47, 922-933.

Gamble, J. (1990). Effect of resting muscle length on the force-interval relationship in right ventricular papillary muscle in the rat. Independent study, University of Windsor, Windsor, Canada.

Ganguly, P.,Pierce, G.N.,and N.S. Dhalla, (1987). Diabetic cardiomyopathy: Membrane dysfunction and therapeutic strategies. J. Appl. Cardio., 2, 323-338.

Ganguly, P., Pierce, G., Dhalla, K., and N. Dhalla, (1983). Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am. J. Physiol., 244, E528-E535.

Garber, D. and J. Neely, (1983). Decreased myocardial function and myosin ATPase in hearts from diabetic rats. Am. J. Physiol., 244, H586-H591.

Garber, D., Everett, A. and J. Neely, (1983). Cardiac function and myosin ATPase in diabetic rats treated with insulin, T_3 and T_4. Am. J. Physiol., 244, H592-H598.

Heyliger, C.E., Prakash, A. and J.H. McNeill (1987). Alterations in cardiac sarcolemmal Ca^{2+} pump activity during diabetes mellitus. Am. J. Physiol., 252, H540-H544.

Horackova, M. and M. Murphy, (1988). Effects of chronic diabetes mellitus on the electrical and contractile activities, $^{45}Ca^{2+}$ transport, fatty acid profiles and ultrastructure of isolated rat ventricular myocytes. Pflugers Arch., 411, 564-572.

Im, W., and C.O. Lee, (1984). Quantitative relation of twitch and tonic tensions to intracellular Na^+ activity in cardiac Purkinje fibers. Am. J. Physiol., 247, C478-C487.

Jewell, B, (1977). A re-examination of the influence of muscle length on myocardial performance. Circ. Res., 40, 221-230.

Johannsson, M. and H. Asgrimsson, (1989). Short-term effects of stimulus interval changes in guinea-pig and rat atrial muscle. Acta. Physiol. Scand., 135, 73-81.

Kentish, J.C., ter Keurs, H.E.D.J., Ricciardi, L., Bucx, J.J.J. and M.I.M. Noble, (1986). Comparison between the sarcomere length-force relations of intact and skinned trabeculae from rat right ventricle: Influence of calcium concentrations on these relations. Circ. Res., 58, 755-768.

Ku, D., and B. Sellers, (1982). Effects of streptozotocin diabetes and insulin treatment on myocardial sodium pump and contractility of the rat heart. J. Pharmacol. Exp. Therap., 222, 395-400.

Lagnado, L., and P.A. McNaughton, (1990). Electrogenic properties of the Na:Ca exchange. J. Membrane Biol., 113, 177-191.

Lopaschuk, G., Katz, S., and J.H. McNeill, (1983). The effect of alloxan- and streptozotocin-induced diabetes on calcium transport in rat cardiac sarcoplasmic reticulum. The possible involvement of long chain acyl carnitines. Can. J. Physiol. Pharmacol., 61, 439-448.

Makino, N., Dhalla, K.S., Elimban, V., and N.S. Dhalla, (1987). Sarcolemmal Ca^{2+} transport in streptozotocin-induced diabetic cardiomyopathy in rats. Am. J. Physiol., 253, E202-E207.

McComb, T.A., (1990). The force-interval relationship in chronic diabetic rat papillary muscle. Independent study, University of Windsor, Windsor, Canada.

Penpargkul, S., Schaible, T., Yipintsoi, T. and J. Scheuer, (1980). The effect of diabetes on performance and metabolism of rat hearts. Circ. Res., 47, 911-921.

Pierce, G.N, Beamish, R.E., and N.S. Dhalla, (1988). Heart dysfunction in diabetes. Boca Raton, Florida: CRC Press Inc..

Pierce, G.N. and N.S. Dhalla, (1983). Sarcolemma $Na^{+}K^{+}$-ATPase activity in diabetic rat heart. Am. J. Physiol., 245, C241-C247.

Poggesi, C., Everts, M., Polla, B., Tanzi, F. and C. Reggiani, (1987). Influence of thyroid state on mechanical restitution of rat myocardium. Circ. Res., 60, 142-151.

Ragnarsdottir, K., Wohlfart, B., and M. Johannsson, (1982). Mechanical restitution of the rat papillary muscle. Acta. Physiol. Scand., 115, 183-191.

Ramanadham, S., Doroudian, A., and J.H. McNeill, (1990). Myocardial and metabolic abnormalities in streptozotocin-diabetic Wistar and Wistar-Kyoto rats. Can. J. Cardiol., 6 (2), 75-82.

Schouten, V., (1985). Excitation-contraction coupling in heart muscle. PhD. thesis, Leiden State University, Netherlands.

Schouten, V., van Deen, J., De Tombe, P. and A. Verveen, (1987). Force-interval relationship in heart muscle of mammals: A calcium compartment model. Biophys. J., 51, 13-26.

Seed, W., and J. Walker, (1988). Review: Relation between beat interval and force of the heartbeat and its clinical implications. Cardiovascular Res., 22, 303-314.

Shattock, M., and D. Bers, (1989). Rat vs rabbit ventricle: Ca flux and intracellular Na assessed by ion-selective microelectrodes. Am. J. Physiol., 256, C813-C822.

Sheu, S., and H.A. Fozzard, (1982). Transmembrane Na+ and Ca2+ electrochemical gradients in cardiac muscle and their relationship to force development. J. Gen. Physiol., 80, 325-351.

Sonn, J., and C. Lee, (1988). Na^{+}-Ca^{++} exchange in regulation of contractility in canine cardiac Purkinje fibers. Am. J. Physiol., 255, C278-C290.

Sutko, J., Bers, D. and J. Reeves, (1986). Postrest inotropy in rabbit ventricle: Na^{+} - Ca^{2+} exchange determines sarcoplasmic reticulum Ca^{2+} content. Am. J. Physiol., 250, H654-H661.

Tahiliani, A.G., and J.H. McNeill, (1984). Lack of effect of thyroid hormone on diabetic rat heart function and biochemistry. Can. J. Physiol. Pharmacol., 62, 617-621.

Tani, M., and J. Neely, (1988). Hearts from diabetic rats are more resistant to In Vitro ischemia: Possible role of altered Ca^{2+} metabolism. Circ. Res., 62(5), 931-940.

Taylor, P., Helbing, R., Rourke, S. and D. Churchill, (1989). Effect of catecholamine-induced cardiac hypertrophy on the force-interval relationship. Can. J. Physiol. Pharmacol., 67, 40-46.

ter Keurs, H.E.D.J., Schouten, V., Bucx, J., Mulder, B., and P. deTombe, (1987). Excitation-contraction coupling in myocardium: Implications of calcium release and Na^{+} - Ca^{2+} exchange. Can. J. Physiol. Pharmacol., 65, 619-626.

ter Keurs, H.E.D.J., Rinjnsburger, W.H., Van Heuningen, R., and M.J. Nagelsmit, (1980). Tension development and sarcomere length in rat cardiac trabeculae: Evidence of length-dependent activation. Circ. Res., 46, 703-714.

Vadlamudi, R., Rodgers, R., and J.H. McNeill, (1982). The effect of chronic alloxan- and streptozotocin-induced diabetes on isolated rat heart performance. Can. J. Physiol. Pharmacol., 60, 902-911.

Walford, G., Gerstenblith, G. and E. Lakatta, (1984). Effect of sodium on calcium-dependent force in unstimulated rat cardiac muscle. Am. J. Physiol., 246, H222-H231.

APPENDIX A

RAW DATA: ANIMAL CHARACTERISTICS, CONTROL

		BW (g)	RV (g)	LV (g)	HW/BW
CONTROL	1	373.00	0.120	0.910	0.0028
	2	411.00	0.116	0.861	0.0024
	3	390.50	0.104	0.842	0.0024
	4	445.00	0.135	0.882	0.0023
	5	421.50	0.128	0.898	0.0024
	6	372.00	0.112	0.880	0.0027
	7	366.00	0.103	0.744	0.0023
	8	367.00	0.131	0.703	0.0023

Note : BW = body weight, RV = right ventricular free wall weight, LV = left ventricular weight, HW/BW = heart weight/body weight

RAW DATA: ANIMAL CHARACTERISTICS, DIABETIC

		BW (g)	RV (g)	LV (g)	HW/BW	BG (mg/dL)
DIABETIC	1	164.00	0.051	0.465	0.0032	547.1
	2	245.50	0.107	0.667	0.0032	468.4
	3	294.50	0.092	0.784	0.0030	525.4
	4	248.00	0.081	0.574	0.0026	592.3
	5	256.00	0.077	0.674	0.0029	475.4

Note : BW = body weight, RV = right ventricular free wall weight, LV = left ventricular weight, HW/BW = heart weight/body weight, BG = blood glucose, n = 5.

APPENDIX B

RAW DATA: TWITCH CHARACTERISTICS, CONTROL

	CONTROL				
	1	2	3	4	5
Force (mN/mm^2)	11.2	27.2	16.0	7.6	8.0
+ dF/dt ($mN/sec/mm^2$)	195.7	411.4	287.8	128.7	129.0
- dF/dt ($mN/sec/mm^2$)	155.1	351.6	260.1	103.7	100.7
Time to Peak Force (msec)	93.4	106.3	86.8	95.3	98.0
Time for Relaxation (msec)	152.3	172.8	122.4	162.3	184.5

RAW DATA: TWITCH CHARACTERISTICS, DIABETIC

	DIABETIC				
	1	2	3	4	5
Force (mN/mm^2)	6.8	4.4	7.6	4.6	6.9
+ dF/dt ($mN/sec/mm^2$)	77.0	51.7	92.9	52.6	139.2
- dF/dt ($mN/sec/mm^2$)	55.9	35.1	65.6	36.9	83.3
Time to Peak Force (msec)	142.38	139.1	131.4	137.0	162.6
Time for Relaxation (msec)	259.1	251.6	262.9	240.0	326.9

APPENDIX C

RAW DATA FOR FORCE-INTERVAL CURVES

FORCE/BASELINE (RATIO)

TIME INTERVAL (SEC)	CONTROL 1	CONTROL 2	CONTROL 3	CONTROL 4	CONTROL 5
0.546	0.692	0.674	0.623	0.580	0.637
0.737	0.742	0.749	0.637	0.616	0.656
0.994	0.798	0.804	0.654	0.648	0.687
1.34	0.859	0.846	0.730	0.686	0.733
1.81	0.905	0.887	0.797	0.759	0.790
2.44	0.931	0.907	0.834	0.826	0.850
3.30	0.953	0.935	0.895	0.895	0.915
4.46	0.990	0.987	0.956	0.949	0.976
6.02	1.037	1.043	1.010	0.977	1.033
8.13	1.105	1.108	1.093	1.043	1.119
10.97	1.177	1.203	1.126	1.102	1.209
14.81	1.267	1.317	1.209	1.172	1.286
20.00	1.354	1.432	1.375	1.256	1.385
27.00	1.453	1.494	1.451	1.340	1.472
36.45	1.593	1.581	1.620	1.425	1.546
49.21	1.751	1.722	1.844	1.526	1.582
66.44	1.891	1.815	2.121	1.609	1.636
89.70	2.046	1.977	2.376	1.799	1.764
121.00	2.258	2.008	2.647	1.903	1.846
130.00	2.311	2.156	2.734	1.928	1.854

RAW DATA FOR FORCE-INTERVAL CURVES

TIME INTERVAL (SEC)	FORCE/BASELINE (RATIO) DIABETIC 1	DIABETIC 2	DIABETIC 3	DIABETIC 4	DIABETIC 5
0.546	0.763	0.775	0.757	0.693	0.721
0.737	0.848	0.864	0.832	0.779	0.804
0.994	0.912	0.814	0.895	0.849	0.897
1.34	0.934	0.928	0.930	0.891	0.956
1.81	0.954	0.958	0.954	0.917	0.979
2.44	0.957	0.979	0.957	0.935	0.986
3.30	0.941	0.980	0.993	0.943	1.006
4.46	0.997	0.983	0.996	0.990	0.982
6.02	1.012	1.017	1.019	1.029	0.992
8.13	1.035	1.050	1.032	1.086	1.012
10.97	1.041	1.079	1.057	1.137	1.019
14.81	1.069	1.124	1.087	1.207	1.036
20.00	1.118	1.182	1.121	1.279	1.082
27.00	1.126	1.229	1.164	1.349	1.120
36.45	1.120	1.187	1.182	1.385	1.140
49.21	1.140	1.177	1.172	1.407	1.190
66.44	1.147	1.208	1.185	1.388	1.241
89.70	1.157	1.193	1.190	1.382	1.243
121.00	1.159	1.161	1.219	1.341	1.233

APPENDIX D

PARAMETERS DERIVED FROM CURVE FITTING PROGRAM

	VARIABLE			
	A	B	M	Z
CONTROL 1	2.00	0.012	0.445	2.60
CONTROL 2	2.00	0.014	0.465	2.30
CONTROL 3	2.00	0.013	0.398	2.80
CONTROL 4	2.00	0.014	0.460	2.10
CONTROL 5	2.00	0.023	0.480	2.00

PARAMETERS DERIVED FROM CURVE FITTING PROGRAM

	VARIABLE			
	A	B	M	Z
DIABETIC 1	2.01	0.044	0.771	1.25
DIABETIC 2	2.00	0.071	0.757	1.24
DIABETIC 3	2.01	0.060	0.773	1.24
DIABETIC 4	2.00	0.046	0.687	1.39
DIABETIC 5	2.00	0.038	0.742	1.26

APPENDIX E

PARAMETERS FOR LOGLIN CURVE PLOTTING PROGRAM

Utilizing the parameters derived from the Eureka program;

A1 = Z * M A2 = Z * (1-M)

A3 = 0

alpha1 = A alpha2 = B

alpha3 = 0

APPENDIX F

CALCULATIONS FOR FORCE-INTERVAL PARAMETERS

Percent Contributions

Alpha = M * 100

Beta = (1-M) * 100

Time Constants

Alpha = 1/A

Beta = 1/B

Rate Constants

Alpha, Beta = Maximum (Change Y/ Change X) , derived from function values of Loglin plotting program

APPENDIX G

RAW DATA: DECAY, CONTROL

	CONTROL				
F/B (ratio)	1	2	3	4	5
Beat 1	1.87	1.77	2.26	2.67	1.74
Beat 2	1.57	1.49	1.80	2.43	1.46
Beat 3	1.36	1.31	1.50	1.69	1.27
Beat 4	1.23	1.19	1.29	1.30	1.16
Beat 5	1.14	1.12	1.15	1.10	1.09
Beat 6	1.08	1.08	1.08	1.22	1.04

RAW DATA: DECAY, DIABETIC

	DIABETIC				
F/B (ratio)	1	2	3	4	5
Beat 1	1.51	1.64	1.42	1.59	1.47
Beat 2	1.15	1.33	1.14	1.33	1.17
Beat 3	1.00	1.13	1.02	1.18	1.01
Beat 4	--	1.01	--	1.11	--

APPENDIX H

RAW DATA: 1.0 mM Ca^{2+} to 40% Na^{+} Reduction, Control

		Force/Baseline	
		1.0 mM Ca^{2+}	40% Na^{+} reduced
CONTROL	1	2.67	5.05
	2	3.33	4.92
	3	1.76	2.63
	4	1.60	2.54
	5	1.87	1.94

RAW DATA: 1.0 mM Ca^{2+} to 0.3 mM Ca^{2+}, Control

		Force/Baseline	
		1.0 mM Ca^{2+}	0.3 mM Ca^{2+}
CONTROL	1	1.74	0.44
	2	2.67	1.58
	3	2.26	1.64
	4	1.77	1.22
	5	1.87	1.01

RAW DATA: 1.0 mM Ca^{2+} to 40% Na^{+} Reduction, Diabetic

		Force/Baseline	
		1.0 mM Ca^{2+}	40% Na^{+} reduced
DIABETIC	1	1.51	2.19
	2	1.64	2.09
	3	1.42	2.00
	4	1.58	1.82
	5	1.47	1.87

RAW DATA: 1.0 mM Ca^{2+} to 0.3 mM Ca^{2+}, Diabetic

		Force/Baseline	
		1.0 mM Ca^{2+}	0.3 mM Ca^{2+}
DIABETIC	1	1.51	0.89
	2	1.64	1.38
	3	1.42	0.78
	4	1.58	1.27
	5	1.47	1.10

APPENDIX I

TWO WAY ANALYSIS OF VARIANCE WITH REPEATED MEASURES

Independent variables: 1. G = group; control, diabetic
2. C = condition; 1.0 mM,60% Na^+

Dependent variable: Force, expressed as Force/Baseline

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
G	5.750	1	5.750	4.995	
C	3.365	1	3.365	17.355	0.01
G/C	0.614	1	0.614	3.166	
Error	1.551	8	0.194		
Total	20.489	19			

TWO WAY ANALYSIS OF VARIANCE WITH REPEATED MEASURES

Independent variables: 1. G = group; control, diabetic
2. C = condition; 1.0 mM, 0.3 mM

Dependent variable: Force, expressed as Force/Baseline

Source	Sum Squares	D.F.	Mean Square	F-ratio	F-prob
G	0.494	1	0.494	2.455	
C	2.192	1	2.192	67.392	0.01
G/C	0.247	1	0.247	7.583	0.05
Error	0.260	8	0.033		
Total	4.802	19			

VITA AUCTORIS

NAME:	Tina Ann McComb
PLACE OF BIRTH:	Owen Sound, Ontario
YEAR OF BIRTH:	1965
EDUCATION:	West Hill Secondary School, Owen Sound 1979-1984
	University of Windsor, Windsor, Ontario 1984-1988, B.H.K.
	University of Windsor, Windsor, Ontario 1988-1990, M.H.K.