THE RELATIONSHIP BETWEEN BETA-BLOCKADE, PLASMA POTASSIUM CONCENTRATIONS AND MUSCLE EXCITABILITY FOLLOWING STATIC EXERCISE

THE RELATIONSHIP BETWEEN BETA-BLOCKADE, PLASMA POTASSIUM CONCENTRATIONS AND MUSCLE EXCITABILITY FOLLOWING STATIC EXERCISE

By

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I am pleased to dedicate this thesis to my mother and father,

with whom I have always enjoyed celebrating my life's journey,

and whose love and encouragement kindles my enthusiasm to reach toward new goals.

PREFACE

This thesis is presented in two chapters. Chapter I is a literature review of the role of potassium as a mechanism of muscle fatigue, the function of the Na^+-K^+ pump and the regulation of Na^+-K^+ transport during exercise. Chapter II presents the thesis research related to exercise-induced hyperkalemia, membrane excitability and force and the adrenergic control of potassium homeostasis. Chapter II is presented in a manuscript sytle suitable for publication.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

REVIEW OF LITERATURE

1.1 INTRODUCTION

Muscle fatigue has been measured and defined in various ways during the long history of its study, yet there is still no consensus as to the underlying mechanisms or the major sites at which fatigue occurs. Recently, it has become increasingly evident that disturbances in electrolyte regulation during physical activity are closely linked to the processes of muscular fatigue. Specifically, the rise in extracellular potassium concentration ($[K^+]$) with exercise is thought to contribute directly to fatigue through K^+-induced depolarization of muscle fibre membranes (Sjøgaard et al. 1985; Hnik et al. 1986; Medbo and Sejersted 1990).

The Na^+-K^+ pump is a membrane-bound protein that attempts to maintain the concentration gradients for sodium (Na^+) and K^+ necessary for the maintenance of resting membrane potential and action potential (Bia and DeFronzo 1981; Clausen and Everts 1989). As a transport mechanism under adrenergic control, Na^+-K^+ pump activity is inhibited by beta-adrenoceptor antagonism (β-blockade) and several studies have demonstrated significantly higher plasma K^+ concentrations during exercise under conditions of β-blockade (Rosa et al. 1980; Linton et al. 1984; Williams et al. 1985; Cleroux et al. 1989). Although changes in excitability may be inferred based on measurement of plasma $[K^+]$, the relationship between plasma $[K^+]$, muscle excitability and

muscular performance may best be understood by actual measurement of muscle excitability during exercise-induced hyperkalemia and during β-blockade.

This review will focus on the role of K^+ as a factor responsible for muscle fatigue. To begin, a brief overview of the fundamental factors responsible for membrane excitability and impulse transmission will be presented. The effect of exercise-induced changes in plasma $[K^+]$ on muscle membrane excitability will then be introduced as an important mechanism responsible for muscle fatigue. This will be followed by a discussion of the role of the Na^+-K^+ pump in preserving K^+ homeostasis. Finally, regulation of active Na^+-K^+ transport by the adrenergic system will be addressed with attention to the effects of β-blockade.

1.2. MUSCLE MEMBRANE EXCITABILITY

Since the "cross-bridge theory of muscle contraction" was published (Huxley 1957), the primary focus in understanding muscle fatigue has been the actin-myosin reaction as the limiting step. However, in order for this reaction to occur in voluntary muscle contractions, there must be an excitable muscle membrane. The excitability depends upon the resting membrane potential, which depends on the distribution of electrolytes across the membrane. The following discussion considers this characteristic of excitability of the muscle membrane.

1.2.1 THE RESTING MEMBRANE POTENTIAL

All neurons have an electrical charge on the membrane that results from a thin cloud of positive and negative ions spread over their intra- and extracellular surfaces. In a

nerve cell at rest, there is a net excess of positive charges on the outside of the membrane and a net excess of negative charges on the inside. The membrane is able to maintain a separation of charge because it acts as a selective permeability barrier to the diffusion of ions. This separation of charge is responsible for the resting membrane potential (E_r) (Koester 1991).

Measurements of E_r with intracellular electrodes and flux studies using radioactive tracers indicate that nerve cells are permeable to Na^+ and Cl^- as well as to K^+ (Koester 1991). An equation derived from basic thermodynamic principles is used to calculate the membrane potential at which each of the ions is in equilibrium (Nernst 1888):

$$E_{ion} = \frac{RT}{ZF} \ln \frac{[ion]_o}{[ion]_i}$$

where E_{ion} is the value of membrane potential at which an ion is in equilibrium, R is the gas constant, T the temperature in degrees Kelvin, Z the valence of the ion, F the Faraday constant, and $[ion]_o$ and $[ion]_i$ the concentrations of the ion on the outside and inside of the cell. At rest, the membrane potential (E_m) is closest to the Nernst potential of K^+ (E_K), the ion to which the membrane is most permeable. However, because the membrane is also somewhat permeable to Na^+, there is an influx of Na^+, which drives E_m slightly positive to E_K. At this membrane potential, the electrical and chemical driving forces acting on K^+ are no longer in balance, so that a steady efflux of K^+ from the cell results (Kimura 1989).

If the passive fluxes (due to diffusion) were allowed to continue unopposed for any appreciable length of time, the ionic gradients would run down gradually, reducing the resting membrane potential. Dissipation of ionic gradients is prevented by a membrane bound Na^+-K^+-ATPase (the Na^+-K^+ pump) which maintains K^+ homeostasis across the cell membrane by extruding Na^+ from the cell while taking in K^+ (Clausen 1986). Typically, three Na^+ ions are extruded in exchange for two K^+ ions during each cycle of the pump (Glynn and Karlish 1975). Since the Na^+-K^+ pump moves Na^+ and K^+ against their net electrochemical gradients, energy from the hydrolysis of ATP is provided to drive these actively transported fluxes. Although the major part of the enzyme activity seems to be associated with the sarcolemma, the transverse tubules (T-tubules) are known to contain Na^+-K^+-ATPase activity, although at a considerably lower density (Lau et al. 1977; Narahara et al. 1979; Seiler and Fleischer 1982).

1.2.2 THE ACTION POTENTIAL

To generate an action potential, the membrane potential must be made less negative by reducing the charge separation across the membrane (i.e. depolarization). An excitatory postsynaptic potential acts as a transient depolarizing potential which causes voltage-gated Na^+ channels to open. The resultant increase in membrane Na^+ permeability allows Na^+ influx to outstrip the K^+ efflux such that the membrane potential approaches E_{Na} at the peak of the action potential (Kimura 1989). Two somewhat slower processes limit the extent of depolarization: 1) there is a delayed opening of voltage-dependent K^+ channels that increases K^+ efflux and 2) there is a slow inactivation of the Na^+ channels

which decreases Na^+ influx. This combination of events continues until the cell has repolarized to its resting value (Koester 1991).

1.3 THE ROLE OF POTASSIUM IN MUSCLE FATIGUE

A prerequisite for action potential propagation along the sarcolemma and into the T-tubule is a membrane potential of approximately -80 mV. Any perturbation in the ionic concentrations would be expected to affect muscle excitability by altering the membrane potential of the single muscle fibres. There has long been speculation that K^+, released into the interstitial spaces by contracting muscle fibres induces a rapid decrease in excitability and subsequently, a reduction in the force generating capacity of the muscle (Sjøgaard et al. 1985; Hnik et al. 1986; Medbo and Sejersted 1990). To understand the proposed role of exercise-induced K^+ loss in the development of muscle fatigue, it is important to gain insight into potassium fluxes during contraction as well as the mechanisms of K^+ release by skeletal muscle.

1.3.1 POTASSIUM EFFLUX DURING EXERCISE

It is well established that a rise in plasma $[K^+]$ accompanies muscular contraction (Keys 1937; Skinner 1961; Laurell and Pernow 1966; Saltin et al. 1987; Medbo and Sejersted 1990; Sjøgaard 1990). A variety of analytical approaches including electron probe technique (Gonzales-Serratos et al. 1978), neutron activation analysis (Lindinger and Heigenhauser 1988), and ion selective electrodes (Hnik et al. 1986) have shown that this K^+ originates from the contracting muscle. This is true in isolated in vivo and in vitro muscle preparations as well as in voluntary contractions of single muscle groups and

whole body exercise in humans (Sjøgaard 1990). Moreover, studies of exercise-induced changes in extracellular [K^+] all confirm the early findings by Fenn (1938) that muscle K^+ loss is proportional to the magnitude and frequency of muscle contraction (Hirche et al. 1980; Vyskocil et al. 1983; Sjøgaard et al. 1985; Hnik et al. 1976, 1986; Sahlin and Broberg 1989; Juel et al. 1990). For example, Wilkerson et al. (1982) sampled blood from the arm antecubital vein during treadmill running exercise at submaximal intensities of 30, 45, 60, 75 and 90% of VO_{2max} and found that plasma [K^+] increased linearly with exercise intensity. Plasma [K^+] measurements as high as 7 mmol/L (arterial) and 8 mmol/L (femoral venous) have been reported following whole body exercise (running, cycling and swimming) at high intensities (Hermansen et al. 1984; Kowalchuk et al. 1988; Medbo and Sejersted 1985, 1990). Conversely, low contraction frequencies permit a more complete reaccumulation of K^+ and result in smaller increases in plasma [K^+] (Vyskocil et al. 1983; Hnik et al. 1986).

Although the magnitude of the increase in plasma [K^+] alone cannot be used to calculate how much K^+ is lost from contracting muscle, it is a good reflection of the rate of K^+ release from muscle. For example, a high femoral venous plasma [K^+] of 5.8 to 7.3 mmol/L at the end of 3.2 minutes of supramaximal exercise corresponded to a total K^+ release from muscle of 7.6 mmol/L (Juel et al. 1990). In contrast, the total K^+ release during 65 minutes of exercise to exhaustion at 67% of VO_{2max} was 22 ± 4 mmol/L and femoral venous [K^+] attained a value of less than 5.6 mmol/L at the point of exhaustion (Sahlin and Broberg 1989).

Studies that employ whole body exercise are, however, limited in calculating fluxes specifically from the exercising muscle since the recruitment pattern of the various muscles may be very complex and specific to each movement or exercise pattern. Thus, isolated in vivo or in vitro muscle preparations provide more precise estimates of the magnitude of K^+ losses relative to different activity patterns. A model which has proven to be especially well suited for the study of "isolated" in vivo exercising muscle in humans is knee-extension. Static contractions have been studied most often with a knee-angle of 90° and a strap around the ankle connected to a force-transducer. Of particular relevance to the current work is that static knee-extensions ranging from 5 to 50% of the maximum voluntary contraction (MVC) have been shown to cause significant changes in arterial as well as femoral venous plasma [K^+] within 0.5 to 3 minutes of sustained contractions (Saltin et al. 1981; Sjøgaard 1988; West et al.1996). Moreover, the highest rate of release has been shown to occur at 25% MVC, while total K^+ loss is largest with 5% MVC because of the longer duration of this contraction (Sjøgaard 1990).

Although plasma [K^+] is considered to reflect interstitial [K^+], measurements of femoral venous plasma [K^+] underestimate the interstitial concentrations during exercise. Microelectrode studies of stimulated dog, cat and mouse muscle have shown the rise in the extracellular [K^+] to be greater than that of the simultaneously collected venous effluent [K^+] (Hnik et al. 1976; Tibes et al. 1977; Hirche et al. 1980). Similar contraction-induced changes in interstitial concentrations in active muscle have been reported in humans. Vyskocil et al. (1983) inserted microelectrodes directly into the

intact human brachioradialis during a maximal static contraction and found that interstitial [K^+] increased from 4.5 mmol/L at rest to an average of 9.5 mmol/L, and exceeded 15 mm/L in one individual.

1.3.2 MECHANISMS OF POTASSIUM EFFLUX

The mechanisms of K^+ loss from contracting skeletal muscle include losses due to electrical activity via three types of K^+ channels (Kolb 1990): (*i*) the delayed rectifier K^+ channels which are responsible for repolarization after the action potential, (*ii*) the ATP-sensitive K^+ channels, and (*iii*) the Ca^{++}-sensitive K^+ channels.

The ATP- and Ca^{++} -sensitive K^+ channels have been largely disregarded as being involved in normal muscle activity because the intracellular calcium concentration [Ca^{++}] was assumed to remain too low, and the ATP concentration too high, to open these channels. However, there is increasing evidence that both channels may in fact contribute directly to the K^+ loss and membrane potential depolarization during muscle contraction. The following summarizes the proposed significance of these K^+ channels as a mechanism of K^+ efflux.

It is well known that mean cellular ATP concentration in muscle samples never reaches the low concentrations required to open the ATP-dependent K^+ channels for a significant period of time (Hultman et al. 1990). However, what has not been considered is that ATP may not exist as a single pool, but may be compartmentalized, with a specific membrane pool that is mainly linked to the Na^+-K^+ pump (Proverbio and Hoffman 1977;

Mercer and Dunham 1981). The ATP-sensitive K^+ channels may respond mainly to the concentration of the membrane ATP pool rather than myofibrillar concentrations of ATP. As proposed by Spruce et al. (1987), during intense activity, the membrane ATP may be sufficiently reduced by ATP-ase activity, resulting in a conformation of the channel protein to permit the efflux of K^+.

The Ca^{++}-sensitive K^+ channels may also open in relation to muscle activity. Fink et al. (1983) have suggested that local shortages of ATP supply may increase the Ca^{++} sensitivity of the Ca^{++}-activated channels in skeletal muscle. This would be important during times of continuous stimulation or repetitive stimulation over a long period when a sustained elevation in cytosolic $[Ca^{++}]$ could damage the cell (Jackson et al. 1984; Edwards 1988; Vollestad and Sejersted 1988). An effective way to lower cytosolic Ca^{++} would be to block the transmission between T-tubule and sarcoplasmic reticulum. The obvious mechanism is for the Ca^{++}-sensitive K^+ channels to increase K^+ conductance.

Thus, involvement of these two K^+ channels in muscle activity would theoretically lead to impaired action potential propagation and a loss in the force of muscle contraction. It follows from this, that these membrane mechanisms of increased K^+ conductance may in fact contribute to a system which continues to allow contraction at reduced rates and forces while preventing catastrophic changes in cellular homeostasis which could lead to irreversible cell damage. Although these theories are very speculative at the present time, it is interesting that, as noted by Sjøgaard (1990), they are consistent with the suggestion

by Bigland-Ritchie et al. (1979) that the critical requirement for energy may not be for the myofilaments but rather may be at the level of the membrane.

1.4 CONTROL OF POTASSIUM HOMEOSTASIS DURING EXERCISE

From the preceding discussion, it is obvious that the K^+ released from the exercising skeletal muscle produces a significant physiological challenge. Theoretically, the loss of K^+ from the working muscles would flood the plasma with K^+ within very short intervals of time and subsequently cause severe interference with excitability and contractile performance. Fortunately, skeletal muscle is equipped with a mechanism that has been shown to play a dominant role in the short term regulation of plasma [K^+]. This mechanism is the Na^+-K^+ pump (Skou 1965; Bia and Defronzo 1981; Clausen 1986; Clausen and Everts 1989).

1.4.1 THE ROLE OF THE NA^+-K^+ PUMP

In most neurons, the Na^+-K^+ pump is not neutral but electrogenic, that is, the pump increases the charge separation across the membrane, making the membrane potential more negative. The resulting hyperpolarization helps to overcome the depolarizing tendency of the increased interstitial [K^+] and to delay the loss of membrane excitability (Koester 1991). At rest, the Na^+-K^+ pump has be shown to add approximately -10 mV to E_m and during muscular activity, the electrogenic contribution from the pump may increase up to as much as -30 mV in an attempt to overcome large increases in extracellular [K^+] (Hicks and McComas 1989).

Physiological evidence for increased Na^+-K^+ pumping during muscle activity has been obtained in a study of rat soleus muscles examined in vivo. Hicks and McComas (1989) observed that repeated tetani at 20 Hz increased the mean resting potential from -79.5 mV to -90.5mV. They attributed this increase to the electrogenic effect of the Na^+-K^+ pump as subsequent experiments repeated in the presence of ouabain (a selective inhibitor of the enzyme), or in the absence of extracellular K^+, failed to produce the hyperpolarizing effect.

Further evidence for increased Na^+-K^+ pumping during exercise is seen in the phenomenon of 'pseudofacilitation'. Studies have demonstrated that during stimulated or voluntary activity, there is little or no decline in the M-wave, but rather, a gradual increase in its amplitude (Hicks and McComas 1989; Hicks et al. 1989; Galea and McComas 1991). The most plausible explanation for this potentiating effect rests in the findings of the aforementioned study (Hicks and McComas 1989), that the individual muscle fibre action potentials are enlarged due to an increase in E_m resulting from enhanced Na^+-K^+ pump activity. The dramatic M-wave enlargement shown in single fast twitch motor units of the cat tibialis posterior muscle (Enoka et al. 1992) lends strong support to this proposal. In this regard, potentiation of the M-wave has led to the utilization of the M-wave as a non-invasive index of Na^+-K^+ pump activity.

1.4.2 REGULATION OF ACTIVE NA^+-K^+ TRANSPORT

The active transport of K^+ and Na^+ is a function of the density of pumps and the activity of each pump (Lindinger and Sjøgaard 1991). The density of pumps has been

shown to change due to factors such as age, muscle activity, and K^+ availability (Kjeldsen et al. 1984, 1985, 1986), but these are slow processes and therefore do not play a role in acute exercise-induced hyperkalemia. The activity of the Na^+-K^+ pump is likely controlled by several mechanisms. During muscle activity, one factor will be the rise in intracellular sodium concentration ($[Na^+]$), as demonstrated by the effects of direct stimulation of single muscle fibres (Hodgkin and Horowicz 1959), and of Na^+ injection into neurons (Thomas 1972). However, Everts et al. (1988) reported a 63% increase in pump activity in rat soleus muscles stimulated at 2 Hz, without a concomitant increase in intracellular $[Na^+]$, which suggests that factors other than intracellular $[Na^+]$ are also responsible for stimulating the pump. Similarly, it has been suggested that a rise in interstitial $[K^+]$ cannot be a major stimulus, since the effect of increasing extracellular $[K^+]$ in resting muscle is to depolarize the fibres (McComas et al. 1993).

In contrast, there is evidence that catecholamines may be a potent stimulus, with the effects mediated by β-adrenoceptors (Clausen 1986; Sejersted and Hallen 1987). Hence, studies into the control of pump activity have made extensive use of β-adrenoceptor agonists and antagonists in an attempt to identify the regulatory role of catecholamines in Na^+-K^+ homeostasis. The following section reviews the role of the adrenergic system as a control mechanism in determining Na^+-K^+ distribution and membrane potential in skeletal muscle.

1.4.2.1 Adrenergic Control of Na^+-K^+ Homeostasis

Epinephrine causes rapid changes in plasma [K^+], a fact that has been known for over 50 years (D'Silva 1934). Our understanding of the in vivo effects of catecholamines on Na^+-K^+ homeostasis is largely based on the analysis of their effects on isolated muscles. In resting skeletal muscle in vitro (animal and human), regardless of whether epinephrine is administered as a single intravenous injection or a continuous infusion, its effect is characterized by decreases in extracellular [K^+] and intracellular [Na^+] and an increase in intracellular [K^+] (D'Silva 1934; Todd and Vick 1971; Evans and Smith 1973; Hays et al. 1974; Lockwood and Lum 1974; Brown et al. 1983).

The mechanism by which epinephrine promotes the cellular uptake of K^+ seems to involve β-adrenergic receptors (Todd and Vick 1971; Wang and Clausen 1976; Lockwood and Lum 1977; Buur et al. 1982; Flatman and Clausen 1989). Although epinephrine is an alpha (α)- as well as a non-specific β-agonist, several studies have demonstrated the ability of isoproterenol (a non-specific β-agonist) to similarly lower plasma [K^+], thus indicating a β-adrenoceptor-mediated effect (Todd and Vick 1971; Lockwood and Lum 1974; Pettit and Vick 1974). This is consistent with the ability of propranolol (a non-specific β-blocker), but not phenoxybenzamine (an α-blocker) to prevent the hypokalemic effect of β-agonists (Todd and Vick 1971; Lum and Lockwood 1972).

Further studies with selective β_1- and β_2- agonists have shown that the effects of epinephrine are elicited specifically via β_2-adrenoceptors in skeletal muscle (Todd and

Vick 1971; Olsson et al. 1978; Clausen and Flatman 1980; Brown et al. 1983; Vincent et al. 1984). For example, Lockwood and Lum (1974) showed in cats that β_2- but not β_1-agonists protected against the lethal effects of K^+ infusion and reduced the rise in plasma $[K^+]$. More recently, Juel (1988a) found that administration of the β_2-agonist terbutaline during electrical stimulation of isolated mouse soleus muscle resulted in 34% smaller depolarisation of the membrane potential, 32% less reduction in intracellular $[K^+]$, and a 27% smaller increase in intracellular $[Na^+]$ compared to stimulated control muscles. Also, muscles treated with terbutaline were somewhat more resistant to fatigue as demonstrated by a 10% smaller reduction in force upon electrical stimulation.

Further to these observations are reports that ouabain blocks the hyperpolarizing effect of β-adrenoceptor stimulation, thus confirming that the actions of epinephrine and β-agonists are the result of increased active Na^+-K^+ transport (Tashiro 1973; Clausen and Flatman 1977; Ballanyi and Grafe 1988; Juel 1988a).

Hence, the physiological relevance of catecholamines in K^+ homeostasis rests in the fact that in skeletal muscle, the most commonly occurring rise in extracellular $[K^+]$ is elicited by exercise and is associated with an elevation of the plasma concentration of catecholamines (Christensen and Galbo 1983; Williams et al. 1985; Clausen et al. 1987). High circulating plasma levels of epinephrine stimulates β-receptors, which in turn produces a marked and rapid activation of the electrogenic Na^+-K^+ transport (Hays et al. 1974; Clausen and Flatman 1977; Rogus et al. 1977; Pfliegler et al. 1983). This favours the net loss of Na^+ and the accumulation of K^+ in the muscle cells, thereby preventing

toxic hyperkalemia. Clearly, this could be of importance for the maintenance of excitability and contractility during exercise.

1.4.2.2 β-Blockade and Potassium Homeostasis

In contrast to the results seen with β-adrenergic stimulation, β-blockade has been shown to inhibit the epinephrine-mediated stimulation of muscle K^+ uptake (Carlsson et al. 1978; Lim et al. 1981; Williams et al. 1985). In this regard, one would expect that inhibition of the Na^+-K^+ pump with β-blockade may be an important limiting factor for physical performance through its effect on K^+ homeostasis and thus muscle excitability. Moreover, because control of the Na^+-K^+ pump is mediated specifically by β_2-adrenoceptors, one might predict a greater effect of $\beta_{1,2}$-antagonism (non-selective β-blockade) versus β_1-antagonsim (selective β-blockade) on the rise in plasma $[K^+]$. Reports of an increased plasma $[K^+]$ response (Carlsson et al. 1978; Lundborg et al. 1981; Brown et al. 1983; Gordon et al. 1985; Williams et al. 1985; Cleroux et al. 1989) and increased fatiguability (Pearson et al. 1979; Lundborg et al. 1981) under non-selective versus selective β-blockade support this prediction. Furthermore, Clausen and Flatman (1980) observed in the rat isolated soleus muscle that stimulation of electrogenic ion transport was completely blocked by the $\beta_{1,2}$ antagonist, propranolol, whereas the β_1-selective antagonist, metoprolol, was found to be at least 50 times less potent. In resting humans, Brown et al. (1983) showed that adrenaline infusion selectively stimulated β_2-adrenoceptors and produced a hypokalemic effect; this effect was abolished when epinephrine was infused together with a β_2-selective adrenoceptor antagonist. In a study

of humans performing progressive exercise to exhaustion, Williams and colleagues (1985) found that β-blockade with propranolol resulted in a larger increase in plasma [K^+] than in controls and a sustained elevation of plasma [K^+] during 30 minutes of recovery.

1.5 THE "K^+ HYPOTHESIS"

The work surrounding this topic of exercise-induced K^+ fluxes has culminated in the "K^+ hypothesis" - a theory suggesting that the depolarization induced by the accumulation of K^+ near the surface membrane is sufficiently large to impair mechanical tension development (Bigland-Ritchie et al. 1979; Sejersted et al. 1982; Sjøgaard et al. 1985; Sjøgaard 1986; Medbo and Sejersted 1990). Both an increase in extracellular [K^+] and a decline in the intracellular [K^+] will independently reduce (i.e.depolarize) the potassium potential and E_m (Adrian 1956; Hodgkin and Horowicz 1959b). A depolarization of the sarcolemma will decrease the amplitude of the action potential (Jones and Bigland-Ritchie 1986; Juel 1988b) by affecting the degree of inactivation of Na^+ channels (Hille 1968; Adrian et al. 1970; Ildefonse and Rougier 1972; Campbell and Hille 1976). This in turn will cause a smaller release of Ca^{++} and diminished tension development, as concluded from voltage clamp studies (Ashley and Ridgway 1970; Vergara et al. 1978).

1.5.1 POTASSIUM, MUSCLE MEMBRANE POTENTIAL AND FATIGUE

Changes in intra- and extracellular [K^+] have been shown to attain a magnitude which may depolarize the membrane significantly. A decline in E_m of approximately 8 to 14 mV has been calculated in human muscles following exhaustive exercise, as well as in

stimulated perfused rat hindlimb muscles (Sjøgaard 1983; Sjøgaard et al. 1985; Lindinger and Heigenhauser 1988, 1991). Even greater declines in E_m of up to 20 mV have been demonstrated using direct microelectrode determinations in mouse extensor digitalis longus (EDL) muscle fibres (Juel 1986) and in frog single muscle fibres (Westerblad and Lannergren 1986). Moreover, this membrane depolarization is probably more pronounced in the T-tubular system, resulting in impairment of action potential propagation through this system (Jones 1981; Jones and Bigland-Ritchie 1986).

1.5.1.2 The Compound Muscle Action Potential

Impairment of electrical propagation is readily evident by examination of the muscle compound action potential (M-wave). The M-wave is the algebraic sum of all of the impulses evoked in a population of muscle fibres and therefore, provides information regarding impulse propagation between the nerve branches and the recording electrodes (Bigland-Ritchie et al. 1979; Duchateau and Hainaut 1985; Enoka and Stuart 1992). The peak-to-peak amplitude of the M-wave is considered representative of membrane excitability in skeletal muscle since it is dependent on both the resting membrane potential and the amplitude of the single fibre action potential. The duration of the M-wave is influenced by the synaptic delay across the neuromuscular junction, the synchronization of the muscle fibre action potentials and the conductance of the inward Na^+ and the outward K^+ channels within the muscle fibre membranes. An increase in duration, producing a broadening of the waveform, reflects a slowing of conduction velocity along the muscle

fibre membrane which may be attributed to a reduction in membrane excitability (Bigland-Ritchie et al. 1979).

M-wave recordings obtained in humans using surface or fine wire electrodes have provided indirect evidence that changes in the action potential shape and propagation velocity play a role in muscle fatigue (Bigland-Ritchie et al. 1979, 1981; Bigland-Ritchie and Woods 1984; DeLuca 1984; Jones and Bigland-Ritchie 1986). Notably, a decline in M-wave amplitude and an increase in M-wave duration has been reported during sustained voluntary contractions in humans (Milner-Brown and Miller 1986; Bellemare and Garzaniti 1988). These observations have contributed to the "K^+ hypothesis" as evidence that fatigue might be due to action potential failure, which is likely to be related to increased extracellular [K^+] (Bigland-Ritchie et al. 1979; Sejersted et al. 1982; Sjøgaard et al. 1985; Sjøgaard 1986; Medbo and Sejersted 1990). Intracellular action potentials recorded directly from isolated muscle preparations provide further support for this hypothesis. For example, an in vitro preparation of non-fatigued skeletal muscle demonstrated a 70% reduction in the muscle action potential following an increase in the [K^+] of the bathing medium from 5 to 10 mmol/L (Jones 1981). As well, a 20 - 40% reduction in action potential conduction velocity in rat muscle fibre bundles (Kossler et al. 1989) and in isolated mouse soleus and EDL (Juel 1988b) was observed when extracellular [K^+] increased from 5 to 10 mmol/L.

However, two additional observations in both human and animal studies support a growing speculation that the site of impaired action potential transmission may not be

located specifically in the sarcolemmal part of the muscle membrane. First, the described changes in the shape of the M-wave are not a consistent observation in human studies of fatigue (Bigland-Ritchie 1981; Merton et al. 1981) and second, most studies on muscle preparations have failed to observe a direct temporal relationship between sarcolemmal action potential amplitude/duration changes and muscle fatigue development or recovery (Sjøgaard 1990). These findings point to the T-tubule as the location of transmission failure. Ionic shifts similar to those across the sarcolemma are also likely to occur across the T-tubule membrane, and because of their restricted volume, larger ionic concentration changes may occur than at the sarcolemma. Hence, it is not unreasonable to propose that transmission failure is more likely to occur in the T-tubular system than along the sarcolemma.

1.6 SUMMARY

The results of animal and human studies have suggested the importance of increased extracellular [K^+] as a mechanism for muscle fatigue during exercise. While the membrane-bound Na^+-K^+-ATPase plays an important role in maintaining K^+ homeostasis during muscular activity, β-blockade has been shown to inhibit the β-adrenoceptor mediated Na^+-K^+ transport, leading to an elevation in plasma [K^+]. An accumulation of extracellular [K^+] along with a reduction in the electrogenic contribution of the Na^+-K^+ pump during β-blockade might be expected to impair muscle membrane excitability and contribute to the development of muscle fatigue. Hence, the relationship between plasma [K^+], muscle excitability and muscular performance may best be understood by the

measurement of muscle excitability during exercise-induced hyperkalemia and during β-blockade.

The M-wave is representative of skeletal muscle membrane excitability (and Na^+-K^+ pump activity) and therefore is useful in determining this relationship. A recent study reported that β-blockade did not exert any specific effect on either force or M-wave characteristics (Cupido et al. 1994), however, the intermittent nature of the fatigue protocol may have allowed sufficient blood flow in between contractions to wash out any significant accumulation of extracellular K^+. A study performed prior to the current work, developed a fatigue protocol that successfully elicited a significant increase in plasma [K^+] (West et al. 1996). Interestingly, the results again demonstrated no evidence of a loss in membrane excitability, providing further support for the role of the electrogenic Na^+-K^+ pump in maintaining excitability of the muscle fibres.

The focus of the following research is to further investigate the role of the Na^+-K^+ pump in preserving muscle excitability during muscular activity. Chapter two will compare the relationship between plasma [K^+], muscle excitability and force under conditions where the Na^+-K^+ pump is supposedly intact (placebo, selective β_1-blockade) with the conditions in which the Na^+-K^+ pump is inhibited (non-selective β-blockade). The hypothesis addressed in chapter two maintains that when the Na^+-K^+ pump is fully functional, it makes such a significant contribution to the membrane potential that muscle excitability is maintained during exercise in spite of the dramatic increase in extracellular [K^+].

CHAPTER II

THE RELATIONSHIP BETWEEN BETA-BLOCKADE, PLASMA POTASSIUM CONCENTRATIONS AND MUSCLE EXCITABILITY FOLLOWING STATIC EXERCISE

2.1 ABSTRACT

The effects of β-blockade on plasma [K^+], muscle excitability and force during exercise were examined. Nine healthy males (mean age 22.3 ± 1.7 yr) performed a 3- min fatigue protocol that consisted of a sustained submaximal contraction (30% MVC) of the right quadriceps muscle. Subjects performed the exercise after treatment with either placebo, β_1-selective (metoprolol, 100 mg) or an equipotent dose of non-selective $\beta_{1,2}$-blockade (propranolol, 80 mg, n = 6; 100 mg, n = 2; 120 mg, n = 1) twice a day for 76 hours before testing according to a randomized double-blind design. Arterial and femoral venous blood samples were drawn at rest, during exercise and during 15-min recovery. Maximal stimulation of the right femoral nerve was performed simultaneously with each blood sample to evoke a twitch and a compound muscle action potential (M-wave). The exercise-induced rise in plasma [K^+] did not differ between treatments, but K^+ uptake during recovery was slower following $\beta_{1,2}$-blockade. The evoked M-waves were unaffected by treatment, suggesting that β-blockade does not affect muscle membrane excitability following fatiguing exercise. However, during the propranolol trial, there was a significantly greater reduction (51.9 ± 7.3 %) in maximal voluntary torque

after the fatigue protocol compared with metoprolol (40.7 ± 3.6 %) or placebo (38.9 ± 3.6 %). Also, evoked torque was lower during the period of increased extracellular [K^+] following $\beta_{1,2}$-blockade. These results suggest that the effect of $\beta_{1,2}$-blockade on K^+ homeostasis during isometric muscle activity may occur at a point distal to surface membrane action potential, most likely in the T-tubular region.

2.2 INTRODUCTION

Exercise causes an elevation of plasma potassium concentration [K^+] as a consequence of a net efflux of K^+ from the working muscle (Sjøgaard et al. 1985). Since the intracellular-to-extracellular [K^+] gradient is crucial in the maintenance of membrane potential and excitability, the rise in extracellular K^+ during muscular activity might contribute to muscle fatigue by depolarizing single muscle fibre membranes and thereby reducing the force generating capacity of the muscle. To prevent K^+-induced membrane depolarization during exercise, the sarcolemmal Na^+-K^+ pump not only opposes the K^+ and Na^+ fluxes across the cell membrane (Bia and Defronzo 1981; Clausen and Everts 1989) but due to its electrogenic nature, also contributes to the membrane potential of skeletal muscle (Hicks and McComas 1989). The ensuing hyperpolarization then helps to overcome the depolarizing tendency of the increased interstitial [K^+] and to delay the loss of membrane excitability.

Short term control of the Na^+-K^+ pump is exerted not only by impulse-mediated alterations in ionic concentration gradients, but as well, through the adrenergic system (Clausen 1986). It has been suggested that improved clearance of exercise-induced increases in extracellular K^+ may result from a β_2-adrenoceptor-mediated effect of endogenous catecholamines on active Na^+-K^+ transport (Clausen and Flatman 1980). Notably, several studies have reported an earlier and more rapid elevation of plasma [K^+] with exercise, following treatment with $\beta_{1,2}$-blockade as opposed to β_1-blockade (Linton 1984; Cleroux 1989). Conversely, β_2-adrenergic agonists have been shown to enhance

cellular K^+ uptake by skeletal muscle in vitro (Lockwood and Lum 1974; Clausen and Flatman 1977; Brown et al. 1983; Juel 1988a). Evidence of this nature has led to the belief that β_2-adrenergic receptor stimulation is an essential element in short-term K^+ homeostasis through the regulation of K^+ uptake by skeletal muscle.

A recent study conducted in our lab that examined the effects of exercise-induced hyperkalemia on muscle excitability and fatiguability reported no evidence of a loss in muscle membrane excitability in spite of very significant increases in plasma [K^+] (West et al. 1996). The authors attributed this finding to the Na^+-K^+ pump-induced hyperpolarization of individual muscle fibres. However, that they also observed a strong relationship between the recovery of force and plasma [K^+] suggests that increased extracellular [K^+] may be exerting its effect at a site distal to surface membrane action potential propagation (i.e. the T-tubules).

An investigation of the effects of both $\beta_{1,2}$-blockade and β_1-blockade on plasma [K^+], muscle excitability and force would help to clarify the role of the Na^+-K^+ pump in preserving muscle membrane excitability during exercise. The primary purpose of the present study, therefore, was to compare the relationship between femoral venous plasma [K^+] and muscle excitability under conditions where the Na^+-K^+ pump was supposedly intact (placebo, β_1-blockade) and when it was inhibited ($\beta_{1,2}$-blockade). It was hypothesized that treatment with $\beta_{1,2}$-blockade would significantly impair the ability of the pump to offset the rise in extracellular [K^+] and this would lead to earlier and more rapid force failure as a result of excitability failure. Evidence of this effect was expected

to be found in both the femoral venous plasma [K^+] and in the assessments of muscle excitability and force during and following fatigue.

2.3 METHODS

2.3.1. SUBJECTS

Nine healthy male university students, whose mean (± SD) age, weight and height were 22.3 ± 1.7 yrs, 77.4 ± 7.8 kg, and 175.0 ± 7.1 cm, respectively, volunteered to participate in this study (Table 1). All were in good health and had no previous history of respiratory or neuromuscular disorders. The subjects were fully informed of the risks associated with the experimental procedures, and gave their written informed consent as requested by the medical ethics committee of McMaster University.

2.3.2 EXPERIMENTAL DESIGN

This study was conducted in two phases. Phase 1 was performed to establish equipotent doses of propranolol ($\beta_{1,2}$-blockade) and metoprolol (β_1-blockade) for each subject. Phase 2 was then undertaken to examine the effects of these drugs on plasma [K^+], muscle excitability and force.

2.3.3 DRUG ADMINISTRATION

For a three day period immediately prior to the scheduled testing day, subjects received twice daily oral doses of either 100 mg of metoprolol or an equipotent dose of propranolol. The final dose was taken on the fourth consecutive day, one hour before testing. Subjects reported to the laboratory following a light breakfast and having abstained from nicotine or caffeine products for a twelve hour period. At least seven days

separated each of the drug trials to ensure complete drug washout. During phase 1 of the experiment, both subjects and investigators were unblinded to the drug intervention. Phase 2 was designed as a randomized double-blind placebo controlled study.

2.3.4 PHASE 1: ESTABLISHMENT OF DRUG DOSES

This stage of the investigation was undertaken to establish the equipotent dose of propranolol required by each subject to produce the effects elicited during exercise by 100 mg twice daily of metoprolol.

Apparatus. Maximal and sub-maximal dynamic exercise tests were performed on an electrically braked cycle ergometer (Monark #868). After securing a noseclip into position, subjects executed the test while breathing through a rubber mouth-piece connected to a Plexiglass open-circuit gas collection system. Expired gases were sampled at 30-second intervals in order to obtain measurements of oxygen (model OM-11 oxygen analyzer, Beckman) and carbon dioxide (model 78356A capnometer, Hewlett-Packard). The gas proportion was read by custom-made software (Vacumetrics) in an IBM computer that computed the oxygen uptakes of the subject over the course of the exercise test.

Silver-silver chloride electrodes (No. 2248, 3M) were used to monitor heart rate (Respironics, Exersentry, IL) continuously during exercise. The chest was carefully prepared (shaved, abraded and wiped clean with rubbing alcohol) before placement of the electrodes in the V5 position.

Experimental Protocol. Maximal oxygen consumption (VO_{2max}) was determined in the control state by a progressive cycle ergometer exercise test. Cycling began at 200 watts and the work load was manually incremented at 2 minute intervals until exhaustion (defined as failure to maintain a cycling rate of 50-60 rpm) was reached. Subsequent drug trials were performed at 70% of each subjects VO_{2max} under two different conditions: 1) metoprolol (100 mg twice daily) and 2) an initial dose of propranolol (80 mg twice daily). For the purposes of this study, the doses were considered equipotent if the heart rate attenuation at 70% of the VO_{2max} in the control state (HR_{70}) differed by less than ±5 beats/min during the two drug trials. Failing this, the propranolol dose was adjusted by 20 mg twice daily (raised or lowered) for three days and a repeat exercise test was performed. The titration process proceeded as necessary until all subjects demonstrated a HR_{70} that was within the required ±5 beats/min of that measured during their metoprolol trial. By limiting the variability in heart rate attenuation to ±5 beats/min, the mean submaximal heart rates between the two drugs were almost identical (111 ± 3 vs. 112 ± 3 beats/min), suggesting that similar degrees of β-blockade were achieved.

2.3.5 PHASE 2: EFFECT OF β-BLOCKADE ON PLASMA [K^+], MUSCLE EXCITABILITY AND FORCE

Catheterization. Indwelling catheters were used to draw blood from the right femoral vein and the right brachial artery for measurement of plasma [K^+] and plasma [La^-]. Catheterization of the brachial artery and femoral vein was performed one hour before the test. After cleansing the inguinal area with betadine and administering a local anaesthetic subcutaneously (5-10 ml of 2% xylocaine without epinephrine; Astra

Pharmaceuticals Inc., Houston, Texas), the Seldinger technique was used to position a catheter (VC FN 7.5-38-J, Cook Canada Inc., Stouffville, Ontario) approximately 13 cm retrograde into the femoral vein. The antecubital area was then prepared in a similar manner and following infiltration of the skin with 1 ml of 2 % xylocaine without epinephrine, a Teflon catheter (20 gauge, 3.2 cm; Becton/Dickinson and Co., Sandy, Utah) was introduced percutaneously into the brachial artery. A slow infusion of nonheparinized isotonic saline (0.9% NaCl, Baxter Healthcare Corp., Deerfield, Illinois) was used to maintain patency of both catheters.

Stimulating and Recording Apparatus. Surface electrical recordings of evoked M-waves were obtained from the vastus medialis muscle. Evoked twitch torques and maximal and submaximal voluntary torques of the quadriceps muscle group were obtained as measurements of mechanical force. Figure 1 *(top)* demonstrates the leg apparatus utilized in this study. The electrode placements, pressure cuff position and femoral catheter site are represented in Figure 1 *(bottom)*.

Subjects sat with their right knee flexed at a 90° angle and their back against an upright support such that the upper leg was positioned at a 100° to the trunk. The leg was prepared for electrode placement by shaving the skin and rubbing it with an abrasive and alcohol. Two 57 mm X 103 mm carbon-impregnated rubber electrodes coated with an electrode jelly were used for transcutaneous stimulation of the right femoral nerve. The cathode was placed in the inguinal crease, over the course of the femoral nerve, and the anode was placed on the anterior aspect of the mid thigh area. Electromyographic

(EMG) recordings were made with two disposable silver-silver chloride monitoring electrodes (No. 2248, 3M) with a recording surface of 5 mm. According to a monopolar derivation, the stigmatic electrode was placed over the belly of the right vastus medialis muscle and the reference electrode was placed approximately 2 cm distal to this and slightly medial to the patella. A silver strip electrode (6 mm X 50 mm) served as the ground and was placed on the lateral aspect of the right thigh, between the anode and the stigmatic electrode. After wrapping a blood pressure cuff (Baumanometer Calibrated V-Lok Cuff; W.A. Baum Co. Ltd., Copiague, New York) loosely around the right leg immediately below the knee, the lower leg was secured in a metal brace by two Velcro straps fastened around the proximal and distal aspects of the lower right limb. Two additional Velcro straps were fastened around the proximal and middle portions of the right thigh to stabilize the upper leg throughout the test. Isometric force produced by the knee extensors was determined from a strain gauge mounted at the level of the knee joint within the metal leg brace.

A high-voltage stimulator (Devices Stimulator 3072, Medical Systems Corp.) was used to deliver single rectangular voltage pulses (pulse width: 200-500 μs) to the femoral nerve. The EMG signals from the recording electrodes were fed into a Honeywell Accudata EMG Amplifier (model #135A) at a sampling rate of 2.7 KHz, filtered (.004-2.5 KHz), and were displayed in real time on a VGA computer monitor (model 2431PO, CTX). The EMG and the evoked and voluntary torques were streamed continuously to

disk by means of a Dataq waveform scrolling board (AT CODAS Interface Card; Dataq Instruments Inc., Akron, Ohio) in an IBM-compatible computer.

Experimental Protocol. The experimental design and timing of the data collection are summarized in Figure 2.

Pre-Fatigue. Once the subject was secured into the testing apparatus, baseline (BL) blood samples were drawn from both the brachial artery and the femoral vein. Following this, the peak torque was determined by progressively increasing the stimulation intensity until no further increase in torque or M-wave occurred. Baseline values of peak twitch torque (Pt) and M-wave were then recorded. This voltage was used to evoke all subsequent twitches throughout the remainder of the experiment. Next, subjects executed three maximal voluntary contractions (MVC) of the right quadriceps muscle group, with 1 minute rest intervals between each 5 second contraction. The highest torque value was used as the outcome measure (MVC1) and to determine the force required for the subsequent fatigue test. An interpolated stimulus was delivered during the voluntary contraction as an indication of the degree of muscle activation achieved by the subjects (Belanger and McComas 1981). The theoretical motor unit activation (MUA) was calculated as follows:

$$\% \text{ MUA} = \frac{\text{Twitch Torque} - \text{Interpolated Twitch Torque}}{\text{Twitch Torque}} \times 100$$

Just prior to commencement of the fatigue protocol, a second baseline assessment was performed that included an arterial and femoral venous blood sample as well as an

evoked M-wave and twitch recording. This time point is referred to as immediately before contraction (IBC).

Fatigue. To induce fatigue in the right quadriceps muscle group, subjects sustained an isometric contraction at a 30% MVC intensity for 3 minutes. Both visual (computer monitor display) and verbal (experimenter) feedback was used to monitor torque output during the exercise period. A single arterial and femoral venous blood sample was drawn during the contraction (DC) at the 2-minute time point. Upon completion of the sustained contraction, subjects were encouraged to attempt an MVC (MVC2) in order to assess the magnitude of the quadriceps fatigue.

Recovery. As the maximal contraction was released, the blood pressure cuff was inflated to 80-100 torr to prevent venous admixture throughout the ensuing 15-minute recovery period. Subjects remained relaxed as simultaneous blood sampling and stimulation (evoked twitch and M-wave) was performed at the following time intervals: once every fifteen seconds for three minutes; once every minute over the next two minutes; and once every five minutes during the final ten minutes of recovery. A tone preceded each stimulation to ensure the blood sampling was timed simultaneously with the twitch. The timing and delivery of the tones and twitches were controlled by a Stoelting Laboratory Controller (Stoelting Laboratory Corp.) interfaced with the computer.

2.3.6 DATA ANALYSES

Blood. All blood samples were drawn into 4.5 ml heparinized syringes (Monovette Li-Heparin plastic syringe, Sarstedt Inc., St. Laurent, Quebec) and

immediately transferred into microcentrifuge tubes (Fisher Scientific, 1.8 ml, cat. no. 05-664-10, Ottawa, Ontario) positioned in an ice tray. The whole blood tubes were centrifuged at 12,400 rpm (Fisher Scientific Micro Centrifuge, model #235C) for separation of plasma, which was subsequently drawn off and stored at -20° until electrolyte analyses were performed. Plasma samples were measured in duplicate for $[K^+]$ and $[La^-]$ using respectively, an automated Na^+/K^+ analyzer (Radiometer KNA2, Copenhagen, Denmark) and an automated lactate analyzer (Yellow Springs Instruments model 23L, Yellow Springs, Ohio).

EMG and Force. A custom-designed computer-based oscillograph and data acquisition system analysis software program (CODAS, release 4.0, Dataq Instruments, Inc., Akron Ohio) were used to analyze all of the electrical and mechanical recordings.

The M-wave parameters measured were peak-to-peak amplitude, duration and area. Analyses of the evoked twitch recordings included peak twitch torque and half relaxation time measurements. The voluntary torques recorded throughout the fatigue protocol were normalized relative to the baseline MVC value.

Statistics. Dependant variables were analyzed for treatment effects with a two factor (drug x time) repeated measures analysis of variance (ANOVA). Significant differences between the means were determined by a Tukey HSD (Tukey a) post hoc test. Polynomial regression analyses were carried out to examine the effects of β-blockade and fatigue on the relationship between plasma $[K^+]$ and force. Statistical significance was accepted at $p < 0.05$. Unless otherwise stated, all values are reported as means ± SEM.

2.4 RESULTS

2.4.1 PHASE 1. DETERMINING EQUIPOTENCY OF DRUG DOSES

Six subjects experienced equipotent effects on submaximal heart rate attenuation while receiving 100 mg metoprolol and 80 mg propranolol. In the remaining three subjects, titration of the propranolol dose to 100 mg in two subjects and 120 mg in one subject was required to achieve equipotency. During submaximal exercise (70% Vo_{2max}), there was no significant difference in heart rate attenuation between the drug trials. The mean heart rates were reduced by 33 ± 3 beats/min and 34 ± 3 beats/min after the metoprolol and propranolol trials, respectively. Individual effects of equipotent doses of metoprolol and propranolol on submaximal heart rates are summarized in Table 2.

2.4.2 PHASE 2. EFFECT OF β-BLOCKADE ON PLASMA [K^+], MUSCLE EXCITABILITY AND FORCE

The focus of these results is to address the differences between control and β-blockade trials. Although time effects will be reported, drug effects and drug by time interactions are the primary interests of this investigation. For clarity, the asterisks in the figures will be used specifically to denote significant differences between the trials.

Plasma Ion Concentrations.

Arterial plasma [La^-]. As illustrated in Figure 3, *(top)* arterial plasma [La^-] increased ($p < 0.01$) from a baseline of 1.2 ± 0.1 mmol/ l (placebo), 1.1 ± 0.2 mmol/l (metoprolol) and 1.1 ± 0.1 mmol/l (propranolol) to a peak value of respectively 5.2 ± 0.5 mmol/l, 5.3 ± 0.3 mmol/l and 5.7 ± 0.5 mmol/l within 2 min 20 sec post-exercise. From

that point on, [La^-] gradually declined but remained significantly higher than baseline at the 15 min mark of recovery. There were no between trial differences in measurements of arterial plasma [La^-].

Femoral Venous plasma [La^-]. During the contraction, femoral venous plasma [La^-] increased significantly from a baseline value of 1.0 mmol/l (placebo), 0.9 ± 0.1 mmol/l (metoprolol) and 1.0 ± 0.1 mmol/l (propranolol) to a concentration of respectively 2.6 ± 0.3 mmol/l, 2.4 ± 0.2 mmol/l, and 2.0 ±0.3 mmol/l. Peak values of 9.4 ± 0.9 mmol/l (placebo), 9.7 ± 0.8 mmol/l (metoprolol) and 9.8 ± 1.0 mmol/l (propranolol) were obtained within 1 min 35 sec of recovery. Femoral venous plasma [La^-] then gradually decreased over the remainder of the recovery period, but remained significantly higher than baseline. As illustrated in Figure 3 *(bottom)*, there was no effect of the metoprolol or propranolol treatments on femoral venous plasma [La^-].

Arterial plasma [K^+]. At baseline, arterial plasma [K^+] was similar for placebo (4.4 ± 0.1 mmol /l), metoprolol (4.4 ± 0.1 mmol/l) and propranolol (4.3 ± 0.1 mmol/l). Within 35 seconds of recovery from the fatiguing contraction, the [K^+] increased ($p < 0.01$) to peak values of 5.1 ± 0.1 mmol/l (placebo), 5.3 ± 0.1 mmol/l (metoprolol) and 5.4 ± 0.1 mmol/l (propranolol), with no significant differences between the trials. However, as shown in Figure 4 *(top)*, a between trial difference was evident from 35 sec to 4 min post-exercise; several [K^+] values over this period of recovery were significantly higher following administration of propranolol than following the placebo treatment. Moreover, the [K^+] returned to resting values during the early stages of recovery during the placebo

and metoprolol trials, whereas the baseline value of arterial plasma [K^+] was never fully restored during the propranolol trial.

Femoral Venous plasma [K^+]. The baseline femoral venous plasma [K^+] was similar for placebo (4.4 ± 0.1 mmol/l), metoprolol (4.4 ± 0.1 mmol/l) and propranolol (4.4 ± 0.2) During the contraction, the [K^+] increased ($p < 0.01$) to values of 5.1 ± 0.1 mmol/l (placebo), 5.3 ± 0.1 (metoprolol) and 5.0 ± .01 (propranolol), with no significant differences among the groups. Peak values of 6.0 ± 0.2 mmol/l (placebo) and 6.3 ± 0.2 mmol/l (metoprolol and propranolol) were obtained at 5 sec post-exercise, representing an increase in concentration of 36 % and 43 %, respectively; there were no between trial differences. Femoral venous plasma [K^+] then began to decrease in all three trials, but the decline occurred much more rapidly during the placebo and metoprolol trials versus the propranolol trial. As shown in Figure 4 *(bottom)*, this difference was significant at several time points throughout the initial 2 min 20 sec of recovery.

During the placebo trial, there was a trend for [K^+] to drop below baseline between 3 and 5 min into the recovery period. Although this did not achieve significance with respect to the baseline measure, it did represent a significant decline (as much as 8.7 %) relative to the β-blockade trials.

Voluntary and Evoked Force.

Table 3 summarizes baseline values of voluntary torque, evoked twitch torque, interpolated twitch torque and theoretical motor unit activation.

Voluntary Torque. Compared with placebo (254.3 ± 17.3 Nm), the torque generated by the baseline MVC was not significantly affected by either metoprolol or propranolol. Similarly, motor unit activation was the same across all conditions with values of 81.8 ± 3.6 %, 83.3 ± 3.7 % and 87.1 ± 1.6 % calculated for placebo, metoprolol and propranolol, respectively.

Figure 5 shows the voluntary torque over the course of fatigue. Torques recorded throughout 2 min 30 sec of the sustained contraction were similar between all trials and ranged from 27.5 ± 1.2 % to 29.9 ± 0.6 % of the baseline MVC values. However, a between trial difference was observed in the final sample obtained at 2 min 53 sec into the contraction. Although the placebo and metoprolol trials remained unchanged, voluntary torque was significantly reduced during the propranolol trial to 23.7 ± 2.8 % of the corresponding baseline MVC value. This fatigue effect was also seen in the MVC force following the sustained submaximal contraction (MVC2). Relative to the respective MVC1 values, a significantly lower torque was achieved during the propranolol trial (48.1 ± 7.3 %) than during the placebo (61.1 ± 3.6 %) and metoprolol (59.3 ± 3.6 %) trials.

Evoked Twitch Torque. Figure 6 *(top)* illustrates the effect of β-blockade treatment on the torques generated by the evoked twitch (Pt) throughout the experimental protocol. At baseline, Pt was slightly higher in the placebo trial (48.9 ± 5.0 Nm) than in the metoprolol (44.8 ± 5.0 Nm) and propranolol (44.3 ± 5.2 Nm) trials, but the difference did not attain significance. Immediately before the fatigue protocol began, significant increases in Pt were observed for the placebo and metoprolol trials (a 13.5 % and a 21.2

% increase, respectively). At 5 sec post-exercise, Pt was reduced ($p < 0.01$) for all three drug trials; notably, the significant difference that was observed between the propranolol condition versus the placebo and metoprolol conditions just prior to the contraction was maintained throughout the initial 3 min of the recovery period.

The evoked twitch demonstrated a significant potentiation during the placebo and metoprolol trials. At 2 min 50 sec post-exercise, Pt was 55.2 ± 5.1 Nm (placebo) and 55.2 ± 6.1 Nm (metoprolol), respresenting a 12.9 % and a 23.2 % increase above baseline, respectively. Although the propranolol trial followed a similar pattern of recovery, it did not attain a significant potentiation.

Half-relaxation time measurements associated with Pt were not significantly affected by the drug treatments (Figure 6, *bottom*). Moreover, there were no significant changes in this twitch characteristic over the course of the experimental protocol.

Evoked EMG.

Table 4 summarizes the M-wave characteristics that were obtained at baseline.

Evoked M-wave. Figure 7 illustrates the effects of the experimental protocol and β-blockade on the amplitude *(top)*, duration *(middle)* and area *(bottom)* of the M-waves. The M-wave characteristics were not significantly affected by the drug treatments.

Relative to baseline, the fatigue task did not elicit any significant changes in M-wave amplitude throughout the early stages of recovery. A significant difference was observed, however, following 10 min of recovery when the M-wave amplitude decreased slightly below baseline (a 5.7 % decrease).

The fatiguing contraction resulted in a significant decrease in M-wave duration at 5 sec post-exercise (a 5.8 % decrease from baseline). Pre-fatigue values were subsequently restored within 15 seconds of recovery. From that point on, the measurements remained stable until the 10 min time point, when duration decreased to a level just below baseline (a 5.8 % decrease).

M-wave area followed a similar pattern to M-wave amplitude, such that there were no significant differences until the latter stages of recovery, when area decreased below baseline (a 11.1 % decrease).

Potassium/Force Relationship.

The relationship between femoral venous plasma [K^+] and Pt was determined for each of the trials (Figure 8). A curvilinear relationship was evident in all three groups, such that the recovery of Pt was significantly related to the recovery of femoral venous plasma [K^+]. The curves providing the best fit for the data points (placebo, $r^2 = .72$; metoprolol, $r^2 = .75$; and propranolol, $r^2 = .83$) are described by the equations:

$$Pt = 269.18 - 80.2071\ K + 7.03\ K^2 \quad \text{(placebo)}$$

$$Pt = 268.44 - 76.66\ K + 6.43\ K^2 \quad \text{(metoprolol)}$$

$$Pt = 198.40 - 50.83\ K + 3.90\ K^2 \quad \text{(propranolol)}$$

where Pt = Twitch torque (Nm) and K = venous [K^+] (mmol/l).

A downward shift in the curve representing this relationship was observed during the propranolol trial, such that a lower Pt was associated with the exercise-induced increases in K^+ compared to the metoprolol or placebo trials.

2.5 DISCUSSION

The hypothesis addressed in this study maintains that inhibition of the Na^+-K^+ pump with $\beta_{1,2}$-blockade may impair exercise performance through its effect on K^+ homeostasis and thus muscle excitability. To date, changes in excitability during β-blockade have only been inferred based on measurement of plasma [K^+]. The methodology employed in this study permitted assessment of muscle excitability during exercise-induced hyperkalemia and during β-blockade in order to gain new insight into the relationship between plasma [K^+], muscle excitability and muscular performance.

2.5.1 THE EFFECT OF β-BLOCKADE ON PLASMA [K^+]

Increases in plasma [K^+] are characteristic of exercise and several studies have demonstrated significantly higher plasma [K^+] under conditions of β-blockade (Rosa et al. 1980; Linton et al. 1984; Williams et al. 1985; Cleroux et al. 1989). In the present study, β-blockade did not modify the exercise-induced rise in plasma [K^+]. The discrepancy between the results of this study and previous work probably relates to differences in the exercise challenge and the strain imposed on Na^+-K^+ pump exchange in the muscle fibres. At the intensity of contraction used in this study, the rise in intramuscular pressure was sufficient to significantly reduce the blood flow through the muscle belly, thereby preventing clearance of K^+ from the interstitial space. It has been suggested that the Na^+-K^+ pump in contracting muscle is not always capable of transporting K^+ back into the cell at sufficient rates to maintain constant ionic balance (Clausen et al. 1987; Clausen and Everts 1988). In this regard, K^+ efflux from intensely contracting skeletal muscle might

well have exceeded the maximal capacity of the Na^+-K^+ pump for K^+ transport during all trials in this study. Thus, any treatment effect on the exercise-induced rise in extracellular [K^+] would likely be masked by the effect of mechanical occlusion, resulting in similar increases in femoral venous plasma [K^+] under all conditions.

However, consistent with previous studies (Carlsson et al. 1978; Lundborg et al. 1981; Laustiola et al. 1983; MacDonald et al. 1984), the results of the present work have shown that $\beta_{1,2}$-blockade delays the recovery of plasma [K^+] to resting levels. This was evident in both active as well as inactive tissues as demonstrated by sustained increases in femoral venous plasma [K^+] (indicating insufficient activation of the Na^+-K^+ pump in the previously active muscle) and arterial plasma [K^+] (suggesting inadequate Na^+-K^+ pump activity in non-contracting fibres).

Conversely, the rapid normalization of arterial and femoral venous plasma [K^+] observed following treatment with β_1-blockade and placebo indicates that the mechanisms acting to restore intracellular [K^+] and lower extracellular [K^+] were intact upon restoration of circulation during these trials. Specifically, several studies have demonstrated that short term control of Na^+-K^+ pump activity during exercise-induced disturbances of K^+ homeostasis is exerted primarily through the adrenergic system, i.e. epinephrine and sarcolemmal β-adrenoceptors (Todd and Vick 1971; Wang and Clausen 1976; Buur et al. 1982; Flatman and Clausen 1989). Epinephrine levels have been shown to remain elevated for up to 5 minutes post-exercise (Kjaer 1989), which would keep the pump stimulated in the early phases of recovery. That the effects of epinephrine are

elicited specifically via β_2-adrenoceptors (Olsson et al. 1978; Brown et al. 1983; Juel 1988b; Clausen and Flatman 1989) is consistent with the results of the present study and may explain the delayed recovery of K^+ homeostasis during β-blockade with propranolol.

2.5.2 THE EFFECT OF β-BLOCKADE ON MUSCLE MEMBRANE EXCITABILITY

The combined effects of exercise-induced increases in extracellular [K^+] and the decline in the intracellular [K^+] will produce a significantly lower intracellular-to-extracellular potassium ratio, and it has been suggested that this may induce significant changes in muscle membrane potential resulting in impaired excitability and contractility (Sjøgaard et al. 1985; Hnik et al. 1986; Medbo and Sejersted 1990). The muscle compound action potential (M-wave) has been shown to provide an accurate index of changes in muscle membrane excitability since it is dependent on both the resting membrane potential and the amplitude of the single fibre action potential (Hicks et al. 1989). Many studies have therefore employed M-wave measurements to examine how fatigue affects the excitability of skeletal muscle (Merton 1954; Bigland-Ritchie et al. 1979; Bigland-Ritchie et al. 1982; West et al. 1996).

Recent work conducted in our lab utilized M-wave measurements during an intermittent voluntary fatigue protocol to investigate whether a failure in muscle excitability contributes to increased fatigue with β-blockade (Cupido 1994). These investigators reported that β-blockade did not exert any specific effect on either force or M-wave characteristics, however, the intermittent nature of the fatigue protocol may have allowed sufficient blood flow in between contractions to wash out any significant build-up

of extracellular K^+. The present study, therefore, utilized M-wave measurements during a sustained voluntary fatigue protocol to further investigate the effect of β-blockade on muscle excitability following exhaustive exercise.

The observation that the M-wave characteristics were not compromised by β-blockade or by increased extracellular [K^+] is an interesting one. Notably, these results confirm and extend the results of a previous investigation by West et al. (1996). The fatigue protocol developed by these investigators for their study of changes in force, EMG and plasma [K^+] following voluntarily-induced fatigue was subsequently utilized in the current work. They also reported a preservation of muscle membrane excitability during recovery from the fatiguing contraction despite very significant increases in plasma [K^+]. Taken together, these results support the hypothesis that through increased electrogenic activity, the Na^+-K^+ pump activity along the sarcolemma can maintain muscle fibre membrane potentials during muscular activity, and thereby compensate for the rise in extracellular [K^+] (Hicks and McComas 1989).

Although the M-wave measurements obtained in the present study do not indicate any effect of β-blockade on peripheral muscle excitability following fatigue, other data from this investigation challenges this position.

2.5.3 THE EFFECT OF β-BLOCKADE ON THE RELATIONSHIP BETWEEN PLASMA [K^+] AND FORCE

The discovery of a strong relationship between femoral venous plasma [K^+] and evoked twitch torque during recovery in all trials, suggests that muscle contractile function is indeed being influenced by the rise in extracellular [K^+]. This relationship (in

the control state) was first reported by West and colleagues (1996); the use of β-antagonists in the present study has extended their observation to show that exercise-induced increases in femoral venous plasma [K^+] are associated with a more attenuated force production following the administration of propranolol versus metoprolol or placebo.

Taken together, these findings support a growing speculation that the site of impaired action potential transmission may not be located specifically in the sarcolemmal part of the muscle membrane (Venosa and Horowicz 1981; Renaud and Light 1992). Although one is inclined to predict a loss of muscle membrane excitability given that muscle extracelluar [K^+] during exercise is even greater than that of the simultaneously collected venous effluent (Hnik et al. 1976; Hirche et al. 1980), the well-maintained M-waves observed in both this study and in the work by West et al. (1996) suggest that the electrical events at the sarcolemma and thereby, also the K^+ gradient across the sarcolemma are not the direct cause of fatigue. Rather, these findings question whether the inhibiting effects of K^+ may be occurring at a site distal to the muscle cell membrane. It has been reported that the T-tubules reach a critically higher [K^+] than at the sarcolemma due to the increased surface to volume ratio, poor diffusion and the decreased density of Na^+-K^+ ATPase in this region (Jones et al. 1979; Venosa and Horowicz 1981). It follows from this that K^+-induced transmission failure of the action potential in the T-tubule may exist as a general fatigue mechanism (Sjøgaard 1990). In this regard, the greater loss of force following treatment with propranolol during the period of increased

femoral venous plasma [K^+] in this study may be attributed to the effect of β-blockade on K^+ homeostasis in the T-tubules, since there was no evidence of a treatment effect at the muscle cell membrane. Further exacerbation of the elevated extracellular [K^+] in the T-tubules due to interference with Na^+-K^+ transport activity following $\beta_{1,2}$-blockade, would have likely prolonged the depolarization of muscle fibre membranes, resulting in a significantly greater reduction in Ca^{++} release and in subsequent muscular tension development than during the metoprolol or placebo trials.

Furthermore, impaired excitation of the T-tubular membranes may also explain the significantly greater attenuation of exercise performance during single limb exercise following $\beta_{1,2}$-blockade treatment versus β_1-blockade or placebo in the present study. Since subjects were capable of achieving normal levels of motor unit activation (as assessed by the interpolated twitch technique), regardless of the treatment, it seems likely that this fatigue effect was associated with hyperkalemia. Although EMG data was not collected during the contraction, in view of the preserved M-wave characteristics immediately following the contraction (at a higher plasma [K^+] level than during the contraction), it is tempting to speculate that alterations in K^+ homeostasis in the T-tubular region may be responsible for the reduced exercise performance following treatment with non-selective β-blockade.

Alternatively, studies regarding muscle metabolism during exercise have shown a fairly consistent association between fatigue and an accumulation of lactate, which simultaneously induces a decrease in pH (Hermansen et al. 1984; Wilkie 1986). However,

this does not prove that acidosis is a cause of fatigue and indeed, in humans there are situations in which fatigue appears to be unrelated to lactate accumulation and/or decrease in pH; this is demonstrated during prolonged exercise at a moderate intensity and in patients with myophosphorylase deficiency who cannot produce H^+ from glycolysis, but can well experience fatigue (Edwards 1983). In the present study, the similar increases in lactate during all three trials despite between trial differences in force generating capacity, supports the notion that pH cannot entirely be responsible for muscle fatigue. Rather, it seems likely that K^+ fluxes within the T-tubule are of major importance, acting not only as a fatigue mechanism to explain impaired mechanical function, but as well, as a safety mechanism protecting the cell against ATP depletion and self-destruction (Edwards 1983).

2.6 SUMMARY

The major significance of this study is that it is the first to report simultaneous changes in force, EMG, and plasma [K^+] during exercise-induced hyperkalemia and during β-blockade. It has been demonstrated that although β-blockade does not appear to augment the exercise-induced rise in plasma [K^+] that accompanies a sustained isometric contraction of the quadriceps muscle group, $\beta_{1,2}$-blockade delays the normalization of plasma [K^+] during the recovery period. Moreover, muscle membrane excitability seems to be well maintained in the presence of significant increases in plasma [K^+]. The latter findings, together with the more attenuated force production relative to plasma [K^+] during recovery with $\beta_{1,2}$-blockade compared with β_1-blockade or placebo, support the

conclusion that the inhibitory effect of $\beta_{1,2}$-blockade on K^+ homeostasis may be occuring in the T-tubular region. Accordingly, the results of this investigation offer suggestive evidence that inhibition of β_2-adrenergic receptors in skeletal muscle induces significant changes in T-tubular membrane potential, resulting in impaired excitability and thus contractility of the muscle fibres.

TABLE 1. SUBJECT CHARACTERISTICS

SUBJECT	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)
SI	26	168	72.0
JM	24	173	72.0
JT	21	172	73.0
MS	22	167	93.0
BR	23	185	85.5
MH	20	181	82.0
MB	21	185	80.5
MG	22	166	69.5
SC	22	178	69.0
Mean	22.3	175.0	77.4
± SD	1.7	7.1	7.8

TABLE 2. SUBJECT SUBMAXIMAL HEART RATES

SUBJECT		TRIAL	HR 70% (bpm)
SI		Control	150
	100 mg	Metoprolol	118
	80 mg	Propranolol	122
JM		Control	144
	100 mg	Metoprolol	110
	80 mg	Propranolol	110
JT		Control	140
	100 mg	Metoprolol	108
	120 mg	Propranolol	112
MS		Control	150
	100 mg	Metoprolol	108
	100 mg	Propranolol	110
BR		Control	128
	100 mg	Metoprolol	98
	80 mg	Propranolol	102
MH		Control	154
	100 mg	Metoprolol	116
	80 mg	Propranolol	112
MB		Control	126
	100 mg	Metoprolol	100
	80 mg	Propranolol	100
MG		Control	168
	100 mg	Metoprolol	124
	100 mg	Propranolol	122
SC		Control	144
	100 mg	Metoprolol	116
	80 mg	Propranolol	118
Mean ± SEM			
		Control	145 ± 4
		Metoprolol	111 ± 3
		Propranolol	112 ± 3

TABLE 3. BASELINE MEASUREMENTS

Trial	MVC (Nm)	Pt (Nm)	ITT (Nm)	MUA (%)
Placebo	254.3 ± 17.3	48.9 ± 5.0	9.1 ± 2.4	81.8 ± 3.6
Metoprolol	267.1 ± 15.6	44.8 ± 5.0	7.2 ± 1.2	83.3 ± 3.7
Propranolol	256.0 ± 10.8	44.3 ± 5.2	6.1 ± 1.3	87.1 ± 1.6

MVC Maximum Voluntary Contraction
Pt Evoked Twitch Torque
ITT Interpolated Twitch
MUA Estimated Motor Unit Activation

Values are group means ± SEM; n = 9

TABLE 4. BASELINE MEASUREMENTS

	M-Wave		
Trial	**Amplitude (mV)**	**Duration (msec)**	**Area (mV·s)**
Placebo	20.2 ± 1.1	37.3 ± 1.1	0.17 ± 0.01
Metoprolol	21.2 ± 1.5	38.4 ± 1.0	0.18 ± 0.01
Propranolol	22.2 ± 1.8	37.8 ± 1.3	0.18 ± 0.01

Values are group means ± SEM; n = 9

FIGURE LEGENDS

FIGURE 1. *Top:* Leg apparatus with restraining straps used in protocol.
Bottom: Electrode placements, pressure cuff position and femoral catheter site.

FIGURE 2. Schematic diagram of the pre-fatigue, fatigue and recovery protocol.
* Blood sample (arterial and venous)
↓ Evoked twitch and M-wave
MVC1 Maximum voluntary contraction prior to the fatigue protocol
MVC2 Maximum voluntary contraction at the end of the fatigue protocol
BL Baseline
IBC Immediately before contraction
DC During contraction

FIGURE 3. *Top:* The effect of placebo (o), metoprolol (∇) and propranolol (□) on arterial plasma lactate concentration at baseline (BL), immediately before contraction (IBC), during fatigue (DC) and over 15 min of recovery.
* indicates mean is significantly different ($p < 0.05$) from control and metoprolol trials. Values are group means ± SEM; n = 9
Bottom: Venous plasma lactate concentration. Details as above.

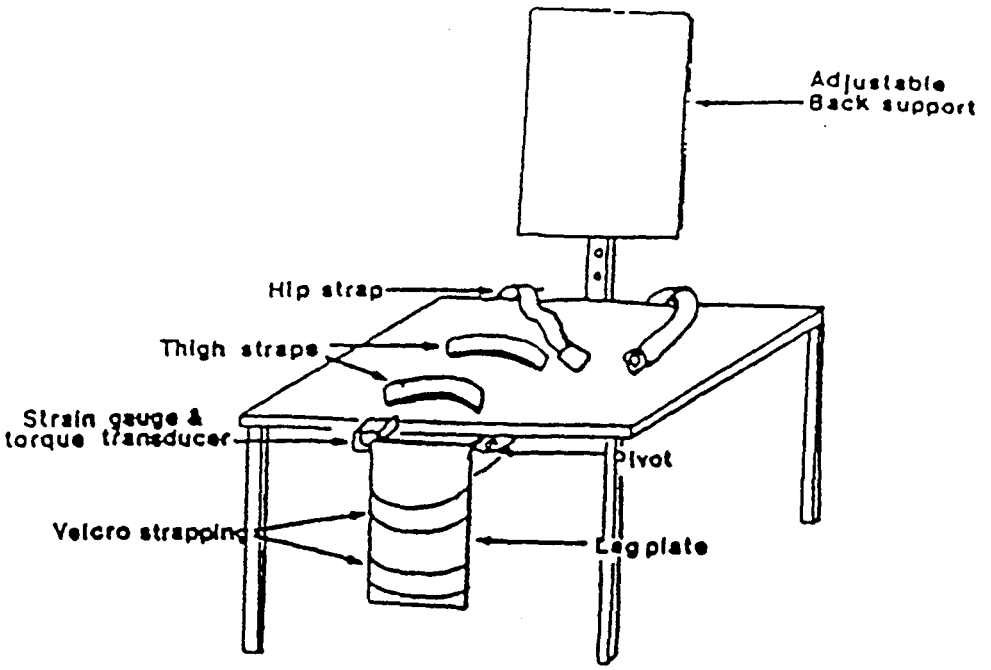
FIGURE 4. The effect of placebo (o), metoprolol(∇)and propranolol (□)on arterial *(top)* and venous *(bottom)* plasma potassium concentration at baseline (BL), immediately before contraction (IBC), during fatigue (DC) and over 15 min of recovery.
* *(top)* indicates mean is significantly different ($p < 0.05$) from control trial.
* *(bottom)* indicates mean is significantly different ($p < 0.05$) from control and metoprolol trials.
Values are group means ± SEM; n = 9.

FIGURE 5. The effect of placebo (o), metoprolol (∇) and propranolol (□) on voluntary torque during the sustained isometric quadriceps contraction and the following maximum voluntary contraction. Significant difference from control and metoprolol trials indicated by * ($p < 0.05$). Values are group means ± SEM; n = 9.

FIGURE 6. Evoked twitch torque *(top)* and half-relaxation time *(bottom)*. Details as in Figure 3 except DC measurement was not performed.

FIGURE 7. M-wave amplitude *(top)*, duration *(middle)* and area *(bottom)*. Values are group means ± SEM; n = 9.

FIGURE 8. The effect of placebo (●) *(top)*, metoprolol (▼) *(middle)* and propranolol (■) *(bottom)* on the relationship between femoral venous plasma potassium concentration and twitch torque during recovery. Values are group means; n = 9.


Adjustable
Back support
Hip strap
Thigh straps
Strain gauge &
torque transducer
Pivot
Velcro strapping
Leg plate

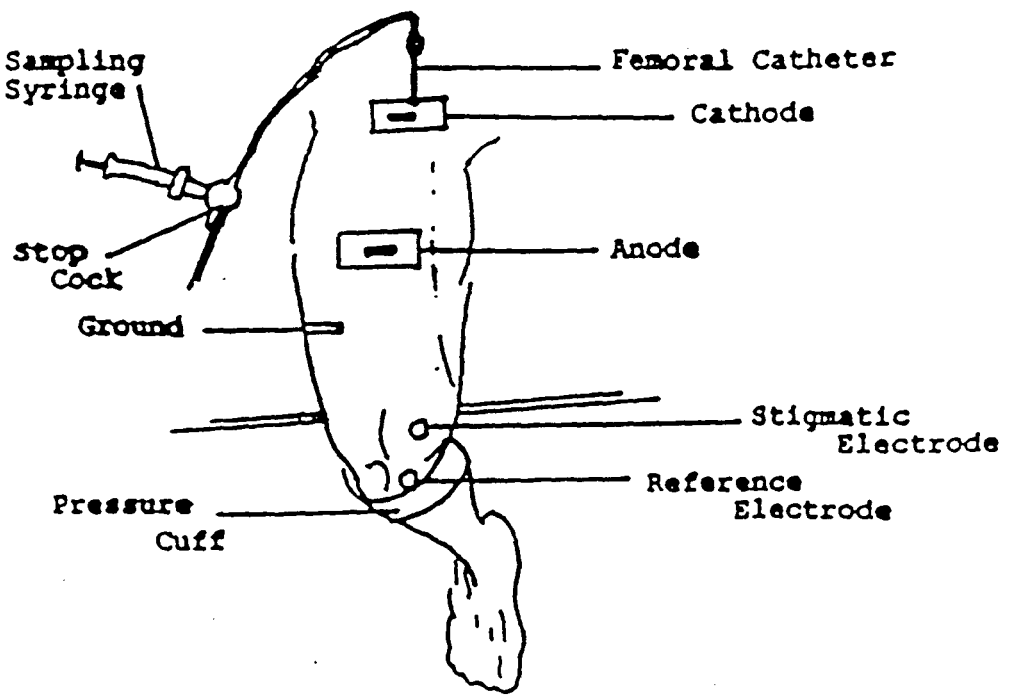

Sampling
Syringe
Femoral Catheter
Cathode
Anode
Stop
Cock
Ground
Stigmatic
Electrode
Reference
Electrode
Pressure
Cuff

FIGURE 2.

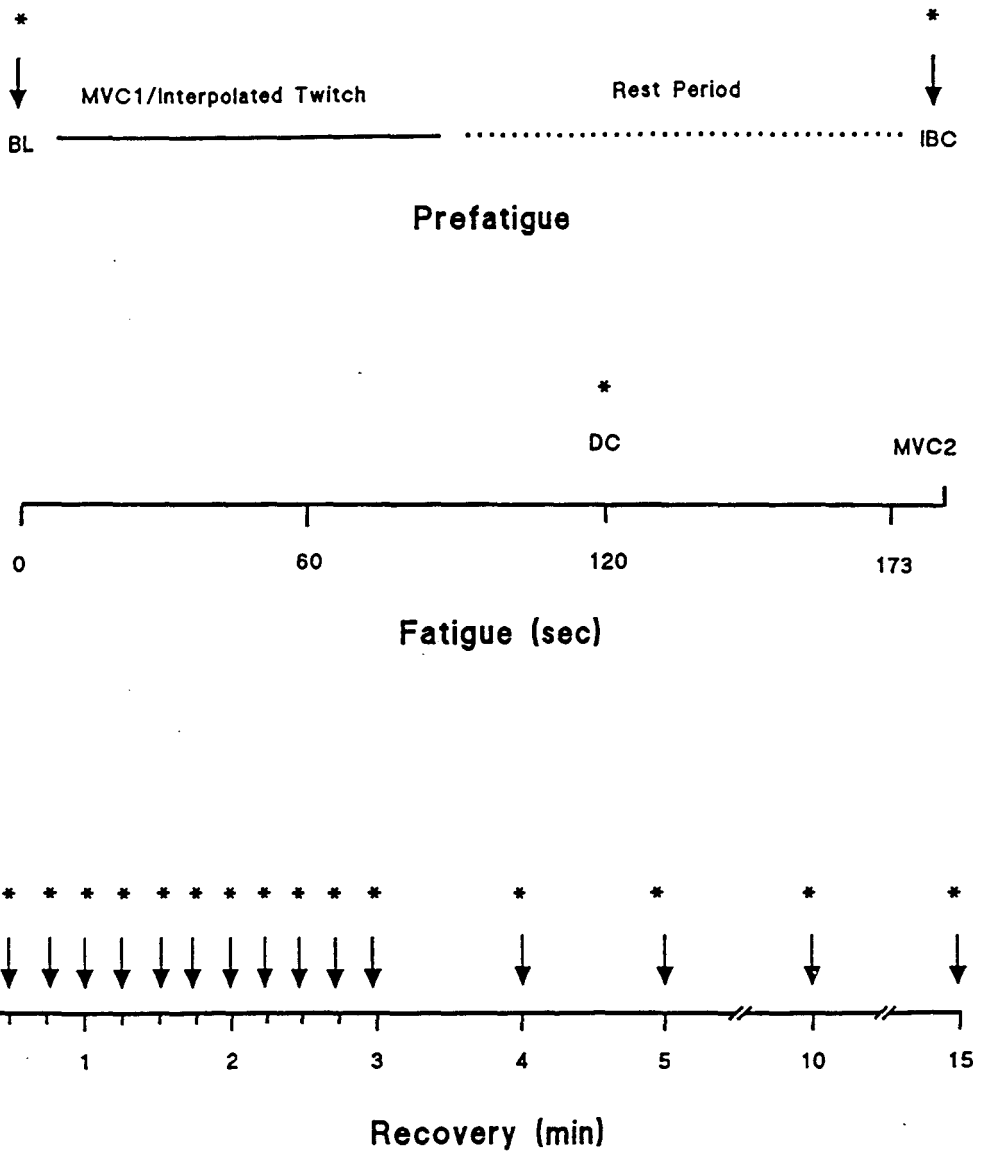

MVC1/Interpolated Twitch
Rest Period
BL
IBC
Prefatigue
DC
MVC2
0
60
120
173
Fatigue (sec)
.083
1
2
3
4
5
10
15
Recovery (min)

FIGURE 3.

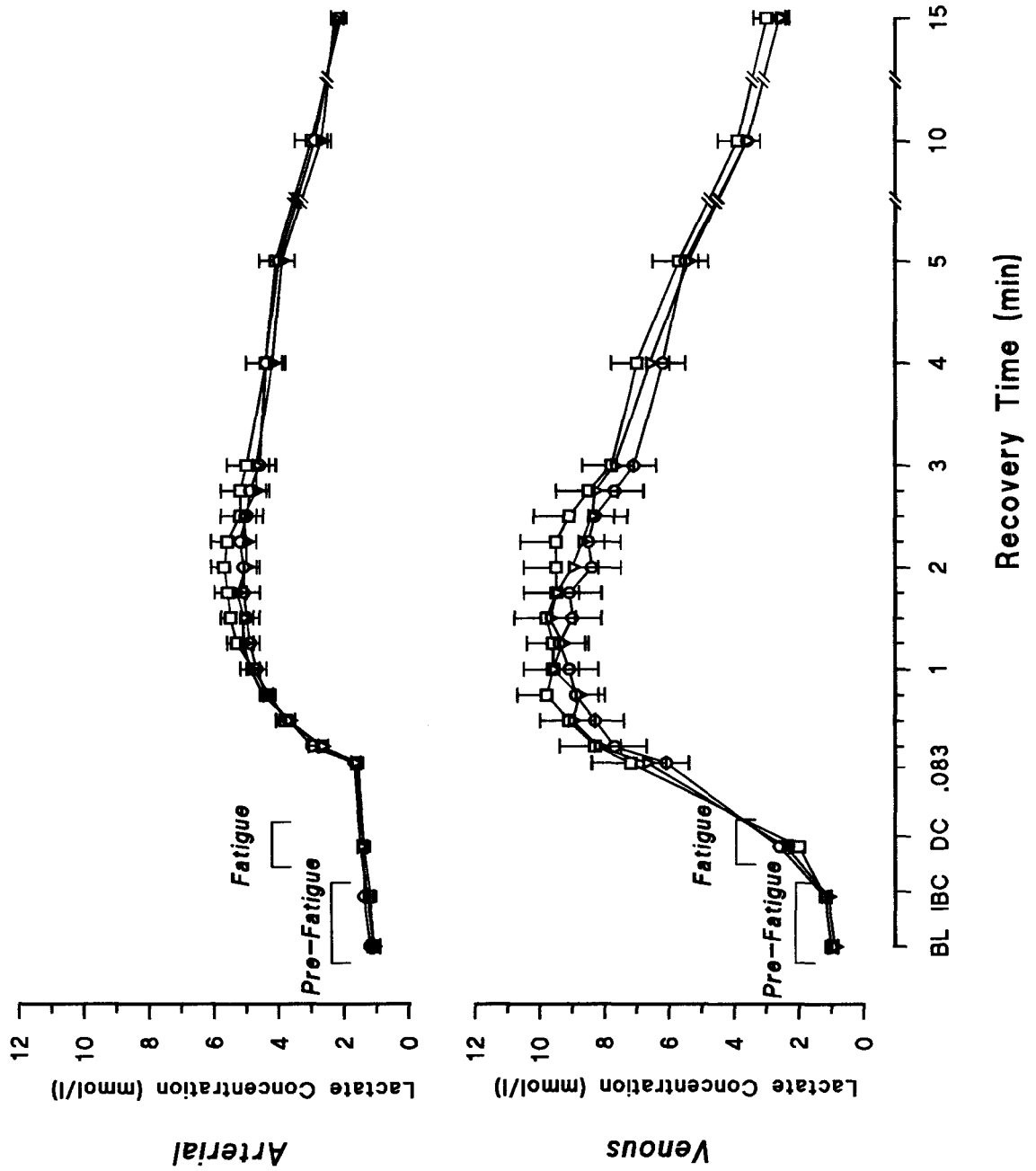

Arterial
Venous
Lactate Concentration (mmol/l)
Lactate Concentration (mmol/l)
0
2
4
6
8
10
12
Pre-Fatigue
Fatigue
Pre-Fatigue
Fatigue
BL IBC DC .083 1 2 3 4 5 10 15
Recovery Time (min)

FIGURE 4.

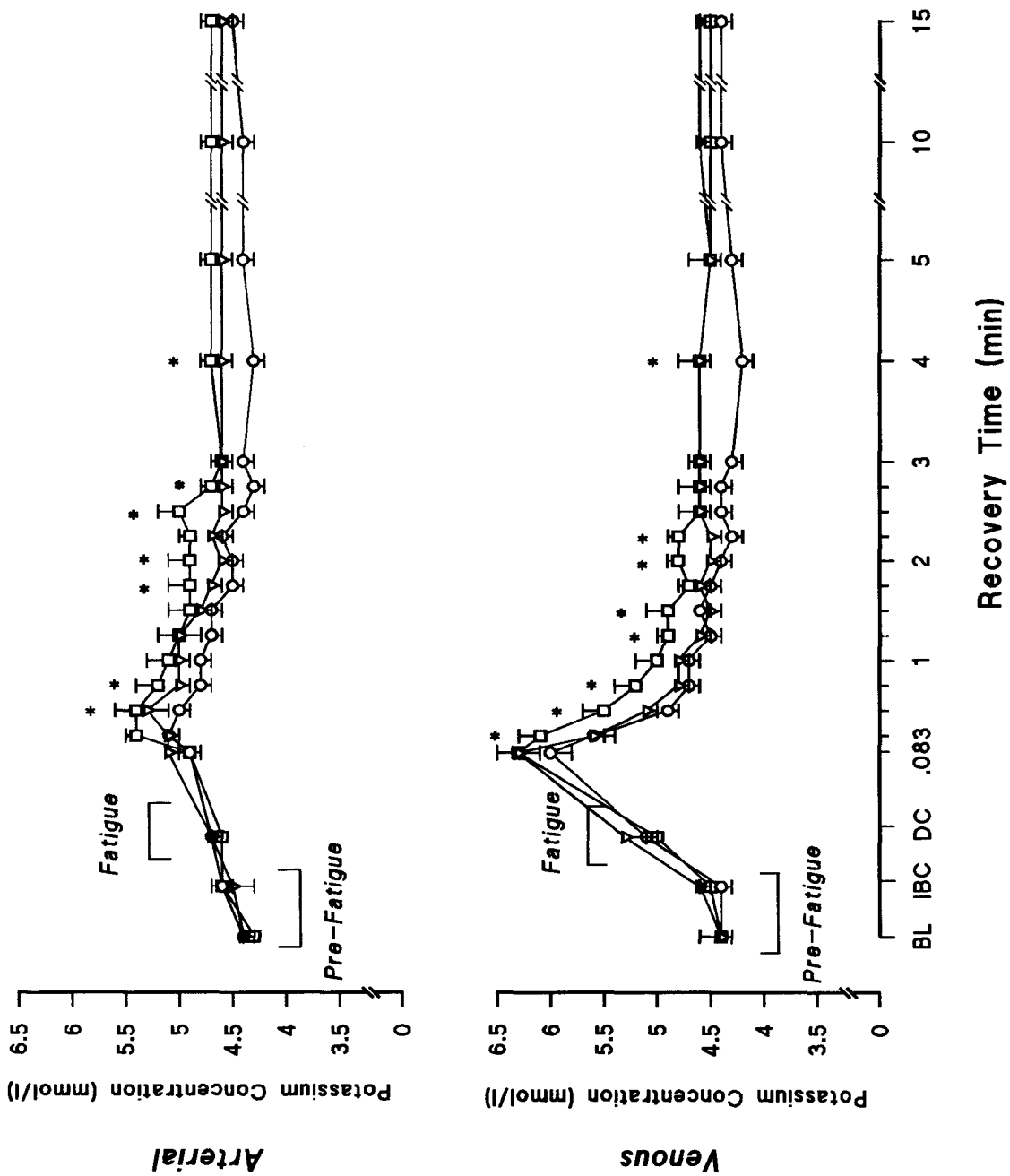

Arterial
Venous
Potassium Concentration (mmol/l)
6.5
6
5.5
5
4.5
4
3.5
0
Fatigue
Pre-Fatigue
BL
IBC
DC
.083
1
2
3
4
5
10
15
Recovery Time (min)

FIGURE 5. 56

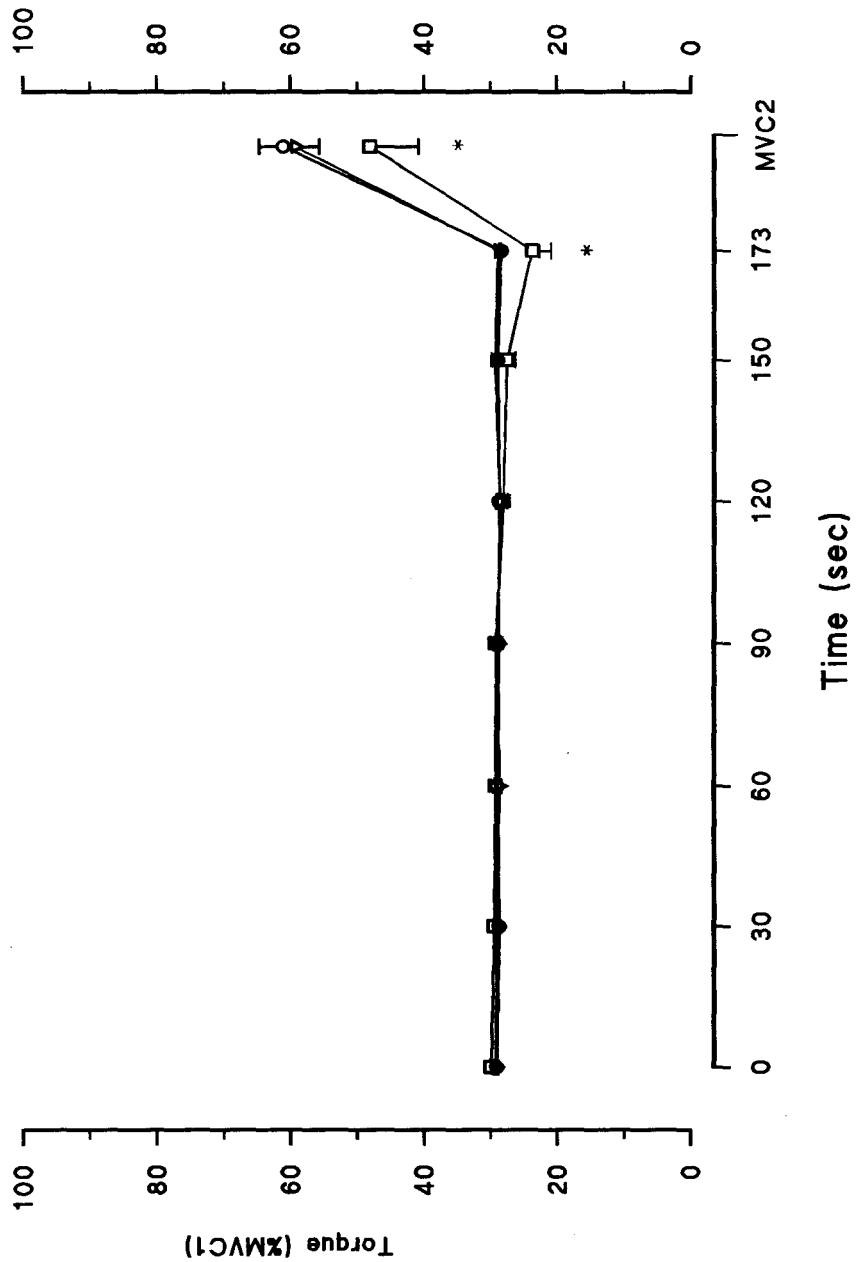


FIGURE 6.

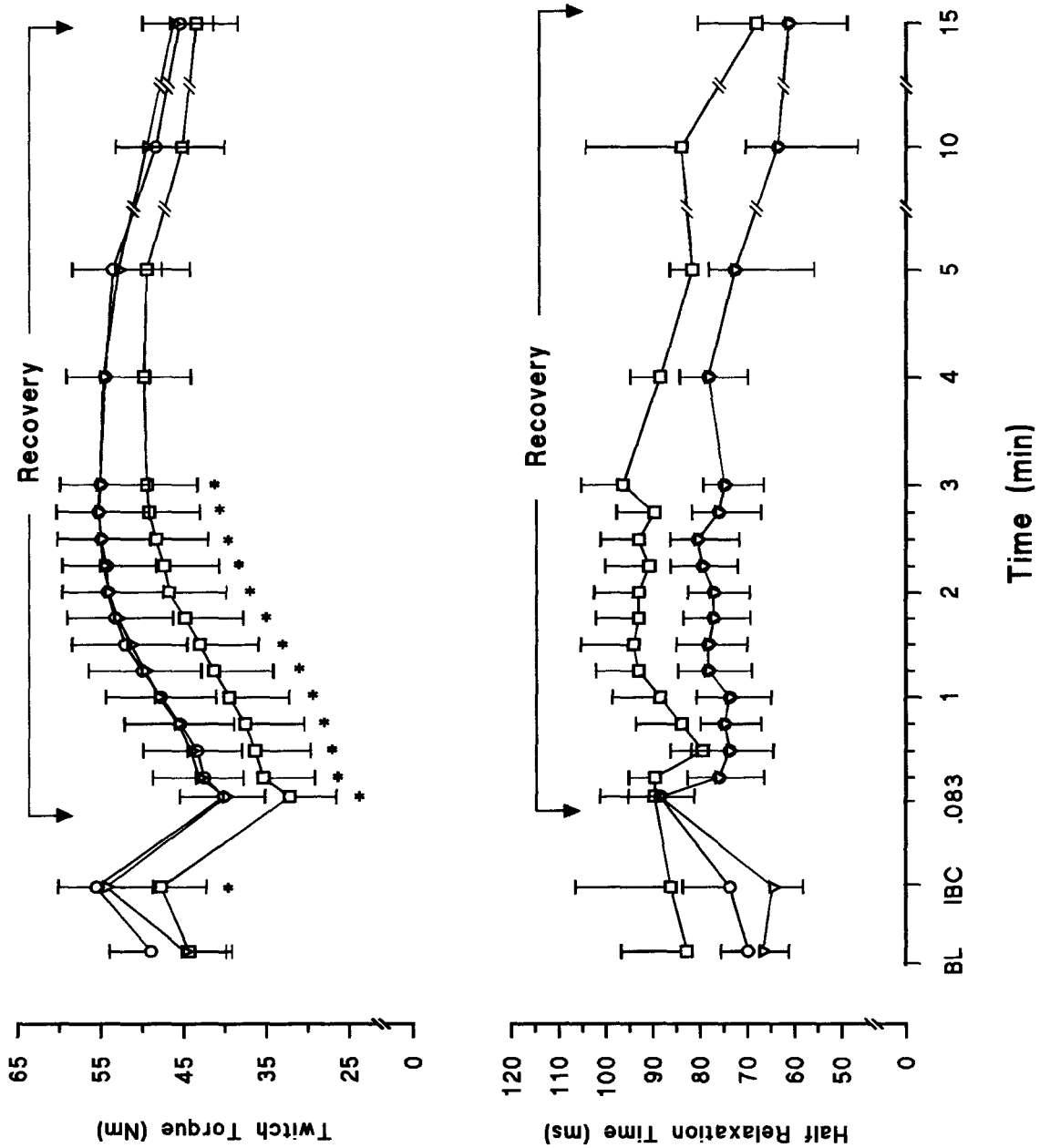
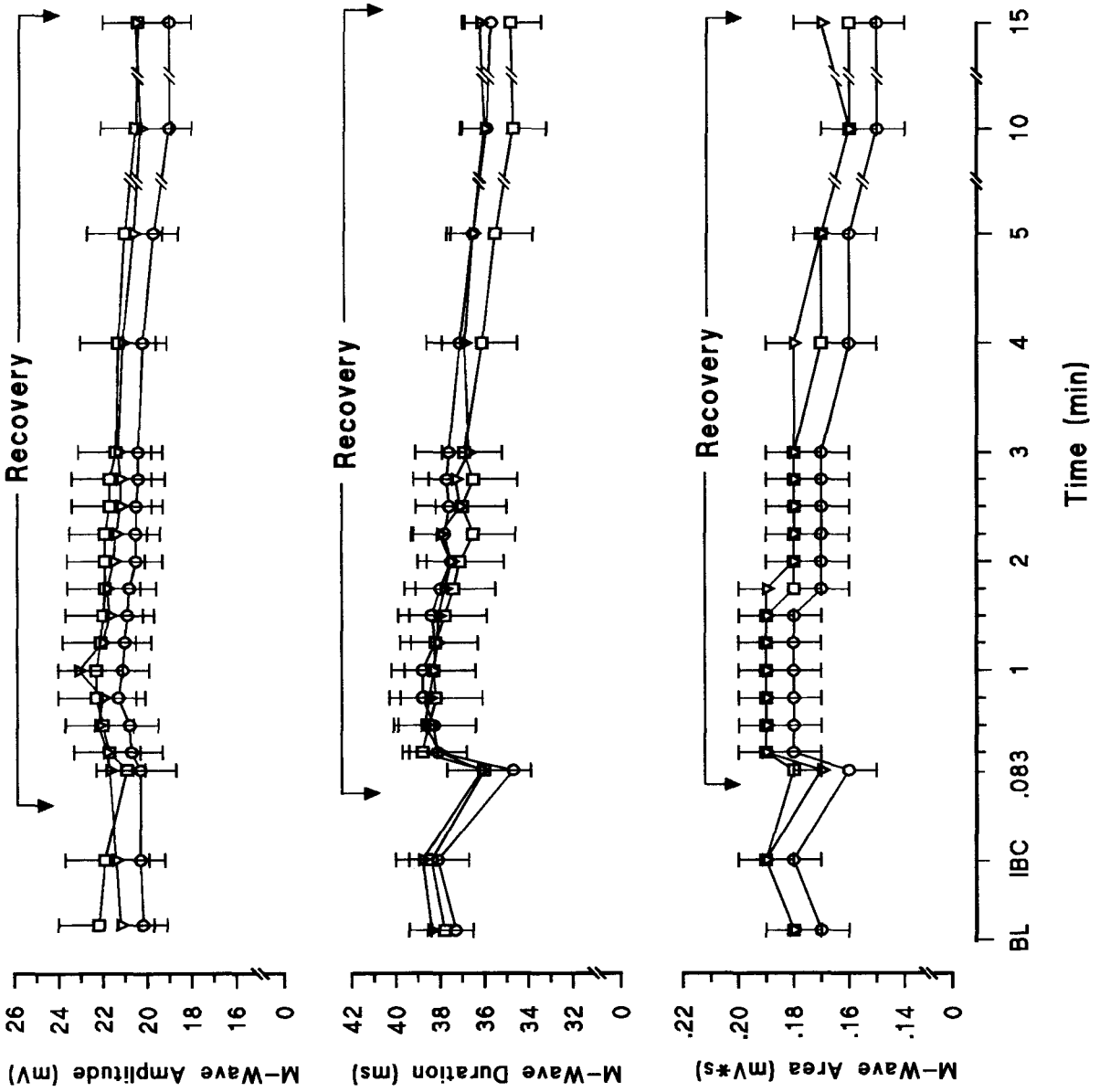

Recovery
Twitch Torque (Nm)
65
55
45
35
25
0
Recovery
Half Relaxation Time (ms)
120
110
100
90
80
70
60
50
0
BL
IBC
.083
1
2
3
4
5
10
15
Time (min)

FIGURE 7.


Recovery
Recovery
Recovery
M-Wave Amplitude (mV)
26
24
22
20
18
16
0
M-Wave Duration (ms)
42
40
38
36
34
32
0
M-Wave Area (mV*s)
.22
.20
.18
.16
.14
0
BL
IBC
.083
1
2
3
4
5
10
15
Time (min)

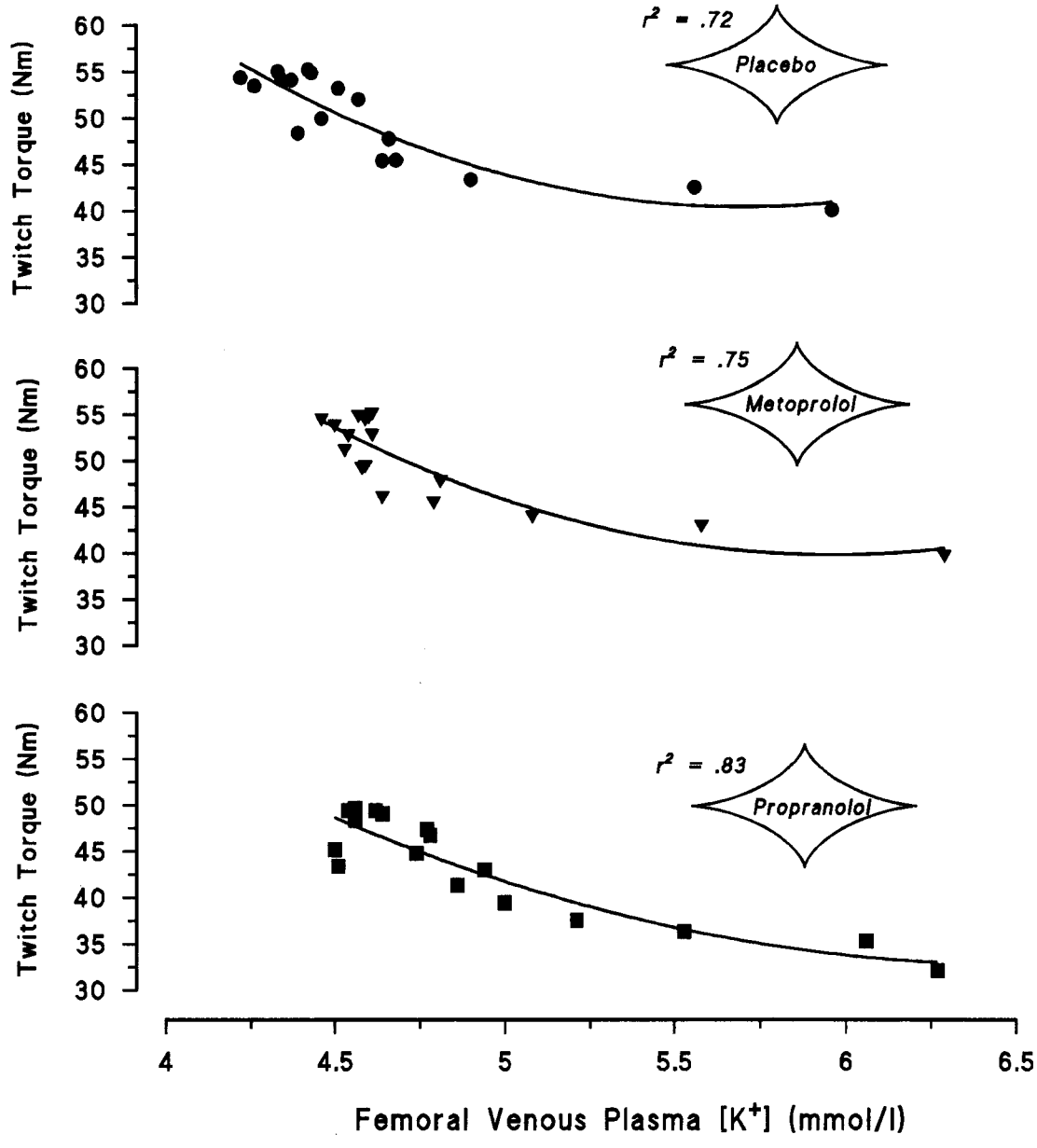

Twitch Torque (Nm)
60
55
50
45
40
35
30
r² = .72
Placebo
r² = .75
Metoprolol
r² = .83
Propranolol
4
4.5
5
5.5
6
6.5
Femoral Venous Plasma [K+] (mmol/l)

FIGURE 8.

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APPENDIX A:

PRE-SCREENING QUESTIONNAIRE
AND
CONSENT FORM

β-BLOCKADE, PLASMA K^+ CONCENTRATIONS AND MUSCLE EXCITABILITY

SUBJECT PRE-SCREENING QUESTIONNAIRE

1. Has your doctor ever told you that you have any type of lung disease?

2. Have you ever been treated for a lung disorder (e.g.: asthma, bronchitis, emphysema, EIB, etc.)?

3. Do you have any allergies?

4. Do you experience frequent coughing, wheezing or shortness of breath:

   a) at rest?

   b) during exercise?

   c) while sleeping?

5. Are you presently taking any type of medication?

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CONSENT FORM

THE RELATIONSHIP BETWEEN β-BLOCKADE, PLASMA POTASSIUM CONCENTRATIONS AND MUSCLE EXCITABILITY DURING STATIC EXERCISE

I, _____, consent to participate in a study directed by Dr. Audrey Hicks and Dr. Robert McKelvie. The purpose of this study is to determine the effects of β-blockade on skeletal muscle function and to investigate some of the mechanisms that may contribute to skeletal muscle fatigue during activity. The results of this study will be made available to the scientific community but participation in this study will offer no direct benefit to me.

For the purposes of this study, I will have two catheters inserted and I will sustain a submaximal isometric contraction of my right quadriceps muscles for 3 min. on three different occasions. During each of these tests, my muscle will be twitched by an electrical stimulation at varying time intervals throughout a 15 minute recovery period and blood samples will be taken.

I am aware that several measurements will be taken during each of the exercise tests. Surface electrodes will be placed over the muscles of my right thigh in order to record their electrical activity. Surface electrodes will also be used to deliver electrical stimuli to my right femoral nerve. Before the exercise begins, catheters will be inserted into my right femoral vein and left brachial artery by a physician (Dr. McKelvie) qualified to perform these procedures. Approximately 5cc (1teaspoon) of blood will be taken from each catheter prior to each test, as a baseline measure, and 16 samples will be taken from each catheter site during the 15 minute recovery period. Throughout the study, a maximum of 200cc of blood will be drawn, which is less than half of a normal blood donation.

I am aware that the protocol during each of the exercise tests is as follows: First, catheters will be inserted by Dr. McKelvie followed by a baseline drawing of blood from both catheters; I will then be strapped into an isometric leg extension chair and surface electrodes will be placed on my right quadriceps muscles and femoral nerve; following this, my maximum twitch torque will be determined by manipulating the intensity of the stimulator and I will then perform 3 maximal voluntary contractions (MVC's) during which an interpolated twitch will be performed to indicate motor unit activation; I will then sustain 30% of my best MVC for 3 minutes at which time I will perform another MVC to indicate my muscle fatigue; finally, at pre-determined times during the ensuing recovery period, I will be twitched by the stimulator and the remaining 32 blood samples will be taken.

I am aware that I will be asked to take two different active drugs (Propranolol and Metroprolol) and a placebo (a capsule without active medication) by mouth, on separate occasions. I will take each drug twice a day for a 4 day period, separated by one week. My exercise tests will be done on the fourth day of taking each drug. I understand that approximately one month prior to the onset of the study period, I will be asked to perform at least three maximal exercise tests on a cycle ergometer to determine appropriate doses of the medications that will be used in the study. The tests will take place after 3 days of one of the drug treatments and each test will be separated by at least one week.

During these tests my heart rate will be monitored with surface electrodes that will be placed on my chest. As well, I will be asked to breathe into a rubber mouth piece. My total time commitment to this study will be approximately 8 weeks.

I understand that there is a slight risk (less than 1 in 1,000) of a blood clot forming at the catheter sites. Also, there is a very slight risk of developing a localized infection at the puncture site, but this has never occurred in similar studies of this kind. There may also be some slight bruising and/or redness around the puncture sites, but these are temporary and should recover within several days of testing. There may be temporary discomfort associated with the muscle stimulation, however this procedure has no apparent side effects. The active drugs that I will be taking will slow my heart rate and will lower my blood pressure. They may also cause me to feel tired and/or dizzy and to experience stomach and/or bowel upset (e.g. nausea, diarrhoea), but these feelings are temporary and will disappear as soon as I stop taking the drugs. I have been assured that the physician (Dr. McKelvie) will be available to respond to any of the side-effects related to the study

Neither my name nor any reference to me will be used in compiling the results nor in publication in any form whatsoever.

I understand that I may withdraw from the study at any time without prejudice, even after signing this form.

Name (print)	Signature	Date

Witness (print)	Signature	Date

I have explained the nature of the study to the subject and believe that he has understood it.

Name (print)	Signature	Date

APPENDIX B:

DATA - CHAPTER II

PLASMA LACTATE CONCENTRATIONS

(mmol/l)

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

			Sample																			
			BL	IBC	DC	RECOVERY																
Subject	Trial	Art./Ven.				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
SI	Placebo	Arterial	1.3	1.3	1.1	1.3	2.2	2.8	3.5	3.7	3.8	3.8	3.4	3.6	3.5	3.4	3.0	3.2	2.7	2.6	1.6	1.2
		Venous	1.1	1.6	3.6	4.1	4.4	5.3	5.9	6.5	6.5	6.7	6.8	6.2	6.0	5.9	5.3	5.4	4.3	4.0	2.5	1.7
	Metoprolol	Arterial	0.8	1.0	1.1	1.3	2.7	3.6	4.5	5.0	5.2	5.3	5.8	5.6	5.5	5.3	5.2	5.0	4.2	4.3	2.8	2.0
		Venous	0.9	1.0	3.7	8.3	8.2	8.4	8.3	7.8	8.5	8.2	8.1	8.0	8.0	7.8	7.7	7.4	6.6	5.8	3.6	2.5
	Propranolol	Arterial	0.9	1.1	1.2	1.4	2.8	3.4	3.8	5.2	5.1	6.1	5.8	5.7	5.9	4.7	5.8	4.6	4.8	4.1	2.5	1.6
		Venous	1.0	1.0	1.5	5.8	7.4	8.1	9.5	9.9	9.2	9.3	8.9	9.4	9.1	8.9	8.6	8.0	7.0	5.4	3.4	2.4
JM	Placebo	Arterial	0.8	1.2	1.1	1.4	3.0	4.2	4.7	5.3	5.7	5.9	5.7	5.4	6.0	6.1	5.8	5.2	5.3	5.0	3.3	2.4
		Venous	0.8	1.0	1.1	5.9	8.2	8.1	8.8	8.4	8.8	8.5	8.4	8.4	8.2	8.2	7.7	7.4	5.8	5.5	3.6	2.3
	Metoprolol	Arterial	2.1	2.5	2.3	2.2	2.9	4.6	5.6	5.7	5.9	6.1	6.5	6.1	6.4	5.9	6.2	6.4	5.8	5.4	4.3	4.3
		Venous	2.0	2.0	2.1	6.5	8.4	8.3	8.3	8.8	8.5	9.2	8.7	9.1	7.9	7.6	7.7	7.5	5.7	5.3	3.9	3.5
	Propranolol	Arterial	0.4	0.6	0.7	0.7	1.3	3.9	5.1	5.2	5.3	5.4	5.9	5.4	6.8	5.7	5.6	5.1	3.7	3.4	2.6	1.7
		Venous	0.7	0.7	0.8	1.9	3.4	5.8	8.7	8.6	8.2	8.3	8.4	8.7	7.5	7.1	5.7	4.9	3.7	3.2	3.5	2.7
JT	Placebo	Arterial	1.3	1.6	2.0	2.2	3.4	4.3	5.2	5.5	5.7	6.4	6.2	6.3	6.3	6.3	6.1	5.9	5.4	5.0	3.4	2.3
		Venous	1.1	1.4	3.1	6.6	8.4	8.8	9.9	9.2	9.7	9.3	9.2	7.7	7.8	7.6	7.2	6.3	5.8	5.9	3.8	2.2
	Metoprolol	Arterial	1.4	1.8	1.8	2.5	3.9	4.4	5.6	6.7	6.0	5.8	5.3	5.7	5.7	5.6	5.4	4.6	4.6	5.3	3.5	2.5
		Venous	0.8	1.0	2.9	6.9	8.3	9.4	10.2	11.5	10.0	9.8	9.7	8.7	8.8	8.2	8.2	8.3	6.6	6.0	3.2	1.8
	Propranolol	Arterial	0.9	1.1	1.5	1.7	1.6	2.6	3.7	3.5	5.0	5.2	5.7	6.0	5.9	5.8	6.3	6.1	6.1	5.5	4.6	3.0
		Venous	1.1	1.7	1.4	5.4	7.5	8.3	7.8	7.3	7.8	9.3	8.8	9.1	9.5	9.5	9.7	8.8	8.4	8.0	6.2	3.6

PLASMA LACTATE CONCENTRATIONS
(mmol/l)

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

			Sample																		
			BL	IBC	DC	RECOVERY															
Subject	Trial	Art./Ven.				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MS	Placebo	Arterial	0.8	1.3	1.1	1.4	3.8	4.4	5.4	6.0	6.0	5.9	7.6	7.5	7.6	7.4	7.8	7.7	7.2	6.6	4.5	3.2
		Venous	1.0	1.2	3.3	5.9	12.6	13.5	14.2	14.3	14.8	14.2	14.6	13.4	14.1	14.0	12.9	10.8	11.2	10.9	6.8	4.7
	Metoprolol	Arterial	1.0	1.2	1.3	1.5	2.5	4.0	4.6	5.2	5.7	5.7	6.7	6.4	6.2	7.6	6.5	6.9	6.3	5.3	3.2	2.2
		Venous	1.1	1.5	2.4	8.2	12.7	12.5	12.2	12.3	13.1	13.5	13.4	13.1	12.9	12.3	12.2	11.4	10.0	6.6	6.5	3.8
	Propranolol	Arterial	1.6	1.8	2.1	2.2	4.7	4.8	4.8	5.4	6.0	6.6	6.8	6.6	6.6	7.0	7.0	6.9	6.9	6.6	5.0	4.0
		Venous	1.4	1.6	2.8	9.2	11.2	12.3	11.5	11.9	11.7	13.0	13.1	12.0	12.3	11.7	11.7	12.0	11.4	11.0	7.1	5.2
BR	Placebo	Arterial	1.1	1.0	1.1	0.9	3.0	3.9	4.3	4.3	4.6	4.4	4.4	4.7	4.6	4.8	4.1	3.9	4.2	3.6	2.6	1.9
		Venous	0.9	1.6	2.8	5.9	6.7	7.0	7.0	7.1	6.6	7.0	6.2	6.4	5.8	6.0	5.9	5.9	4.6	4.1	2.7	2.5
	Metoprolol	Arterial	0.8	1.1	1.1	1.6	3.9	4.2	4.7	5.0	5.2	5.5	4.7	4.1	4.6	4.9	4.7	4.2	3.8	3.2	2.0	1.6
		Venous	0.5	0.8	1.8	4.1	6.0	7.6	7.5	7.2	8.7	9.7	9.3	9.3	8.8	7.6	8.0	7.4	6.6	5.6	3.3	2.3
	Propranolol	Arterial	1.2	1.2	1.3	1.6	2.5	3.4	3.9	4.1	4.2	4.5	4.2	4.2	4.2	4.3	3.9	3.7	3.1	2.9	1.6	1.3
		Venous	1.1	1.4	1.6	5.9	6.1	7.4	8.1	7.4	7.8	6.4	5.8	5.8	5.4	5.4	4.7	4.4	4.7	4.1	2.3	2.0
MH	Placebo	Arterial	1.4	1.4	1.3	1.7	2.7	2.8	3.2	3.3	3.6	3.4	3.3	3.2	2.7	2.7	2.9	2.1	2.2	1.6	1.5	1.3
		Venous	1.1	1.1	2.2	2.7	2.8	3.9	5.6	6.3	6.9	5.2	5.3	4.7	4.9	4.6	3.9	3.5	3.6	3.2	2.6	2.1
	Metoprolol	Arterial	0.8	1.1	1.7	1.7	2.1	2.7	3.3	3.7	3.1	3.1	3.6	3.4	3.3	3.2	2.9	3.1	2.6	2.5	2.1	1.7
		Venous	0.6	0.7	1.9	5.4	6.3	6.8	5.8	5.9	5.2	5.7	5.6	5.4	5.6	5.1	5.4	5.1	4.1	4.1	2.7	1.5
	Propranolol	Arterial	1.3	1.3	1.4	1.7	2.2	3.3	3.4	3.2	4.1	4.3	4.0	4.3	2.5	1.4	1.8	1.6	1.2	1.3	0.9	0.7
		Venous	0.8	1.2	2.0	7.2	8.6	8.4	8.7	7.6	8.0	7.9	7.8	6.5	7.3	6.4	6.6	5.4	5.3	4.5	1.7	1.8

PLASMA LACTATE CONCENTRATIONS

(mmol/l)

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

			Sample																		
			BL	IBC	DC	RECOVERY															
Subject	Trial	Art./Ven.				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MB	Placebo	Arterial	1.0	1.3	1.3	1.7	2.8	3.4	3.9	4.3	4.6	4.6	4.7	4.5	4.5	4.6	4.6	4.3	3.7	4.0	2.6	2.0
		Venous	0.8	0.9	2.2	7.3	8.0	9.2	8.6	8.9	9.4	8.9	9.3	9.0	9.0	8.5	7.6	7.4	6.1	5.5	3.1	2.3
	Metoprolol	Arterial	1.5	1.3	1.5	1.9	2.0	4.0	4.4	4.5	5.3	5.0	5.0	4.6	4.9	4.3	4.0	4.0	3.8	3.3	2.4	2.0
		Venous	0.8	1.1	2.2	5.0	6.0	6.8	7.3	8.9	8.4	8.2	8.5	6.7	7.0	7.5	5.9	6.1	5.0	4.5	3.0	2.4
	Propranolol	Arterial	0.9	1.0	1.0	1.5	2.5	4.5	4.1	5.7	5.9	6.2	6.3	7.4	6.5	6.4	6.2	6.5	5.1	5.2	3.2	1.9
		Venous	0.8	0.7	1.8	7.0	8.4	8.9	9.2	9.4	10.1	10.6	9.6	9.7	10.1	8.8	9.5	9.6	8.3	6.9	3.5	2.7
MG	Placebo	Arterial	1.2	1.2	1.4	1.7	3.2	3.8	4.9	5.3	5.8	6.4	6.1	6.0	6.5	5.7	5.5	5.3	4.9	4.7	4.4	3.0
		Venous	1.1	0.8	1.9	10.7	10.9	10.1	10.7	12.0	11.8	11.6	11.9	11.1	11.4	11.6	9.9	8.3	6.6	5.4	3.8	2.8
	Metoprolol	Arterial	0.5	0.8	0.8	0.8	2.0	3.4	4.0	4.3	5.1	5.1	5.2	4.5	4.6	4.9	4.1	4.2	3.5	3.2	2.2	1.8
		Venous	0.7	0.8	1.7	6.0	7.5	9.9	9.2	10.3	10.7	11.5	10.4	11.3	9.6	9.7	9.9	7.6	7.3	6.6	3.9	2.8
	Propranolol	Arterial	0.9	1.2	1.1	1.4	4.0	5.3	5.7	6.4	7.3	7.0	7.2	7.6	7.5	7.0	6.5	6.1	5.5	4.9	4.2	2.7
		Venous	0.9	1.1	2.1	15.5	15.0	15.1	16.5	15.3	15.2	15.8	15.8	16.2	16.4	16.7	13.5	10.4	8.1	4.0	4.4	3.9
SC	Placebo	Arterial	1.6	2.2	2.3	2.6	2.7	3.8	4.3	4.3	4.7	4.3	4.8	4.6	4.7	4.3	4.5	4.1	3.7	3.2	2.2	2.4
		Venous	1.0	1.5	3.1	6.1	7.2	8.4	9.4	9.1	10.0	9.2	9.8	9.1	9.5	8.6	9.3	8.7	7.5	4.7	3.2	3.2
	Metoprolol	Arterial	1.0	1.3	1.7	1.4	2.5	2.6	3.9	4.0	4.7	4.6	4.8	4.4	4.2	3.9	3.5	3.7	3.3	2.9	1.9	2.4
		Venous	0.7	1.4	3.2	9.9	10.5	11.4	10.2	13.7	10.6	11.5	11.7	9.1	9.5	9.7	9.6	8.9	7.3	4.2	2.7	2.4
	Propranolol	Arterial	1.5	1.9	2.0	2.2	2.5	3.4	4.2	4.1	4.8	4.1	4.1	4.5	4.6	4.5	4.0	4.1	3.4	3.2	2.3	2.1
		Venous	0.8	1.3	3.9	6.5	6.7	7.9	8.0	9.0	8.0	7.5	7.4	7.9	7.7	7.7	6.3	6.9	5.9	4.3	2.7	2.4

Plasma [La-]
(mmol/l)

Mean Values

BL - Baseline
IBC - Immediately before contraction
DC - During contraction

		Sample																			
		BL	IBC	DC	RECOVERY																
					:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Arterial	1.2	1.4	1.4	1.7	3.0	3.7	4.4	4.7	4.9	5.0	5.1	5.1	5.2	5.0	4.9	4.6	4.4	4.0	2.9	2.2
	Venous	1.0	1.2	2.6	6.1	7.7	8.3	8.9	9.1	9.4	9.0	9.1	8.4	8.5	8.3	7.7	7.1	6.2	5.5	3.6	2.6
Metoprolol	Arterial	1.1	1.3	1.5	1.7	2.7	3.7	4.5	4.9	5.1	5.1	5.3	5.0	5.0	5.1	4.7	4.7	4.2	3.9	2.7	2.3
	Venous	0.9	1.1	2.4	6.7	8.2	9.0	8.8	9.6	9.3	9.7	9.5	9.0	8.7	8.4	8.3	7.7	6.6	5.4	3.6	2.6
Propranolol	Arterial	1.1	1.2	1.4	1.6	2.7	3.8	4.3	4.8	5.3	5.5	5.6	5.7	5.6	5.2	5.2	5.0	4.4	4.1	3.0	2.1
	Venous	1.0	1.2	2.0	7.2	8.3	9.1	9.8	9.6	9.6	9.8	9.5	9.5	9.5	9.1	8.5	7.8	7.0	5.7	3.9	3.0

Plasma [La-]
(mmol/l)

Standard Deviation

BL - Baseline
IBC - Immediately before contraction
DC - During contraction

		Sample																			
		BL	IBC	DC	RECOVERY																
					:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Arterial	0.3	0.3	0.4	0.5	0.4	0.6	0.7	0.8	0.8	1.1	1.3	1.3	1.5	1.4	1.5	1.5	1.4	1.4	1.0	0.6
	Venous	0.1	0.3	0.8	2.1	2.8	2.6	2.5	2.5	2.5	2.5	2.7	2.5	2.8	2.8	2.5	2.0	2.1	2.1	1.2	0.8
Metoprolol	Arterial	0.5	0.5	0.4	0.5	0.7	0.7	0.7	0.9	0.8	0.8	0.9	1.0	0.9	1.2	1.1	1.2	1.1	1.1	0.8	0.8
	Venous	0.4	0.4	0.6	1.7	2.1	1.9	1.8	2.4	2.1	2.2	2.1	2.1	1.9	1.9	2.0	1.7	1.6	0.9	1.1	0.7
Propranolol	Arterial	0.3	0.4	0.4	0.4	1.0	0.8	0.7	1.0	0.9	1.0	1.1	1.2	1.5	1.7	1.6	1.6	1.6	1.5	1.3	0.9
	Venous	0.2	0.3	0.9	3.5	3.1	2.7	2.6	2.4	2.4	2.8	2.9	2.9	3.1	3.2	2.8	2.5	2.2	2.4	1.7	1.0

Plasma [La-]
(mmol/l)

Standard Error of the Mean

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

		Sample																			
		BL	IBC	DC	RECOVERY																
					:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Arterial	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.2
	Venous	0.0	0.1	0.3	0.7	1.0	0.9	0.9	0.9	0.9	0.9	1.0	0.9	1.0	1.0	0.9	0.7	0.7	0.7	0.4	0.3
Metoprolol	Arterial	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.3	0.3
	Venous	0.1	0.1	0.2	0.6	0.7	0.7	0.6	0.8	0.7	0.8	0.7	0.8	0.7	0.7	0.7	0.6	0.6	0.3	0.4	0.2
Propranolol	Arterial	0.1	0.1	0.2	0.2	0.4	0.3	0.2	0.4	0.3	0.3	0.4	0.4	0.5	0.6	0.6	0.6	0.6	0.5	0.5	0.3
	Venous	0.1	0.1	0.3	1.2	1.1	0.9	0.9	0.9	0.8	1.0	1.0	1.0	1.1	1.1	1.0	0.9	0.8	0.8	0.6	0.4

PLASMA POTASSIUM CONCENTRATIONS

(mmol/l)

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

			Sample																			
			BL	IBC	DC	RECOVERY																
Subject	Trial	Art./Ven.				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
SI	Placebo	Arterial	4.6	4.3	4.2	4.9	4.8	4.6	4.9	4.7	4.7	4.6	4.3	4.6	4.5	4.4	4.3	4.6	4.2	4.4	4.3	4.5
		Venous	4.4	4.2	5.2	5.3	5.1	4.7	4.6	4.3	4.4	4.1	4.2	4.1	4.2	4.2	4.2	4.1	4.1	4.0	4.2	4.4
	Metoprolol	Arterial	4.7	4.1	4.7	5.5	5.6	5.5	5.0	4.8	4.8	4.8	4.8	4.6	4.6	4.6	4.4	4.5	4.6	4.7	4.6	4.5
		Venous	4.6	4.3	5.9	7.2	5.4	5.3	5.0	4.6	4.5	4.6	4.5	4.4	4.4	4.3	4.4	4.2	4.3	4.5	4.6	4.7
	Propranolol	Arterial	4.4	4.7	4.4	4.8	5.7	5.6	5.0	5.3	5.0	4.8	4.8	4.7	4.7	4.8	4.6	4.7	4.6	4.6	4.6	4.5
		Venous	4.6	4.4	4.8	6.2	6.0	5.4	5.1	4.9	4.9	4.7	4.7	4.8	4.8	4.7	4.7	4.7	4.4	4.6	4.6	4.7
JM	Placebo	Arterial	4.0	3.9	4.2	4.5	4.9	4.8	4.6	4.6	4.3	4.5	4.4	4.2	4.2	4.2	4.2	4.0	3.9	3.8	4.0	3.9
		Venous	3.8	4.1	4.5	5.8	5.5	5.1	4.8	4.4	4.3	4.4	4.3	4.2	4.2	4.1	4.0	3.9	3.8	3.6	3.6	3.9
	Metoprolol	Arterial	4.3	5.3	5.0	5.0	4.8	5.0	5.3	5.3	6.0	5.7	4.9	4.7	5.1	5.1	5.0	5.3	5.2	5.0	5.1	4.4
		Venous	4.8	4.7	5.0	6.0	5.6	4.8	5.1	4.5	4.7	4.3	4.5	4.7	4.4	4.6	4.3	4.1	4.5	4.5	4.4	4.4
	Propranolol	Arterial	3.7	3.7	4.0	4.5	5.7	6.1	5.7	5.8	5.6	4.7	4.6	5.0	5.1	5.3	4.3	4.8	4.3	4.4	4.9	4.9
		Venous	5.2	4.8	4.9	5.5	6.6	5.3	5.8	5.3	4.8	5.1	5.0	5.1	5.0	4.3	5.1	5.0	4.4	4.1	4.8	4.9
JT	Placebo	Arterial	4.2	4.6	5.2	5.6	5.4	5.2	5.3	5.5	5.1	5.5	5.1	5.0	5.0	4.7	4.7	4.7	4.5	4.7	4.7	4.7
		Venous	4.9	5.0	5.7	6.2	6.3	5.3	5.2	4.6	4.8	4.7	4.6	4.7	4.6	4.5	4.4	4.2	4.1	4.3	4.4	4.4
	Metoprolol	Arterial	4.2	4.0	4.2	5.3	5.0	4.8	4.6	5.0	4.5	4.9	4.4	4.3	5.0	4.4	4.3	4.3	4.3	4.3	3.9	4.5
		Venous	4.0	4.7	5.1	6.4	5.8	5.1	4.8	5.3	4.4	4.2	4.3	4.1	4.3	4.1	4.1	5.1	4.0	3.9	4.0	4.0
	Propranolol	Arterial	4.5	4.5	4.2	4.5	5.8	5.4	6.1	6.0	6.1	5.8	6.1	5.9	5.4	5.6	5.4	5.4	5.2	4.6	5.1	5.0
		Venous	5.2	5.2	5.3	7.0	7.3	6.5	5.9	5.8	5.5	5.8	5.2	5.2	5.2	5.3	5.5	4.8	5.3	5.5	4.9	4.5

PLASMA POTASSIUM CONCENTRATIONS

(mmol/l)

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

			Sample																		
			BL	IBC	DC	RECOVERY															
Subject	Trial	Art./Ven.				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MS	Placebo	Arterial	4.2	5.0	5.1	4.6	5.4	5.1	4.7	4.4	4.8	4.4	4.8	4.2	4.2	4.3	4.1	4.2	4.1	4.2	4.3	4.5
		Venous	4.8	4.5	4.9	5.7	5.8	4.8	4.4	4.4	4.3	4.1	4.2	4.1	4.0	4.1	4.3	3.9	3.7	4.2	4.4	4.4
	Metoprolol	Arterial	4.2	5.0	5.3	5.0	5.4	6.0	5.4	5.4	5.5	5.0	5.1	5.2	4.7	4.9	5.0	4.3	4.7	4.9	5.0	5.0
		Venous	4.0	4.7	6.5	7.0	6.7	5.9	5.0	5.3	4.9	4.6	5.0	4.6	4.8	4.9	4.3	4.9	4.5	4.8	4.7	4.8
	Propranolol	Arterial	4.1	4.3	4.3	4.5	4.9	5.3	4.9	4.8	4.3	4.6	4.5	4.7	4.5	4.5	4.5	4.3	4.6	4.5	4.2	4.5
		Venous	4.1	3.9	4.7	6.4	5.4	4.8	4.4	4.1	4.6	4.3	4.3	3.9	4.2	3.9	4.0	4.0	3.7	3.8	4.0	4.0
BR	Placebo	Arterial	4.4	4.3	4.7	5.0	5.2	5.2	4.8	4.8	4.7	4.5	4.3	4.9	4.6	4.9	4.4	4.2	4.4	4.5	4.4	4.1
		Venous	4.7	4.5	5.3	6.2	6.1	5.3	5.1	4.8	4.4	5.0	4.3	4.8	4.3	4.3	4.6	4.4	4.5	4.3	4.6	4.6
	Metoprolol	Arterial	4.7	4.1	4.4	4.7	5.0	5.0	5.3	4.8	4.9	4.8	4.3	4.2	4.6	4.4	4.4	4.4	4.3	4.5	4.5	4.7
		Venous	4.6	4.4	5.1	6.1	5.3	5.1	4.9	4.6	4.6	4.4	4.3	4.7	4.5	5.0	4.7	4.2	5.2	5.2	4.4	4.0
	Propranolol	Arterial	4.8	4.7	5.1	5.2	5.3	5.5	5.4	5.3	5.4	5.4	5.1	5.2	5.4	5.4	5.2	4.9	5.0	5.2	4.8	4.9
		Venous	4.7	4.8	5.5	6.4	6.0	5.6	5.0	5.1	5.5	5.3	5.1	5.3	5.1	4.6	4.8	4.9	4.8	4.2	4.6	4.6
MH	Placebo	Arterial	4.5	4.9	4.4	4.8	4.8	5.0	4.5	4.5	4.3	4.4	4.6	4.3	4.3	4.2	4.3	4.6	4.1	4.2	4.3	4.5
		Venous	4.2	4.6	4.9	5.0	4.9	4.4	4.5	4.9	4.7	4.5	4.7	4.3	4.5	4.7	4.2	4.5	4.5	4.7	4.5	4.1
	Metoprolol	Arterial	4.2	4.6	4.5	5.0	5.2	5.6	5.1	5.0	4.6	4.2	4.8	4.8	4.8	4.7	4.5	4.7	4.5	4.6	4.9	4.8
		Venous	3.8	4.4	4.6	5.5	4.9	4.5	4.6	4.3	4.2	4.6	4.6	4.1	4.8	4.2	4.6	4.7	4.8	4.4	4.5	4.7
	Propranolol	Arterial	4.5	5.0	4.4	5.0	4.9	5.1	4.6	4.8	4.5	4.8	4.6	4.7	4.8	4.3	4.6	4.5	4.5	5.0	4.7	4.5
		Venous	4.2	4.5	4.1	6.3	6.0	5.4	5.7	4.5	4.4	4.6	4.6	4.7	4.3	4.7	4.3	4.2	4.2	4.7	4.1	4.2

PLASMA POTASSIUM CONCENTRATIONS

(mmol/l)

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

			Sample																			
			BL	IBC	DC	RECOVERY																
Subject	Trial	Art./Ven.				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MB	Placebo	Arterial	4.1	4.3	4.3	4.5	5.0	5.0	4.9	4.5	4.7	4.6	4.5	4.6	4.5	4.3	4.3	4.2	4.1	4.0	4.2	4.4
		Venous	4.3	3.9	4.8	6.3	5.4	5.3	4.8	4.7	4.7	4.6	4.5	4.4	4.6	4.5	4.2	4.4	4.2	4.3	4.3	4.3
	Metoprolol	Arterial	4.9	4.6	4.6	5.2	4.7	5.6	4.7	4.9	4.6	4.7	4.5	5.0	4.5	4.4	4.5	4.4	4.7	4.5	4.5	4.6
		Venous	4.8	5.1	5.6	5.3	5.2	5.1	4.7	4.5	5.2	4.3	4.7	4.9	4.3	5.2	5.3	4.4	4.3	4.7	5.0	5.3
	Propranolol	Arterial	4.2	4.9	4.4	5.0	5.3	4.7	4.9	4.4	4.6	4.4	4.6	4.5	4.3	4.5	4.5	4.0	4.2	4.0	4.3	4.4
		Venous	3.9	4.1	5.1	5.6	5.4	5.0	4.8	4.5	4.5	4.6	4.3	4.4	4.5	4.2	4.6	4.6	4.8	4.6	4.1	4.6
MG	Placebo	Arterial	4.5	4.8	4.7	4.8	4.8	4.9	4.7	4.9	4.8	5.0	4.3	4.2	4.9	4.2	4.0	4.1	4.6	4.7	4.5	4.7
		Venous	4.2	4.4	5.2	6.5	5.4	4.7	4.5	4.4	4.2	4.7	4.7	4.5	4.5	4.8	4.8	4.8	4.2	4.8	5.0	4.9
	Metoprolol	Arterial	4.1	5.0	5.1	5.4	5.4	5.4	5.0	5.2	5.1	4.8	4.9	4.8	4.8	4.8	4.7	4.6	4.5	4.7	5.0	4.7
		Venous	4.2	4.6	4.7	6.5	5.8	5.4	4.8	4.8	4.5	4.8	4.5	4.8	4.4	4.4	4.7	4.7	4.8	4.8	5.1	5.0
	Propranolol	Arterial	4.8	5.0	5.3	5.8	5.9	6.0	5.3	5.2	5.0	5.2	5.2	5.0	5.1	5.1	4.8	4.3	4.9	4.6	5.0	5.0
		Venous	4.2	4.9	5.1	7.2	6.2	6.4	5.2	5.5	4.8	5.2	5.0	4.7	4.6	4.2	4.3	4.4	4.4	4.8	5.0	4.5
SC	Placebo	Arterial	4.7	5.2	5.2	5.4	5.2	5.4	5.1	5.0	5.0	4.8	4.6	4.8	4.8	4.5	4.2	4.6	4.5	4.8	4.9	4.9
		Venous	4.6	4.8	5.1	6.6	5.5	4.5	4.2	5.4	4.3	5.0	5.1	4.2	4.2	4.7	5.1	4.8	4.9	4.1	4.5	4.9
	Metoprolol	Arterial	4.0	4.1	4.4	4.4	4.6	4.4	4.9	4.7	4.9	4.5	4.8	4.2	4.6	4.5	4.2	4.7	4.7	4.3	4.0	4.3
		Venous	4.6	4.8	5.1	6.6	5.5	4.5	4.2	5.4	4.3	5.0	5.1	4.2	4.2	4.7	5.1	4.8	4.9	4.1	4.5	4.9
	Propranolol	Arterial	3.6	4.8	4.9	4.8	5.4	4.8	4.9	4.5	4.5	4.3	4.8	4.3	4.9	5.1	4.5	4.8	4.8	5.0	4.5	4.9
		Venous	3.7	4.2	5.3	5.8	5.6	5.4	5.0	5.3	4.7	4.9	4.5	4.9	5.2	5.1	4.5	5.0	5.0	4.6	4.4	4.6

Plasma [K+]
(mmol/l)

Mean Values

BL - Baseline
IBC - Immediately before contraction
DC - During contraction

		Sample																		
		BL	IBC	DC	RECOVERY															
					:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Arterial	4.4	4.6	4.7	4.9	5.1	5.0	4.8	4.8	4.7	4.7	4.5	4.5	4.6	4.4	4.3	4.4	4.3	4.4	4.4	4.5
	Venous	4.4	4.4	5.1	6.0	5.6	4.9	4.7	4.7	4.5	4.6	4.5	4.4	4.3	4.4	4.4	4.3	4.2	4.3	4.4	4.4
Metoprolol	Arterial	4.4	4.5	4.7	5.1	5.1	5.3	5.0	5.0	5.0	4.8	4.7	4.6	4.7	4.6	4.6	4.6	4.6	4.6	4.6	4.6
	Venous	4.4	4.6	5.3	6.3	5.6	5.1	4.8	4.8	4.6	4.5	4.6	4.5	4.5	4.6	4.6	4.6	4.6	4.5	4.6	4.6
Propranolol	Arterial	4.3	4.6	4.6	4.9	5.4	5.4	5.2	5.1	5.0	4.9	4.9	4.9	4.9	5.0	4.7	4.6	4.7	4.7	4.7	4.7
	Venous	4.4	4.5	5.0	6.3	6.1	5.5	5.2	5.0	4.9	4.9	4.7	4.8	4.8	4.6	4.6	4.6	4.6	4.5	4.5	4.5

Plasma [K+]
(mmol/l)

Standard Deviation

BL - Baseline
IBC - Immediately before contraction
DC - During contraction

		Sample																		
		BL	IBC	DC	RECOVERY															
					:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Arterial	0.2	0.4	0.4	0.4	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.3	0.3	0.3
	Venous	0.3	0.3	0.3	0.5	0.4	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.3
Metoprolol	Arterial	0.3	0.5	0.3	0.3	0.3	0.5	0.3	0.2	0.5	0.4	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.4	0.2
	Venous	0.4	0.2	0.6	0.6	0.5	0.4	0.3	0.4	0.3	0.2	0.3	0.3	0.2	0.4	0.4	0.3	0.3	0.4	0.3	0.4
Propranolol	Arterial	0.4	0.4	0.4	0.4	0.3	0.5	0.4	0.5	0.6	0.5	0.5	0.4	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.2
	Venous	0.5	0.4	0.4	0.5	0.6	0.5	0.5	0.5	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.5	0.3	0.3

Plasma [K+]
(mmol/l)

Standard Error of the Mean

BL - Baseline

IBC - Immediately before contraction

DC - During contraction

		Sample																			
		BL	IBC	DC	RECOVERY																
					:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Arterial	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Venous	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Metoprolol	Arterial	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Venous	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propranolol	Arterial	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
	Venous	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.1

FATIGUE

Sustained Voluntary Contraction

MVC - Maximum Voluntary Contraction

		BASELINE		CONTRACTION							
Subject	Trial	MVC1 (Nm)	Measurement	0	:30	1:00	1:30	2:00	2:30	2:53	2:57 (MVC2)
SI	Placebo	205.7									
			%MVC1	29.6	29.5	28.4	28.8	28.3	29.4	28.9	70.9
	Metoprolol	258.9									
			%MVC1	31.2	30.4	30.0	30.1	29.3	29.6	30.0	38.0
	Propranolol	245.1									
			%MVC1	32.7	30.5	30.6	30.0	29.7	29.4	29.1	47.9
JM	Placebo	313.8									
			%MVC1	28.4	28.6	28.3	29.2	28.4	29.1	28.1	53.1
	Metoprolol	322.1									
			%MVC1	29.5	29.3	29.2	28.4	27.5	31.5	29.0	64.1
	Propranolol	285.4									
			%MVC1	30.6	30.0	30.3	28.6	26.3	29.4	29.2	57.0
JT	Placebo	282.9									
			%MVC1	30.7	29.3	28.8	27.6	28.1	28.8	28.8	57.2
	Metoprolol	298.0									
			%MVC1	29.9	26.8	27.0	27.9	28.9	29.5	29.0	62.3
	Propranolol	259.4									
			%MVC1	31.8	29.9	29.7	30.5	29.1	30.2	29.9	54.3

FATIGUE

Sustained Voluntary Contraction

MVC - Maximum Voluntary Contraction

		BASELINE		CONTRACTION							
Subject	Trial	MVC1 (Nm)	Measurement	0	:30	1:00	1:30	2:00	2:30	2:53	2:57 (MVC2)
MS	Placebo	279.5									
			%MVC1	28.2	25.2	28.9	29.2	28.7	29.1	28.8	44.8
	Metoprolol	274.1									
			%MVC1	29.0	30.2	29.4	29.8	30.1	29.9	29.8	47.1
	Propranolol	286.9									
			%MVC1	30.1	29.2	29.2	28.8	27.6	28.0	20.3	20.7
BR	Placebo	326.3									
			%MVC1	28.6	29.2	29.3	28.3	27.0	26.6	26.7	65.0
	Metoprolol	329.4									
			%MVC1	29.4	30.3	29.3	28.6	27.7	26.9	27.6	55.0
	Propranolol	300.7									
			%MVC1	28.7	29.1	28.7	29.6	29.7	25.0	22.3	55.3
MH	Placebo	221.0									
			%MVC1	29.0	29.2	28.6	27.2	29.7	28.6	28.1	80.6
	Metoprolol	266.0									
			%MVC1	27.6	28.6	28.5	29.6	27.0	29.6	27.5	69.0
	Propranolol	256.5									
			%MVC1	28.7	29.5	28.8	28.2	28.4	29.1	29.2	77.2

FATIGUE

Sustained Voluntary Contraction

MVC - Maximum Voluntary Contraction

		BASELINE		CONTRACTION							
Subject	Trial	MVC1 (Nm)	Measurement	0	:30	1:00	1:30	2:00	2:30	2:53	2:57 (MVC2)
MB	Placebo	269.5									
			%MVC1	29.6	28.2	29.5	29.3	28.1	28.7	27.7	60.3
	Metoprolol	259.5									
			%MVC1	27.4	28.3	28.6	29.1	29.7	29.0	28.0	63.0
	Propranolol	237.0									
			%MVC1	27.2	27.2	27.7	26.5	23.8	19.1	6.0	26.5
MG	Placebo	211.0									
			%MVC1	28.5	29.4	29.7	29.3	29.1	29.5	28.5	52.6
	Metoprolol	197.9									
			%MVC1	30.5	28.4	28.3	28.0	28.0	28.4	28.9	63.0
	Propranolol	237.6									
			%MVC1	29.8	29.7	29.3	30.0	28.6	27.0	16.5	18.9
SC	Placebo	178.7									
			%MVC1	29.7	29.9	29.3	30.1	30.7	30.3	30.1	65.5
	Metoprolol	198.4									
			%MVC1	29.4	28.4	27.9	28.4	28.8	29.3	29.6	72.6
	Propranolol	195.3									
			%MVC1	29.3	29.8	29.6	30.3	29.4	30.6	30.7	74.7

FATIGUE
Sustained Voluntary Contraction

MEAN VALUES

MVC - Maximum Voluntary Contraction

	BASELINE		CONTRACTION							
	MVC1 (Nm)	Measurement	0	:30	1:00	1:30	2:00	2:30	2:53	2:57 (MVC2)
Placebo	254.3									
		%MVC1	29.1	28.7	29.0	28.8	28.7	28.9	28.4	61.1
Metoprolol	267.1									
		%MVC1	29.3	29.0	28.7	28.9	28.6	29.3	28.8	59.3
Propranolol	256.0									
		%MVC1	29.9	29.4	29.3	29.2	28.1	27.5	23.7	48.1

STANDARD DEVIATION

MVC - Maximum Voluntary Contraction

	BASELINE		CONTRACTION							
	MVC1 (Nm)	Measurement	0	:30	1:00	1:30	2:00	2:30	2:53	2:57 (MVC2)
Placebo	48.9									
		%MVC1	0.8	1.3	0.5	0.9	1.0	0.9	0.9	10.2
Metoprolol	44.1									
		%MVC1	1.2	1.1	0.9	0.8	1.0	1.2	0.9	10.3
Propranolol	30.4									
		%MVC1	1.6	0.9	0.8	1.2	1.8	3.4	7.9	20.5

STANDARD ERROR OF THE MEAN

MVC - Maximum Voluntary Contraction

	BASELINE		CONTRACTION							
	MVC1	Measurement	0	:30	1:00	1:30	2:00	2:30	2:53	2:57 (MVC2)
Placebo	17.3									
		%MVC1	0.3	0.5	0.2	0.3	0.4	0.3	0.3	3.6
Metoprolol	15.6									
		%MVC1	0.4	0.4	0.3	0.3	0.4	0.4	0.3	3.6
Propranolol	10.8									
		%MVC1	0.6	0.3	0.3	0.4	0.6	1.2	2.8	7.3

EVOKED TWITCH

Pt - EvokedTwitch Torque (Nm)

HRT - Half Relaxation Time (msec)

			Sample																	
			BL	IBC	RECOVERY															
Subject	Trial	Mst.			:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
SI	Placebo	Pt	57.5	62.5	54.1	58.4	57.8	58.5	60.2	61.5	63.0	64.0	64.1	63.0	62.8	63.2	63.1	60.8	58.9	52.7	50.2
		HRT	58.3	40.8	51.0	40.8	40.8	51.0	51.0	51.0	51.0	51.0	51.0	51.0	51.0	51.0	51.0	40.8	40.8	61.2	51.0
	Metoprolol	Pt	42.1	55.7	28.5	34.4	33.1	34.5	36.3	39.8	42.1	44.9	47.2	49.4	51.6	52.0	53.1	53.5	51.6	48.3	44.5
		HRT	51.7	61.2	61.2	40.8	40.8	40.8	51.0	61.2	61.2	61.2	61.2	71.4	61.2	61.2	61.2	61.2	71.4	40.8	51.0
	Propranolol	Pt	44.1	49.5	34.0	37.6	38.2	38.7	40.3	42.7	45.5	47.0	47.8	48.9	50.5	51.2	51.7	50.4	49.5	45.8	42.7
		HRT	48.3	71.4	61.2	71.4	51.0	51.0	61.2	61.2	61.2	61.2	71.4	61.2	71.4	71.4	71.4	71.4	71.4	61.2	51.0
JM	Placebo	Pt	65.3	65.7	43.5	44.1	43.9	46.8	48.9	50.6	53.2	54.9	55.8	57.7	59.7	59.7	61.8	61.3	63.0	59.2	54.2
		HRT	93.3	142.9	102.0	81.6	91.8	91.8	91.8	91.8	91.8	91.8	91.8	91.8	102.0	61.2	61.2	71.4	71.4	112.2	102.0
	Metoprolol	Pt	53.9	76.3	60.0	62.3	64.6	66.1	70.4	69.5	70.1	72.6	73.0	75.2	73.4	72.0	73.3	73.2	66.8	63.4	63.6
		HRT	100.0	102.0	51.0	71.4	71.4	61.2	81.6	81.6	112.2	102.0	112.2	122.5	122.5	132.7	122.5	122.5	214.3	204.1	163.3
	Propranolol	Pt	48.5	57.2	45.8	48.2	47.8	47.5	48.1	50.9	52.7	54.3	56.4	56.6	57.3	58.0	57.1	57.1	57.3	54.9	51.1
		HRT	178.8	244.9	91.8	102.0	91.8	81.6	91.8	102.0	102.0	112.2	71.4	71.4	71.4	71.4	122.5	71.4	71.4	244.9	163.3
JT	Placebo	Pt	40.9	56.5	33.3	33.8	33.7	35.2	37.5	39.5	42.9	44.0	45.7	45.3	45.8	46.3	45.7	45.3	43.4	38.2	36.0
		HRT	50.0	61.2	71.4	61.2	61.2	71.4	51.0	61.2	51.0	51.0	51.0	51.0	61.2	61.2	61.2	61.2	61.2	51.0	51.0
	Metoprolol	Pt	27.5	36.8	32.5	31.8	30.0	29.7	30.3	31.0	32.6	34.4	34.4	34.9	35.9	35.3	35.0	34.8	34.5	30.6	28.1
		HRT	60.0	51.0	71.4	91.8	91.8	91.8	112.2	112.2	61.2	71.4	71.4	71.4	61.2	61.2	61.2	61.2	61.2	61.2	51.0
	Propranolol	Pt	34.5	38.9	25.2	24.2	22.8	21.3	22.1	23.0	24.2	26.1	28.2	29.7	32.8	34.2	35.0	36.6	38.7	35.5	34.4
		HRT	65.0	71.4	81.6	71.4	71.4	81.6	81.6	81.6	51.0	61.2	61.2	61.2	71.4	61.2	61.2	71.4	71.4	71.4	61.2

EVOKED TWITCH

Pt - EvokedTwitch Torque (Nm)

HRT - Half Relaxation Time (msec)

			Sample																	
			BL	IBC	RECOVERY															
Subject	Trial	Mst.			:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MS	Placebo	Pt	39.7	41.8	17.5	15.9	15.1	16.0	18.4	20.4	24.1	27.3	31.5	33.0	36.8	39.9	42.0	45.8	47.1	39.9	35.7
		HRT	65.0	61.2	91.8	81.6	71.4	81.6	51.0	61.2	61.2	61.2	71.4	71.4	71.4	81.6	81.6	81.6	81.6	61.2	61.2
	Metoprolol	Pt	38.6	41.9	20.8	21.3	19.7	19.8	21.8	24.1	25.3	28.7	31.1	35.1	38.1	41.7	42.7	48.2	48.0	43.5	40.2
		HRT	85.0	81.6	102.0	91.8	81.6	81.6	102.0	91.8	91.8	102.0	102.0	102.0	102.0	102.0	102.0	112.2	112.2	81.6	81.6
	Propranolol	Pt	34.9	35.3	7.9	10.1	10.2	9.2	11.3	12.9	15.9	17.0	22.0	24.5	28.0	30.2	32.6	37.8	41.0	36.0	33.0
		HRT	108.3	71.4	122.5	81.3	61.2	71.4	71.4	91.8	91.8	81.6	91.8	91.8	91.8	91.8	91.8	91.8	81.6	61.2	61.2
BR	Placebo	Pt	54.6	59.4	47.5	50.2	51.9	51.7	55.5	60.2	61.0	64.5	65.8	63.4	63.4	62.4	61.2	59.5	61.1	51.2	49.8
		HRT	58.3	81.6	81.6	102.0	122.5	71.4	71.4	91.8	81.6	91.8	81.6	81.6	81.6	71.4	81.6	81.6	71.4	51.0	51.0
	Metoprolol	Pt	50.2	59.4	47.5	53.5	54.4	60.2	63.3	64.3	70.0	68.4	70.3	70.0	68.4	68.0	67.1	65.0	63.2	56.7	52.8
		HRT	65.0	71.4	91.8	142.9	132.7	102.0	112.2	112.2	122.5	122.5	122.5	122.5	132.7	122.5	112.2	102.0	71.4	61.2	71.4
	Propranolol	Pt	41.3	49.1	41.3	44.1	45.3	49.0	53.1	53.1	51.8	55.2	55.1	53.2	53.9	54.3	52.0	53.3	51.2	45.9	44.0
		HRT	103.3	81.6	81.6	81.6	81.6	112.2	122.5	122.5	132.7	132.7	132.7	132.7	132.7	132.7	132.7	122.5	102.0	71.4	61.2
MH	Placebo	Pt	74.3	80.6	63.3	73.8	78.7	82.1	84.1	84.9	88.4	84.1	84.2	84.7	85.5	85.4	83.8	82.2	80.8	77.6	73.1
		HRT	73.3	91.8	183.7	71.4	91.8	102.0	112.2	112.2	112.2	102.0	91.8	112.2	102.0	102.0	81.6	91.8	81.6	71.4	71.4
	Metoprolol	Pt	77.2	82.9	56.2	66.6	74.3	79.2	81.7	84.5	84.1	88.0	87.9	85.7	86.1	89.0	85.7	82.5	80.7	76.6	71.2
		HRT	75.0	71.4	112.2	91.8	102.0	112.2	112.2	132.7	112.2	102.0	102.0	102.0	91.8	102.0	91.8	81.6	71.4	61.2	71.4
	Propranolol	Pt	81.7	86.2	57.5	68.7	75.0	79.2	81.6	84.1	85.3	85.4	88.6	87.5	86.9	86.5	88.3	86.9	83.4	78.5	75.6
		HRT	70.0	71.4	102.0	91.8	112.2	132.7	132.7	132.7	142.9	112.2	122.5	122.5	112.2	112.2	122.5	91.8	91.8	71.4	61.2

EVOKED TWITCH

Pt - EvokedTwitch Torque (Nm)

HRT - Half Relaxation Time (msec)

			Sample																	
			BL	IBC	RECOVERY															
Subject	Trial	Mst.			:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MB	Placebo	Pt	44.8	54.8	49.8	52.4	52.8	58.6	60.0	62.7	61.8	62.3	60.8	61.1	60.2	60.1	59.3	57.8	55.4	49.4	46.5
		HRT	101.7	51.0	71.4	81.6	61.2	71.4	71.4	71.4	81.6	71.4	81.6	71.4	81.6	71.4	81.6	81.6	61.2	40.8	51.0
	Metoprolol	Pt	45.7	56.7	46.6	50.8	54.0	54.4	57.4	57.9	59.2	59.0	59.7	58.9	58.7	57.9	57.1	55.9	54.4	52.8	47.0
		HRT	50.0	40.8	102.0	102.0	61.2	71.4	71.4	81.6	81.6	81.6	81.6	91.8	91.8	81.6	81.6	71.4	61.2	51.0	51.0
	Propranolol	Pt	48.5	37.9	29.1	35.9	39.1	42.9	44.3	46.6	49.5	53.7	54.1	55.7	55.2	56.1	56.3	53.9	53.6	47.4	50.3
		HRT	53.3	40.8	81.6	102.0	61.2	61.2	61.2	81.6	81.6	81.6	81.6	81.6	91.8	81.6	81.6	91.8	81.6	51.0	40.8
MG	Placebo	Pt	29.6	37.2	16.9	19.5	21.5	25.0	28.4	32.2	34.3	36.7	37.6	37.6	38.4	38.4	37.6	37.6	33.1	30.7	28.8
		HRT	61.7	61.2	71.4	102.0	61.2	71.4	81.6	81.6	91.8	91.8	91.8	91.8	91.8	91.8	91.8	91.8	91.8	61.2	51.0
	Metoprolol	Pt	29.8	36.1	23.3	25.8	25.9	26.9	29.8	31.7	33.4	34.6	35.6	35.8	35.7	35.7	35.5	34.1	33.3	31.5	29.5
		HRT	56.7	51.0	71.4	61.2	51.0	51.0	61.2	61.2	71.4	71.4	71.4	81.6	81.6	81.6	81.6	71.4	61.2	51.0	51.0
	Propranolol	Pt	28.9	32.2	6.8	9.8	10.2	12.9	15.4	18.3	19.9	21.5	23.8	25.3	25.9	26.4	26.8	28.8	27.2	24.7	23.5
		HRT	60.0	71.4	91.8	122.5	102.0	112.2	122.5	112.2	122.5	122.5	132.7	122.5	122.5	112.2	112.2	112.2	102.0	71.4	71.4
SC	Placebo	Pt	33.8	41.4	34.6	34.9	34.8	35.2	36.6	37.4	39.7	41.1	41.3	41.5	41.0	41.5	40.9	38.9	38.6	36.3	34.3
		HRT	66.7	71.4	71.4	61.2	61.2	61.2	81.6	81.6	81.6	81.6	81.6	91.8	81.6	91.8	81.6	102.0	91.8	61.2	61.2
	Metoprolol	Pt	38.7	42.7	42.5	41.1	40.6	39.9	40.6	43.4	44.7	45.8	46.5	46.6	46.0	45.6	45.3	44.1	43.3	40.7	38.9
		HRT	56.7	51.0	102.0	102.0	71.4	71.4	51.0	51.0	61.2	51.0	61.2	61.2	61.2	61.2	51.0	51.0	61.2	40.8	40.8
	Propranolol	Pt	36.1	43.4	41.1	39.0	38.0	37.1	38.6	40.2	42.1	42.9	44.2	44.4	44.5	44.7	44.9	42.4	42.5	37.5	35.7
		HRT	58.3	51.0	91.8	81.6	81.6	51.0	51.0	51.0	61.2	71.4	71.4	71.4	71.4	71.4	71.4	71.4	61.2	51.0	40.8

Evoked Twitch — Mean Values

Pt - EvokedTwitch Torque (Nm)

HRT - Half Relaxation Time (msec)

		Sample																	
		BL	IBC	RECOVERY															
				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Pt	48.9	55.5	40.1	42.5	43.3	45.4	47.7	49.9	52.0	53.2	54.1	54.2	54.9	55.2	55.0	54.4	53.5	48.3	45.4
	HRT	69.8	73.7	88.4	76.0	73.7	74.8	73.7	78.2	78.2	77.1	77.1	79.4	80.5	76.0	74.8	78.2	72.6	63.5	61.2
Metoprolol	Pt	44.8	54.3	39.8	43.1	44.1	45.6	48.0	49.6	51.3	52.9	54.0	54.6	54.9	55.2	55.0	54.6	52.8	49.4	46.2
	HRT	66.7	64.6	85.0	88.4	78.2	76.0	83.9	87.3	86.2	85.0	87.3	91.8	89.6	89.6	85.0	81.6	87.3	72.6	70.3
Propranolol	Pt	44.3	47.7	32.1	35.3	36.3	37.5	39.4	41.3	43.0	44.8	46.7	47.3	48.3	49.1	49.4	49.7	49.4	45.1	43.4
	HRT	82.8	86.2	89.6	89.5	79.4	83.9	88.4	93.0	94.1	93.0	93.0	90.7	93.0	89.6	96.4	88.4	81.6	83.9	68.0

Evoked Twitch — Standard Deviation

Pt - EvokedTwitch Torque (Nm)

HRT - Half Relaxation Time (msec)

		Sample																	
		BL	IBC	RECOVERY															
				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Pt	14.1	13.0	15.0	17.5	18.5	18.9	18.7	18.5	18.1	16.5	15.7	15.3	14.9	14.3	14.0	13.2	13.8	13.5	12.8
	HRT	16.1	28.4	36.3	18.7	23.0	14.4	19.7	18.0	19.2	18.1	15.3	19.1	16.3	16.0	12.7	17.3	15.5	19.1	16.0
Metoprolol	Pt	14.0	15.8	13.3	15.2	17.6	19.0	19.7	19.3	19.2	19.1	18.7	17.8	17.0	17.2	16.5	15.6	14.6	14.1	13.6
	HRT	15.9	18.0	20.4	27.2	26.3	22.1	24.9	26.0	23.1	22.0	21.6	21.0	24.9	25.4	23.1	23.6	47.4	48.0	35.2
Propranolol	Pt	14.7	15.5	15.9	17.6	19.1	20.2	20.4	20.5	20.1	20.0	19.6	18.6	17.7	17.3	17.2	15.9	14.8	14.4	14.1
	HRT	39.4	57.3	15.8	15.8	19.1	27.5	28.9	25.7	31.5	25.7	27.0	26.5	22.8	23.0	25.0	18.0	13.6	57.5	35.0

Evoked Twitch

Standard Error of the Mean

Pt - EvokedTwitch Torque (Nm)

HRT - Half Relaxation Time (msec)

		Sample																		
		BL	IBC	RECOVERY																
				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Pt	5.0	4.6	5.3	6.2	6.5	6.7	6.6	6.5	6.4	5.8	5.5	5.4	5.3	5.1	4.9	4.7	4.9	4.8	4.5
	HRT	5.7	10.0	12.8	6.6	8.1	5.1	7.0	6.4	6.8	6.4	5.4	6.8	5.8	5.7	4.5	6.1	5.5	6.8	5.6
Metoprolol	Pt	5.0	5.6	4.7	5.4	6.2	6.7	7.0	6.8	6.8	6.7	6.6	6.3	6.0	6.1	5.8	5.5	5.2	5.0	4.8
	HRT	5.6	6.4	7.2	9.6	9.3	7.8	8.8	9.2	8.2	7.8	7.6	7.4	8.8	9.0	8.2	8.3	16.8	16.9	12.4
Propranolol	Pt	5.2	5.5	5.6	6.2	6.7	7.1	7.2	7.2	7.1	7.1	6.9	6.6	6.3	6.1	6.1	5.6	5.2	5.1	5.0
	HRT	13.9	20.3	5.6	5.6	6.8	9.7	10.2	9.1	11.1	9.1	9.5	9.4	8.1	8.1	8.9	6.4	4.8	20.3	12.4

EVOKED M-WAVE

M-Wave Amplitude (mV)
M-Wave Duration (msec)
M-Wave Area (mV·s)

			Sample																	
			BL	IBC	RECOVERY															
Subject	Trial	Mst.			:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
SI	Placebo	Amplitude	26.4	27.0	29.8	29.0	28.8	28.6	28.3	28.0	27.4	27.5	27.4	26.7	26.8	26.8	26.4	26.2	25.8	25.1	25.0
		Duration	30.6	31.5	27.0	30.3	30.7	31.5	31.5	31.5	31.8	32.2	31.8	32.2	31.1	31.5	31.1	30.7	31.1	30.7	30.3
		Area	0.17	0.18	0.17	0.18	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16
	Metoprolol	Amplitude	20.9	21.8	24.7	24.0	23.5	23.0	23.0	22.6	22.3	22.1	21.6	21.4	21.1	20.9	20.9	20.2	19.9	19.7	19.4
		Duration	34.1	35.6	31.1	34.4	35.2	34.4	35.9	35.6	35.2	35.6	34.1	35.6	34.1	33.7	33.7	34.4	34.1	33.3	33.3
		Area	0.15	0.16	0.16	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.13	0.13
	Propranolol	Amplitude	19.9	19.4	22.1	21.6	21.6	20.9	20.6	19.7	19.4	19.4	19.2	19.0	18.7	18.7	18.5	18.5	18.2	18.0	18.2
		Duration	35.6	34.8	29.6	33.0	33.3	30.7	32.6	30.7	32.2	29.6	29.6	33.0	32.2	29.6	33.7	31.8	31.5	31.1	31.8
		Area	0.13	0.13	0.14	0.14	0.13	0.13	0.13	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.12	0.11	0.11	0.11	0.11
JM	Placebo	Amplitude	19.9	20.4	19.0	19.7	20.2	20.2	19.9	20.9	20.6	20.2	19.9	20.2	20.2	20.2	20.9	19.9	19.9	18.7	19.0
		Duration	36.9	38.6	34.8	38.6	38.9	38.9	40.1	39.3	38.2	38.9	39.7	37.4	38.2	38.6	37.4	37.4	37.1	36.3	35.6
		Area	0.14	0.15	0.13	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.12	0.12
	Metoprolol	Amplitude	26.3	27.6	24.9	25.1	28.6	27.8	35.3	27.8	27.6	27.8	27.1	27.8	27.1	27.1	27.4	27.6	24.8	24.9	26.6
		Duration	39.3	37.4	34.4	41.2	40.4	39.3	36.7	38.2	37.1	35.6	37.1	37.8	34.4	36.3	34.4	36.3	36.7	35.6	35.2
		Area	0.22	0.23	0.19	0.21	0.25	0.23	0.22	0.22	0.22	0.22	0.21	0.21	0.21	0.21	0.21	0.20	0.18	0.19	0.20
	Propranolol	Amplitude	26.9	27.6	27.1	27.8	28.1	28.3	28.6	28.1	28.3	27.8	27.4	27.6	27.6	27.4	27.1	27.1	26.6	26.4	25.7
		Duration	34.9	34.8	33.3	36.3	35.6	35.2	34.8	35.2	34.4	34.8	33.7	32.6	33.0	34.4	33.3	33.3	31.8	32.2	32.2
		Area	0.21	0.21	0.22	0.22	0.22	0.22	0.21	0.21	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.18	0.19	0.19
JT	Placebo	Amplitude	20.4	18.8	19.4	19.9	20.1	20.0	19.8	20.0	19.4	19.3	18.9	19.9	20.2	19.9	19.7	19.4	19.4	18.7	18.7
		Duration	34.9	35.6	31.1	35.9	36.3	37.4	38.2	37.4	37.8	37.4	36.7	38.6	38.2	38.6	38.9	38.6	38.6	37.8	37.1
		Area	0.17	0.15	0.15	0.17	0.18	0.18	0.18	0.17	0.17	0.17	0.16	0.18	0.17	0.17	0.17	0.17	0.16	0.16	0.16
	Metoprolol	Amplitude	18.0	18.5	19.0	19.2	19.4	19.2	19.2	19.0	19.0	18.7	18.5	18.5	18.2	18.2	18.2	18.0	17.8	17.3	17.3
		Duration	38.3	41.9	41.9	41.6	41.9	42.3	43.1	43.4	42.3	41.9	40.1	41.9	40.1	41.2	40.8	39.3	38.9	38.9	38.9
		Area	0.17	0.18	0.16	0.19	0.19	0.20	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.16	0.16
	Propranolol	Amplitude	20.4	20.6	19.7	19.9	20.6	20.9	21.1	21.6	21.4	21.4	21.4	21.6	21.6	21.6	21.6	21.6	22.1	21.6	21.6
		Duration	33.0	35.2	32.2	38.2	38.6	38.9	38.6	36.7	35.2	35.2	33.7	33.7	35.2	34.8	34.1	33.7	32.2	32.2	31.5
		Area	0.17	0.18	0.17	0.20	0.21	0.21	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.17	0.16

EVOKED M-WAVE

M-Wave Amplitude (mV)
M-Wave Duration (msec)
M-Wave Area (mV·s)

			Sample																	
			BL	IBC	RECOVERY															
Subject	Trial	Mst.			:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MS	Placebo	Amplitude	21.8	22.6	21.6	22.1	22.3	24.0	24.2	24.2	24.2	24.2	24.2	24.0	24.2	24.0	24.0	23.8	22.6	21.6	21.6
		Duration	40.6	42.3	42.3	45.3	44.9	44.6	43.8	44.6	43.8	43.8	42.7	42.7	42.3	42.3	41.9	40.4	39.7	37.1	37.4
		Area	0.22	0.23	0.23	0.26	0.25	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.26	0.25	0.25	0.24	0.22	0.20	0.20
	Metoprolol	Amplitude	17.5	17.5	17.8	18.5	18.5	18.5	18.7	18.5	18.2	18.2	18.2	18.5	19.0	18.7	18.5	18.2	17.8	16.6	16.6
		Duration	41.9	44.6	44.2	45.3	46.1	45.7	44.6	43.4	45.3	45.3	43.4	44.2	42.3	43.4	43.4	41.9	41.9	40.4	38.6
		Area	0.16	0.17	0.18	0.19	0.20	0.20	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.16	0.14	0.14
	Propranolol	Amplitude	24.0	22.3	21.6	23.2	23.7	23.3	23.2	22.9	22.7	22.4	22.1	22.0	22.0	22.1	22.0	22.2	21.9	19.6	19.9
		Duration	42.6	46.8	50.9	51.3	52.4	50.9	50.5	50.5	50.2	49.1	49.4	49.1	49.8	48.3	47.9	44.9	45.3	41.6	40.1
		Area	0.24	0.24	0.26	0.28	0.28	0.28	0.27	0.27	0.27	0.26	0.26	0.25	0.25	0.25	0.24	0.24	0.23	0.19	0.19
BR	Placebo	Amplitude	18.7	18.0	16.3	17.0	17.8	18.7	18.7	18.2	17.8	17.5	17.5	17.5	17.0	17.3	17.3	17.3	16.8	17.0	16.6
		Duration	38.6	36.7	35.6	37.1	37.8	37.1	36.3	35.9	37.1	36.3	35.2	35.9	35.9	35.2	35.2	35.2	35.2	34.8	35.2
		Area	0.18	0.17	0.14	0.16	0.17	0.17	0.17	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.14	0.15	0.14	0.14	0.15
	Metoprolol	Amplitude	16.1	15.4	15.6	15.6	15.6	15.6	15.8	16.1	15.4	15.4	15.4	15.1	15.1	15.4	15.4	15.4	15.4	15.1	15.1
		Duration	34.9	34.8	31.1	33.3	33.7	34.4	34.1	33.3	33.7	34.4	33.3	34.1	33.7	33.7	33.7	33.0	32.6	32.6	33.7
		Area	0.13	0.13	0.11	0.12	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.12
	Propranolol	Amplitude	15.4	15.1	15.6	14.6	14.9	15.4	15.1	14.6	14.6	14.4	14.6	14.6	14.4	14.6	14.6	14.2	14.4	14.9	14.4
		Duration	42.3	42.7	37.4	41.2	40.4	39.7	40.4	40.8	39.3	40.1	39.7	38.9	39.3	39.3	38.9	40.4	39.3	39.7	40.8
		Area	0.16	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
MH	Placebo	Amplitude	18.0	18.2	17.3	18.5	18.7	19.2	19.0	18.7	19.0	19.0	18.7	18.5	18.5	18.0	18.5	18.7	18.5	18.2	18.0
		Duration	36.9	35.9	29.6	35.9	33.3	37.4	37.1	33.3	35.2	32.2	31.1	33.0	32.6	32.6	32.6	31.8	32.6	31.8	32.2
		Area	0.18	0.17	0.13	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
	Metoprolol	Amplitude	24.5	23.8	23.8	24.0	24.5	24.5	27.1	25.2	24.2	26.2	26.4	25.9	25.9	26.2	25.7	25.4	25.4	25.4	25.2
		Duration	41.3	40.8	36.3	37.4	38.2	38.6	38.2	38.2	38.9	38.6	38.2	38.2	38.6	38.2	37.8	38.2	38.6	37.8	38.6
		Area	0.26	0.24	0.21	0.22	0.23	0.24	0.24	0.24	0.24	0.25	0.25	0.24	0.25	0.25	0.24	0.24	0.24	0.24	0.25
	Propranolol	Amplitude	33.1	32.4	27.4	29.3	30.7	31.2	31.0	31.4	30.7	30.7	31.0	30.5	30.7	30.7	31.2	30.7	30.7	29.3	29.5
		Duration	36.9	35.6	34.1	32.6	34.1	34.1	33.7	34.4	33.7	33.7	33.3	33.3	33.0	32.2	33.7	34.1	34.1	33.3	34.4
		Area	0.25	0.26	0.20	0.22	0.24	0.24	0.24	0.24	0.23	0.24	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.23

EVOKED M-WAVE

M-Wave Amplitude (mV)
M-Wave Duration (msec)
M-Wave Area (mV·s)

			Sample																	
			BL	IBC	RECOVERY															
Subject	Trial	Mst.			:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
MB	Placebo	Amplitude	23.8	23.5	25.9	24.7	23.8	24.5	24.2	24.0	23.8	23.5	23.3	23.3	23.0	22.8	22.6	22.3	21.8	20.4	20.9
		Duration	36.6	37.4	34.4	36.3	36.7	35.9	35.9	35.9	35.9	35.2	37.1	34.4	34.4	34.4	34.8	34.8	34.8	34.1	33.7
		Area	0.20	0.20	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.16
	Metoprolol	Amplitude	28.1	27.8	30.7	29.3	28.6	28.3	27.8	28.1	27.8	27.1	25.3	25.0	24.9	25.3	26.4	26.4	25.3	25.4	25.7
		Duration	35.6	35.2	33.7	33.0	34.4	34.4	34.4	34.8	33.3	33.3	35.6	34.8	35.2	33.7	33.7	34.8	33.7	32.6	34.8
		Area	0.22	0.23	0.21	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.21	0.21	0.21	0.21	0.21	0.22	0.21	0.21	0.21
	Propranolol	Amplitude	23.3	23.0	20.2	23.0	21.8	22.3	22.6	22.3	22.6	22.6	22.3	22.3	22.1	22.1	21.4	21.8	21.6	20.3	21.4
		Duration	34.3	33.7	31.8	32.7	32.2	33.0	33.3	34.8	34.4	34.8	33.7	33.0	33.0	32.7	33.7	32.2	30.7	31.5	32.6
		Area	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.15	0.15	0.15	0.15
MG	Placebo	Amplitude	17.0	18.0	18.2	19.4	19.2	19.7	18.7	18.7	19.2	19.0	18.7	18.5	18.5	18.2	18.2	17.8	17.5	16.8	16.8
		Duration	39.7	40.4	37.8	38.9	40.4	39.7	40.4	39.7	40.1	39.3	38.9	39.7	41.2	40.8	41.9	41.6	37.1	38.9	36.7
		Area	0.15	0.16	0.14	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.14	0.13	0.13	0.12
	Metoprolol	Amplitude	22.8	23.0	21.8	22.8	23.3	23.3	23.0	23.0	22.8	23.0	22.8	22.6	21.8	21.8	22.1	21.8	21.8	21.6	21.6
		Duration	40.9	41.9	39.7	40.4	41.2	40.4	41.2	39.7	40.4	39.7	40.4	40.4	40.1	40.4	38.9	39.7	38.2	38.9	37.8
		Area	0.20	0.20	0.18	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17
	Propranolol	Amplitude	20.6	19.7	18.7	19.7	20.4	21.4	21.6	21.6	21.8	21.8	22.1	22.3	21.6	21.6	20.2	19.4	18.0	18.5	17.3
		Duration	36.6	37.1	36.7	40.1	37.8	37.4	37.4	38.2	37.1	36.7	37.4	33.3	35.2	34.4	34.4	34.1	33.0	30.3	29.6
		Area	0.17	0.16	0.17	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.15	0.14	0.12	0.11
SC	Placebo	Amplitude	16.1	16.6	15.4	15.6	16.1	16.6	16.8	16.6	16.8	16.6	16.3	16.3	16.3	16.1	16.1	16.1	15.1	14.6	14.6
		Duration	40.9	44.2	40.1	44.9	45.7	46.8	45.7	46.4	46.1	46.8	44.6	46.4	44.9	44.9	44.2	43.8	42.7	41.9	42.7
		Area	0.17	0.18	0.15	0.18	0.18	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.16	0.15	0.14	0.14
	Metoprolol	Amplitude	16.8	17.5	16.6	17.3	17.8	18.0	18.0	18.0	18.0	18.0	17.8	17.5	17.3	17.3	17.3	17.0	16.8	16.6	16.8
		Duration	39.6	37.4	32.2	35.2	37.1	37.1	36.3	35.9	36.7	35.6	35.2	35.2	35.2	35.2	35.2	34.1	34.1	33.7	34.1	34.8
		Area	0.15	0.16	0.13	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.13	0.13	0.13
	Propranolol	Amplitude	16.6	16.8	15.6	16.1	16.6	16.8	16.8	17.0	16.8	16.8	16.8	16.8	16.6	16.6	16.3	16.3	15.8	15.6	15.8
		Duration	43.6	44.9	37.8	43.4	41.9	43.8	43.4	42.7	43.4	42.7	43.1	41.9	42.3	42.7	42.3	40.8	41.2	40.8	40.1
		Area	0.17	0.17	0.14	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.14

M-Wave

Mean Values

M-Wave Amplitude (mV)
M-Wave Duration (msec)
M-Wave Area (mV·s)

		Sample																		
		BL	IBC	RECOVERY																
				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Amplitude	20.2	20.3	20.3	20.7	20.8	21.3	21.1	21.0	20.9	20.8	20.5	20.5	20.5	20.4	20.4	20.2	19.7	19.0	19.0
	Duration	37.3	38.1	34.7	38.1	38.3	38.8	38.8	38.2	38.4	38.0	37.5	37.8	37.6	37.7	37.6	37.1	36.5	35.9	35.7
	Area	0.17	0.18	0.16	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.15	0.15
Metoprolol	Amplitude	21.2	21.4	21.7	21.8	22.2	22.0	23.1	22.0	21.7	21.8	21.5	21.4	21.2	21.2	21.3	21.1	20.6	20.3	20.5
	Duration	38.4	38.8	36.1	38.0	38.7	38.5	38.3	38.1	38.1	37.8	37.5	38.0	37.1	37.3	36.7	36.9	36.5	36.0	36.2
	Area	0.18	0.19	0.17	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.16	0.17
Propranolol	Amplitude	22.2	21.9	20.9	21.7	22.0	22.3	22.3	22.1	22.0	21.9	21.9	21.9	21.7	21.7	21.4	21.3	21.0	20.5	20.4
	Duration	37.8	38.4	36.0	38.8	38.5	38.2	38.3	38.2	37.8	37.4	37.1	36.5	37.0	36.5	36.9	36.1	35.5	34.7	34.8
	Area	0.18	0.19	0.18	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.16	0.16

M-Wave

Standard Deviation

M-Wave Amplitude (mV)
M-Wave Duration (msec)
M-Wave Area (mV·s)

		Sample																		
		BL	IBC	RECOVERY																
				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Amplitude	3.1	3.2	4.5	3.9	3.6	3.5	3.5	3.4	3.3	3.4	3.4	3.2	3.3	3.3	3.2	3.1	3.1	2.9	2.9
	Duration	3.0	3.6	4.7	4.4	4.6	4.3	4.1	4.6	4.1	4.6	4.3	4.4	4.3	4.3	4.3	4.2	3.4	3.3	3.3
	Area	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02
Metoprolol	Amplitude	4.2	4.3	4.6	4.1	4.4	4.2	5.8	4.1	4.1	4.3	4.0	4.0	3.9	4.0	4.1	4.2	3.7	3.9	4.2
	Duration	2.7	3.3	4.5	4.1	3.8	3.7	3.6	3.4	3.8	3.7	3.1	3.3	3.0	3.5	3.4	2.9	3.0	2.9	2.1
	Area	0.04	0.04	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Propranolol	Amplitude	5.1	5.0	4.0	4.6	4.7	4.7	4.8	4.8	4.8	4.7	4.7	4.6	4.8	4.7	4.8	4.8	4.9	4.5	4.5
	Duration	3.8	4.7	5.9	5.8	5.8	5.8	5.5	5.5	5.4	5.4	5.8	5.4	5.5	5.6	4.9	4.4	4.9	4.3	4.1
	Area	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.04

M-Wave

Standard Error of the Mean

M-Wave Amplitude (mV)

M-Wave Duration (msec)

M-Wave Area (mV·s)

		Sample																		
		BL	IBC	RECOVERY																
				:05	:20	:35	:50	1:05	1:20	1:35	1:50	2:05	2:20	2:35	2:50	3:05	4:00	5:00	10:00	15:00
Placebo	Amplitude	1.1	1.1	1.6	1.4	1.3	1.2	1.2	1.2	1.2	1.2	1.2	1.1	1.2	1.2	1.1	1.1	1.1	1.0	1.0
	Duration	1.1	1.3	1.6	1.6	1.6	1.5	1.4	1.6	1.5	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.2	1.2	1.2
	Area	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Metoprolol	Amplitude	1.5	1.5	1.6	1.5	1.6	1.5	2.0	1.5	1.5	1.5	1.4	1.4	1.4	1.4	1.5	1.5	1.3	1.4	1.5
	Duration	1.0	1.2	1.6	1.4	1.4	1.3	1.3	1.2	1.3	1.3	1.1	1.2	1.1	1.2	1.2	1.0	1.0	1.0	0.8
	Area	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Propranolol	Amplitude	1.8	1.8	1.4	1.6	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.6	1.7	1.7	1.7	1.7	1.7	1.6	1.6
	Duration	1.3	1.7	2.1	2.0	2.1	2.1	1.9	1.9	1.9	1.9	2.0	1.9	2.0	2.0	1.7	1.6	1.7	1.5	1.4
	Area	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

APPENDIX C:

ANALYSIS OF VARIANCE TABLES - CHAPTER II

The effect of the drug interventions and sustained voluntary contraction on the arterial plasma [La-]

Filename: La- art
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	263.5	8			
Drug (D)	2.5	2	1.3	0.24	
Error	84.3	16	5.3		
Time (T)	1192.4	19	62.8	68.89	< 0.001
Error	138.5	152	0.9		
D x T	8.7	38	0.2	0.93	
Error	74.7	304	0.2		

The effect of the drug interventions and sustained voluntary contraction on the venous plasma [La-]

Filename: La- ven
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	1328.7	8			
Drug (D)	23.9	2	11.9	0.59	
Error	326.2	16	20.4		
Time (T)	4492.4	19	236.4	80.67	< 0.001
Error	445.5	152	2.9		
D x T	22.6	38	0.6	0.51	
Error	353.3	304	1.2		

The effect of the drug interventions and sustained voluntary contraction on the arterial plasma [K+]

Filename: K+ art
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	6.9	8	0.9		
Drug (D)	6.4	2	3.2	2.37	0.124
Error	21.6	16	1.4		
Time (T)	29.1	19	1.5	17.04	< 0.001
Error	13.6	152	0.1		
D x T	3.2	38	0.1	1.18	0.225
Error	21.5	304	0.1		

The effect of the drug interventions and sustained voluntary contraction on the venous plasma [K+]

Filename: K+ ven
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	9.0	8	1.1		
Drug (D)	7.1	2	3.6	2.72	0.095
Error	21.0	16	1.3		
Time (T)	108.8	19	5.7	43.85	< 0.001
Error	19.8	152	0.1		
D x T	5.5	38	0.1	1.57	0.021
Error	27.9	304	0.1		

The effect of the drug interventions on the absolute voluntary torque during the fatigue protocol

Filename: abs3mtor
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	33797.4	8	4224.7		
Drug (D)	2923.8	2	1461.9	3.35	0.059
Error	6985.6	16	436.6		
Time (T)	116861.6	7	16694.5	45.40	< 0.001
Error	20602.8	56	367.9		
D x T	5750.1	14	410.7	4.06	< 0.001
Error	11324.3	112	101.1		

The effect of the drug interventions on the relative voluntary torque (%MVC1) during the fatigue protocol

Filename: rel3mtor
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	700.4	8	87.5		
Drug (D)	221.0	2	110.5	2.51	0.111
Error	703.1	16	43.9		
Time (T)	18022.6	7	2574.7	47.15	< 0.001
Error	3058.0	56	54.6		
D x T	853.3	14	61.0	3.30	< 0.001
Error	2068.4	112	18.5		

The effect of the drug interventions and sustained voluntary contraction on the evoked twitch torque

Filename: twtor
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	128496.6	8	16062.1		
Drug (D)	4689.9	2	2344.9	5.41	0.016
Error	6938.0	16	433.6		
Time (T)	11836.8	18	657.6	14.58	< 0.001
Error	6497.0	144	45.1		
D x T	537.1	36	14.9	1.80	0.005
Error	2382.6	288	8.3		

The effect of the drug interventions and sustained voluntary contraction on the evoked twitch half-relaxation time

Filename: twtor
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	132821.1	8			
Drug (D)	13516.4	2	6758.2	3.41	0.057
Error	31679.1	16	1979.9		
Time (T)	17958.2	18	997.7	1.34	0.170
Error	107004.4	144	743.1		
D x T	5827.0	36	161.9	0.57	
Error	82151.2	288	285.2		

The effect of the drug interventions and sustained voluntary contraction on the absolute M-wave amplitude

Filename: m-w amp
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	5097.9	8	637.2		
Drug (D)	154.4	2	77.2	0.36	0.706
Error	3402.9	16	212.7		
Time (T)	163.3	18	9.1	7.78	< 0.001
Error	168.0	144	1.2		
D x T	19.7	36	0.5	1.02	0.439
Error	154.0	288	0.5		

The effect of the drug interventions and sustained voluntary contraction on the absolute M-wave duration

Filename: m-w dur
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	5944.4	8	743.1		
Drug (D)	21.3	2	10.6	0.06	0.931
Error	2751.3	16	172.0		
Time (T)	513.0	18	28.5	9.03	< 0.001
Error	454.4	144	3.2		
D x T	62.6	36	1.7	1.26	0.152
Error	395.9	288	1.4		

The effect of the drug interventions and sustained voluntary contraction on the absolute M-wave area

Filename: m-w area
9 subjects
ANALYSIS OF VARIANCE TABLE
2-Way Within Subjects - Randomized Block Design

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	p Value
Subjects	0.343	8	0.043		
Drug (D)	0.010	2	0.005	0.28	0.761
Error	0.289	16	0.018		
Time (T)	0.044	18	0.002	15.01	< 0.001
Error	0.024	144	0.000		
D x T	0.001	36	0.000	1.08	0.351
Error	0.010	288	0.000		

The effect of placebo and sustained voluntary contraction on the relationship between venous plasma potassium concentration and evoked twitch torque

Filename: tor/potpl
CURVILINEAR REGRESSION

Trend	R-Squared	F (increment)	df	Probability
Linear	0.655	28.49	1, 15	< 0.001
Quadratic	0.718	3.10	1, 14	0.097

The effect of metoprolol and sustained voluntary contraction on the relationship between venous plasma potassium concentration and evoked twitch torque

Filename: tor/potmet
CURVILINEAR REGRESSION

Trend	R-Squared	F (increment)	df	Probability
Linear	0.661	29.29	1, 15	< 0.001
Quadratic	0.749	4.91	1, 14	0.042

The effect of propranolol and sustained voluntary contraction on the relationship between venous plasma potassium concentration and evoked twitch torque

Filename: tor/potpro
CURVILINEAR REGRESSION

Trend	R-Squared	F (increment)	df	Probability
Linear	0.793	57.61	1, 15	< 0.001
Quadratic	0.825	2.49	1, 14	0.134