The Effects of Isometric Exercise Training on Resting Blood Pressure With Specific Reference to Selected Cardiovascular, Neuromuscular, and Metabolic Variables

by

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Abstract

There were two purposes to the work of this thesis (a) to identify the role of isometric training intensity in the training-induced reductions in resting blood pressure, and (b) to identify whether the mechanism for the reduced resting blood pressure is best reflected in what can be broadly termed cardiovascular, neuromuscular or metabolic markers of that training. Firstly, in a cross-sectional study, the only strong correlation was found between heart rate variability (a cardiovascular marker) and resting blood pressure. Secondly, this cardiovascular marker was also significantly affected by a single session of isometric exercise, an effect that persisted for at least 4 hours after exercise. However, thirdly, this marker and other cardiovascular markers (such as cardiac output and stroke volume) did not correlate with reductions in blood pressure seen after 4 weeks of isometric training. Instead, the training-induced reductions in blood pressure correlated strongly with neuromuscular and metabolic markers of isometric training. The extent to which local muscle fatigue was induced during isometric training correlated with the reductions in resting blood pressure. Therefore (a) isometric training intensity appears to be of utmost importance in the reductions in resting blood pressure (when bilateral-leg exercise is performed in 2 minute bouts), and (b) the mechanism whereby the adaptations in resting blood pressure occur is best reflected in neuromuscular and metabolic markers of local muscle fatigue during that training. These findings are discussed with a particular focus on the possible role of muscle metaboreceptor stimulation, during isometric training in the mechanism of training-induced reduction in resting blood pressure.

Contents

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Commonly Used Abbreviations

Δ = change

2min-Torque$_{peak}$ = the highest mean torque that could be sustained for 2 minutes

ANOVA = analysis of variance

ANCOVA = analysis of covariance

BMR = basal metabolic rate

BP = blood pressure

BRS = baroreflex sensitivity

C of V % = coefficient of variance

CV = cardiovascular

DBP = diastolic blood pressure

ECG = electrocardiography

EMG = electromyography

EMG$_{peak}$ = peak of electromyography signal

EMG$_{amp}$ = electromyography signal amplitude

EMG$_{freq}$ = electromyography signal frequency

HF = high frequency spectral component of heart rate variability

HFnu = normalised units of high frequency spectral component of heart rate variability

HR = heart rate

HR$_{peak}$ = peak heart rate(s)

HR$_{train}$ = exercise training heart rate(s)

HRV = heart rate variability

IET = isometric exercise training

IHG = isometric handgrip

La = lactate

La_{peak} = peak lactate value

La_{train} = derived training intensity as a lactate value

LF = low frequency spectral component of heart rate variability

LF/HF = ratio of low and high frequency spectral components of heart rate variability

LFnu = normalised units of low frequency spectral component of heart rate variability

MAP = mean arterial blood pressure

mmHg = millimetres of mercury

$mmol.L^{-1}$ = millimole(s) per litre

MSNA = muscle sympathetic nerve activity

mV = millivolt(s)

MVC = maximal voluntary contraction

N·m = Newton metres

NM = neuromuscular

Q = cardiac output

SBP = systolic blood pressure

SV = stroke volume

TP = total power of spectral frequencies of heart rate variability

TPR = total peripheral resistance

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Chapter 1

Introduction

1.1 Introduction

Blood pressure (BP) can be defined as the hydrostatic pressure blood exerts on the walls of the vasculature, ranging between maximal (systolic) and minimal (diastolic) levels between each heartbeat (Booth, 1977). Hypertension is generally diagnosed when the average of 2 or more diastolic (DBP) measurements is ≥90 mmHg, and the average of 2 or more systolic (SBP) measurements is ≥140 mmHg, at the brachial artery (Carretero and Oparil, 2000). Hypertension is acknowledged as one of the most prevalent and powerful risk factors for the development of cardiovascular disease (Chobanian *et al*. 2003). Recently, the Framingham Heart Study, one of the most prominent epidemiological research entities in The United States predicted a 90% residual lifetime risk for developing hypertension, in healthy middle-aged and elderly individuals (Vasan *et al*. 2002). According to the National Institute for Health and Clinical Excellence in the UK, at the turn of the 21st century, the National Health Service funded approximately 90 million prescriptions for antihypertensive medications annually (North of England Hypertension Guideline Development Group, 2004). The same report claimed that the total cost of these prescriptions equated to ≈15% of the total annual NHS budget for primary care drugs. Furthermore, the incidence of hypertension is predicted to increase 60% by 2025 in developed Western societies (Kanavos *et al*. 2007).

Previous research is unequivocal, in that treatment of hypertension reduces the risk of cardiovascular (CV) disease and mortality (Chobanian *et al*. 2003). Typically, initial intervention has involved the prescription of antihypertensive medications, which involves significant fiscal issues as aforementioned. Furthermore, it has been reported that poor adherence to medicated treatment results in low rates of BP control in the general population (Fitz-Simon *et al*. 2005). As such, it is now widely accepted that initial therapy should involve lifestyle modification, of which exercise is an important facet. It is generally accepted that an intervention of dynamic, endurance-based exercise is a viable means of reducing resting BP (Pescatello *et al*. 2004; Fagard, 2001; Fagard, 2006). However, as current recommendations state that ≥3 hours per week of endurance exercise is most effective in altering resting BP (Pescatello *et al*. 2004), and given that time is a major determinant for rates of exercise adherence (Chao *et al*. 2000), there is a strong case for finding an alternative exercise regimen to assist in the first-line treatment of hypertension.

There exists a limited body of knowledge that affirms an intervention of isometric exercise to be an effective means of reducing resting BP, typically involving just over 30 minutes of exercise per week (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Peters *et al*. 2006; Millar *et al*. 2007; Wiles *et al*. 2010). However, whilst isometric exercise training (IET) is known to reduce resting BP (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Peters *et al*. 2006; Millar *et al*. 2007; Wiles *et al*. 2010), the explanations for the physiological changes associated with these reductions are not clear (Wiles *et al*. 2010). Also, the role that isometric exercise intensity plays in inducing these BP reductions is elusive (Wiles *et al*. 2007; Wiles *et al*. 2010). Therefore, it would seem appropriate to focus research effort on these two aspects of the topic. The work of this thesis will centre on these by (a) measuring selected physiological variables before and after IET, which might explain the reductions in resting BP and (b) exploring the role of exercise intensity during IET in bringing about these reductions in resting BP.

Previous training studies have indicated the training-induced reduction in resting BP is associated with changes in autonomic nervous system control at rest (Taylor *et al*. 2003) and therefore it would seem appropriate to make some measurements of this, before and after IET. It has also been shown that IET induces changes in vascular function (Green *et al*. 1994; Alomari and Welsch, 2007) and therefore some measure of this would also be useful. The changes in vascular function would, in theory, modify total peripheral resistance to blood flow around the circulation, and therefore it would be appropriate to measure total peripheral resistance (TPR) before and after training in an attempt to provide some insight into its contribution to the reduced resting BP. This is usually done by derivation from measures of cardiac output and mean arterial pressure ($Q = MAP \times TPR$) and therefore there is some additional justification for measurement of these variables. Finally, there has been a focus of research attention on the acute effects of isometric exercise on baroreflex function (Iellamo *et al*. 1994; Kamiya *et al*. 2001; Carrington and White, 2001; Carrington *et al*. 2003; Fisher *et al*. 2006; Fisher *et al*. 2007) and therefore a further attempt to quantify the effects of IET on this measure will be made.

In exploring the role of IET intensity in causing the training-induced reductions in resting BP, there is growing evidence that this aspect of isometric exercise is important in the reductions seen after training. Recent findings (Wiles *et al*. 2010) have suggested

that IET at higher relative heart rates, compared to lower intensities caused similar reductions in resting BP, but the BP reductions in the higher intensity group occurred earlier during the training programme. Therefore, it appears that intensity, as reflected by heart rate (HR) during IET, might be important in the training induced reductions in resting BP.

In relation to training, it is plausible to expect that the pressor response to isometric exercise might be a contributing factor in the training-induced changes in resting BP. This is because the pressor response modifies BP so markedly during exercise (Lind and McNicol, 1967; Lind, 1970). Therefore, when attempting to elucidate the role of exercise intensity in the reductions in resting BP after IET, it would be useful to be able to precisely quantify the magnitude of the pressor response. This is one of the aims of the novel approach to isometric training provided by the methods of Wiles *et al*. (2007; 2010), who used an incremental exercise test and electromyographic (EMG) activity to set isometric training intensity. Furthermore, it is known that the pressor response during isometric exercise is mediated via stimulation of mechanically (mechanoreceptor) and chemically (metaboreceptor) sensitive nerve cells in the muscle (McCloskey and Mitchell; 1972; Freund *et al*. 1979; Kaufman *et al*. 1983; Mitchell *et al*. 1989; Iellamo *et al*. 1999b; Ichinose *et al*. 2006), and this deserves attention.

The role of metaboreceptor stimulation during isometric exercise has especially received growing attention in recent studies (Mostoufi-Moab *et al*. 1998; Fisher and White, 1999; Houssiere *et al*. 2005; López-Barneo *et al*. 2008). It might be expected that the physiological actions associated with these structures may be modified because of their repeated stimulation during isometric exercise (Fisher and White, 1999) and therefore it would be pertinent to explore possible markers which reflect the muscle environment that is responsible for stimulation of these receptors. Blood lactate is thought to be a possible candidate for such a marker of muscle metaboreceptor stimulation during isometric exercise (Rotto and Kaufman, 1988; Mostoufi-Moab *et al*. 1998). Furthermore, new markers of muscle 'fatigue' have been used during isometric exercise, which might also be useful in this context (Fisher and White, 1999; Fisher and White, 2004). These are derived from changes in EMG activity during isometric exercise (Person and Mishin 1964; Person and Kudina 1968; Vredenbregt and Rau, 1973; Moritani *et al*. 1982).

Therefore, there is justification, when attempting to explain the reduced BP after IET, for inclusion of measures such as cardiac output (Q; heart rate and stroke volume), mean arterial pressure (MAP), total peripheral resistance (TPR), heart rate variability (HRV) and baroreflex sensitivity (BRS). For the purposes of this thesis these measures are collectively referred to as cardiovascular variables. Also, in determining the role of isometric exercise intensity, there is justification for the inclusion of measures of EMG which might be termed neuromuscular variables. Finally, measures which reflect the intensity of isometric exercise and the associated changes in muscle metabolic environment during isometric exercise (such as blood lactate) could be referred to as metabolic variables. For these reasons, selected cardiovascular (CV), neuromuscular (NM), and metabolic variables will be the focus of the work of this thesis. Indeed, the majority of studies that have attempted to advance knowledge and understanding of this adaptive mechanism have shown changes in variables that can be broadly categorised into one or more of these three physiological systems. These studies will be reviewed in the present chapter (section 1.7).

Of course, before making measurements of this type in relation to IET (Chapters 4 and 5), the selected CV, NM and metabolic variables need to be explored in various ways. For example, published reports of blood lactate assessment during isometric exercise are scarce. Therefore appropriate methods for assessment of blood lactate during isometric exercise need to be developed especially at a range of isometric exercise intensities (this is described in Chapter 2, pages 50-60). Also, since 'training' is an adaptation to repeated 'acute responses' to each isometric exercise period, it is possible that acute changes in the selected variables might offer some explanation of what eventually becomes an adaptation to training. Therefore, measurement of acute responses to isometric exercise and their persistence during the post-exercise period might offer some insight into the physiological mechanisms associated with training (and this is included in Chapter 3, pages 78-97). For example, there is some evidence for statistically significant post-exercise hypotension following isometric exercise (Stewart *et al*. 2007; Millar *et al*. 2009).

Before detailing the previous research that has explored the effects of isometric exercise on resting BP by using CV, NM, and metabolic variables, published work relating to the underpinning concepts of BP regulation during isometric exercise will be reviewed. For

the reasons outlined previously in this section, this will be done in three sections: CV, NM and metabolic.

1.2 Cardiovascular components of blood pressure regulation

The predominant CV factors that affect BP involve the structure and function of both the heart and the conduit vessels through which the blood must flow, which may be dependent upon the prevailing level of BP itself (Oparil *et al*. 2003). Heart rate (HR), Q, and SV provide quantifiable measures of the functional output of the heart, both at rest and during exertion. The measurement of Q together with MAP allows for the calculation of TPR. Blood pressure regulation is governed by changes in BP which are sensed by baroreceptors and thence (via the brain), adjustments are made to the CV system, using the autonomic nervous system. The adjustments can be achieved by changes in HR and SV, but are predominantly achieved by altering TPR (Ogoh *et al*. 2003). Both HR and SV (Q = HR x SV) are modified by the autonomic nervous system, so if indeed these variables were to be significant in the adaptation of resting BP following a course of isometric exercise, they would not act in isolation.

Baroreceptor control of BP is achieved via the arterial baroreflex, which provides an influential negative feedback regulation system (Billman *et al*. 1982). The baroreflex is a regulatory process whereby baroreceptor cells monitor the extent of stretch applied to the walls of the aortic arch and carotid arteries, especially during systole. The baroreceptors located in both the wall of the ascending aorta and the aortic arch are predominantly responsible for regulating systemic BP (Shoukas *et al*. 1987). Prevailing BP levels therefore affect baroreceptor regulation of BP, as the rate of the nerve impulses which are sent by the baroreceptors are determined by the amount of stretch applied to the baroreceptor cells, which is in turn determined by the pressure exerted on the vasculature. Higher BP results in greater stretching and thus stimulation of the baroreceptors, and conversely, lower BP results in less stretching of the baroreceptors and so results in reduced stimulation. The rate of the nerve impulses sent by the baroreceptors determines the balance of sympathetic and parasympathetic stimulation, which is controlled by the CV centre in the medulla oblongata. The afferent discharge of these receptor cells can elicit increased or decreased HR, cardiac contractility, vascular

resistance, and venous return, leading to concomitant increased/decreased BP (Pang, 2001). Baroreflex sensitivity has been said to limit variations of BP for a given BP range, rather than exclusively setting BP status. As such, it is believed that other control mechanisms would have to act in concert with BRS to significantly adjust a given BP level (Liard, 1980).

Another indicator of CV autonomic balance may be found by analysing the oscillations of both the interval between consecutive heartbeats, and between consecutive instantaneous heart rates. This method is termed heart rate variability, HRV (Lopes and White, 2006). By separating spectral frequencies of electrocardiogram recordings it has been suggested that the prevailing dominance of vagal/parasympathetic or sympathetic autonomic function can be detected (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996), and that the efferent activity is synchronous with oscillations in BP itself (Malliani *et al*. 1991). Blood pressure is said to both affect, and be strongly influenced by autonomic function (Piccirillo *et al*. 1996a, 1996b). The two agonistic components of the autonomic nervous system act directly on the function of the heart, thus affecting BP. The sympathetic tone has chronotropic, dromotropic, and inotropic actions, increasing HR, conduction, and contractility respectively, thus increasing BP. The second, parasympathetic tone, acts via the vagus nerve to decrease HR and conduction, thus usually decreasing BP, via negative chronotropic and dromotropic actions, with no effect on contractility (Médigue *et al*. 2004). The spectral frequencies are categorised as high frequency (representing vagal parasympathetic modulation) and low frequency (representing sympathetic activity) components, with the ratio of the two (LF/HF) said to represent sympathovagal balance (Malliani *et al*. 1991).

1.3 Neuromuscular components of blood pressure regulation and the use of electromyographic measurement during isometric exercise

Along with the aforementioned exercise pressor reflex, BP may also be regulated during isometric exercise by central command, although central command may also regulate BP at rest (Rowell and O'Leary, 1990). Central command and the exercise pressor response (the latter having been mentioned already) can be influenced by both mechanically and

chemically sensitive afferent nervous activity originating from muscle tissue, which provides feedback to the CV centres in the brainstem. Central command then activates the CV and somatomotor systems though irradiation of the CV centres (Goodwin *et al*. 1972). Currently, it is suggested that the increased central command leads to the heightened CV response typically seen during isometric exercise (Seals, 1993; Franke *et al*. 2000). Furthermore, the increased central command during an isometric exercise stimulus supposedly increases both motor unit recruitment and the firing rates of those units (Vaz *et al*. 1996).

Consequently, this means that when isometric exercise is performed at a constant force, as is typical in most IET studies (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Taylor *et al*. 2007), central command will continue to rise in an attempt to maintain force output in the presence of ever increasing fatigue (Vaz *et al*. 1996). Given the effects of central command on the HR and BP response (total CV response) to isometric exercise, this will mean that the CV response to an isometric exercise stimulus will not be stable if performed at constant force (Schibye *et al*. 1981; Franke *et al*. 2000).

However, the use of surface EMG during isometric exercise, with exercise prescribed at a constant EMG value, results in both a relatively stable and predictable HR response during the exercise stimulus (Franke *et al*. 2000; Wiles *et al*. 2007). Force production typically decreases as fatigue levels increase during isometric exertion (Sadamoto *et al*. 1983). Surface EMG measures muscle activity non-invasively, on the skin overlying the active muscle, and is an ideal technique for prolonged motor unit recruitment measurement (Pullman *et al*. 2000), allowing for objective processing of collected data (Abbink *et al*. 1998).

Isometric exercise is ideal for EMG analysis, as muscle length and velocity are strictly controlled (Vredenbregt and Rau, 1973), and through the use of a dynamometer, muscle generated force is accurately measured (Potvin, 1997). A stable CV response to isometric exercise permits precise quantification of the 'CV stimulus' experienced, at a given intensity, in each exercise session of a training programme. It has been argued that this allows a clearer understanding of the components of IET (such as intensity) that are

responsible for BP reduction (Wiles *et al*. 2007; Wiles *et al*. 2010). Surface EMG measurement also allows for analysis of the fatigue-related EMG variables, which will be discussed in section 1.6 describing the effects of isometric exercise on those variables.

1.4 Metabolic components of blood pressure regulation

Blood pressure may be affected by the chemical composition of the blood, whereby changes in blood O_2, CO_2, and pH, along with other metabolite concentrations stimulate metabolic sensitive cells called chemoreceptors, often when exercise induced lactic acidaemia occurs (Poole *et al*. 1988). These sensory receptor cells act similarly to baroreceptor cells, in that nerve impulses are sent to the CV centre in the medulla oblongata where increased sympathetic activity causes vasoconstriction in arterioles and venoconstriction in veins leading to increased BP. The chemoreceptive response can also be responsible for changes in blood volume distribution (Heistad and Abboud, 1971). Heart rate, SV, and Q are also indirectly affected by the chemical composition of the blood, as chemoreceptor activation leads to an increase in respiration rate, caused by the relative decrease in O_2 content of the blood, leading the vasomotor centre in the medulla oblongata to modify HR, SV, and Q, thus affecting BP (López-Barneo *et al*. 2008).

Evidence also suggests that the chemoreceptive response interacts with the baroreflex system (discussed in section 1.2) to heavily influence circulatory dynamics, especially when changes in BP occur (Somers *et al*. 1991). It is believed that repeated stimulation of the chemoreceptive cells leads to a change in the sensitivity and/or operating point of the reflex action, and that an individual's prevailing BP may also affect the sensitivity of the chemoreceptor response (Prabhakar and Peng, 2004). It has been suggested that repeated chemoreceptor stimulation is likely present during isometric exercise, although the mechanism(s) responsible for sensitivity and operating point changes remain unknown (Fisher and White, 1999). Propositions include changes in the vascular reaction to nitric oxide (Sun *et al*. 1999) along with other metabolites and neurotransmitters (Prabhakar, 1994) responsible for altering vasoconstriction and vasodilatation activity.

Peripheral metabolic conditions present in exercising muscle also contribute to the regulation of BP during an isometric exercise stimulus (Fisher and White, 1999). Afferent nervous activity from type III and IV afferents originating in the active muscle provide feedback to the regulatory processes of CV control (Goodwin *et al*. 1972). The type IV muscle afferents are said to be more specifically chemically sensitive (Mense and Stahnke, 1983), and are commonly termed metaboreceptors and/or chemoreceptors (Kaufman and Forster, 1996). The peripheral afferent activity originating from the chemically sensitive receptor cells combines with central command in higher brain centres to produce the enhanced CV pressor response experienced during isometric exercise (Lind *et al*. 1964; Goodwin *et al*. 1972; Victor *et al*. 1988; Saito *et al*. 1991; Seals *et al*. 1991; Thornton *et al*. 2002).

1.5 Summary of cardiovascular, neuromuscular, and metabolic regulation of blood pressure

The previous sections have introduced how BP may be regulated by a host of variables that may be broadly assigned into CV, NM, and metabolic components. The CV variables are predominantly focused on the structure and function of the heart and vasculature, together with fluctuations of the autonomic nervous system and arterial baroreflex feedback. Neuromuscular regulation of BP has been described as the direct role of central command on the CV system, involving CV centres in the brain accounting for the heightened pressor response evident during isometric exercise. Central command is reflected in EMG activity (Schibye *et al*. 1981; Franke *et al*. 2000). The section detailing metabolic regulation of BP has introduced the concept of peripherally located receptor cells, which are sensitive to the chemical composition of the blood. Changes in metabolite balance during isometric exercise leading to a peripherally modulated response, coupled with increased central command, resulting in the enhanced CV pressor response.

1.6 The acute effects of isometric exercise on blood pressure; cardiovascular, neuromuscular, and metabolic components

Isometric exercise is commonplace in everyday life, required to simply maintain an upright body position. Occurrences that require static effort of some kind involve lifting, carrying, holding, pushing, and pulling objects. Often the maintenance of limb position (e.g. arms held overhead) alone requires static or isometric effort (Lind, 1970). The human CV response during isometric exercise is well documented. Heart rate increases linearly with isometric exercise intensity (Wiles *et al*. 2007), although the increases are more modest than those during dynamic forms of exercise (Lind and McNicol, 1967). Stroke volume does not increase during isometric exercise as markedly as it does during dynamic exercise, so Q demands are predominantly met by increases in HR (Donald *et al*. 1967). Systemic vascular resistance, or TPR, increases during isometric exercise (Kivowitz *et al*. 1971) due to the physical occlusion of blood flow to exercising muscle (Barcroft and Millen, 1939; Mark *et al*. 1985). Heart rate increases during sustained isometric exercise are said to be dependent on both the intensity and the duration of the exercise (Martin *et al*. 1974), and is said to be due to a gradual increase in central command (Franke *et al*. 2000).

The amount of muscle mass used in isometric exercise has also been suggested to modulate the central command and subsequent CV responses during isometric exercise (Freund *et al*. 1978; Franke *et al*. 2000). It has also been shown that 'steady state' HR response to typical intensities used in isometric exercise studies is reached after 60 seconds, in a 120 second exertion period (Wiles *et al*. 2007). Systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures all increase rapidly, more so than during dynamic exercise, likely due to the occlusion of muscle blood flow evident during isometric exercise, meaning any rise in Q leads to elevated BP (Lind and McNicol, 1967; Lind, 1970).

In a resting state, any rise in BP leads to a baroreflex-mediated bradycardia. However, as aforementioned, during isometric exercise both HR and BP rise simultaneously. As such baroreflex control of HR and BP during isometric exercise is said to be reset by both central command (Querry *et al*. 2001; Ogoh *et al*. 2002) and the exercise pressor reflex

(Gallagher *et al*. 2001; Smith *et al*. 2003). More recent work has reported that both central command and the isometric exercise pressor reflex act simultaneously and in seemingly equal measures (Gallagher *et al*. 2006), so that as BP rises during the exercise stimulus, BRS may continue to operate around the increasing pressures.

It has also been reported that the arterial baroreflex may be heavily involved in the recovery of HR following a bout of isometric exercise (Iellamo *et al*. 1999b). During isometric exercise, changes in BRS are also associated with changes in measures of HRV, where an increase in the low frequency spectral component can be observed during exercise (Cooley *et al*. 1998), together with a decrease in the high frequency spectral component (Iellamo *et al*. 1999b). This has been said to be associated with the central command changes during isometric exercise, and is representative of increased sympathetic modulation (Malliani *et al*. 1991; Pagani *et al*. 1997; Montano *et al*. 1998; Cooley *et al*. 1998) during isometric exercise. Recently, Millar *et al*. (2009) reported altered cardiac autonomic regulation 5 minutes post isometric handgrip (IHG) exercise.

In terms of NM elements, the measurement of surface EMG activity of muscle recruitment during isometric exercise provides an index of NM fatigue, via analysis of the signal amplitude and frequency. The increased signal amplitude evident at the onset of fatigue is suggested to occur from either increased spatial or temporal motor unit recruitment (Vredenbregt and Rau, 1973; Moritani *et al*. 1982), or increased spatial/temporal motor unit recruitment in concert with increased motor unit synchronisation (Person and Mishin 1964; Person and Kudina 1968). The decreased EMG signal frequency at the onset of fatigue, or spectral compression, is said to arise principally from decreased conduction velocities of action potentials (Bigland-Ritchie *et al*. 1981; Sadoyama *et al*. 1983). At the point where NM fatigue begins and then increases, force output typically reduces due to the less efficient excitation/contraction coupling process (Moritani *et al*. 1986; Moritani *et al*. 1993; St Clair Gibson *et al*. 2001). Utilising NM measures allows for the analysis of the physiological stress during isometric exercise itself, to investigate the importance of the level of training stimulus on resultant BP adaptation.

Previous studies investigating metabolic variables during short-term (5 to 8 minutes) fatiguing isometric exercise have reported anaerobiosis at the muscle site, with increasing lactate, hydrogen ion (H^+), and other metabolite concentrations due to occlusion of the vascular beds of 'active' muscles (Ahlborg *et al*. 1972; Edwards *et al*. 1972; Funderburk *et al*. 1972; Karlsson and Ollander, 1972; Karlsson *et al*. 1975; Tesch and Karlsson, 1979). It is hypothesized that peripheral chemoreceptor stimulation in the active muscle augments muscle sympathetic nerve activity, leading to an increased pressor response to any given exercise stimulus, resulting in an amplified total CV reaction (both HR and BP) during isometric exercise (McCloskey and Mitchell; 1972; Freund *et al*. 1979; Kaufman *et al*. 1983; Mitchell *et al*. 1989; Iellamo *et al*. 1999b; Ichinose *et al*. 2006).

Similarly to CV responses, it has been proposed that muscle chemoreceptor stimulation is directly related to the duration of isometric effort (Gandevia and Hobbs, 1990), and the muscle mass utilised in the isometric exercise (Freund *et al*. 1978). Studies have also investigated the effects of occluding blood flow from an exercised limb immediately following a period of isometric exercise. It has been found that by confining the blood with increased metabolite concentrations, BP remains elevated significantly beyond an equivalent control period, and that concomitant delayed decreases in the low frequency HRV component are also evident (Iellamo *et al*. 1999b). This supports the notion that the chemoreceptors significantly contribute to the immediate and acute responses of BP regulation both during and following isometric exercise.

The problem arising from occluded vascular beds during isometric exercise is that lactate concentration cannot be detected unless an indwelling catheter is used at the muscle site. Therefore, lactate measured by commonly used finger/thumb prick samples can only be assessed after the cessation of isometric exercise, allowing accumulated lactate to circulate in the systemic vasculature (Poole *et al*. 1988). Consequently, a novel procedure was required for this research, enabling lactate measurement to be incorporated into a discontinuous incremental isometric exercise test, to assess lactate metabolism at progressively increasing intensities (see Chapter 2, pages 50-60 for details of test procedure and findings).

The previous paragraphs of this section have discussed the immediate and/or acute CV, NM, and metabolic responses to isometric exercise. There also exists a body of work that has investigated the effects of chronic exposure to IET. These studies report significant reductions in resting BP following a course of IET, using either handgrip/forearm (Wiley *et al*. 1992; Ray and Carrasco, 2000; Taylor *et al*. 2003; McGowan *et al*. 2004; Millar *et al*. 2007) or leg (Howden *et al*. 2002; Wiles *et al*. 2010) based training methods.

1.7 The effects of isometric exercise training

Training duration is most commonly conducted over a period of 8 weeks (Wiley *et al*. 1992; McGowan *et al*. 2004; Millar *et al*. 2007; Wiles *et al*. 2010), although 10 (Taylor *et al*. 2003) and as little as 5 weeks has been used (Ray and Carrasco, 2000; Howden *et al*. 2002). Typically, BP reductions are between 16 mmHg and 5 mmHg (SBP), 13 and 5 (DBP), and around 5 mmHg for MAP. Mean arterial pressure results are less commonly reported than either SBP or DBP, which is surprising given that it represents the average pressure during each cardiac cycle (Oblouck, 1987). Also, MAP is known to be interrelated with other CV variables, such as TPR and Q (Walker *et al*. 1992; Turner *et al*. 1996). As such, MAP will be analysed in both acute response (Chapter 3) and chronic adaptation (Chapters 4 and 5) data within this thesis.

These previous studies customarily use normotensive participants, although an amalgamated review conducted by Millar *et al*. (2007) investigated the effects of IHG training on hypertensive individuals. Interestingly, the reported BP reductions in these hypertensive individuals (SBP = -6 mmHg, DBP = -3 mmHg) were considerably less than those commonly reported in normotensive individuals (SBP up to -16 mmHg and DBP up to -14.9 mmHg), using the same time course (8 weeks) and training intensities (30% of maximal voluntary contraction, MVC) of previous studies (Wiley *et al*. 1992; McGowan *et al*. 2004).

What also remains unclear is/are the critical element(s) of isometric exercise that are essential to elicit adaptations in resting BP. Usually, IET is performed at a percentage of

maximal isometric force (MVC; Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002), although this method has only ever been reported to be important in eliciting strength gains following isometric exercise (Lind, 1970), not BP reductions. The use of EMG measurement with the analysis of metabolic and fatigue-related variables within this thesis is an attempt to provide quantifiable data to uncover the critical element(s) of IET-induced BP reductions.

Previous studies have sometimes investigated possible causes for BP reductions. Initially, Ray and Carrasco (2000) reported that reductions in DBP and MAP were not associated with any changes in muscle sympathetic nerve activity. Indeed in this study, sympathetic activity was unaltered after 5 weeks of training. Importantly, SBP was not significantly altered following the IET regimen. It led Ray and Carrasco (2000) to postulate that reductions in BP following chronic exposure to isometric exercise are brought about by changes to systemic vascular resistance. Work conducted by McGowan *et al*. (2004) offered some quantitative data to support ideas related to changes in the peripheral vasculature. After 8 weeks of IHG training that resulted in significant reductions in resting BP, it was reported that increases in flow mediated dilatation and decreased reactive hyperaemic flow were also present. This led McGowan *et al*. (2004) to suggest that isometric training brings about heightened vasoactive sensitivity, which may be the important factor in the resultant BP changes evidenced after 8 weeks of isometric training. However, resting blood flow remained unchanged in this study.

Taylor *et al*. (2003) presented data suggesting that it may in fact be nervous activity balance affecting resultant BP levels because they found that, after 10 weeks of IHG training, the LF/HF ratio component of HRV tended to decrease. They suggested that sympathetic activity was reduced, relatively, to parasympathetic modulation at rest. Peters *et al*. (2006) have suggested that reduced SBP following 6 weeks of IET was interrelated with changes in markers of oxidative stress during isometric exercise. Finally, Millar *et al*. (2007) suggest that BP decline is linearly related with the time course of training, and that individuals with greater initial baseline SBP values will experience greater SBP reductions, perhaps emphasizing the potential benefits of an isometric training regimen.

Studies investigating the effects of dynamic and strength/resistance exercise on CV variables are numerous and concurring. Adherence to an exercise regimen commonly results in an increased SV at rest, with simultaneous decreases in HR, reducing the stress on the heart and vasculature. The effects of resistance/strength based training programmes, more akin to isometric exercise, are often less pronounced than those of a dynamic, endurance type (Fagard, 1996; 1997).

Previously, it has been postulated that TPR may be altered by physical activity through the increased muscle afferent activity present during exercise, which via sympathoexcitation and the muscle sympathetic nerve activity modifies vascular resistance to blood flow, thus altering BP (Fisher and White, 1999). Persistent physical activity is reported to reduce baroreflex mediated sympathetic activation (Coats *et al.* 1992; Grassi *et al.* 1994) invariably leading to a decrease in BP. It is also suggested that arterial compliance is enhanced with physical training, most likely due to improved endothelial function (Cameron and Dart, 1994; Silva *et al.* 1997) and increased elastic properties of the aortic and carotid arterial vessels (Kingwell *et al.* 1997), leading to altered afferent discharge of the baroreceptor cells.

Dynamic exercise training has previously been reported to modify the autonomic balance detectable with HRV measures (Arai *et al*. 1989; Furlan *et al*. 1993). This has been evidenced by typical increased sympathetic (low frequency, LF) and reduced vagal (high frequency, HF) activity during exertion, to increase heart contractility and other factors to meet the demands of exercise, and then reversing the balance following cessation of exercise and a period of recovery to favour parasympathetic dominance (La Rovere *et al*. 1992). Whether physiological modifications following isometric exercise closely associate with those typically seen following dynamic based exercise remains to be seen.

1.8 Summary of the effects of isometric exercise on blood pressure; cardiovascular, neuromuscular, and metabolic components

The previous sections have introduced how BP, together with selected CV, NM, and

metabolic variables are affected, in both the acute short term ('responses' to isometric exercise) and the chronic long term ('adaptations' to IET) by an isometric exercise stimulus. The section covering the effects of isometric exercise on NM factors has detailed how central command drives motor unit recruitment during isometric exercise and increases HR and Q at the onset of isometric exercise. Muscle blood flow occlusion in the presence of increased Q results in the elevated BP evident during isometric exercise and this raises TPR. During prolonged isometric exercise NM fatigue is detectable and this affects metabolic variables, possibly associated with exercise-induced acidosis. This seems to be as a result of vascular bed occlusion during isometric exercise, which when coupled with increased central command results in the enhanced CV pressor response.

The previous section also covered previous attempts to explain IET-induced BP reductions, including changes in autonomic nervous function and systemic vascular resistance. Figure 1 provides an illustration of the sequence of events caused by isometric exercise, and links the physiological mechanisms and isometric exercise intensity factors investigated within this thesis. This is based upon aforementioned research investigating the effects of an isometric exercise stimulus (Barcroft and Millen, 1939; Goodwin *et al*. 1972; McCloskey and Mitchell, 1972; Mark *et al*. 1985; Fisher and White, 2004).

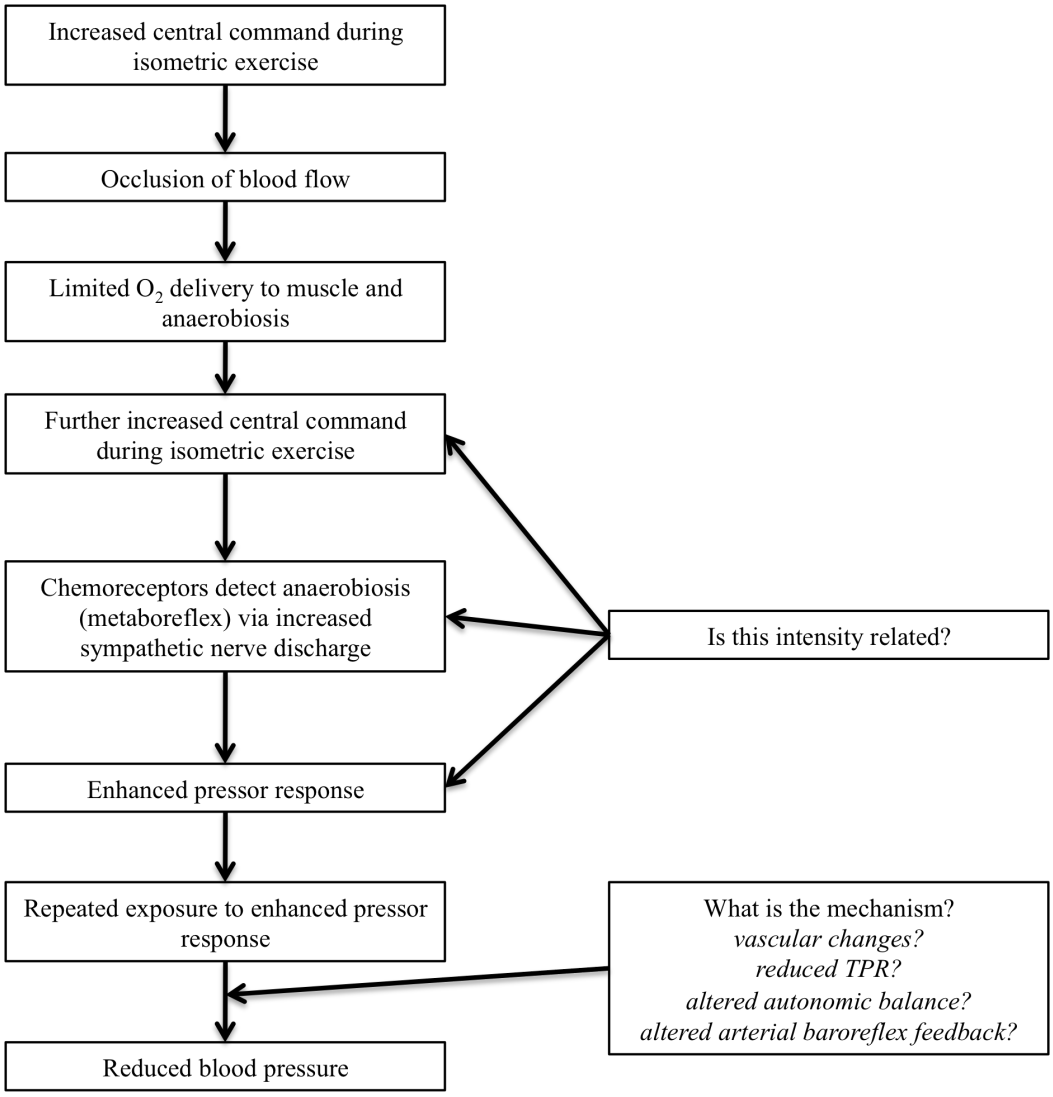

Figure 1. Sequence of events resulting from exposure to an isometric exercise stimulus

1.9 Study aims

Human CV responses and adaptations to isometric exercise require further investigation before they can be fully understood. This thesis will focus on three important elements of human CV responses and adaptations to isometric exercise, including the acute responses of CV regulation to isometric exercise, the chronic adaptations of CV regulation to IET, and the critical elements of this training which result in modified resting BP. The aim of investigating these elements in relation to BP homeostasis is to improve the understanding of BP regulation following isometric exercise, and the significant factor(s) of this exercise that cause the reductions in resting BP.

The principal effect of isometric exercise is to cause BP, HR, and sympathetic nerve activity to progressively rise during exercise, and it is not known how, why, or indeed if repeated exposure to higher HR and BP leads to resting BP adaptation. There is a lack of understanding of the important elements of CV regulation (CV, neural, and metabolic), both centrally and peripherally moderated, and their roles in causing or assisting BP reductions following a programme of IET. Evidence of MAP changes together with changes in associated CV variables is not fully understood following exposure to an IET stimulus. There is also uncertainty regarding the length of time for which the training-induced reductions in resting BP persist after cessation of training. It is possible that BP measures recorded at the end of an isometric exercise intervention may be an acute response to the final training session(s), rather than a chronic adaptation brought about by the entire training course.

Previous studies that have used IET to study BP adaptation have often only used small muscle groups, through IHG training (Wiley *et al*. 1992; Ray and Carrasco, 2000; Taylor *et al*. 2003; McGowan *et al*. 2004). Fewer studies have used larger muscle groups, and so theoretically increased muscle sympathoexcitation, by the way of bilateral-leg isometric exercise (Howden *et al*. 2002; Wiles *et al*. 2007; Wiles *et al*. 2010). However, all of the previous research in this field (with the exception of the work of Wiles *et al*. 2010) has prescribed isometric exercise as a percentage of maximal force, without fully understanding the critical elements of isometric exercise, and if indeed training at a percentage of maximal force is the prerequisite for invoking BP adaptation.

To conclude, the overall aim of this thesis is to improve the understanding of the physiological changes associated with reductions in resting BP following IET, and to uncover the role that isometric exercise intensity plays in inducing these BP reductions. The hypotheses of this thesis are that firstly, BP manipulation following IET must initially be either centrally or peripherally mediated. And secondly, IET intensity must in some way be directly implicated in the subsequent CV adaptation, and that the traditionally used torque sustained at a percentage MVC will not be the determining factor.

Therefore the objectives of the work of this thesis were to assess:

1. The relative significance of CV variables in the control of BP regulation.
2. The acute responses of BP and other CV variables to a single session of isometric exercise.
3. The chronic adaptations in BP to 4 weeks of IET and associated measures, including CV, NM, and metabolic variables.
4. The key elements of IET (especially intensity), and its relationship with selected NM and metabolic variables, thought to be responsible for invoking reductions in resting BP. And, whether these factor(s) could be utilised for future exercise intensity prescription methods.
5. The measurement of blood lactate during isometric exercise without invasive indwelling catheters, overcoming the problems of vascular bed occlusion present during isometric exercise.

Chapter 2

General Methods

2.1 Introduction

This chapter details the general methods used in this thesis which include: the procedures for the operation of the Biodex System 3 isokinetic dynamometer, the measurement and recording of electrocardiography, electromyography, blood pressure, baroreflex sensitivity, cardiac output, stroke volume, and total peripheral resistance. This chapter also includes reliability data for each measured variable. Lastly, it details the formalisation of the discontinuous incremental isometric exercise test.

2.2 The research approach

Study 1 provided cross-sectional data to evaluate the relative significance of a number of cardiovascular (CV) variables in relation to resting blood pressure (BP) levels and will be detailed in Chapter 3 (Part A). In study 2 the acute responses of these variables, together with resting BP, were analysed following a single session of bilateral-leg isometric exercise, and this will be detailed in Chapter 3 (Part B). The third study was designed to provide an insight into the chronic adaptations of these CV variables in relation to BP change following 4 weeks of bilateral-leg isometric exercise training for 3 days.wk^{-1} and this will be detailed in Chapter 4. The data (EMG and blood lactate) from this study was also used subsequently with an aim of relating markers of fatigue (intensity-related) to training-induced BP reductions. This EMG and blood lactate data will be presented separately to the CV variables of Chapter 4, in Chapter 5, Parts A and B respectively. Study 3 involved a 4 week training intervention period which was chosen on the basis of previous work conducted by Wiles *et al*. (2010) who indicated that the majority of BP adaptations evident after an 8 week training programme had been discernible after just 4 weeks, although it had not reached significance. Earlier work conducted (Wiley *et al*. 1992; Howden *et al*. 2002), using differing isometric exercise techniques and protocols had reported significant change to some BP components, but not all SBP, DBP, and MAP, after 3 weeks, during the course of a longer intervention study (8 and 5 weeks respectively). Therefore, a 4 week intervention was selected to see whether all resting BP components (SBP, MAP and DBP) would show adaptations upon completion of this relatively short-term training intervention.

Before the implementation of the bilateral-leg isometric exercise ('acute responses' and 'training') studies, a methodological study was needed to assess the reproducibility of an incremental discontinuous bilateral-leg isometric test and this will be detailed in the present chapter. This test will be used for the setting of exercise intensities, similar to that described by Wiles *et al*. (2007 and 2010) and would allow for metabolic variables to be measured in later studies. The research design is illustrated in Figure 2.1.

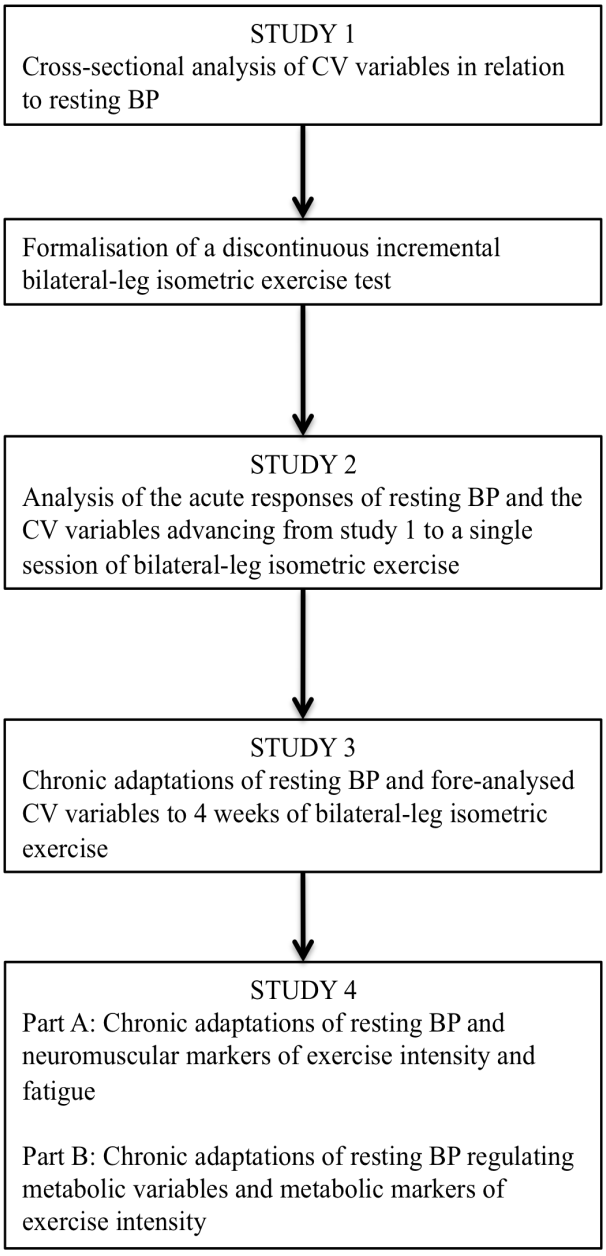

Figure 2.1. Schematic of the design and process of studies contained within the thesis

2.3 Participants and recruitment

Healthy males (18 to 35 years of age) were recruited for the studies described within this thesis. 'Healthy' was defined as those free from any clinically diagnosed CV (including hypertension), respiratory, and/or metabolic conditions/disorders. The selection of young healthy males reduced the risk of possible confounding variables affecting data outcomes, as 'health' status, gender and age have been shown to affect the individual response to an isometric exercise stimulus (Petrofsky *et al*. 1975; Kent-Braun *et al*. 2002). The incidences of clinically diagnosed disorders that have been mentioned are statistically lower in younger males, compared to older males (Department of Health, 2006a; 2006b). Furthermore, all prospective participants were screened before study data collection, including 3 BP measurements (to warrant normotensive status), in accordance with protocols described in section 2.7, together with a personal and family heredity health questionnaire. Women were excluded from the studies due to known confounding variations in CV variables during the menstrual cycle (Sato *et al*. 1995), and gender differences in the associations between physical activity and BP status which have been shown in large population sample studies (MacAuley *et al*. 1996).

In addition to 'healthy' status, participants were required to have been free from any condition that might have hindered exercise performance, and thus subsequent study adherence. Participants were not selected who were, or had ever been smokers, were taking any medications that would affect CV function, or who donated blood on either a regular basis or those who intended to do so during the course of study participation. Blood donation would very likely confound results in studies of this nature due to acute blood volume changes and disturbances in blood chemistry (Bouchard *et al*. 1995).

Young healthy active males were recruited from an undergraduate Sport and Exercise Science degree at Canterbury Christ Church University. Participation was voluntary and included no monetary benefits, but did offer additional experience of research activity beyond modular academic provision. A number of participants volunteered for multiple studies. Prospective participants were supplied with information sheets for the specific study/studies they wished to volunteer for. An example of an information sheet can be found in the Appendix (pages 212-213). Participants were then invited to the laboratory

for a familiarisation session, where the protocols of the study they had volunteered for were further described and then conducted. At this point, the BP measures were checked for normotensive status, together with the responses collected on their personal and hereditary health questionnaire. If measured BP was hypertensive (SBP > 140 mmHg, and DBP > 90 mmHg), or if any complications were highlighted by the questionnaire, then the participant could not be invited to partake in the study. Before any familiarisation protocols were conducted, an informed consent form was signed by the participant (see Appendix, page 214 for an example).

2.4 Biodex System 3 isokinetic dynamometer

All exercise tests were conducted using the same Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY), which has been shown to have very good mechanical intra-day and inter-day reliability for both position and torque (Drouin, *et al*. 2004). The dynamometer was interfaced with a computer using Biodex Advantage software for Windows XP (Microsoft Corporation), which allows the operator to program in commands relating to specific test protocols. A data link from the Biodex remote access to a 16-channel chart recorder (Powerlab, ADInstruments Ltd., Australia) was used to synchronize the time component of surface electromyographic (EMG) activity and force recordings during tests. The Biodex was fitted with a modified hip attachment that was inserted into the standard knee attachment, to allow for bilateral-leg extension exercise to be performed. The dynamometer allows for isometric force to be applied to a movement arm, using a hydraulic servo-controlled mechanism to create resistance against applied force. The interfacing of the dynamometer with microprocessors allows for the measurement of human muscle function, including torque and endurance variables.

Participants sat in the dynamometer seat in an upright position, with 90 degrees of flexion at the hip with the thighs supported. The seat position was then adjusted for each individual according to their lower limb lengths and girth. The lateral femoral condyle of the participants' right leg was aligned with the centre of rotation of the dynamometer head. The backrest was moved until the forward edge of the seat base fitted into the backs of the knees and the lumbar support pressed firmly into the lower back, thus

avoiding unnecessary strain on these regions. The movement arm (modified hip attachment) was secured 1 cm superior to the medial malleoli of the ankles. The strap was padded with 3 cm thick high-density foam facing the anterior portion of the shin, pressing the leg against more high-density foam on the posterior lower leg on the dynamometer arm. Participants were instructed to maintain a relaxed upper body during all leg contractions in order to satisfy standardised levels of stabilisation, so not to affect force outputs achieved (Magnusson, *et al*. 1993; Mendler, 1976). Participants were also instructed to breathe at a normal rhythm and depth at all times to prevent Valsalva manoeuvres. Figure 2.2 illustrates the dynamometer, modified leg attachment, and participant position. For all studies involving the Biodex System 3 isokinetic dynamometer, individual participant machine settings and positions were recorded and maintained throughout the duration of the study to enable standardisation.

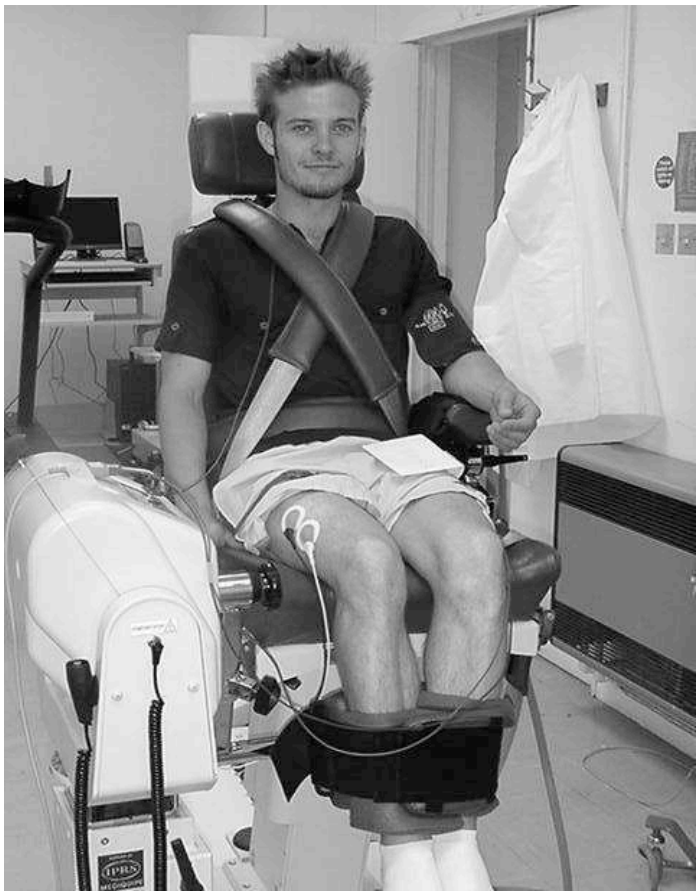

Figure 2.2. Participant position on the dynamometer

2.5 Electromyography recording

A dual bio-amplifier was used to enable surface EMG measurement from both legs, along with the 16-channel chart recorder. The root mean square of the raw EMG signal was computed using the chart recording software (Chart 5 for Windows XP, ADInstruments Ltd., Australia). EMG was smoothed at 1 second using both high and low pass digital filters.

Surface EMG was recorded from both the right and left vastus lateralis, as this muscle has been shown to exhibit a linear relationship ($r = 0.9961$, $p < 0.01$) between EMG and force when performing isometric leg extension exercise (Alkner *et al*. 2000). Electrode placements were consistent among all participants using the vastus lateralis location as described by SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles, www.seniam.org). Skin preparation was also performed in accordance with SENIAM recommendations, which involved shaving the area to remove hair from the sensor location area, cleaning the skin with alcohol, and allowing the alcohol to vaporise to leave dry skin before electrodes were fitted. Skin impedance was checked after all electrode placements, with impedance values of below 10 kΩ being accepted (Hermens *et al*. 2000). The skin preparation and impedance checks were performed prior to all testing procedures on every laboratory visit.

Positive and negative electrodes (sensor T ECG pads, Ambu Inc., Maryland, USA) were placed at two-thirds on the line from the anterior spina iliaca superior to the lateral side of the patella, in the direction of the muscle fibres. An earth electrode was placed on the right olecranon at the proximal end of the ulna. Figures 2.3 and 2.4 illustrate the EMG electrode placements. EMG from each vastus lateralis were combined and averaged to give a single EMG output, representing the combined activity of both muscles. This average was displayed to the participants during all exercise tests, and was used as the 'target' value as the basis for exercise prescription.

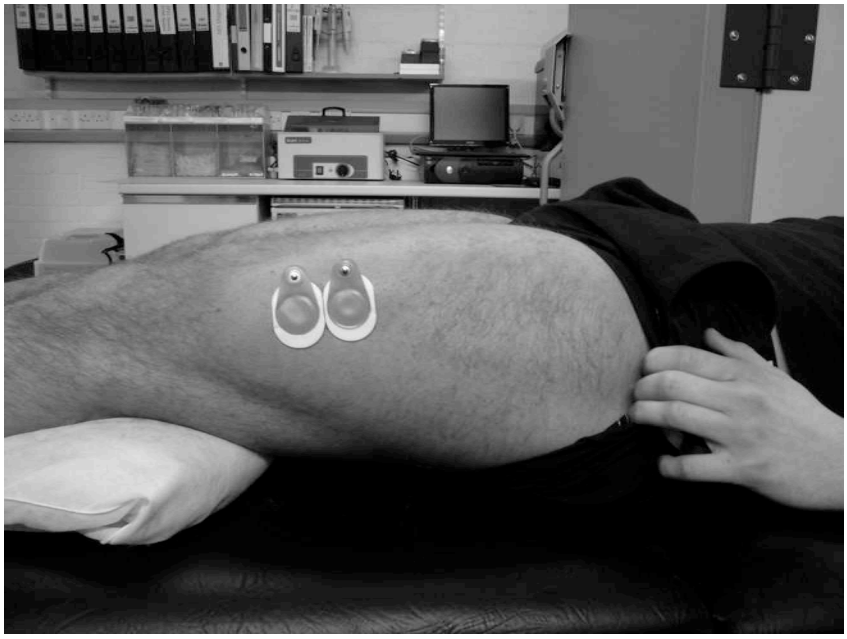

Figure 2.3. Positive and negative electrodes over the vastus lateralis.

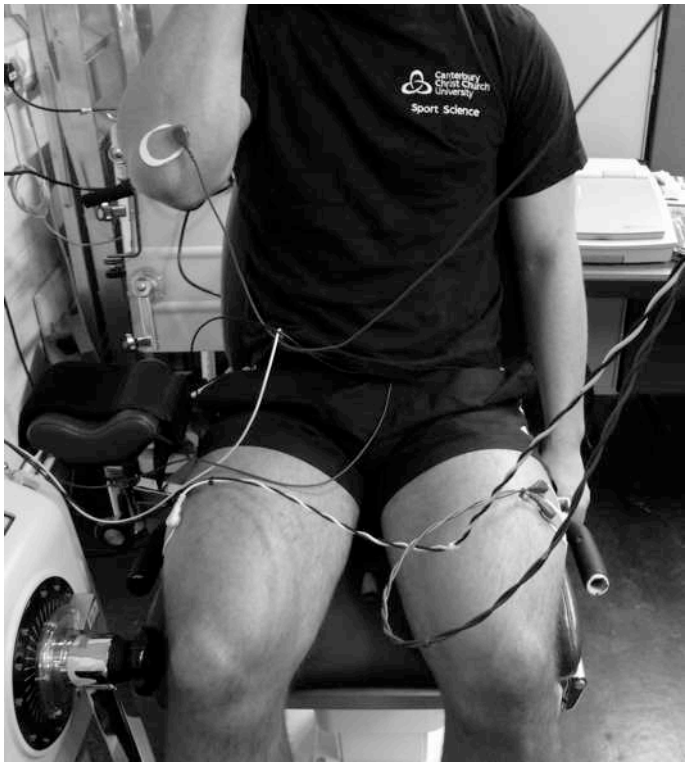

Figure 2.4. Electromyography earth electrode on the right olecranon.

EMG signal amplitude and frequency were recorded and analysed using Chart 6 software. Electromyography signal amplitude was calculated by subtracting the minimum signal voltage from the maximum signal voltage (maximum mV – minimum mV). For the purpose of signal amplitude analysis, all EMG recordings were segregated into 5 second blocks, so that each 2 minute bilateral leg isometric exercise repetition provided 24 EMG amplitude values. Similarly, EMG signal frequencies were also segregated into 5 second blocks, so that direct amplitude and frequency comparisons could be made for each exercise bout. Electromyography signal frequency was analysed by utilising an additional separate channel, set up for cyclic frequency measurement, for each vastus lateralis EMG reading. The two EMG amplitude and two EMG frequency channels (representing the left and right vastus lateralis') were averaged to provide a single amplitude and frequency value for each 5 second period.

2.6 Electrocardiography

Heart rate and HRV components were measured and recorded via electrocardiography (ECG) using a 16-channel chart recorder, PowerLab/SP16 with Chart 6 software (AD Instruments, Castle Hill, Australia). Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., Maryland, U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by AD Instruments was used. The electrodes were placed inferior to the right (earth) and left (negative) clavicle, midway between the conoid tubercle and the costal tuberosity, and over the tenth rib (positive) on the left side. Heart rate was continuously measured and recorded during all exercise tests. Heart rate variability components were only measured and recorded at every resting measures test session. Resting HR was ascertained from the HRV protocol.

Resting HR and HRV were measured and recorded after a standardised resting period of 15 minutes in a supine position in a silent, dimly lit room at an ambient temperature between 22 and 26°C. Previously it has been noted that in order for high frequency and low frequency components of HRV to be suitably distinguished in short-term investigations of CV regulation, breathing rate must be paced, in order to avoid indeterminate spectral components (Pinna *et al*. 2006). As such, breathing was paced at 12 breaths.min^{-1} (0.2 Hz) using a metronome during all resting HR and HRV protocols,

as suggested (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Depth of breathing could not be standardised, so participants were instructed to breath at a depth that was comfortable at the standardised breathing frequency.

A period of 60 seconds (the final 60 seconds of the 15 minute rest period) immediately prior to the start of HRV recording, the metronome was started to allow time for breathing synchronisation to occur. Breathing synchronisation was assessed by visually observing the movement of the participant's abdomen. Participants were not spoken to during the 15 minute rest period or the 5 minute recording period. Participants were instructed before the rest period about breathing synchronisation with the metronome, and to remain still and silent during the entire 20 minute combined protocol.

Heart rate variability was measured using the parametric frequency domain method (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996), whereby power spectral density analysis demonstrates how power distributes as a function of frequency. A signal for each QRS complex detected during ECG recording allows Chart software to identify heartbeats (and so resting HR) before any spectral analysis has taken place. An R wave detection threshold is set for each individual participant, by sampling online ECG immediately prior to the testing protocol (as shown in Figure 2.5). Heart rate variability data was divided into consecutive sections comprising 1024 data points, as recommended (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996), with a Fast Fourier Transform (FFT) algorithm being applied to each of the sections in turn. If complete divisions between 1024 data points could not be made, incomplete segments (< 1024 data points) were padded with zeros before the FFT algorithm was applied (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

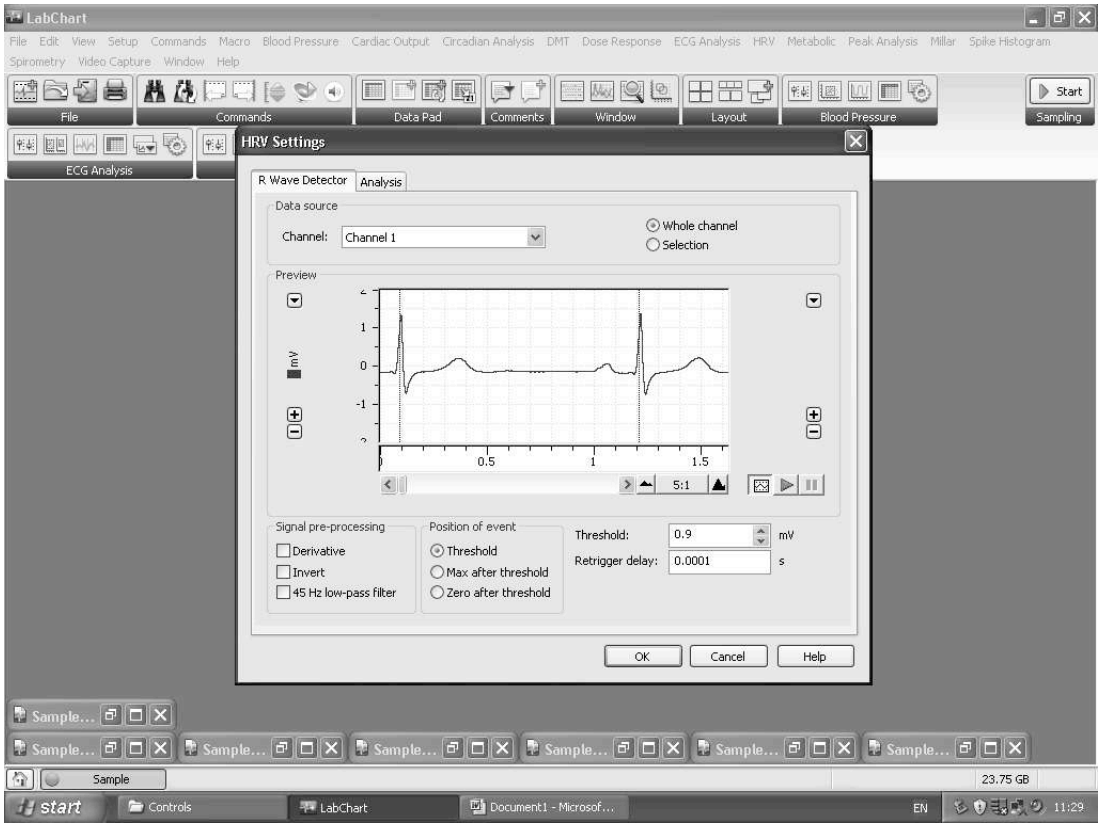

Figure 2.5. R wave detection threshold, with vertical lines indicating detection of QRS complex from current setting (0.9 mV in this example)

Ectopic heartbeats, described as abnormal heartbeats originating from a self-excitable site in the atria or ventricles, rather than the sinoatrial node, can produce erroneous HRV data. Ectopic heart beats result in an abnormal QRS complex, with the R-R interval usually extended in comparison to a sinoatrial node derived normal heartbeat. These lengthened R-R intervals subsequently affect the oscillations of both the interval between consecutive heartbeats, and between consecutive instantaneous heart rates, the basis of HRV analysis (Mateo and Laguna, 2003).

To limit the risk of ectopic beats affecting HRV analysis a 'manual' visual check of ECG traces was conducted offline after each of the 5 minute readings, together with Chart software's automatic linear interpolation check. A standard limit of less than 10% ectopic beats is said to be acceptable for HRV analysis from a 5 minute ECG recording (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). To further ensure that ectopic beats did not infiltrate the analysed ECG data, Chart software replaced any ectopic incidences with

the nearest normal R-R interval, ensuring continuity of ECG data and constant duration of ECG measurement. Ectopic beats are more frequently common in individuals with abnormal sinus rhythm, such as post myocardial infarction patients. The participants used within this thesis were young healthy adults, and ectopic instigated errors were not evident in any participant sample from any of the proceeding studies.

A sample rate of 1000 Hz was used (also used for exercise HR measurement), as recommended to reduce the risk of signal morphology, whereby signal noise in erroneously interpreted and included within the HRV spectrum (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Chart 6 software uses a FFT algorithm to convert time domain data into spectral frequency domains, which is the most commonly used mathematical method for analysing spectral HRV. There is an often-stated criticism of FFT, in that the mathematical algorithm assumes that the physiological mechanisms controlling HRV within any frequency band remain constant throughout the ECG measurement period. This can be a fundamental issue if analysing long term (24 hour) HRV data, as is often done, due to circadian rhythms having been evident in HR modulation in extended HRV analysis (Malliani, 2005). However, the use of short-term 5 minute ECG recordings within this thesis should limit the effect of any circadian rhythms (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

The spectral frequency bands for HRV analysis were pre-selected and standardised for all participants, following universal guidelines. The bands used were < 0.04 Hz for very low frequency (VLF), 0.04 – 0.15 Hz for low frequency (LF), and 0.15 – 0.4 Hz for high frequency (HF) (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Figure 2.6 provides an illustration of typical HRV spectra following a 5 minute ECG recording. Very low frequency component was not analysed due to the 5 minute recording time period, as this has been shown to provide erroneous results, as it is affected by the baseline and trend removal algorithms used, resulting in a non-harmonic component with incoherent properties (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

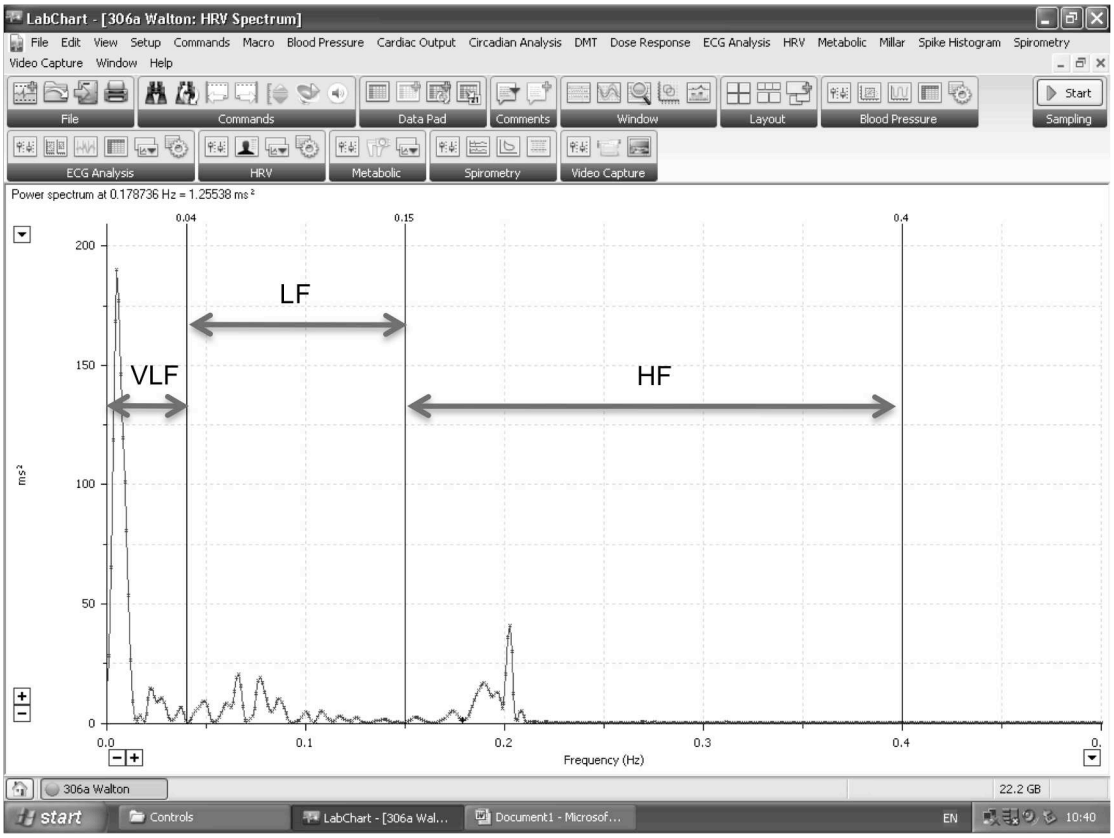

Figure 2.6. Typical heart rate variability spectra from a 5 minute resting electrocardiogram reading, showing very low (VLF), low (LF), and high (HF) frequency bands. Each spectral component power is calculated as the area under the curve within the respective spectrum band. VLF data is discarded

Both absolute and relative HRV parameters are offered by ECG analysis. Absolute parameters include total power (TP), LF (0.04 – 0.15 Hz), and HF (0.15 – 0.4 Hz) components. Total power represents the variance of all R-R intervals over the 5 minute recorded ECG sequence. Low frequency and HF represent the power in each of their respective frequencies during the 5 minute ECG recording. All absolute HRV parameters are expressed in units of ms^2. Relative parameters of HRV include LF/HF ratio, and normalised units of LF and HF power (LFnu and HFnu respectively).

The LF/HF ratio is calculated using the following formula:

$$LF/HF = \text{Ratio of LF } (ms^2) / \text{HF } (ms^2)$$

The LFnu and HFnu are calculated once the VLF power is subtracted from the TP of the ECG recording, and units are expressed as normalised units (nu):

LFnu = LF / (TP – VLF) * 100

HFnu = HF / (TP – VLF) * 100

It is these relative HRV parameters that are said to represent the balance of CV control by the sympathetic and parasympathetic branches of the autonomic nervous system (Malliani, 1999). The use of calculated normalised units supposedly further reduces signal noise and minimises the effect of changes in TP on the LF and HF components. They have also been purported to be more practicable when evaluating the effect of an intervention on participants, especially when large differences in TP are evident (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996; Malliani, 1999).

The main spectral components that were considered within this thesis were the relative components HFnu, LFnu, and LF/HF ratio, in order to gain an insight into autonomic nervous balance and its role in BP and CV regulation and manipulation. However, in accordance with recommendations outlined by Task Force (1996), absolute values of TP, LF, and HF power are presented to describe in total, the distribution of power in spectral components. Resting HR is also provided by the 5 minute ECG recording. See pages 46-49 for HR and HRV variable measurement reliability data.

2.7 Blood pressure recording

Resting BP (SBP, DBP, and MAP) measurements were made using an automated BP monitor (Dinamap Pro 300 Critikon, GEMedical Systems, Slough, Berks, UK). This device is operated by the use of an oscillometric technique, and a pneumatic cuff placed around the participant's upper left arm, approximately 1.5 cm above the antecubital fossa, roughly level with the participant's heart. Participants' arm circumferences were measured to ensure the appropriate sized cuff was used for measurement. Blood pressure was measured over the left brachial artery after 15 minutes rest in a seated position with the back and left arm supported, and with feet placed flat on the floor with legs uncrossed. The lowest of three measures (for each SBP, DBP, and MAP) was used for

analysis, as done so by Wiles *et al*., (2010), since it has been previously stated that initial measurements are often higher than subsequent measures, and so are not reflective of true resting arterial pressures (Katzel *et al*. 1995). Measurements were separated by a period of 60 seconds, in accordance with previous recommendations and studies (Pickering *et al*. 2005; Wiles *et al*. 2010).

A microprocessor controls the sequence of cuff inflation and deflation. The inflation-measurement cycle took approximately 20 seconds. The oscillometric technique measures the changes in pressure waves in the brachial artery that are exerted on the pneumatic cuff. The cuff is initially inflated to a suprasystolic pressure, and is then deflated progressively until oscillation amplitudes cease to exist below DBP. A transducer senses minute pressure oscillations within the cuff and the changes are processed by the microprocessor. Systolic blood pressure is detected where oscillation amplitudes increase most rapidly, resulting from the point where blood begins to pass down the previously occluded brachial artery. Diastolic blood pressure, conversely, is detected where oscillation amplitudes decrease most rapidly. Mean arterial pressure is identified as the lowest cuff pressure at which the greatest average oscillation occurs (Sherwood and Carels, 2000).

The device used has been assessed for accuracy and reliability of measurement. The Dinamap has been validated in comparison to invasive central aorta catheter measurement, and has met the American National Standards Institute/Association for the Advancement of Medical Instrumentation SP10 1992 requirements for accuracy (Baker, 1986). It has though been found to overestimate DBP compared with mercury sphygmomanometer readings (Beaubien *et al*. 2002). However, the device appears to have similar inaccuracies to other semi-automated devices (Lewis *et al*. 2002) and was not used for the diagnosis of hypertension. It has previously been suggested that the use of a semi-automatic device eliminates investigator bias, where readings taken using the manual auscultatory method could be rounded up or down, depending on what the observer expected to measure (Coe and Houghton, 2002). Any discrepancies in comparison to alternative measurement techniques should not be prohibitive to the proceeding studies of this thesis, as long as readings are consistent across the population sample.

As many other studies investigating the validity and reliability of the Dinamap device have focused on elderly and/or hypertensive patients, a test of inter-day reproducibility using the Dinamap Pro 300 on young, healthy male adults was conducted for the purpose of the proceeding studies. See pages 46-49 for SBP, DBP, and MAP variable measurement reliability data.

2.8 Baroreflex sensitivity

Baroreflex sensitivity (BRS) was measured non-invasively by correlating BP variance with HR variance, using a Finometer (Finapres, TNO Instruments, Amsterdam, The Netherlands). Baroreflex sensitivity is calculated accurately by the Finometer (Gizdulich *et al*. 1996), by the regression slope of the beat-to-beat and inter-beat variables. This is therefore a time-domain analysis of spontaneous BRS. Specifically, it is the SBP waveform and the interbeat interval time series that are cross-correlated and interpolated, and then resampled at 1Hz. The correlation and regression slopes between SBP and interbeat intervals are computed every 10 seconds. The delay in interbeat interval with the highest positive coefficient of correlation is then selected. Assuming that the coefficient of correlation is significant ($p < 0.01$), the slope between SBP and interbeat interval is then recorded as a BRS estimate, and incorporated into a geometric mean value.

In order for phase shift calculations of BRS within Finometer software to be made, three consecutive R-R intervals in the same direction are necessary. As BRS varies with time, a recording of ten minutes is advised (Chesterton *et al*. 2005), so was administered in all proceeding studies. The Finometer measures finger BP noninvasively and gives waveform measurements similar to intra-arterial recordings. The Finometer measures brachial pressure and corrects for finger pressure accordingly, whilst correcting for the hydrostatic height of the finger with respect to the level of the heart. Heart rate is measured at the same finger cuff, from pulsations detected at the digital artery (Gizdulich *et al*. 1996). Blood pressure was recorded from the left arm and middle (long) finger of the left hand using appropriate sized cuffs according to finger circumference. All measurements were taken from the same arm and finger for consistency.

The Finometer uses a fast servo system to apply pressure changes to the finger cuff, in order that pulsations in cuff pressure are in precise opposition to intra-arterial pressure at the digital artery. This dynamic unloading process ensures that the artery remains at a constant cross-sectional diameter, set at a point below the maximally elastically expanded diameter (Wesseling *et al*. 1995). The finger cuff comprised of a 50 μM thin plastic bladder, inflated by the means of a short air hose connected to a microprocessor block strapped to the participants' wrist. The microprocessor block also contains preamplifiers, an air valve, and a manometer, in order that pressure changes necessary to maintain digital artery diameter are detected and then applied.

To constantly monitor digital artery diameter the finger cuff uses infrared transmission plethysmography, as it houses a light source of LED infrared, emitting at a wavelength of 950 nM, and an infrared sensing photodiode photocell. It is the fluid blood tissue itself inside the artery that is predominantly monitored, as blood absorbs the infrared light significantly more than other surrounding tissues (Wesseling *et al*. 1995). The Finometer's microprocessors control artery diameter using a differential amplifier to constantly compare the current from the infrared transmission plethysmograph, to a fixed level that represents the desired maintained artery size. As well as monitoring and amplifying this current difference, cuff pressure is also constantly monitored, and together the amplified signal difference plethysmogram is fed to a gain and frequency response adaptive servo controller, in order that rapid changes may be made. This constant evaluation of pressure differences utilizes the volume clamp theory of Peñàz (1969; 1973; 1976; 1992), whereby:

$$P_T = P_A - P_C$$

Where:

P_T = Transmural pressure (difference between intra-arterial and cuff pressure)

P_A = Intra-arterial pressure

P_C = Read cuff pressure

Therefore, it is proposed that when transmural pressure (P_T) equals zero, the applied cuff pressure must equal intra-arterial pressure itself and thus represent prevailing BP levels

(after recognition of the hydrostatic height sensor for brachial and digital artery height discrepancy).

For all resting BRS measurements the participant was in a seated position with the midpoint of the upper left arm approximately in line with the fourth intercostal space. Resting BRS measurements were preceded by a standard period of rest of 15 minutes in the same seated position as subsequent measurements were taken, in a seated position with the back and left arm supported, and with feet placed flat on the floor with legs uncrossed. Figures 2.7 and 2.8 show the participant position and cuff placements for all resting BRS tests. The geometric mean BRS value (ms/mmHg) was used for analysis. See pages 46-49 for BRS variable measurement reliability data.

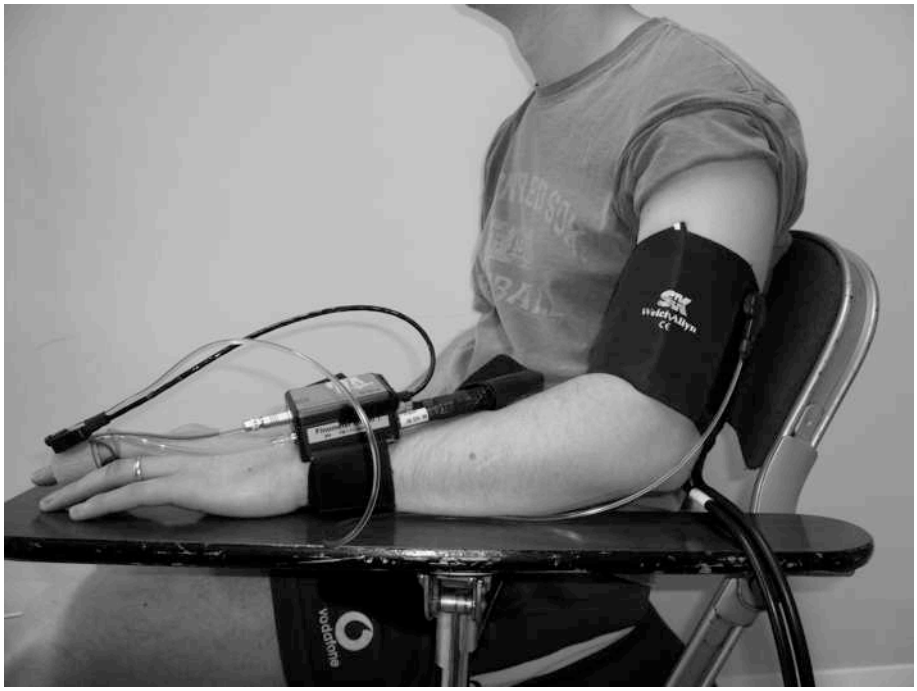

Figure 2.7. Upper arm cuff, microprocessor wrist block, finger cuff, and hydrostatic height sensor arrangement

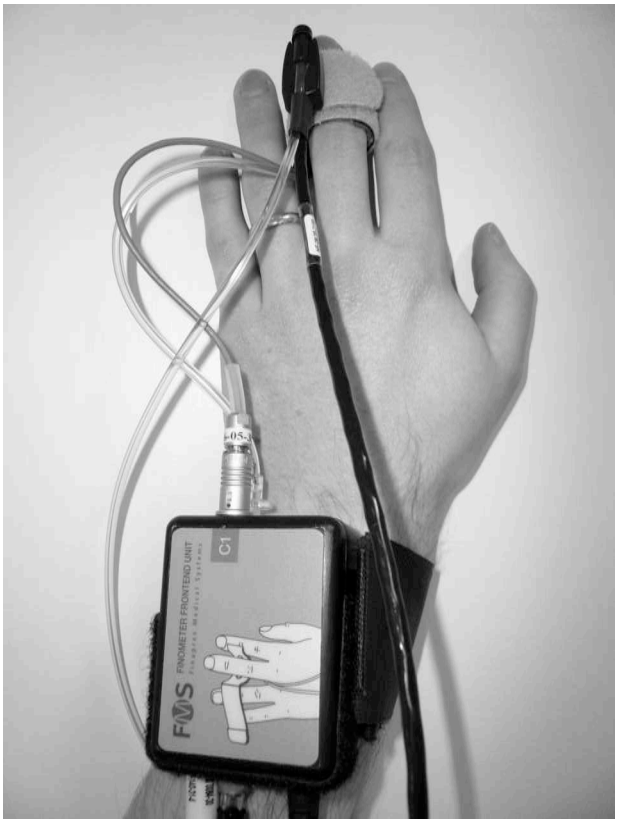

Figure 2.8. Finger cuff and short air hose connected to the microprocessor block. The hydrostatic height sensor can be seen attached to the finger cuff

2.9 Cardiac output and stroke volume

The Innocor non-invasive cardiac output (Q) monitor (Innovision A/S, Odense S, Denmark) was used to calculate stroke volume (SV) and Q at rest via inert gas re-breathing. Innocor calculates cardiopulmonary parameters based on the single alveolar lung model. The O_2 enriched gas mixed used, as supplied by Innovision (A/S, Odense, Denmark), was comprised of a blood soluble gas (N_2O, at 0.5% concentration) and a blood insoluble gas (SF_6 at 0.1% concentration). Calculations made by this system assume that there is complete and instantaneous mixing of all gases. The relative levels of the two inhaled inert gases of differing solubility in blood are measured, over four to five respirations. The calculation system also assumes that there is instantaneous equilibration of the soluble gas between the alveoli and blood, and between alveoli and tissue respectively. N_2O concentration decreases with every rebreathing manoeuvre, at a rate proportional to pulmonary blood flow. The calculation system assumes that pulmonary blood flow is constant, as well as assuming constant lung tissue volume.

Cardiac output is then calculated as the rate of N_2O uptake into the blood over time. This calculation is performed using the slope of the regression line through the logarithmically transformed end-expiratory N_2O concentrations plotted against time (once corrections have been made for lung volume using end-expiratory SF_6 concentrations, normalising pulmonary blood flow to lung size). The Innocor then calculates pulmonary blood flow. Figure 2.9 presents a typical logarithmic plot for soluble gas during the rebreathing protocol, for the calculation of pulmonary blood flow.

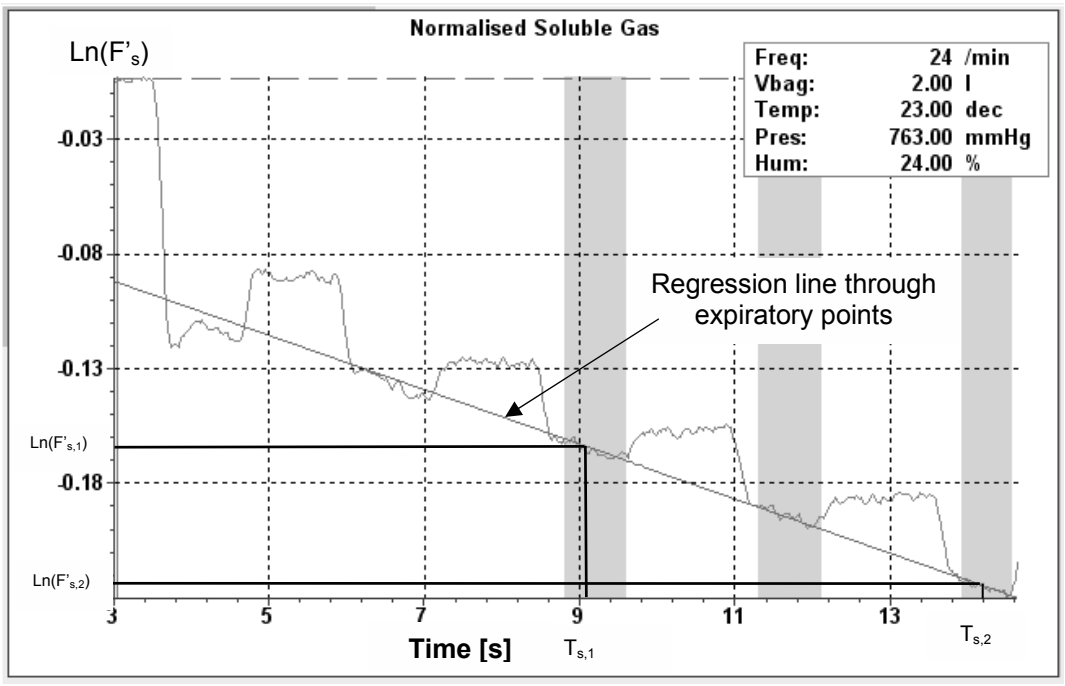

Figure 2.9. Logarithmic plot for soluble gas (N_2O) concentration during the rebreathing method with regression line through expiratory points

The slope of the regression line as depicted in Figure 2.9 is calculated using the following formula:

$$\ln (F'_S(t)) = \ln (F_S(t) * F_i^0 / F_i(t) * F_S^0)$$

Where:

$F'_S(t)$ = normalised fractional concentrations of soluble gas

$F_S(t)$ = fractional soluble gas concentration as a function of time

F_i^0 = initial fractional insoluble gas concentration in the rebreathing bag

$F_i(t)$ = fractional insoluble gas concentration as a function of time

F_S^0 = initial fractional concentration of soluble gas in the rebreathing bag

To calculate Q ($l.min^{-1}$) the Innocor utilises Fick's Principle (1870) of mass conservation and applies it to the pulmonary circulation, through online measurement of the gas uptake together with arterial and mixed venous oxyhaemoglobin saturation. This method has been previously validated (Davies *et al*. 1987; Doi *et al*. 1990; Keinanen *et al*. 1992). The process comprises of three formulae:

(1) Based on simple mass conservation:

$$Q = PBF + Q'_S$$

Where:

PBF = pulmonary blood flow

Q'_S = shunt flow ($l.min^{-1}$)

(2) From Fick's Principle:

$$Q = VO_2 / (C_AO_2 - C_VO_2)$$

Where:

VO_2 = oxygen uptake ($l.min^{-1}$)

C_AO_2 = oxygen content in arterial blood

$C_AO_2 = 0.000139 \cdot Hb \cdot \%S_PO_2$

$\%S_PO_2$ = arterial oxygen saturation

Hb = haemoglobin concentration (g/dl)

C_VO_2 = oxygen content in mixed venous blood

(3) Fick's Principle applied to the pulmonary circulation:

$$PBF = VO_2 / (C_CO_2 - C_VO_2)$$

Where:

C_CO_2 = oxygen content in end capillary blood

$C_CO_2 = 0.000139 \cdot Hb \cdot \%S_CO_2$

$\%S_CO_2$ = end capillary oxygen saturation (98% assumed)

Subsequently, Q can then be calculated, as the three previous formulae contain three unknown parameters (Q, Q'S, and C_vO_2), and so Q is calculated using:

$$Q\ (l.min^{-1}) = 1 / ((1 / PBF) + (C_AO_2 - C_CO_2) / VO_2)) \qquad \text{(Innovision A/S, 2008)}$$

In the absence of significant intrapulmonary shunt, pulmonary blood flow is equal to Q. Where arterial oxygen saturation ($\%S_PO_2$) is aforementioned in Q formulae; this is determined by incorporated pulse oximetry, measured by infrared absorption differences between oxygenated and reduced haemoglobin in the blood. Figure 2.10 provides a schematic illustration of measured/calculated parameters in the cardiopulmonary system.

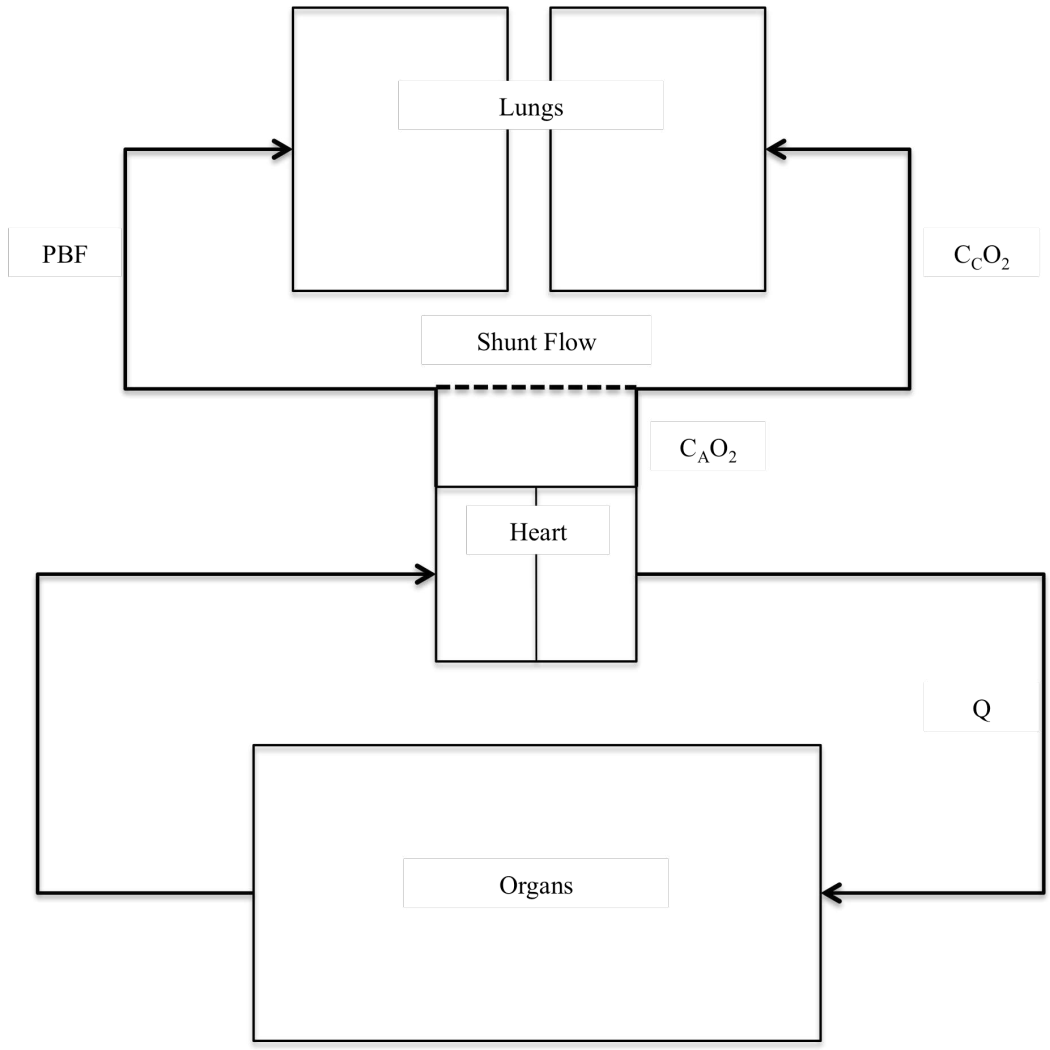

Figure 2.10. Cardiac output (Q) defined as the output in litres of the left ventricle per minute ($l.min^{-1}$)

The Innocor houses a three-way respiratory valve connected to a photoacoustic gas analyser. A mouthpiece, nose clip and an anti-static rebreathing bag create a closed loop system, allowing the participant to respire the gas mixture exclusively, with no atmospheric air contaminating the analysed mixture. Heart rate was measured during tests using the incorporated pulse oximeter. The average value of two Q and SV measures were used for analysis. Measures were separated by a period of 5 minutes to ensure removal of previously inhaled gases, as calculations presume negligible mixed venous concentration of soluble gas throughout the rebreathing period. Participants sat in an upright position to prevent any interference with normal breathing (Damgaard and Norsk, 2005). Ideal breathing rate and depth during Q calculation is displayed to participants on the Innocor display screen during the rebreathing protocol. See pages 46-

49 for Q and SV variable measurement reliability data. Figure 2.11 illustrates the participant position and Innocor/participant interface.

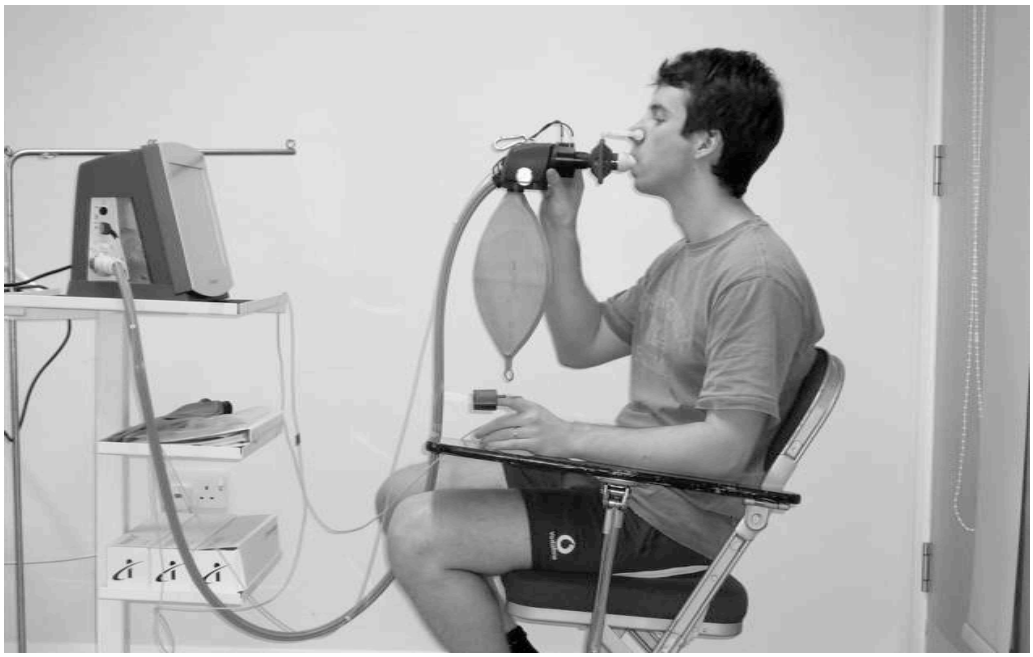

Figure 2.11. Resting cardiac output (Q) and stroke volume (SV) measurement with the Innocor device

2.10 Total peripheral resistance

Total peripheral resistnace was calculated using MAP from Dinamap BP readings, and Q from the Innocor device: TPR = MAP/Q (Walker *et al*. 1992; Turner *et al*. 1996). See pages 46-49 for TPR variable measurement reliability data.

2.11 Blood lactate concentration

Blood lactate concentrations were determined using a Biosen 5030 lactate analyzer (EKF Diagnostic, Barleben/Magdeburg). The Biosen 5030 lactate analyzer has been shown to provide quick reliable lactate concentration measures over the full range of typically anticipated values up to and including severe exercise levels (Davison *et al*.

2000). The Biosen 5030 lactate analyzer uses the enzymatic and amperometric measurement principles to provide lactate concentration values. This principle of measurement involves enzymatic reactions, in which the analyte (in this case lactate) must participate in an enzymatic reaction to produce a substrate detectable by amperometry (detection of electron-transfer process). The amperometric signal is attained by measuring the current generated by electron exchange (changes in oxidation state at an electrode) in an electrochemical cell. The concentration level of a substrate is proportional to the electron flux caused by the electrochemical transformation (Belluzo *et al*. 2008). Figure 2.12 illustrates the process of an amperometric biosensor.

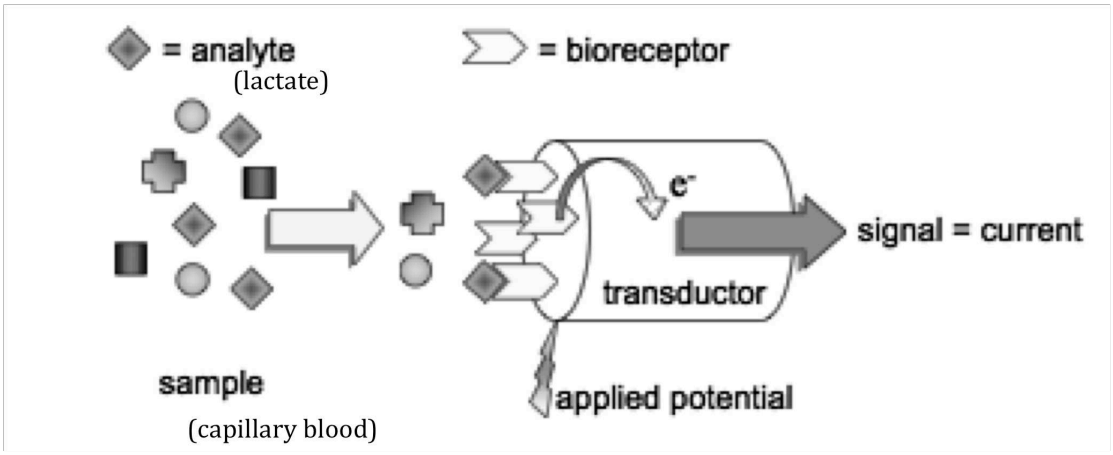

Figure 2.12. The functional principles of an amperometric biosensor, as used in the Biosen 5030 lactate analyzer (adapted from Belluzo *et al*. 2008, p.1367)

The measurement technique uses redox enzymes, which catalyse the oxidation of lactate. The redox enzyme involved in lactate concentration analysis is lactate oxidase, which allows for the detection of the amount of oxidation of lactate in the blood sample. This is achieved by the conversion of lactate by catalysis into two substrates in the presence of the lactate oxidase enzyme. The production rate of these substrates is electrochemically determined and is used as an indirect indicator of the concentration level of lactate in the analysed blood sample (Belluzo *et al*. 2008).

The amperometric bioelectrode (as depicted in Figure 2.12) is driven at a constant potential, in the presence of a reagent solution containing lactate oxidase. The current then varies depending on the amount of the detected substrate present after the catalysis

of lactate in the blood sample. It is this current change that is registered and indirectly calculated to determine the blood lactate concentration of the sample. The bioreceptors present in the Biosen lactate cell membrane (EKF Diagnostic) must maintain stable biorecognition activity to produce reliable concentration results, and so recommended lifetime cycles of membranes were followed according to the manufacturers recommendations. The use of this methodology for indirect lactate concentration determination has been previously validated (Durliat *et al*. 1990; Aduen *et al*. 1994; Kost *et al*. 2000).

Capillary blood samples for the determination of lactate concentration during the discontinuous incremental isometric exercise test were collected from either the left thumb, left index finger, or left middle (long) finger (Zavorsky *et al*. 2005). A 20 μl capillary blood sample was immediately mixed with 200 μl of reagent solution in a safe-lock vial. Before analysis the Biosen 5030 was calibrated using a standard EKF Diagnostic 12.0 $mmol.L^{-1}$ solution, which was repeated when requested by the analyzer. All samples were analysed immediately after the completion of each discontinuous incremental isometric exercise test. Skin punctures were made using a lancet. The skin on the aforementioned fingers and thumb were prepared by alcohol wipe and allowed to air-dry prior to the start of the discontinuous incremental exercise test.

2.12 Reproducibility of measured cardiovascular variables, minimum detectable change, and sample size power calculations

Sixteen healthy normotensive males (mean age 22.8 ± 3.1 years; body mass 85.1 ± 11.6 kg; height 1.79 ± 0.1 m) volunteered to participate in an inter-day reproducibility study, visiting the laboratory on three separate occasions. Participants underwent SBP, DBP, MAP, HRV, BRS, and then Q and SV measurements as previously described in this chapter. Total peripheral resistance was calculated using MAP and Q measurements as previously described. The initial tests were followed by tests at 7 and 28 days later, to replicate future planned intervention study protocols. Each test was performed at the same time of day for each visit. All participants were moderately physically active (5.9 hours at 7.5 multiple of basal metabolic rate, BMR, per week).

Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed an exercise readiness questionnaire, and provided written informed consent, thereby adhering to the guidelines set by the 1964 Declaration of Helsinki. All participants were non-smokers and were not taking any medication. Participants fasted for 4 hours, and abstained from caffeine and alcohol for at least 12 hours prior to testing procedures (Jáuregui-Renaud *et al*. 2001) and were instructed to maintain dietary and physical activity behaviours prior to each visit. All participants completed identical familiarization sessions prior to the first of the three analysed visits.

The coefficient of variation (C of V %), 95% confidence intervals for the difference in means, and intra-class correlation coefficient (ICC r) were calculated between trials A (1-2) and B (2-3). Data was assessed for conformity with parametric assumptions (Field, 2000). A single C of V % was derived by log-transformed two-way analyses of variance, ANOVA (Atkinson and Neville, 2001). Optimal confidence intervals were ascertained for a normal distribution using the methods of Tate and Klett (1959).

Table 2.1 illustrates the measured CV variable reliability results. Also included are calculations for the minimum detectable change (MDC). Minimum detectable change was calculated at the 95% confidence level ($MDC_{95\%}$), using the unbiased estimate of standard error of measurement (SEM) in the following equation:

$$MDC_{95\%} = 1.96 \times \sqrt{2} \times SEM \qquad \text{(Schmitt and Di Fabio, 2004)}$$

As BP was the focus of this thesis, SBP, DBP, and MAP were considered to be the dependent variables, and so participant sample size decisions were based on the BP component with the smallest minimum detectable change at the 95% confidence level (Schoenfeld, 2001). For a study with a power of 0.8 (80%) at the 0.05 significance (5%) level, a sample size of eight participants was required in a crossover design study (Chapter 3 part B, Chapter 4 and Chapter 5 part A and part B), given the $MDC_{95\%}$ of DBP at 3.17 (see Table 2.1). Absolute HRV measures are not included due to their recognised descriptive nature (see pages 46-49), and are included in results of the proceeding studies as a supplement to relative HRV measures.

It can be seen in Table 2.1 that some CV measures present very high C of V %. Considerable day-to-day variations in HRV indices using healthy participants are commonly reported (Van Hoogenhuyze *et al*. 1991; Sandercock *et al*. 2004). Coefficients of variance for HRV measures have been reported to vary between <1% and >100%, so given the heterogeneous nature of the measure, it is recommended not to suggest homogeneity with participant populations not directly measured in a study (Sandercock *et al*. 2004; Sandercock *et al*. 2005). The same high levels of test-retest variance can also be seen in the BRS data. Therefore, it must be recognised that using the available CV measures, the high C of V % and 95% confidence intervals increase the likelihood of not detecting significant results that may aid the understanding of physiological change in association with BP reductions following IET.

Table 2.1. Cardiovascular variable reliability results

	HR	LF/HF	LFnu	HFnu	BRS
C of V (%)	8.67	86.08	46.17	31.36	21.80
95% CI for Δmean	6.53 – 11.29	64.81 – 112.13	34.76 – 60.14	23.61 – 40.85	16.41 – 28.40
ICC (r)	0.68	0.61	0.66	0.65	0.68
$MDC_{95\%}$	4.19	0.62	14.30	14.57	2.90
	SBP	DBP	MAP	Q	SV
C of V (%)	1.84	2.60	2.09	6.64	11.11
95% CI for Δmean	1.39 – 2.40	1.97 – 3.39	1.57 – 2.72	5.00 – 8.65	8.36 – 14.47
ICC (r)	0.97	0.86	0.88	0.89	0.84
$MDC_{95\%}$	5.74	3.17	3.89	0.86	17.81

Establishment of a discontinuous incremental isometric exercise protocol

2.13 Introduction

Previously, Wiles *et al*. (2007) described the need to be able to precisely control the intensity of isometric exercise. Traditionally, isometric exercise training studies have prescribed exercise intensity based on the principle of constant force (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Millar *et al*. 2007). This is defined as a percentage of the maximum force an individual can produce isometrically, referred to as a percentage of maximal voluntary contraction (% MVC). The issue regarding the prescription of isometric exercise intensity using the constant force principle is that it results in a variable CV state during exercise, as HR will increase with time (Seals, 1993; Smolander *et al*. 1998; Galvez *et al*. 2000).

It has been argued that the method of training intensities based on HR, commonly used in other modes of exercise (Karvonen and Vuorimaa, 1988) affords an indication of the pressor response, and optimizes any subsequent training adaptation (Jeukendrup and Van Diemen, 1998). Furthermore, the prescription of isometric exercise intensity based on the principle of constant force production has not been identified as being an important factor in eliciting either CV adaptation, or BP reduction specifically. Constant force production techniques have only ever been specifically associated with improvements in maximal force production after a period of training (Lind, 1970).

The work conducted by Wiles *et al*. (2007) reported that during a continuous bilateral-leg incremental isometric exercise test, HR displayed a linear relationship with EMG activity. This led the authors to propose that isometric exercise training intensities may be prescribed using an individual EMG activity value for each participant, that would correspond to a precise HR, and so control the CV stimulus more precisely during training periods. The protocol created by Wiles *et al*. (2007) involved up to 10 minutes of continuous isometric exercise at increasing intensity up to the point of fatigue. This can potentially involve severe discomfort for participants, and may not be suitable if this protocol were to be used for hypertensive and/or elderly participants.

Performing a discontinuous protocol instead can alleviate this, which is commonly used in dynamic modes of exercise (Gilman and Wells, 1993; Karvonen and Vuorimaa, 1988; Swain *et al*. 2004). Another drawback of using a continuous incremental protocol is that blood lactate concentration measurements are unattainable, unless an indwelling catheter is used, due to the occluded vascular beds of the active muscles preventing clearance and then systemic circulation of accumulated metabolites. Measurement of blood lactate concentration will allow for the analysis of lactate metabolism during isometric exercise at different intensities, and may offer an insight into chemoreceptor stimulation and CV response to that stimulation 'during' isometric exercise.

Therefore, the aim of the present study was to assess the linearity of the relationship between EMG and HR, during a discontinuous bilateral-leg isometric exercise test, and to compare this relationship using repeated tests for the purpose of establishing inter-day test re-test reliability. The present study would also assess the lactate response time-course during rest periods between incremental intervals, and to establish the reliability of that response. This study also intended to assess the HR steady state response time during a discontinuous protocol, to confirm the 60 second 'steady state' finding by Wiles *et al*. (2007) when using a continuous protocol.

2.14 Methods

Seventeen healthy normotensive males (mean age 21.5 ± 2.5 years; body mass 78.1 ± 16.4 kg; height 178.0 ± 4.9 cm) volunteered to participate in a repeated measures design study. All participants were moderately physically active (8.0 hours at 7.4 multiple of BMR, per week). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed an exercise readiness questionnaire, and provided written informed consent, thereby adhering to the guidelines set by the 1964 Declaration of Helsinki. All participants were non-smokers and were not taking any medication. Participants fasted for 4 hours and abstained from caffeine and alcohol for at least 12 hours prior to testing procedures (Jáuregui-Renaud *et al*. 2001), and were instructed to maintain physical and dietary activity behaviours prior to each visit. All participants completed identical familiarization sessions. Time between repeated discontinuous incremental test conditions was separated by at least 7 days.

Determination of the maximal voluntary contraction and EMG_{peak}

Maximal voluntary contraction (MVC) and EMG_{peak} were determined prior to the discontinuous incremental test. Participants performed three maximal effort contractions against the immoveable leg brace. Each MVC was terminated after 2 seconds, and each was interspersed by a 120 second rest period. The isometric leg extension exercise was performed at a knee angle of 90 degrees (180 degrees corresponds to full knee extension) on the isokinetic dynamometer (Alkner *et al*. 2000). Participants were instructed to avoid using their upper body musculature to assist in generating force during isometric bilateral-leg exercise in order to standardize the level of stabilization and to avoid this affecting force outputs (Magnusson *et al*. 1993; Mendler 1976).

EMG_{peak} was determined from the MVC attempt producing the highest torque. EMG_{peak} was established from the mean of the EMG activity recorded 0.25 seconds immediately prior to peak torque, as used by Wiles *et al*. (2010). The EMG_{peak} value was then used to create $\%EMG_{peak}$ 'targets' for the subsequent incremental exercise test.

Discontinuous incremental isometric exercise test

Participants began bilateral-leg isometric exercise at 10% EMG_{peak} for a period of 2 minutes. Thereafter, the intensity increased in 5% increments, interspersed by 5 minute rest periods, up to volitional fatigue (or failure to maintain EMG signal within +/- 5% of the 'target' value). Electromyography was continuously monitored and recorded. Heart rate was continuously recorded. Lactate samples were taken at the point of cessation of exercise, and then at 60 second intervals during each 5 minute rest period, up to the start of the next exercise increment. Peak lactate for each 5 minute rest period was determined using non-linear regression curve fitting analysis on GraphPad Prism software (GraphPad Software Inc., La Jolla, USA).

Equipment

Isometric exercise: All tests were conducted using a Biodex System 3 Pro, isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY), which is described in detail in section 2.4. The Biodex was fitted with a modified hip attachment that was inserted into the standard knee attachment, to allow for bilateral-leg extension exercise to be performed.

Participants sat in the dynamometer in an upright position, with 90 degrees of flexion at the hip and with the thighs supported.

Electromyography recording: A dual bio-amplifier was used to enable surface EMG measurement from both vastus lateralis', to give a combined average, along with the 16-channel chart recorder. This is described in detail in section 2.5.

Exercising heart rate: Was continuously recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., Maryland, U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments (NSW, Australia) was used. Heart rate was sampled at a frequency of 1000 Hz.

Blood lactate concentration: Lactate samples were taken from the left thumb at 1 minute intervals during each 5 minute rest period reached before volitional fatigue. Each 20 μl capillary sample was immediately mixed with 200 μl of reagent solution for subsequent lactate determination. Lactate was determined using a Biosen 5030 lactate analyzer (EKF Diagnostic, Barleben/Magdeburg). A total of 6 samples were collected for each rest period, which were then analysed using non-linear curve fitting (Microsoft Prism software) to determine peak lactate (LA_{peak}) for each rest period of the discontinuous test. After all the calculated LA_{peak} values had been determined for each rest period, a second non-linear curve fitting procedure was conducted to assume the whole test lactate response curve. Electromyography activity was then calculated at 2, 3, and 4 $mmol.L^{-1}$ to assess lactate metabolism at specific neuromuscular activity states.

Data analyses: All data were assessed for conformity with parametric assumptions (Field, 2000). A paired t-test was used to compare the repeated maximal voluntary contractions of sessions 1 and 2. Pearson's product-moment correlation coefficient was used to explore the relationship between EMG and HR. Comparisons of these relationships between sessions 1 and 2 were conducted using analysis of covariance (ANCOVA) by Microsoft Prism software, whereby the two linear regression lines (slopes and intercepts) were compared. Within-participant variation expressed as a coefficient of variation was derived by log-transformed

two-way analysis of variance 'ANOVA' (Atkinson and Nevill, 2001), together with the confidence intervals for a normal distribution (Tate and Klett, 1959). Repeated measures ANOVA was used to assess for differences in each 30 second HR period during the discontinuous increments, and for differences in lactate concentrations at the different EMG activity states between the repeated tests.

2.15 Results

Mean MVC for the 17 participants was 500.7 ± 119.4 N·m and 495.3 ± 137.3 N·m, for the first and second tests respectively, which represented no significant change between the two MVC trials ($p > 0.05$).

The differences between successive 30 second HR intervals were: 1.6 $b.min^{-1}$ (± 1.8) for 30 – 60 s (90.6 vs. 92.2 $b.min^{-1}$, $p = 0.01$), 1.7 $b.min^{-1}$ (± 1.8) for 60 – 90 s (92.2 vs. 93.9 $b.min^{-1}$, $p = 0.01$), and 0.3 $b.min^{-1}$ (± 1.5) for 90 – 120 s (93.9 vs. 94.2 $b.min^{-1}$, $p > 0.05$). Therefore, the final 60 seconds of each increment (mean of 60 – 90 s and 90 – 120 s values) were averaged and used in subsequent analyses.

The relationship between EMG and HR was linear for all participants (r-values = 0.93 – 1.00; $p < 0.05$ in all cases) during all discontinuous incremental tests (test 1 versus test 2). Comparison of whole group test 1 and test 2 revealed no significant differences in the slope ($p = 0.42$) or elevation/intercept ($p = 0.68$) of the regression lines. The r-values were 0.63 [$p < 0.0001$; SEE(95%) = 5.38%] and 0.70 [$p < 0.0001$; SEE(95%) = 3.44%] for test 1 and test 2 respectively. Figure 2.13 illustrates the group mean linear relationships between EMG and HR.

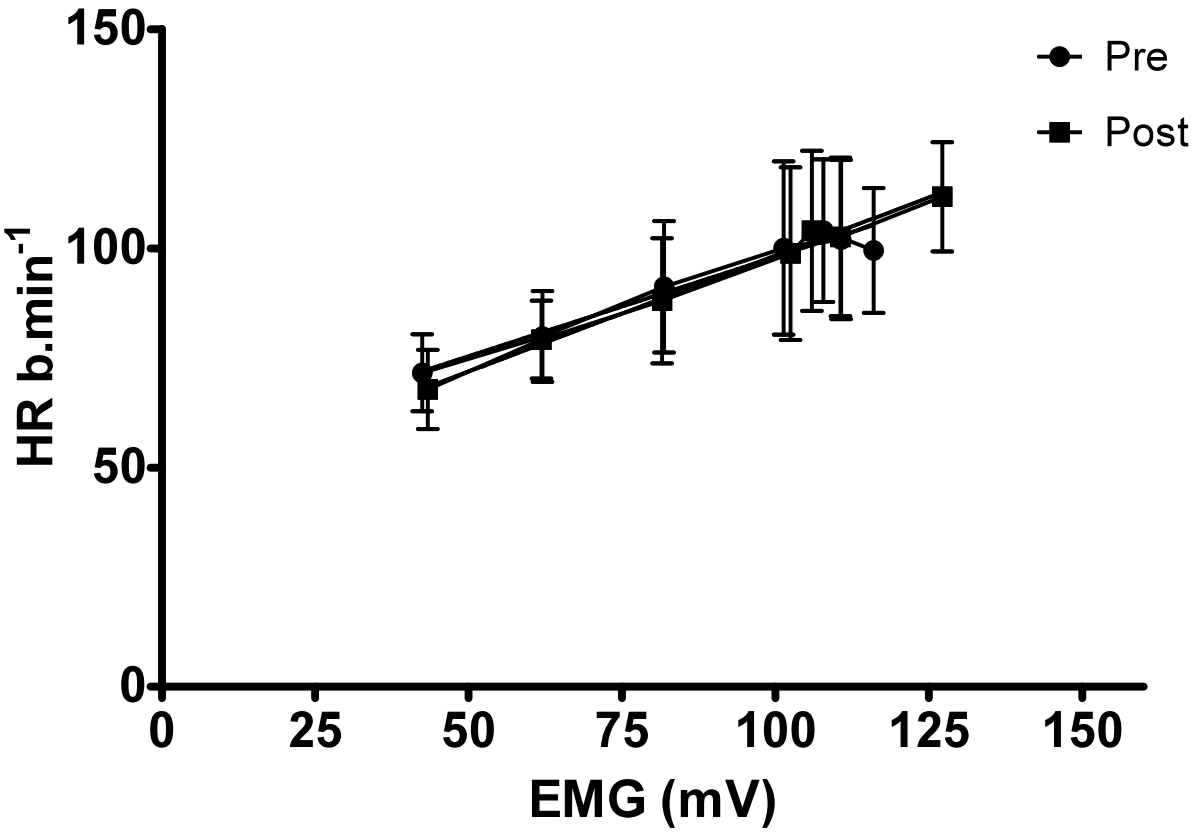

Figure 2.13. Comparison of group mean linear relationships between average surface electromyography (EMG) at the left and right vastus lateralis' and heart rate (HR) during repeated discontinuous incremental isometric exercise tests: test 1 (pre) vs. test 2 (post).

LA_{peak} was reached within the 5 minute rest period during all rest periods for all participants during each discontinuous test. The group mean peak lactates that were achieved were 3.9 ± 1.1 mmol.L^{-1} and 3.7 ± 1.0 mmol.L^{-1} for tests 1 and 2 respectively. Figure 2.14 presents a typical participant lactate response curve from the discontinuous protocol.

The EMG values at 2, 3 and 4 mmol.L^{-1} tests 1 and 2 are given in Table 2.2. These values demonstrated no significant shifts between tests 1 and 2 (all = $p > 0.05$). Figure 2.15 provides a graphical illustration of the EMG and blood lactate relationships for tests 1 and 2.

Table 2.2. Group mean values for EMG activity (mV) at 4, 3, and 2 mmol.L^{-1} lactate concentrations, for repeated discontinuous incremental isometric exercise tests.

Lactate Concentration (mmol.L^{-1})	Test 1 EMG (mV)	Test 2 EMG (mV)
4	136.2 ± 37.7	136.2 ± 17.9
3	113.8 ± 29.7	115.4 ± 15.6
2	84.2 ± 19.0	86.7 ± 15.0

Group mean values (± SD)

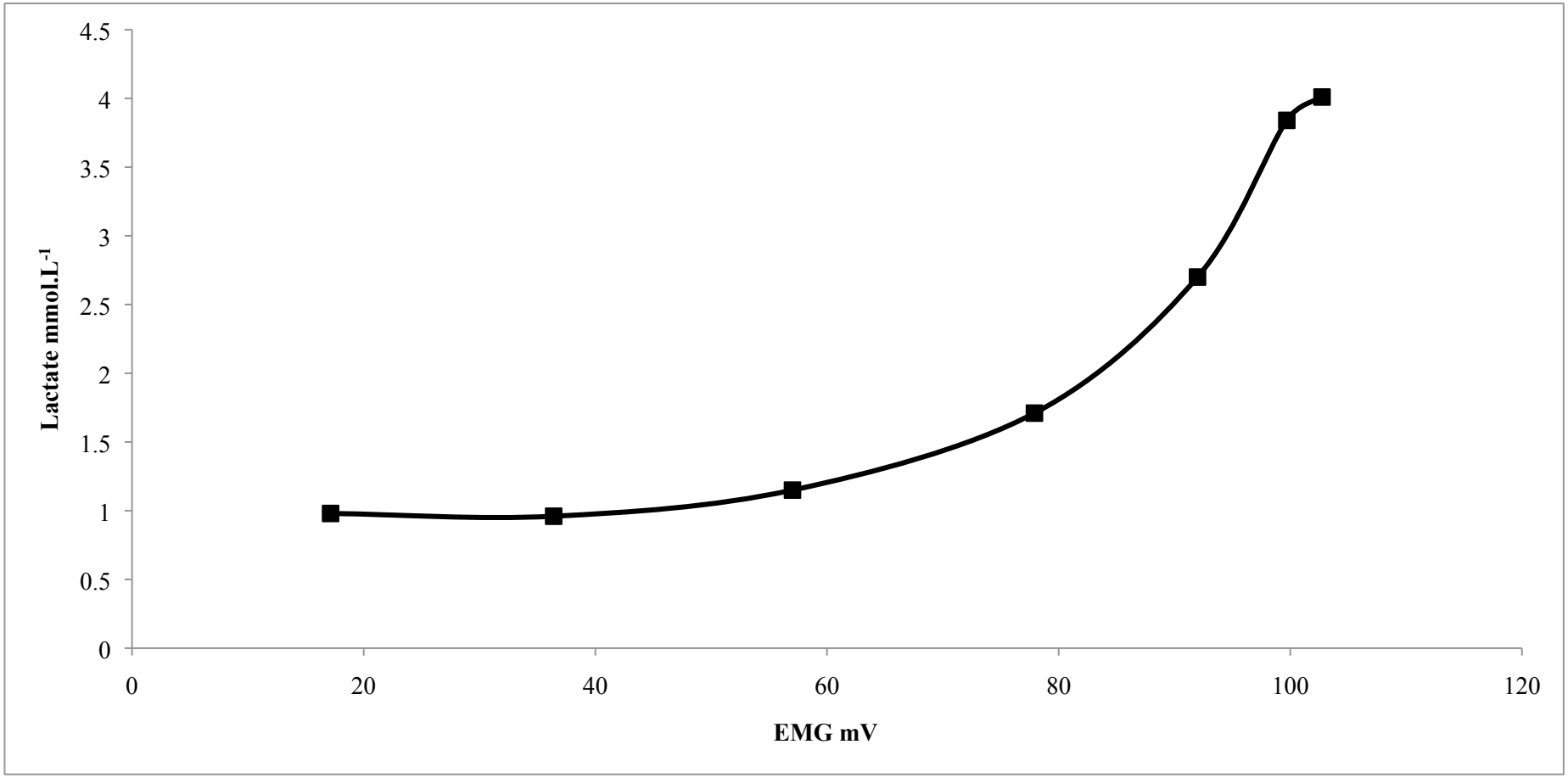

Figure 2.14. Example of lactate response from one participant during the discontinuous incremental isometric exercise test

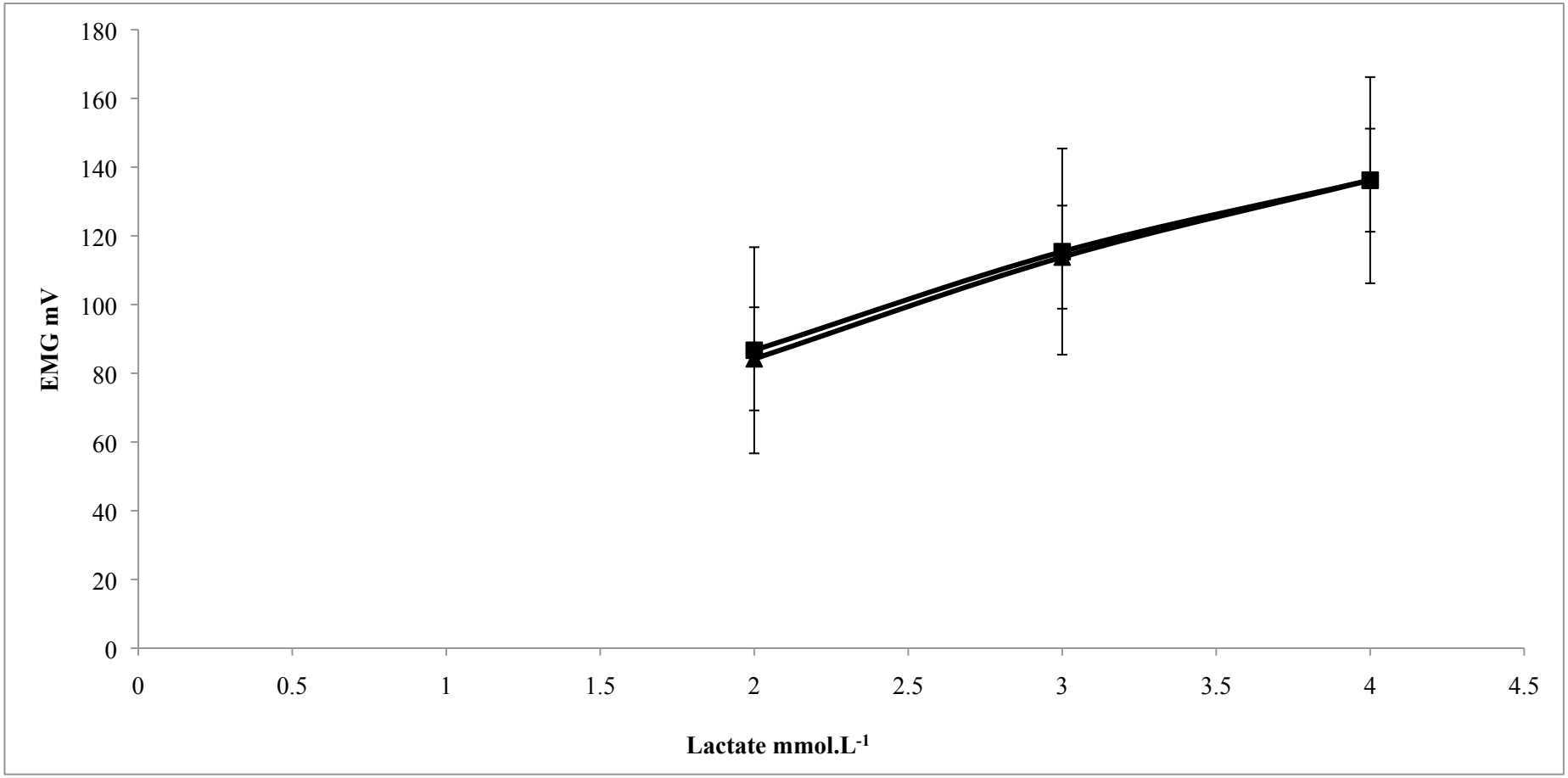

Figure 2.15. Group mean EMG values at 2, 3, and 4 mmol.L^{-1} lactate concentrations for test 1 (▲) and test 2 (■) of the establishment of a discontinuous incremental isometric exercise test

Chapter 5 further utilises the discontinuous incremental protocol in a 4 week bilateral-leg isometric exercise training study, reporting significant shifts in EMG values at 3 and 4 mmol.L^{-1} blood lactate concentrations.

2.16 Discussion

The main finding of the present study was that EMG retains a strong linear relationship with HR when using a discontinuous bilateral-leg isometric exercise protocol, and that lactate metabolism remains stable when compared to EMG muscle activity. Whilst it is difficult to directly compare these results to the majority of previous studies, using either smaller muscle mass during IHG (Wiley *et al*. 1992; Kahn *et al*. 2000; Taylor *et al*. 2003), or constant force alone (Kahn *et al*. 2000; Koltyn *et al*. 2001; Petrofsky and Laymon, 2002), it is possible to draw more meaningful comparisons to the work of Wiles *et al*. (2007), where a continuous, rather than a discontinuous protocol was utilized. This is possible as the steady state HR response times were consistent with those reported previously by Wiles *et al*. (2007). The confirmation of a steady state HR response is essential if analysis of the linear dependence between EMG and HR is to be explored. The reason for this is likely due to the isometric exercise being performed at constant EMG (for each increment), which has previously been shown to educe a more stable CV response to the exercise stimulus (Schibye *et al*. 1981; Wiles *et al*. 2007).

If comparing the absolute HR response (each 30 second segment) of this discontinuous incremental test, to Wiles *et al*. (2007) continuous protocol, it can be seen that mean heart rates were lower during this discontinuous format (average -6.8 ± 0.6 b.min^{-1} across the 0 to 120 second mean values). This is likely due to the inclusion of the 5 minute rest periods of the discontinuous protocol, allowing a HR recovery period between increments. However, this apparent brief HR recovery did not affect the linearity of the overall EMG and HR relationship. The standard errors of the estimate (SEE) of EMG vs. HR in the discontinuous test (5.38% and 3.44%, tests 1 and 2 respectively) are slightly higher than the continuous SEE (2.86% and 4.00%, tests 1 and 2 respectively) reported by Wiles *et al*. (2007), although statistical significance and repeatability of the linear relationship was maintained. The SEE values of this discontinuous protocol fall between the continuous isometric protocol

aforementioned, and those reported from incremental dynamic exercise studies, such as Becque *et al*. (1993) when using cycle ergometry (3.2% and 7.3%).

The linear relationship of EMG and HR during bilateral-leg isometric exercise, and the stability of that relationship with relatively minor error allows for subsequent prescription of isometric exercise intensity to be based on this discontinuous protocol. The stability of retest lactate measurements taken during the rest periods of the discontinuous protocol also permits lactate metabolism and/or chemoreceptor stimulation factors to be investigated following a prolonged intervention of isometric exercise.

Chapter 3

(A). Prominence of cardiovascular variables in the regulation of resting blood pressure within normotensive young males

(B). Acute responses of resting blood pressure and associated cardiovascular variables to a single session of bilateral-leg isometric exercise

(A) Prominence of cardiovascular variables in the regulation of resting blood pressure within normotensive young males

3.1 Introduction

As discussed in Chapter 1, pages 6-10, blood pressure (BP) may be regulated by a host of controlling factors, broadly assigned in this thesis into cardiovascular (CV), neuromuscular (NM), and metabolic components. The proceeding chapters aim to investigate the respective roles of these components in BP responses and adaptations following acute and chronic exposure to isometric exercise, with additional exploration of NM and metabolic indicators of exercise intensity. Therefore it is important to initially understand the relative importance of a variety of resting CV variables in relation to prevailing BP levels.

The aim of this study was therefore to assess the relationships between baroreflex sensitivity (BRS), components of heart rate variability (HRV), cardiac output (Q), stroke volume (SV), and total peripheral resistance (TPR), with prevailing BP levels. It has previously been stated that the structure and function of both the heart and the conduit vessels through which the blood must flow are of critical importance in determining resting BP levels (Oparil *et al*. 2003). BP regulation has been said to be governed by changes in HR, SV, and variance in TPR (Ogoh *et al*. 2003). Baroreflex sensitivity, an indicator of the activity of baroreceptors, has also been proposed as an integral mechanism in the manipulation and regulation of systemic BP (Shoukas *et al*. 1987). The afferent discharge of the baroreceptor cells can elicit changes in HR, cardiac contractility, vascular resistance, and venous return, thus supposedly regulating BP (Pang, 2001).

Resting HRV measures make it possible to compartmentalize the effects of the autonomic nervous system on heart rate (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996), and the efferent autonomic activity is synchronous with oscillations in BP itself (Malliani *et al*. 1991). Blood pressure is said to both affect, and be strongly influenced

by autonomic function (Piccirillo et al. 1996a, 1996b). The two agonistic components of the autonomic nervous system have been reported to act directly on the function of the heart, thus affecting BP (Médigue *et al*. 2004).

However, previous research conducted investigating CV control of BP status has commonly been conducted by comparing individuals with differing resting BP status (i.e. hypertensive, borderline hypertensive, and normotensive subject groups). The studies contained within this thesis use normotensive healthy males exclusively, in order that confounding variables may be reduced. This cross-sectional study therefore attempted to illustrate the significance of a variety of CV variables on BP status within a homogeneous normotensive participant group, and to assess the rationale for each in its use for the proceeding studies of this thesis. This was done by correlating numerous CV variables that have previously been associated with total CV control, and identified as possible factors in the mechanism of BP regulation.

3.2 Methods

Thirty five healthy normotensive males (mean age 22.8 ± 3.1 years; body mass 85.1 ± 11.6 kg; height 179.0 ± 3.1 cm) volunteered to participate in a cross-sectional design study. All participants were moderately physically active (6.1 hours at 6.8 multiple of BMR, per week). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed an exercise readiness questionnaire, and provided written informed consent, thereby adhering to the guidelines set by the 1964 Declaration of Helsinki. All participants conformed to the selection criteria outlined in Chapter 2, pages 24-25. All participants completed identical familiarization sessions to the protocols used for data collection.

3.2.1 Equipment

Resting heart rate and heart rate variability: Was recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments was used. Heart rate was sampled at a frequency of

1000 Hz. Both were recorded after a standardized resting period of 15 minutes in a supine position in a silent, comfortably warm, dimly lit room. Resting HRV was measured using the parametric frequency domain method whereby power spectral density analysis demonstrates how power distributes as a function of frequency, as described in detail in Chapter 2, pages 29-34.

Arterial blood pressure: Resting BP measurements were made using an automated BP monitor (Dinamap Pro 300 Critikon, GEMedical Systems, Slough, Berks, UK). Blood pressure was measured after 15 minutes rest in a seated position. The lowest of three measures, separated by 60 seconds, was used for analysis. The process of automated BP measurement is described in detail in Chapter 2, pages 34-36.

Cardiac Output, Stroke Volume, and Total Peripheral Resistance: The Innocor non-invasive Q monitor (Innovision A/S, Odense S, Denmark) was used to measure SV and Q at rest via inert gas re-breathing. The relative levels of two inhaled inert gases of differing solubility in blood are measured, over four to five respirations. The average value of two measures was used for analysis. Measures were separated by 5 minutes to ensure removal of previously inhaled gases. Participants sat in an upright position to prevent any interference with normal breathing (Damgaard and Norsk 2005). Cardiac output and SV measurement processes are discussed in detail in Chapter 2, pages 39-44. Total peripheral resistance was subsequently calculated using MAP from Dinamap BP readings, recorded immediately prior to Q, and Q from the Innocor device: TPR = MAP/CO (Turner *et al*. 1996; Walker *et al*. 1992).

Baroreflex sensitivity: Resting BRS was measured non-invasively by correlating BP variance with HR variance, using a Finometer (Finapres, TNO Instruments, Amsterdam, Netherlands). The Finometer allows for measurement of constant beat-to-beat and inter-beat BP changes. Resting BRS is calculated accurately (Gizdulich et al. 1996), by the regression slope of the beat-to-beat and inter-beat variables. In order for phase shift calculations of BRS within Finometer software to be made, three consecutive R-R intervals in the same direction are necessary. As BRS varies with time, a recording of ten minutes is advised (Chesterton et al. 2005), and so was administered in this study. Resting BRS measurement processes are discussed in detail in Chapter 2, pages 36-39.

3.2.2 Procedures

Resting Measures: All resting measure variables were conducted in one laboratory visit, starting at 08:30 am. Participants fasted for 4 hours, and abstained from caffeine and alcohol for at least 12 hours prior to testing procedures (Jáuregui-Renaud *et al*. 2001). All testing procedures were conducted prior to actual data collection, to allow for participant familiarization, at 08:30 am on a laboratory visit prior to the data collection visit.

3.2.3 Data Analyses

All data were assessed for conformity with parametric assumptions (Field 2000). An alpha level of < 0.05 was set as the threshold for statistical significance. Pearson product moment correlation was administered to assess for linear dependence between SBP, DBP, and MAP with other resting CV measures, with Bonferroni correction applied for multiple correlations made.

3.3 Results

Resting SBP, DBP and MAP were significantly correlated with resting indices of HRV LFnu (see figures 3.1, 3.2, and 3.3 respectively), and HRV HFnu (see figures 3.4, 3.5, and 3.6 respectively). Resting SBP and MAP were significantly correlated with HRV LF/HF ratio (see figures 3.7 and 3.8 respectively). Table 3.1 presents all Pearson Product – Moment correlation results.

Further analysis of correlations highlighted significant relationships between BRS and HR ($r = 0.40$, $p = 0.01$), and BRS and the following absolute HRV indices: TP ($r = 0.69$, $p < 0.001$), LF ($r = 0.68$, $p < 0.001$), and HF ($r = 0.61$, $p < 0.001$).

Table 3.1. Pearson Product – Moment correlations (r) of systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures with resting heart rate (HR), cardiac output (Q), stroke volume (SV), total peripheral resistance (TPR), heart rate variability absolute parameters of total power (TP), low frequency power (LF), high frequency power (HF), and heart rate variability relative parameters of normalised units of low frequency power (LFnu), normalised units of high frequency power (HFnu), and ratio of low and high frequency spectral power (LF/HF).

	SBP	DBP	MAP
HR	0.37*	-0.05	0.19
CO	0.14	-0.12	0.05
SV	0.15	0.01	0.14
TPR	0.07	0.32	0.18
BRS	-0.01	0.30	0.19
TP	-0.10	0.36*	0.14
LF	0.03	0.44*	0.25
HF	-0.25	0.18	-0.04
LFnu	0.71*	0.47*	0.69*
HFnu	-0.71*	-0.46*	-0.69*
LF/HF	0.66*	0.33	0.60*

* significant at $p < 0.05$

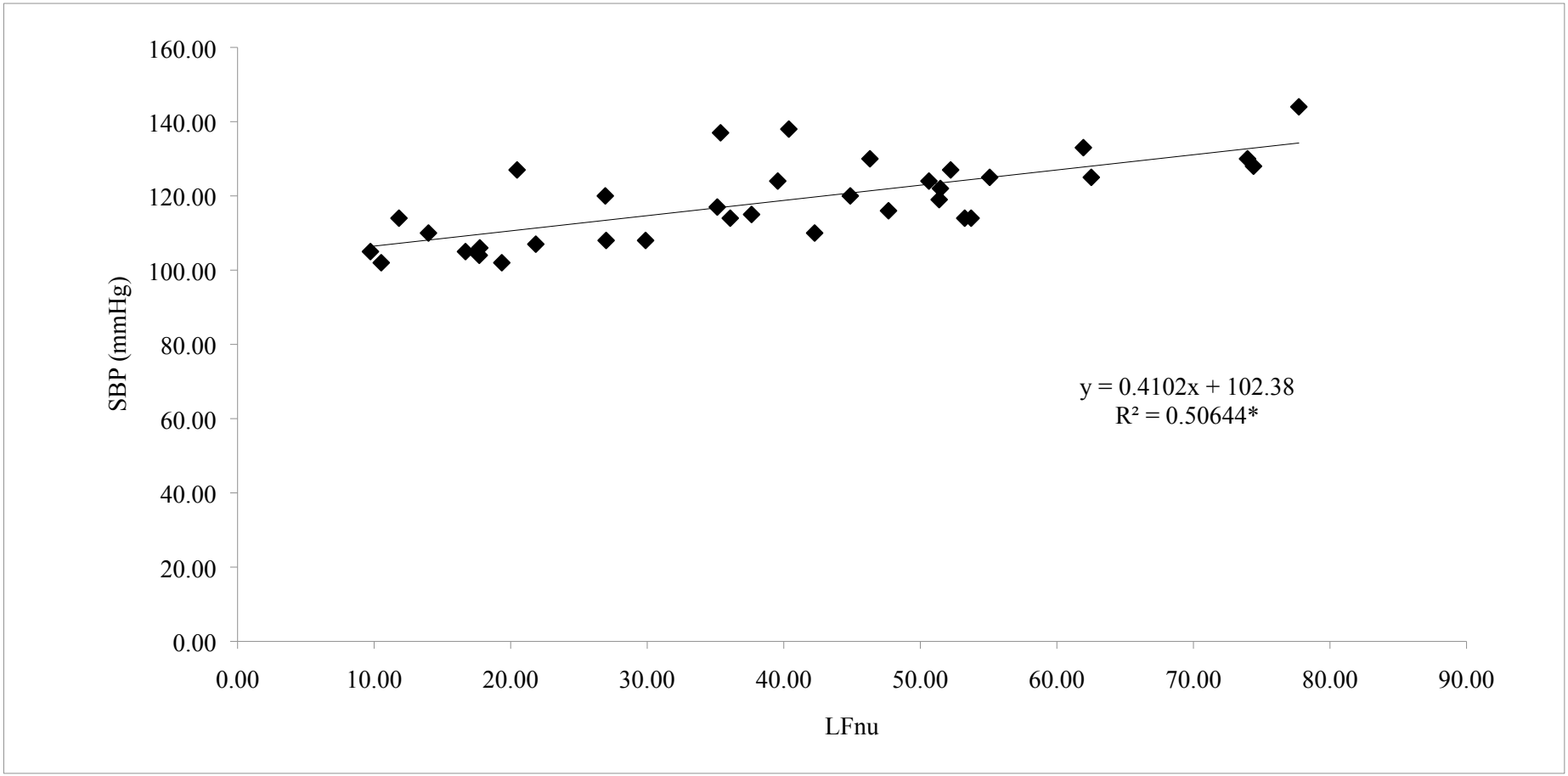

Figure 3.1. Systolic blood pressure (SBP) correlation with the relative heart rate variability component of low frequency normalised units (LFnu) at rest (* = significance, $p < 0.05$)

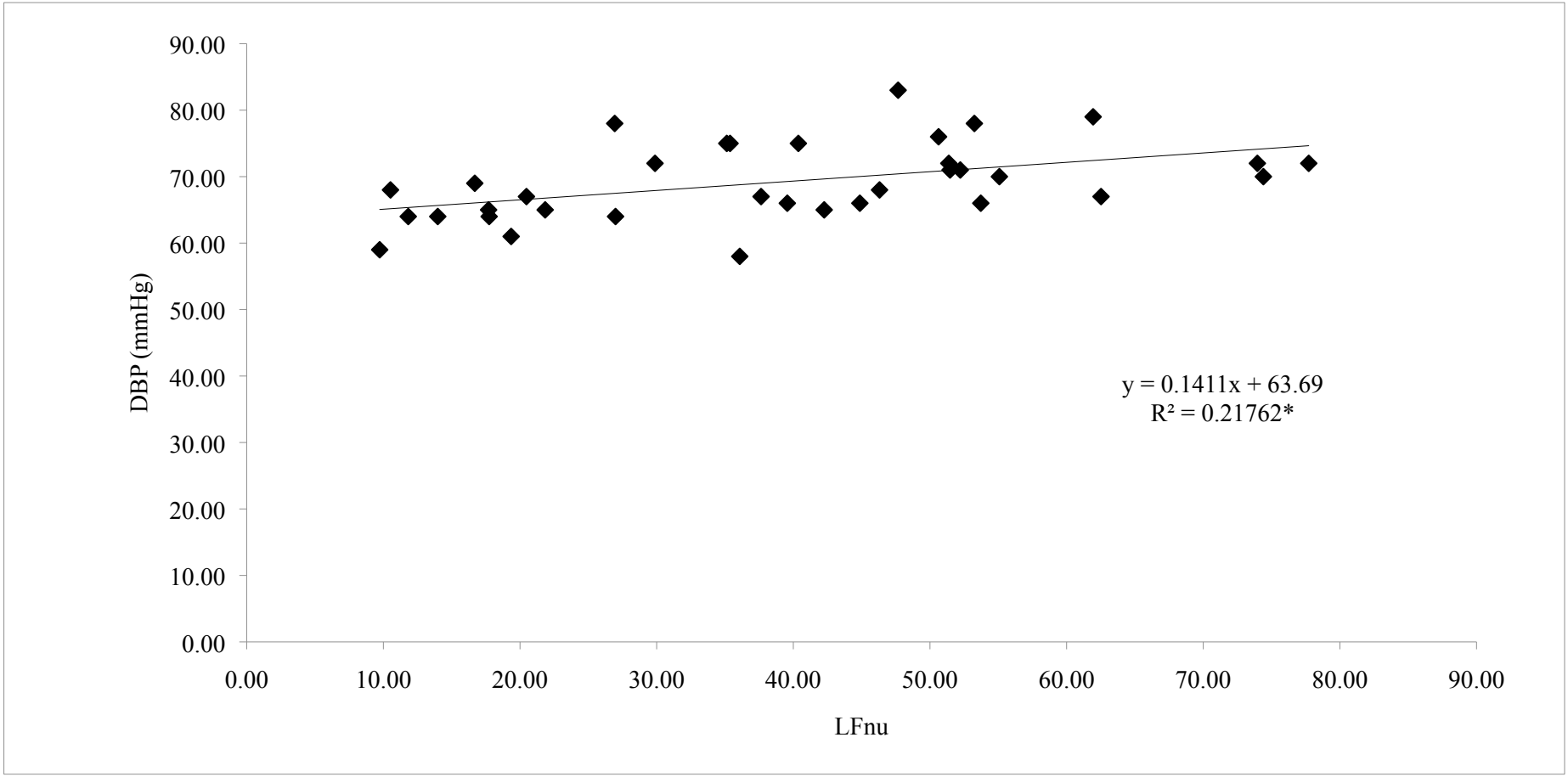

Figure 3.2. Diastolic blood pressure (DBP) correlation with the relative heart rate variability component of low frequency normalised units (LFnu) at rest (* = significance, $p < 0.05$)

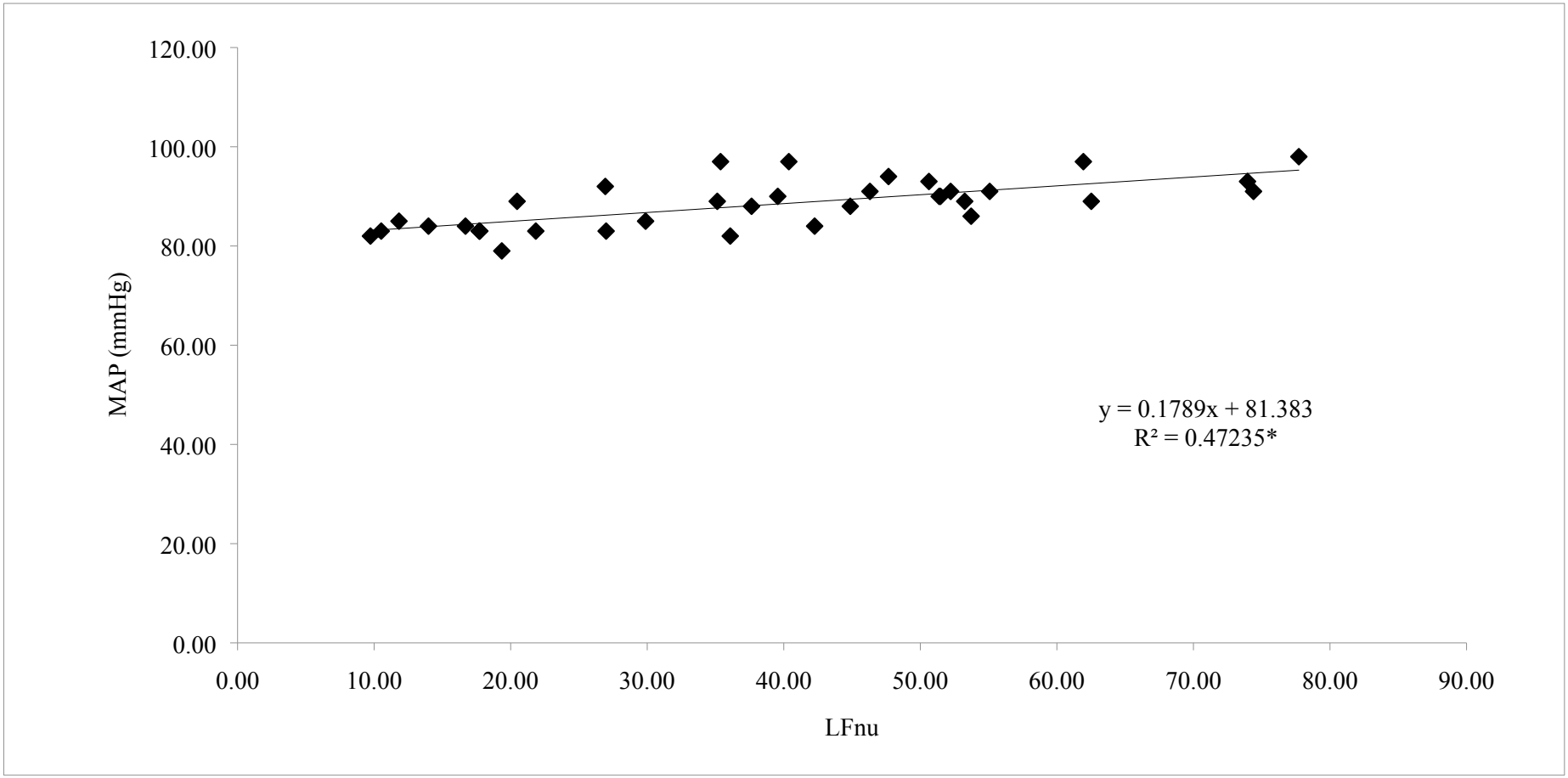

Figure 3.3. Mean arterial blood pressure (MAP) correlation with the relative heart rate variability component of low frequency normalised units (LFnu) at rest (* = significance, $p < 0.05$)

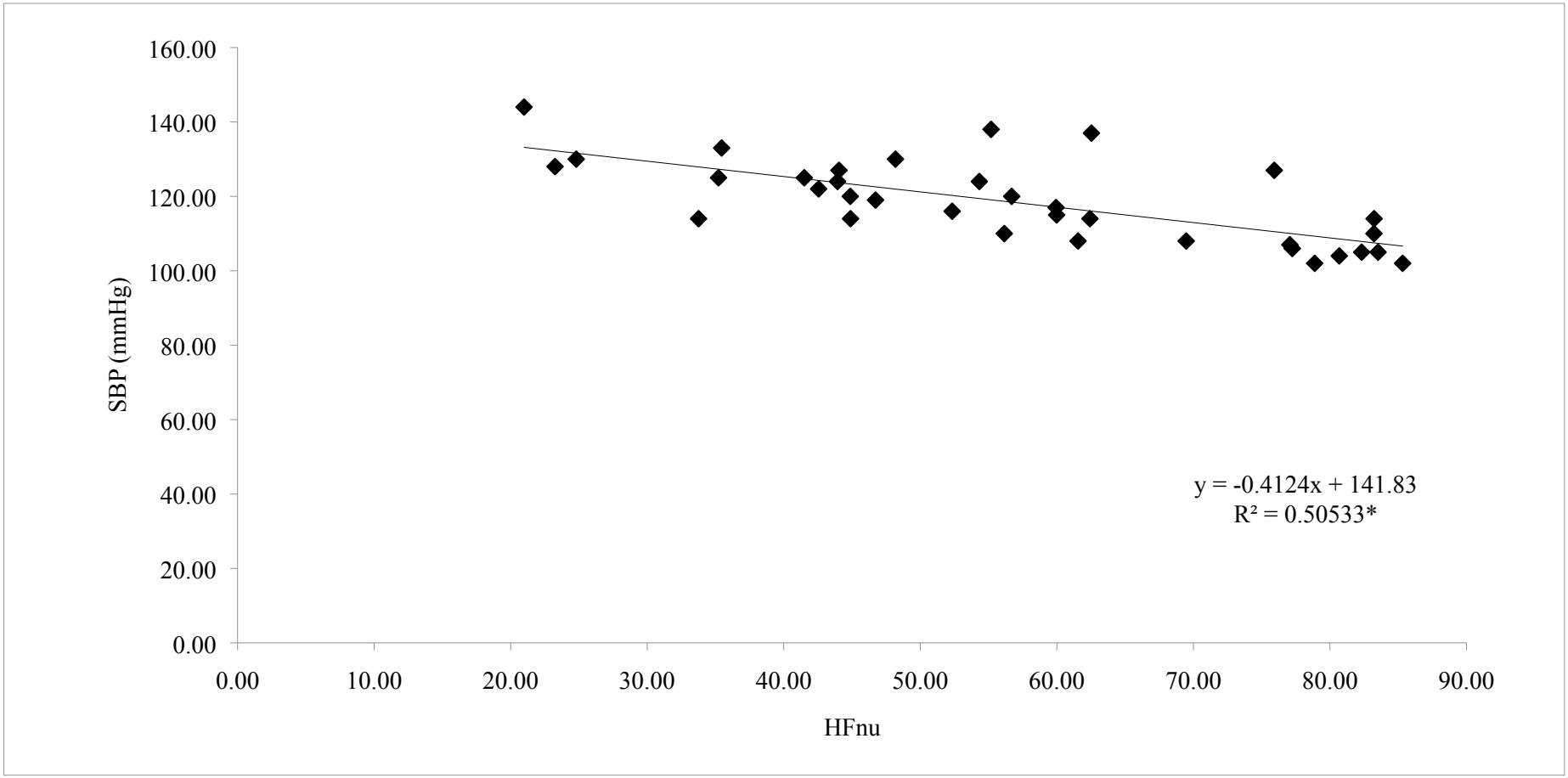

Figure 3.4. Systolic blood pressure (SBP) correlation with the relative heart rate variability component of high frequency normalised units (HFnu) at rest (* = significance, $p < 0.05$)

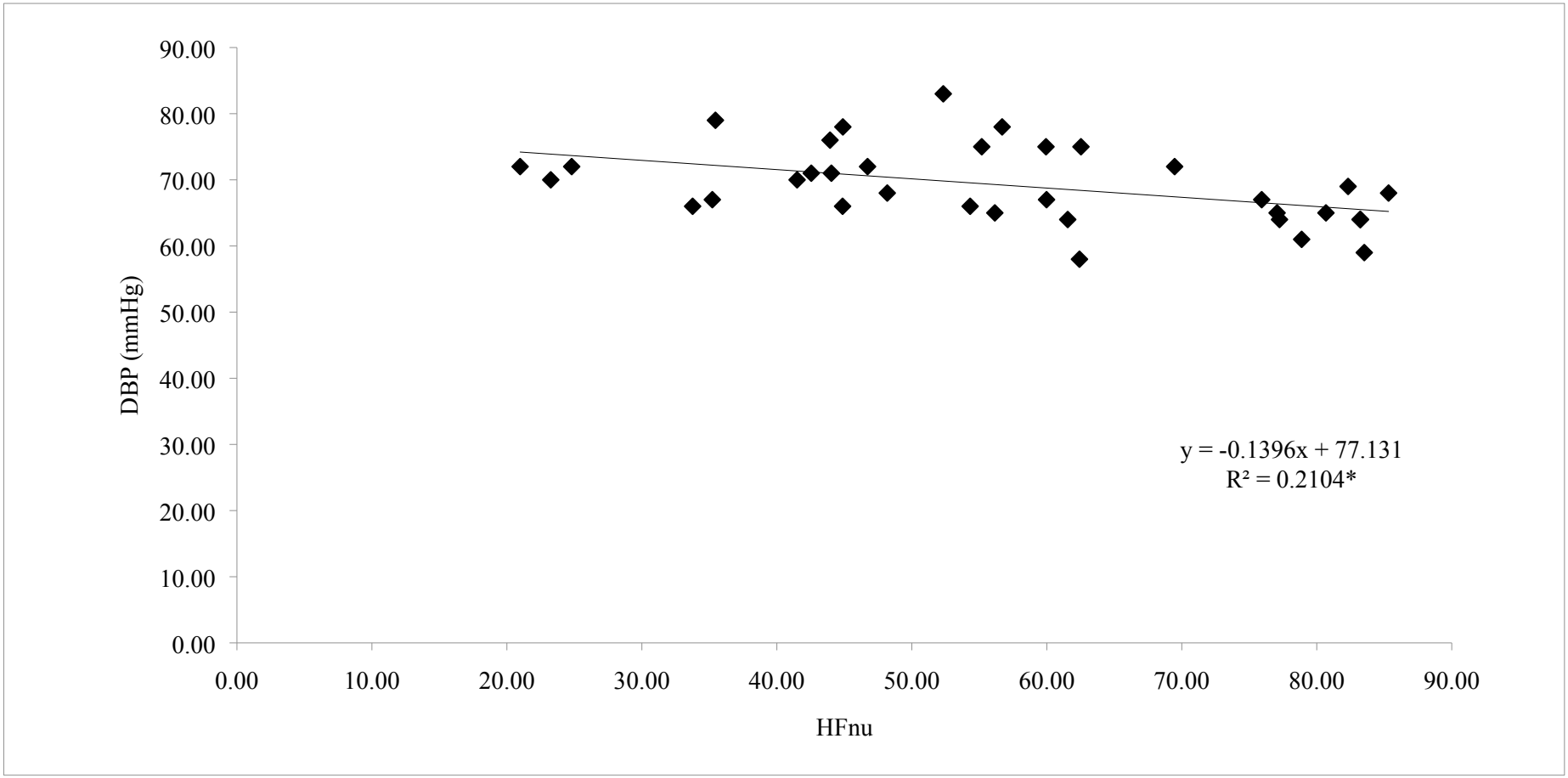

Figure 3.5. Diastolic blood pressure (DBP) correlation with the relative heart rate variability component of high frequency normalised units (HFnu) at rest (* = significance, $p < 0.05$)

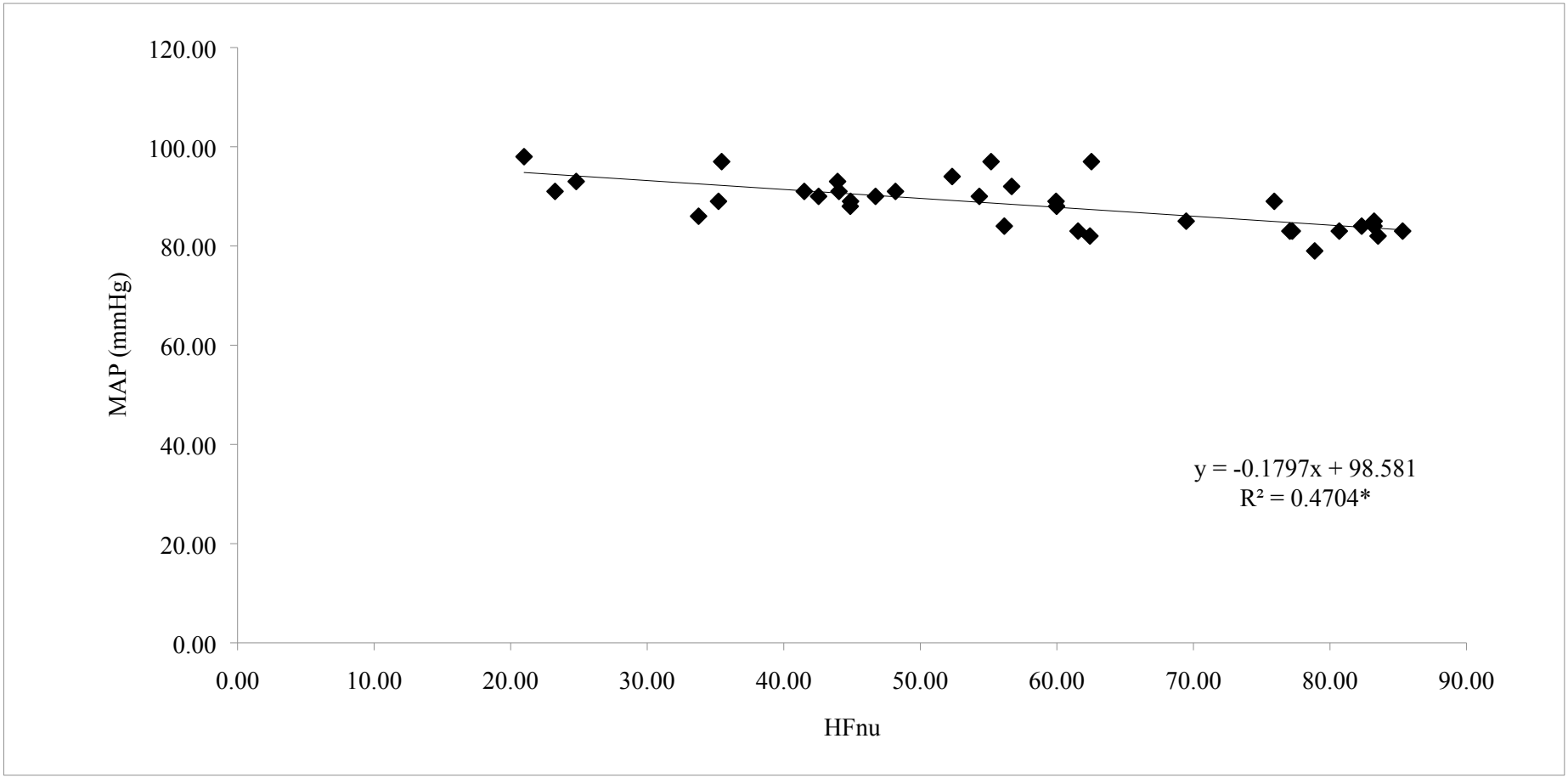

Figure 3.6. Mean arterial blood pressure (MAP) correlation with the relative heart rate variability component of high frequency normalised units (HFnu) at rest (* = significance, $p < 0.05$)

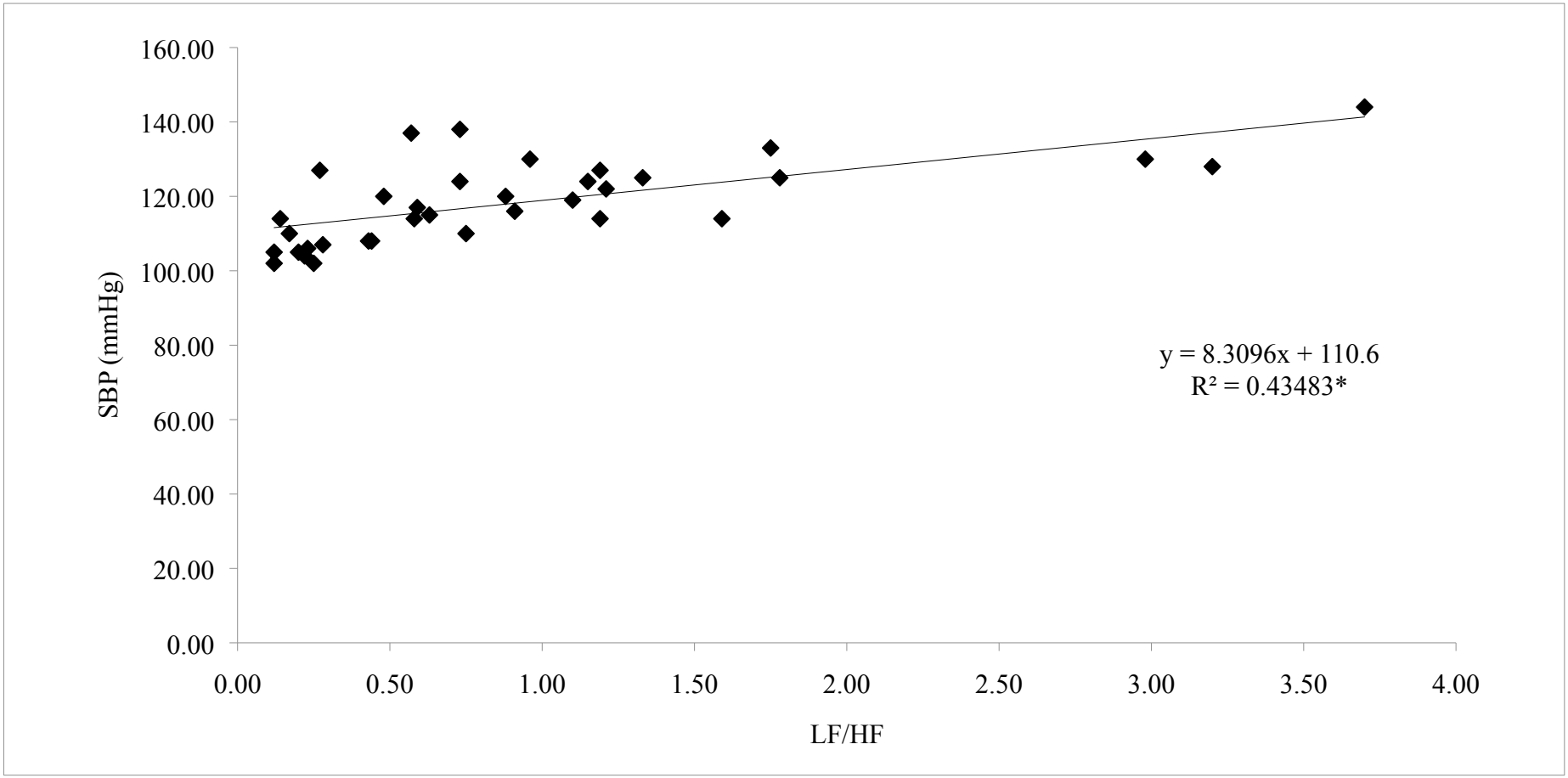

Figure 3.7. Systolic blood pressure (SBP) correlation with the relative heart rate variability component of low to high frequency ratio (LF/HF) at rest (* = significance, $p < 0.05$)

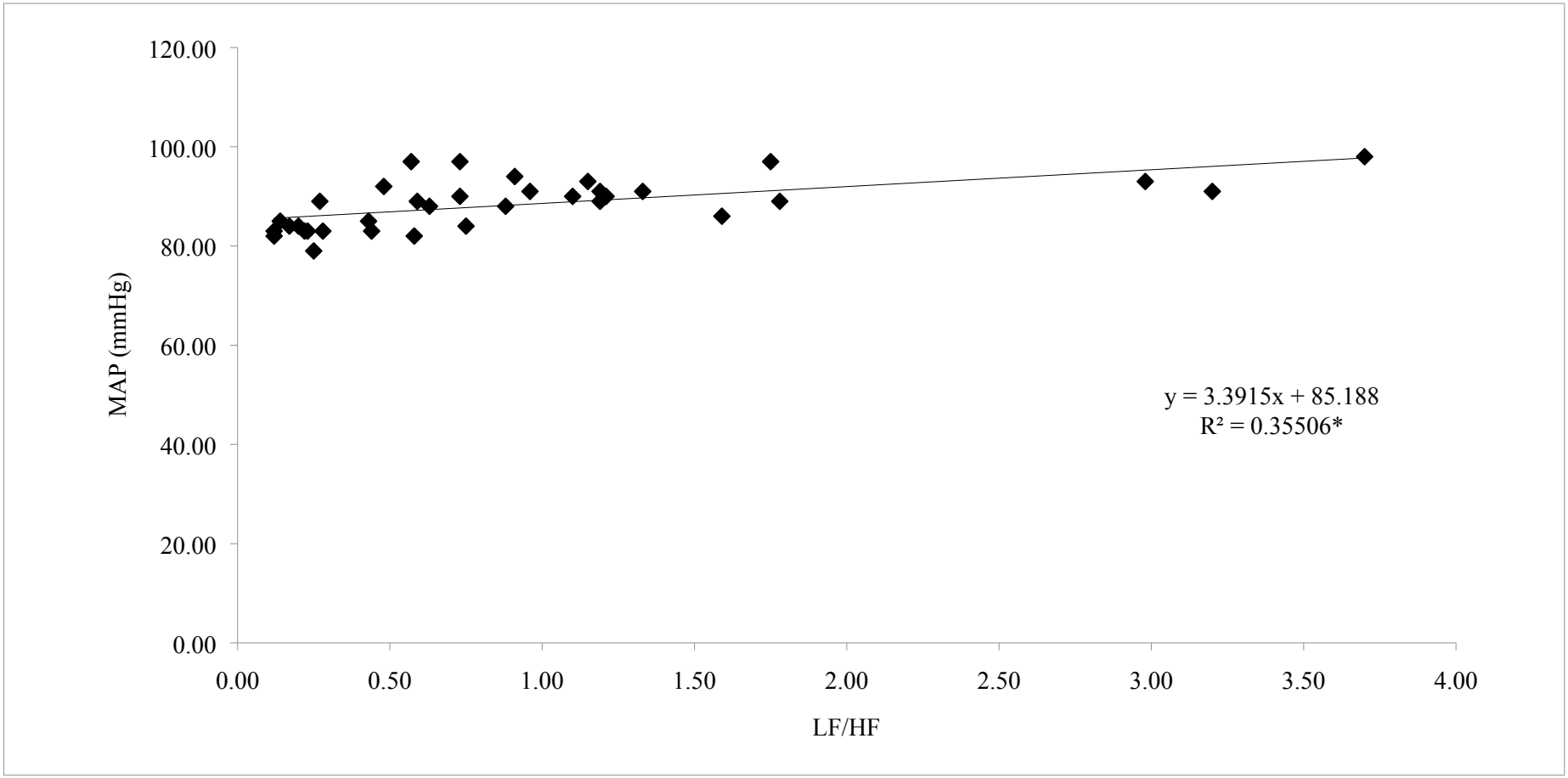

Figure 3.8. Mean arterial blood pressure (MAP) correlation with the relative heart rate variability component of low to high frequency ratio (LF/HF) at rest (* = significance, $p < 0.05$)

3.4 Discussion

The aim of this study was to explore the relative roles of a number of CV variables in the maintenance and control of resting BP in normotensive young males. This study has revealed significant correlations between resting SBP and MAP with resting indices of LFnu, HFnu, and LF/HF ratio components of HRV. The data may suggest that these indicators of autonomic nervous activity could have an active role in the regulation of BP for normotensive young males in a rested state. Although these components may be linked, they should be viewed with caution until confirmation using intervention studies is available.

These correlation findings support previous studies (Langewitz *et al*. 1994; Dietrich *et al*. 2006) where HRV indices have been found to correlate with BP status, reporting reduced parasympathetic frequency activity in individuals whose BP classifies as stage 1 hypertensive (SBP > 140 mmHg) and above, compared to normotensive individuals. This finding concurred with earlier work conducted by Guzzetti *et al*. (1991), where increased sympathetic activity (indicated by increased LFnu spectral component) was evident in hypertensive individuals compared to their normotensive counterparts at rest. Autonomic dysregulation has been reported in early stage hypertension (Singh *et al*. 1998), and it has previously been proposed that initial chronic elevations in arterial pressure are actually caused by a sustained increase in sympathetic activity alone (Goldstein, 1983). Whilst these two earlier studies have compared the effects of hypertension on HRV indices at rest, the data reported within the present study suggests that BP status may be associated with the sympathetic and parasympathetic indicators of HRV within normotensive individuals.

Earlier studies have associated changes in HRV with changes in BP variability using beat-to-beat analysis techniques (Pagani *et al*. 1986). Suggestions for the underlying mechanism responsible for this association have included direct central neural fluctuations (Koh *et al*. 1994; Cooley *et al*. 1998; Montano *et al*. 2000) and peripheral baroreflex feedback (DeBoer *et al*. 1987; Cevese *et al*. 2001). Further analysis of the current results did in fact show correlations between BRS and absolute HRV indices of TP, LF, and HF spectral power, although BRS did not correlate with any of SBP, DBP,

or MAP. This supports previous work suggesting that perhaps within a young normotensive subject sample, fluctuations in HRV are indeed intrinsically aligned with peripheral baroreflex action (DeBoer *et al*. 1987; Cevese *et al*. 2001). It has also been suggested that the increased levels of sympathetic activity evident with elevated BP may directly alter the vascular beds of the systemic circulation, resulting in vasoconstriction and thus increased systemic vascular resistance, or TPR (Jacobsen *et al*. 1992). The present results show that TPR did not correlate with levels of LFnu, taken to represent sympathetic autonomic nervous activity (Malliani *et al*. 1991; Pagani *et al*. 1997; Cooley *et al*. 1998), or with any component of resting BP.

Whilst the present data shows a correlation between CV variables such as HRV and BRS, these variables have not been consistently associated with resting BP measures. It may be that the associations of CV variables such as BRS and TPR with resting BP components reported from previous studies are not evident within the current data due to the normotensive status of the participants in the present study. For instance, BRS has previously been proposed to limit variations of BP within a given BP range, rather than exclusively setting BP status (Liard, 1980). Previous studies have commonly compared CV mechanisms likely responsible for BP regulation between individuals who fall into either hypertensive or normotensive participant groups, finding and discussing dissimilarities between individuals in different resting BP categories.

Figures 3.7 and 3.8, illustrating correlations between SBP and MAP with the relative ratio of LF and HF activity are clearly being influenced by three outliers. Therefore, until greater numbers of participants have been assessed, this correlation should be viewed with caution. However, it is possible that these data points are not atypical, but more extreme values than are otherwise presently represented. It could be expected that with a larger participant sample a more even spread of data points would be evident. When considering the present data, factors such as measurement sensitivity may affect definitive conclusions in homogeneous participant groups. Another consideration may be the definite role each variable has in regulating BP.

Furthermore, it must be recognised that cross-sectional design studies by their nature often require excessive sample size numbers to confidently infer non-significant results.

This is particularly the case for selected CV variables used within this thesis, given the high variance in some reproducibility measures (see Chapter 2, pages 46-49). Therefore, although correlations were not evident between some CV variables and resting BP within this study, their inclusion in proceeding studies may prove prudent. This is because the addition of an isometric exercise stimulus may offer an alternative insight into their respective roles in BP regulation and/or manipulation following an isometric exercise stimulus.

3.5 Conclusion

This study suggests that within a normotensive group of young healthy males, HRV derived markers of autonomic nervous function correlate with resting BP. Other CV variables do not appear to be as prominent in a resting state, although this may be due to measurement sensitivity at rest, or subtle differences in their regulatory processes around a given BP level category.

(B) Acute responses of resting blood pressure and associated cardiovascular variables to a single session of bilateral-leg isometric exercise

3.6 Introduction

The effects of a single session of bilateral-leg isometric exercise on CV function are unknown. Recent studies conducted by Stewart *et al*. (2007) and Millar *et al*. (2009) have reported immediate post-exercise SBP hypotensive responses to IHG exercise. Stewart *et al*. (2007) have reported significant reductions in SBP (-2 mmHg) 1 minute after bilateral-arm IHG exercise of 2 minutes duration at an intensity of 35% MVC. Similarly, Millar *et al*. (2009) reported significant reductions in SBP (-3 mmHg) 5 minutes after the completion of bilateral-arm IHG exercise of 4 x 2 minute contractions at 30% MVC. What remains unclear is not only the effect of utilising a greater muscle mass during isometric exercise (as in bilateral-leg exercise), but also the recovery duration of the SBP responses. Furthermore, it is essential to ascertain if indeed they would return to baseline levels prior to re-testing resting BP in a chronic adaptation study, typically measured 48 hours after the final training session (Howden *et al*. 2002; Wiles *et al*. 2010)

If intervention studies report chronic adaptations to BP (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Wiles *et al*. 2010) it is important to ascertain whether those reported changes are indeed chronic adaptations, rather than an acute response to the most recent training session. Furthermore, analysis of short-term effects of BP regulatory factors to bilateral-leg isometric exercise may aid in the understanding of the underlying mechanisms responsible for BP manipulation, if evident following chronic exposure to an exercise stimulus. The CV components of BP regulation studied in this thesis (HRV, Q, SV, BRS, and TPR), are all reported to undergo short-term variation (Robotham *et al*. 1979; Ichinose *et al*. 2007), as are autonomic nervous components (Taylor *et al*. 1999; Malpas, 2002), especially HRV (Askelrod *et al*. 1981; Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). However, the acute responses of BP and these CV variables to a single session of bilateral-leg isometric exercise currently remain unknown.

Therefore, the purpose of this study was to examine the acute responses of the CV system to a single session of bilateral leg isometric exercise. For this acute response study, exercise intensity was prescribed using the same linear relationship between EMG and HR that would be used in a subsequent 4 week intervention study. This would allow for discussions to be made in relation to the intervention studies that had been planned. An additional lower intensity was also prescribed to allow for reflective discussions with respect to previous work conducted by Wiles *et al*. (2010). Furthermore, in light of correlations present between resting BP with components of HRV reported in Part A of this chapter, the acute effects of differing intensities of bilateral-leg isometric exercise on HRV derived indicators of autonomic nervous function were a particular focus.

3.7 Methods

Eight healthy normotensive males (mean age 26.0 ± 4.9 years; body mass 80.5 ± 14.1 kg; height 178.2 ± 4.9 cm) completed a 3-way crossover design study. Ten participants originally volunteered to participate. One unspecified voluntary cancellation and one case of personal illness were the reasons for the two cases of participant withdrawal. All participants were moderately physically active (7.9 hours at 8.2 multiple of BMR, per week). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed an exercise readiness questionnaire, and provided written informed consent, thereby adhering to the guidelines set by the 1964 Declaration of Helsinki. All participants conformed to the selection criteria outlined in Chapter 2, pages 24-25. All participants completed identical familiarization sessions prior to data collection. Participants fasted for 4 hours, and abstained from caffeine and alcohol for at least 12 hours prior to testing procedures (Jáuregui-Renaud *et al*. 2001), and were instructed to maintain physical and dietary activity behaviours prior to each visit.

3.7.1 Equipment

Isometric exercise: All tests were conducted using a Biodex System 3 Pro, isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY), which is described in detail in Chapter 2, pages 25-26. The Biodex was fitted with a modified hip attachment that was inserted into the standard knee attachment, to allow for double leg extension

exercise to be performed. Participants sat in the dynamometer in an upright position, with 90 degrees of flexion at the hip and with the thighs supported. Participant positions and machine settings were recorded and maintained throughout the study for the purpose of standardisation.

Electromyography recording: A dual bio-amplifier was used to enable surface EMG measurement from both vastus lateralis', to give a combined average, along with the 16-channel chart recorder. This is described in detail in Chapter 2, pages 27-29.

Exercising heart rate: Heart rate was recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments was used. Heart rate was sampled at a frequency of 1000 Hz.

Resting heart rate and heart rate variability: Was recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments was used. Heart rate was sampled at a frequency of 1000 Hz. Both were recorded after a standardized resting period of 15 minutes in a supine position in a silent, dimly lit room. Resting HRV was measured using the parametric frequency domain method whereby power spectral density analysis demonstrates how power distributes as a function of frequency, as described in detail in Chapter 2, pages 29-34.

Arterial blood pressure: Resting BP measurements were made using an automated BP monitor (Dinamap Pro 300 Critikon, GEMedical Systems, Slough, Berks, UK). Blood pressure was measured after 15 minutes rest in a seated position. The lowest of three measures, separated by 60 seconds, was used for analysis. The process of automated BP measurement is described in detail in Chapter 2, pages 34-36.

Cardiac Output, Stroke Volume, and Total Peripheral Resistance: The Innocor non-invasive Q monitor (Innovision A/S, Odense S, Denmark) was used to measure SV and Q at rest via inert gas re-breathing. The relative levels of two inhaled inert gases of differing solubility in blood are measured, over four to five respirations. The average value of two measures was used for analysis. Measures were separated by 5 minutes to ensure removal of previously inhaled gases. Participants sat in an upright position to prevent any interference with normal breathing (Damgaard and Norsk 2005). Cardiac output and SV measurement processes are discussed in detail in Chapter 2, pages 39-44. Total peripheral resistnace was subsequently calculated using MAP from Dinamap BP readings, and Q from the Innocor device: TPR = MAP/Q (Turner *et al*. 1996; Walker *et al*. 1992).

Baroreflex Sensitivity: Resting BRS was measured non-invasively by correlating BP variance with HR variance, using a Finometer (Finapres, TNO Instruments, Amsterdam, Netherlands). The Finometer allows for measurement of constant beat-to-beat and inter-beat BP changes. Resting BRS is calculated accurately (Gizdulich *et al*. 1996), by the regression slope of the beat-to-beat and inter-beat variables. In order for phase shift calculations of BRS within Finometer software to be made, three consecutive R-R intervals in the same direction are necessary. As BRS varies with time, a recording of ten minutes is advised (Chesterton *et al*. 2005), and so was administered in this study. BRS measurement processes are discussed in detail in Chapter 2, pages 36-39.

3.7.2 Procedures

Testing organisation: The three crossover sessions were conducted within one week for each participant, on a Monday, Wednesday, and Friday, at the same time of day for each visit. This ensured that 48 hours separated any crossover. The order of the testing schedule was randomised for each participant, between control, low intensity, and high intensity protocols. A discontinuous isometric incremental exercise test was conducted to allow for subsequent prescription of exercise intensities, on the Friday prior to the start of the 3 crossover sessions week. Figure 3.9 provides a schematic overview of the testing schedule.

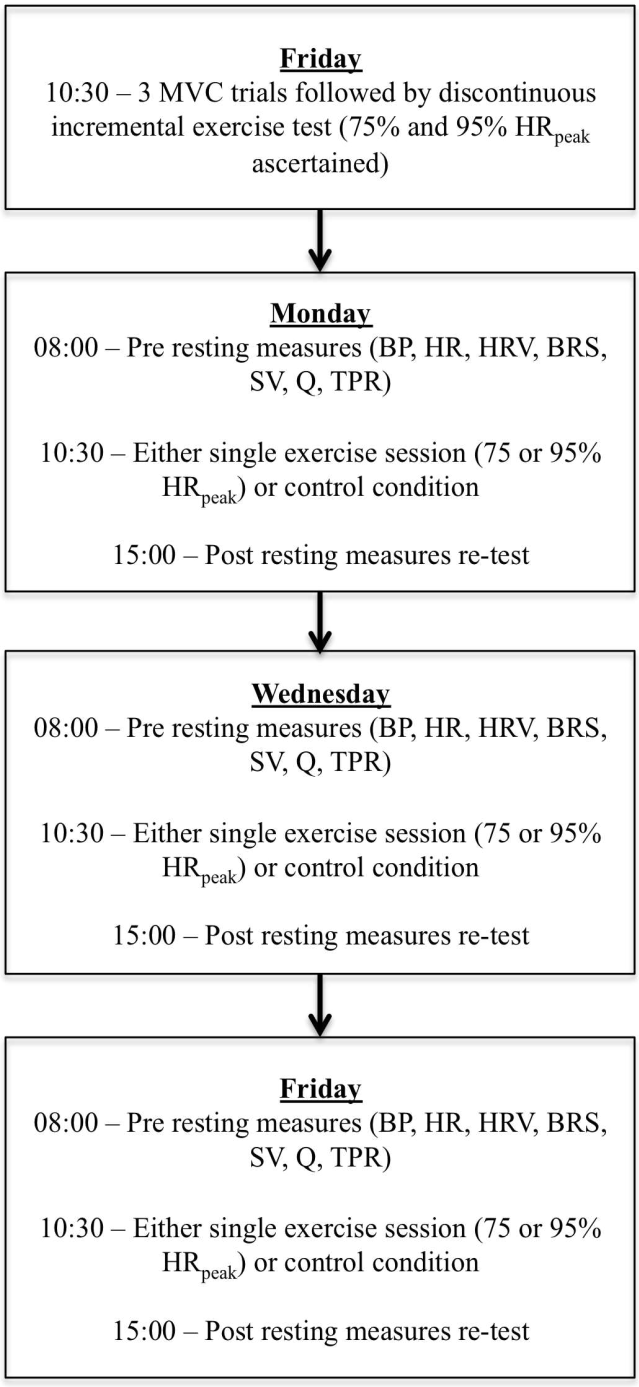

Figure 3.9. Outline of three-way crossover acute response study design

Maximal voluntary contraction and EMG_{peak}: Maximal voluntary contraction (MVC) and EMG_{peak} were determined prior to the discontinuous incremental test. Participants performed three maximal effort contractions against the immoveable leg brace. Each MVC was terminated after 2 seconds, and each was interspersed by a 120 second rest period, previously shown to allow for full recovery (Friedman *et al*. 1992; Ferguson and

Brown, 1997). The isometric leg extension exercise was performed at a knee angle of 90 degrees (180 degrees corresponds to full knee extension) on the isokinetic dynamometer (Alkner *et al*. 2000). Participants were instructed to avoid using their upper body musculature to assist in generating force during isometric double-leg exercise in order to standardize the level of stabilization and to avoid this affecting force outputs (Magnusson *et al*. 1993; Mendler 1976).

EMG_{peak} was determined from the MVC producing the highest torque. EMG_{peak} was established from the mean of the EMG activity recorded 0.25 seconds immediately prior to peak torque, as used by Wiles *et al*. (2010). The EMG_{peak} value was then used to create $\%EMG_{peak}$ 'targets' for the subsequent incremental exercise test.

Discontinuous incremental isometric exercise test: Participants began double-leg isometric exercise at 10% EMG_{peak} for a period of 2 minutes. Thereafter, the intensity increased in 5% increments, interspersed by 5 minute rest periods, up to volitional fatigue (or failure to maintain EMG signal within +/- 5% of the 'target' value). Electromyography was continuously monitored and recorded. Heart rate was continuously recorded.

Exercise Sessions: Participants exercise sessions were at an individual participant-specific EMG 'target' that equated to both 75% HR_{peak} and 95% HR_{peak}, interpolated from the regression line of HR versus EMG ascertained during the initial incremental test. Participants exercised at both the low and high intensities once, in a randomised order together with a control condition that involved no exercise session between the recorded resting measures. Exercise sessions were separated by 48 hours if an exercise session condition succeeded another training session condition, or by 96 hours if the control condition separated the training condition sessions. Participants performed four bouts involving 2 minutes of isometric exercise separated by 3 minute rest periods. Electromyography, HR, and torque were measured and recorded continuously throughout both the low and high intensity training sessions. Participants were instructed to breathe at a normal rhythm and depth at all times to avoid Valsalva manoeuvres. Training intensity set and maintained at constant EMG allows for a stable HR response

to isometric exercise, whereas constant torque results in HR drift during an exercise bout (Schibye *et al*. 1981).

Resting Measures: Resting SBP, DBP, MAP, HR, HRV, Q, SV, TPR, and BRS were measured prior to and following both the low and high training session conditions, and at the same time of day for the control condition. The control condition followed exactly the same protocol as the single isometric exercise sessions, in terms of resting measure protocols (Chapter 2), and the time of day those measures were taken. The only difference being the absence of an isometric exercise session in the control condition.

3.7.3 Data Analyses

All data were assessed for conformity with parametric assumptions (Field 2000). Pre-post exercise and control differences, and differences in BP changes between the experimental and control condition were assessed using ANCOVA. An alpha level of < 0.05 was set as the threshold for statistical significance, and the Bonferroni post-hoc procedure was used to explore any significant differences detected. Group mean percentage changes were explored using Friedman's non-parametric test of difference for the pre- post training and control conditions.

3.8 Results

Resting blood pressure: A single session of low intensity (75% HR_{peak}) bilateral-leg isometric exercise did not cause a 4 hour response in resting SBP (-1.5 ± 2.4 mmHg, $p = 0.13$), DBP (-0.6 ± 2.5 mmHg, $p = 0.50$), or MAP (-0.5 ± 2.6 mmHg, $p = 0.61$). A single session of high intensity (95% HR_{peak}) bilateral-leg isometric exercise did not cause a significant 4 hour response in resting SBP (-0.9 ± 2.4 mmHg, $p = 0.34$), DBP (-1.1 ± 2.3 mmHg, $p = 0.21$), or MAP (0.3 ± 1.6 mmHg, $p = 0.67$). No differences in the control data (SBP = 0.3 ± 1.9 mmHg, $p = 0.72$; DBP = -0.3 ± 2.0 mmHg, $p = 0.73$; MAP = -0.1 ± 2.4 mmHg, $p = 0.89$) were evident. Resting SBP, DBP and MAP responses to both low and high intensity exercise were not different to the control condition ($p > 0.05$). Table 3.2 demonstrates BP group mean values before and 4 hours after the two isometric exercise sessions together with control data.

Table 3.2. Group mean values for systolic (SBP), diastolic (DBP) and mean arterial (MAP) pressure before and 4 hours after isometric exercise.

Condition	BP component	Pre (mmHg)	Post (mmHg)
Control	SBP	112.8 ± 7.4	113.0 ± 7.9
	DBP	69.0 ± 6.9	68.8 ± 5.5
	MAP	86.5 ± 4.5	86.4 ± 3.9
Low	SBP	112.8 ± 7.1	111.3 ± 7.8
	DBP	68.8 ± 6.2	68.1 ± 5.5
	MAP	85.5 ± 2.7	85.0 ± 4.6
High	SBP	112.4 ± 6.0	111.5 ± 7.3
	DBP	67.1 ± 4.7	66.0 ± 4.8
	MAP	84.9 ± 3.8	85.1 ± 4.5

Group mean values (± SD)

Resting heart rate variability measures: A single session of either low (75% HR_{peak}) or high intensity (95% HR_{peak}) bilateral-leg isometric exercise did not cause a significant 4 hour response in any resting HRV measure (all $p > 0.05$, pre vs. post). However, differences were observed in the control data for all relative components of HRV: HFnu (11.8 ± 12.6), LFnu (-12.1 ± 14.1, $p = 0.046$) and HRV LF/HF (-0.7 ± 0.8, $p = 0.03$), comparing morning versus afternoon measures.

Other resting measures: A single session of either low (75% HR_{peak}) or high intensity (95% HR_{peak}) bilateral-leg isometric exercise did not cause a significant 4-hour response in any other resting measure (HR, BRS, Q, SV, TPR, all $p > 0.05$, pre vs. post), as was also the case for all resting measure control data. Tables 3.3, 3.4, and 3.5 present HRV and other resting measure group mean values in the control condition, and before and 4 hours after both the isometric exercise training stimulus conditions.

ANOVA showed that SBP, DBP and MAP responses to both low and high intensity bilateral-leg isometric exercise (at 4 hours post-exercise) were not different to the control condition ($p > 0.05$). Also, analysis showed that SBP, DBP, and MAP were not significantly altered throughout the week (3 laboratory visits; $p > 0.05$ in all cases). Figure 3.10 provides an illustration of SBP, DBP, and MAP stability across the three laboratory visits (which took 1 week overall).

Table 3.3. Group mean values for resting heart rate (HR), baroreflex sensitivity (BRS), heart rate variability absolute measures (TP, HF, LF), heart rate variability relative measures (HFnu, LFnu, LF/HF), cardiac output (Q), stroke volume (SV), and total peripheral resistance (TPR) before and 4 hours after control condition.

Resting Measure	Control	
	Pre	Post
HR ($b.min^{-1}$)	57.5 ± 9.9	55.8 ± 8.1
BRS (ms/mmHg)	12.8 ± 6.0	13.6 ± 3.0
HFnu	50.3 ± 20.2	62.1 ± 15.1*
LFnu	47.8 ± 19.9	35.8 ± 13.9*
LF/HF	1.4 ± 1.1	0.7 ± 0.6*
TP (ms^2)	8663 ± 11445	9045 ± 7968
HF (ms^2)	4066 ± 6285	4955 ± 5122
LF (ms^2)	2625 ± 3635	2118 ± 1620
Q ($l.min^{-1}$)	5.6 ± 0.6	5.7 ± 0.5
SV (ml)	89.6 ± 19.4	92.0 ± 16.9
TPR	15.7 ± 1.9	15.3 ± 1.7

Group mean values (± SD), * significant at $p < 0.05$

Table 3.4. Group mean values for resting heart rate (HR), baroreflex sensitivity (BRS), heart rate variability absolute measures (TP, HF, LF), heart rate variability relative measures (HFnu, LFnu, LF/HF), cardiac output (Q), stroke volume (SV), and total peripheral resistance (TPR) before and 4 hours after low intensity (75% HR_{peak}) isometric exercise condition.

Resting Measure	Low intensity (75% HR_{peak})	
	Pre	Post
HR ($b.min^{-1}$)	57.2 ± 10.1	59.9 ± 8.7
BRS (ms/mmHg)	13.9 ± 8.4	12.9 ± 4.6
HFnu	53.9 ± 18.5	54.8 ± 16.4
LFnu	44.6 ± 18.6	42.6 ± 14.8
LF/HF	1.1 ± 0.9	0.9 ± 0.7
TP (ms^2)	7590 ± 11482	7599 ± 8191
HF (ms^2)	3432 ± 4775	3493 ± 5106
LF (ms^2)	2356 ± 4171	2078 ± 2124
Q ($l.min^{-1}$)	5.5 ± 1.0	5.7 ± 0.9
SV (ml)	91.8 ± 25.3	90.9 ± 21.2
TPR	15.9 ± 3.0	15.4 ± 2.8

Group mean values (± SD)

Table 3.5. Group mean values for resting heart rate (HR), baroreflex sensitivity (BRS), heart rate variability absolute measures (TP, HF, LF), heart rate variability relative measures (HFnu, LFnu, LF/HF), cardiac output (Q), stroke volume (SV), and total peripheral resistance (TPR) before and 4 hours after high intensity (95% HR_{peak}) isometric exercise condition.

Resting Measure	High intensity (95% HR_{peak})	
	Pre	Post
HR ($b.min^{-1}$)	54.8 ± 5.5	56.5 ± 8.6
BRS (ms/mmHg)	12.6 ± 2.5	12.9 ± 3.8
HFnu	63.1 ± 17.0	56.8 ± 19.2
LFnu	35.7 ± 17.0	41.3 ± 18.9
LF/HF	0.7 ± 0.5	1.0 ± 0.8
TP (ms^2)	9632 ± 9812	10489 ± 10591
HF (ms^2)	4402 ± 4441	3516 ± 3561
LF (ms^2)	2206 ± 2831	3129 ± 4881
Q ($l.min^{-1}$)	5.7 ± 0.8	5.8 ± 0.9
SV (ml)	94.1 ± 17.2	89.8 ± 20.6
TPR	15.3 ± 2.4	15.1 ± 2.5

Group mean values (± SD)

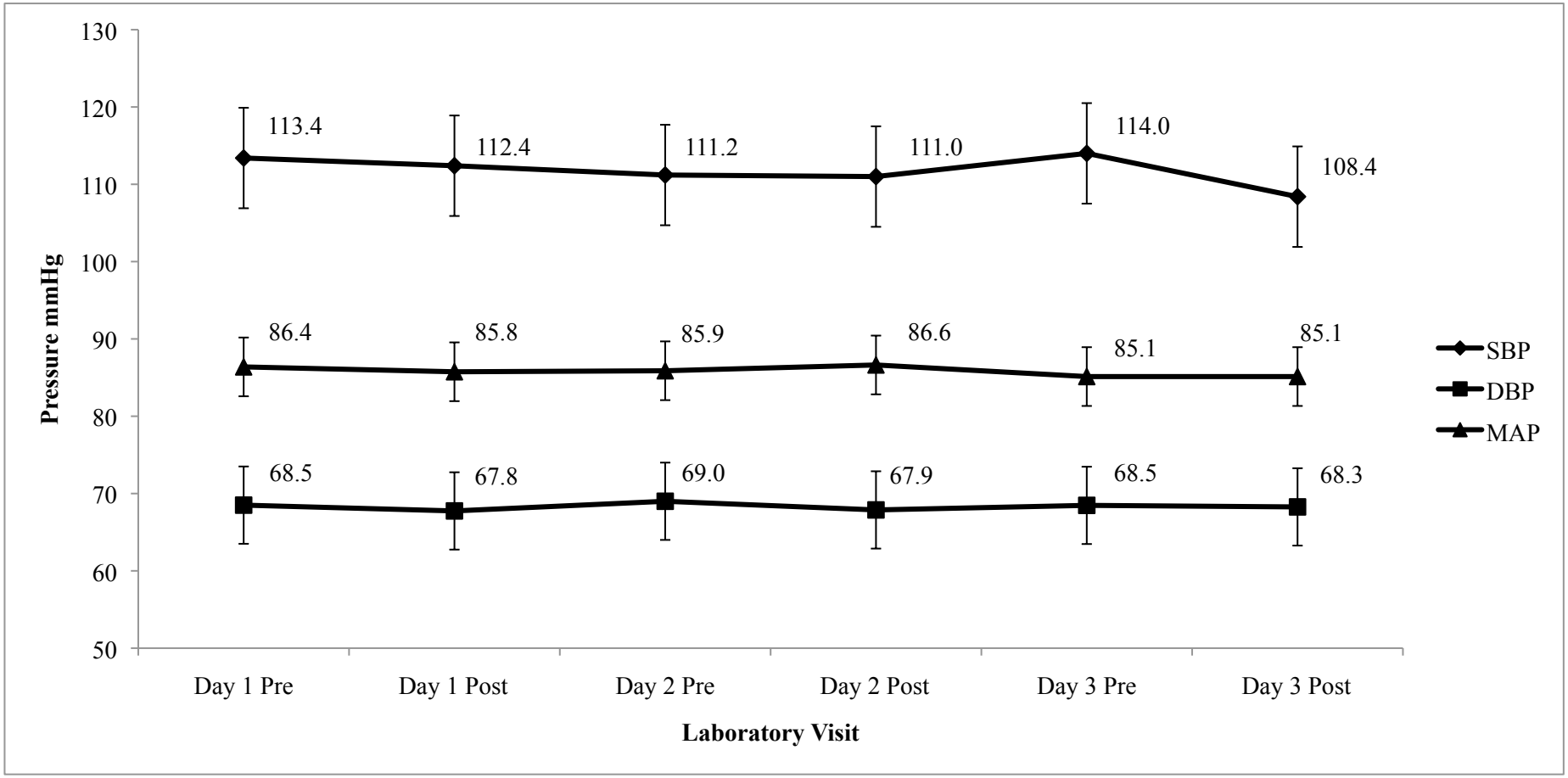

Figure 3.10. Systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressure stability throughout all resting measure protocols

Group mean percentage changes: Table 3.6 demonstrates SBP, DBP, and MAP, together with the relative HRV group mean changes from all three conditions, previously shown in tables 3.3, 3.4, and 3.5, as percentage of change values. Friedman's non-parametric test reported significant differences between the high intensity (95% HR_{peak}) and control condition for all relative HRV indices: HFnu ($p = 0.03$), HRV LFnu ($p < 0.001$), and HRV LF/HF ($p = 0.01$). All other resting measure Friedman's comparisons were non-significant ($p > 0.05$).

Figures 3.11, 3.12 and 3.13 show the group percentage change results for HRV HFnu, HRV LFnu, and HRV LF/HF variables respectively, pre and post all three test conditions.

Table 3.6. Percentage change values for systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressure, heart rate variability high frequency normalised units (HRV HFnu), heart rate variability low frequency normalised units (HRV LFnu), and heart rate variability low-to-high frequency ratio (HRV LF/HF), before and 4 hours after control condition, low intensity (75% HR_{peak}), and high intensity (95% HR_{peak}) isometric exercise conditions.

Resting measure	Control % change	Low % change	High % change
SBP	0.18	-1.33	-0.8
DBP	-0.29	-1.02	-1.64
MAP	-0.12	-0.58	0.24
HFnu	34.39	1.67	-9.98*
LFnu	-25.10	-4.48	15.69*
LF/HF	-50.00	-18.18	42.86*

* = significant difference to control condition ($p < 0.05$)

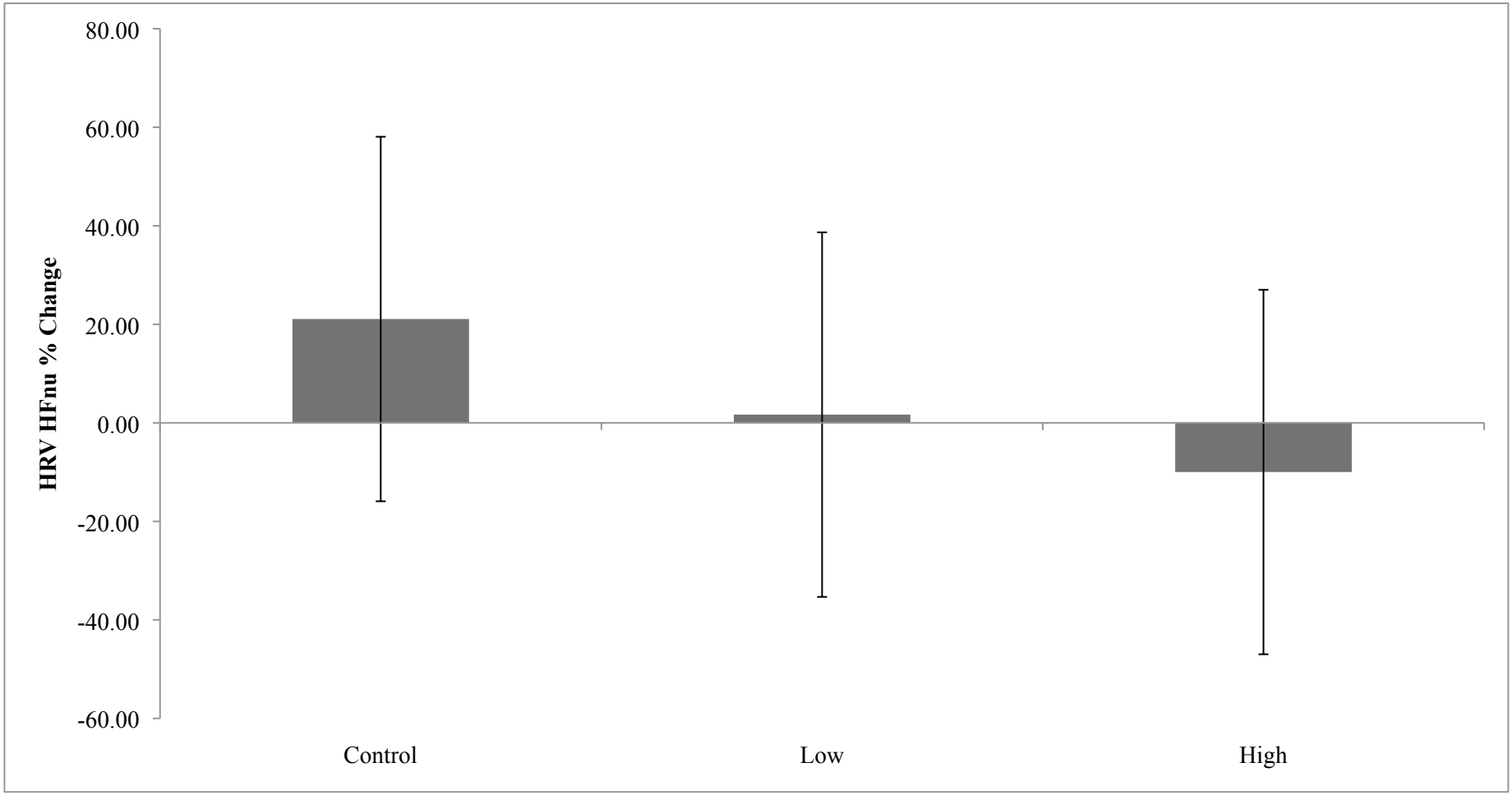

Figure 3.11. Heart rate variability high frequency normalised unit (HRV HFnu) group percentage 4 hour changes for the control condition, and low intensity (75% HR_{peak}), and high intensity (95% HR_{peak}) isometric exercise conditions

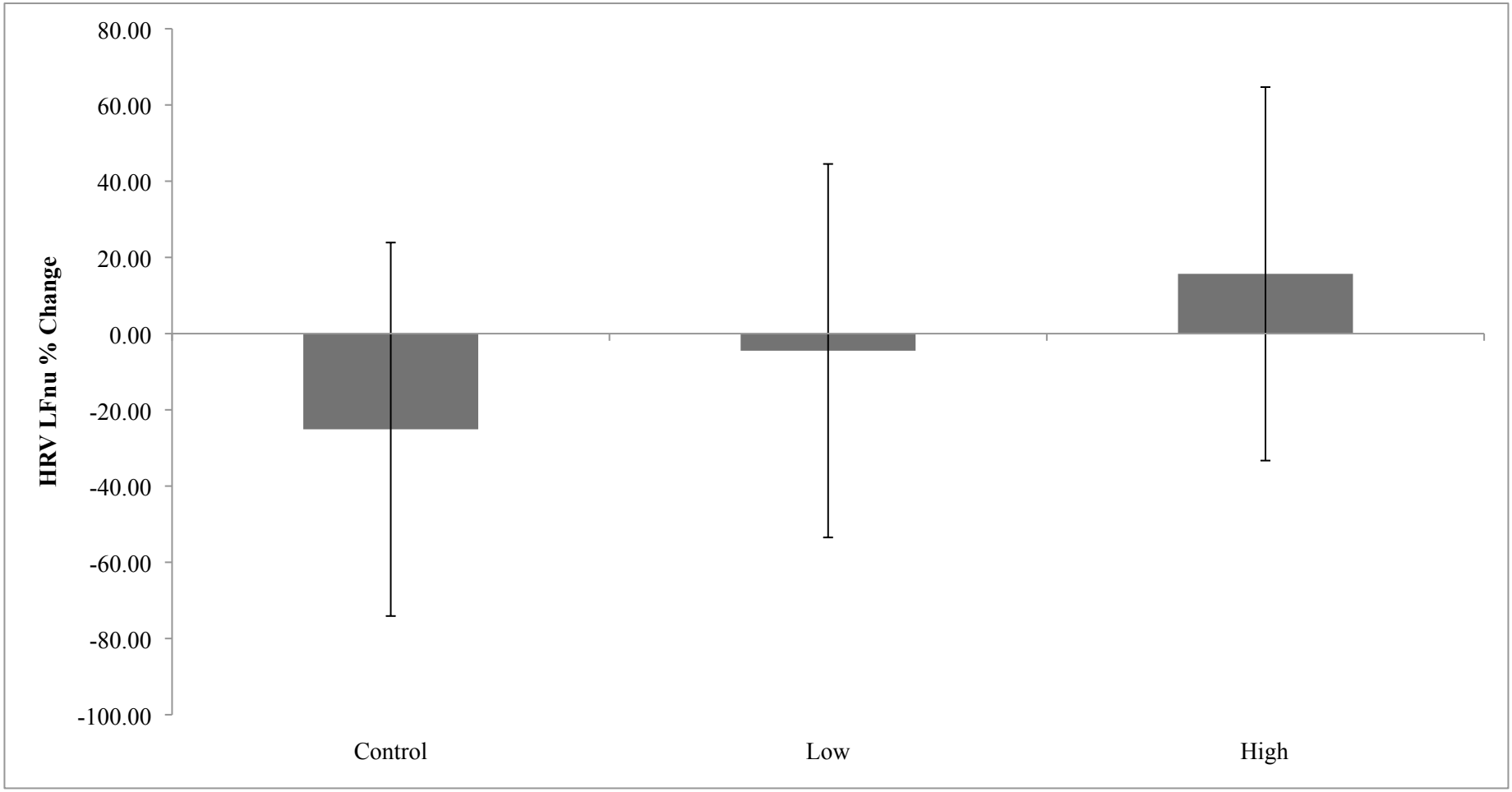

Figure 3.12. Heart rate variability low frequency normalised unit (HRV LFnu) group percentage 4 hour changes for the control condition, and low intensity (75% HR_{peak}), and high intensity (95% HR_{peak}) isometric exercise conditions

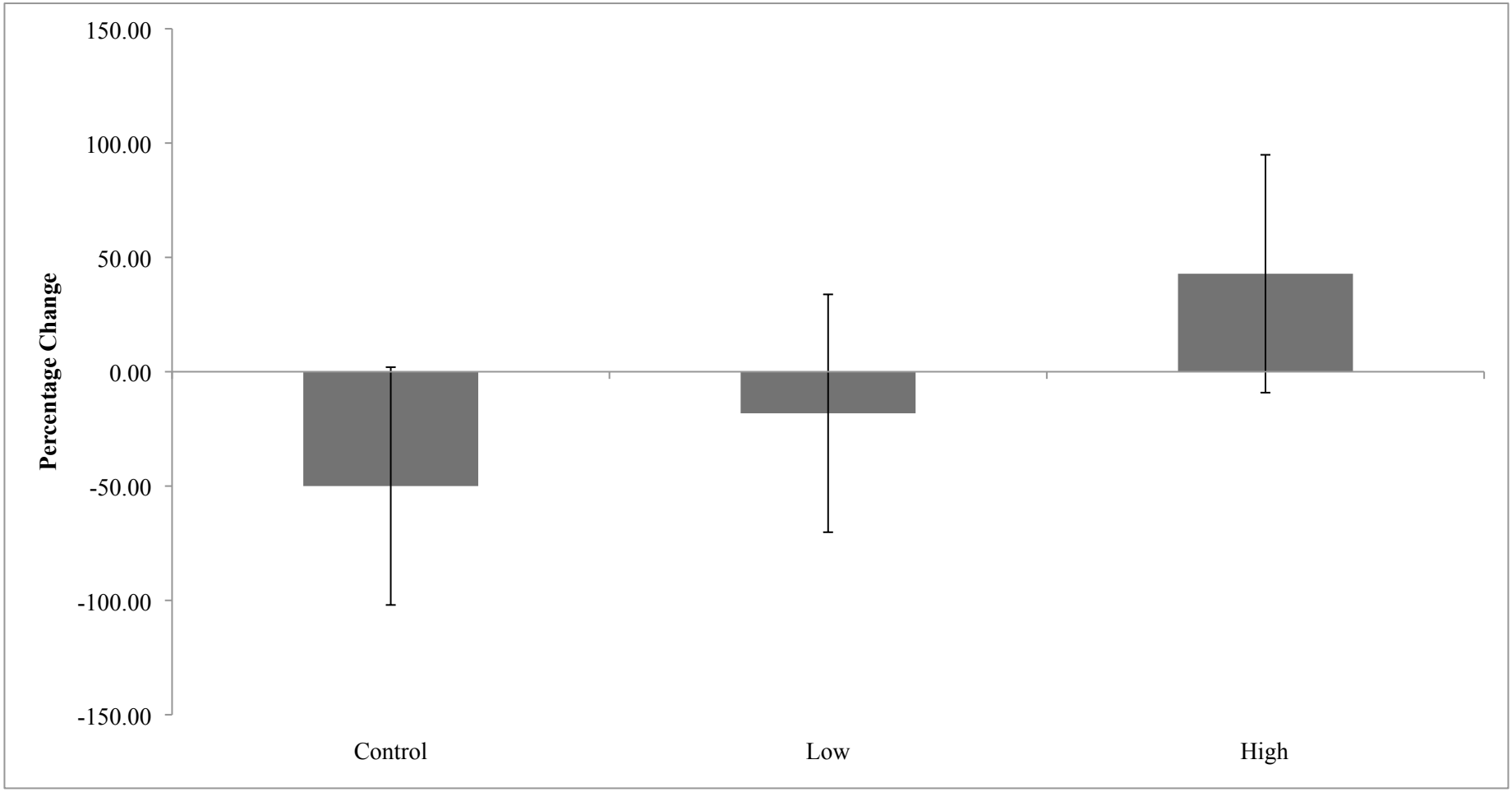

Figure 3.13. Heart rate variability low-to-high frequency ratio (HRV LF/HF) group percentage 4 hour changes for the control condition, and low intensity (75% HR_{peak}), and high intensity (95% HR_{peak}) isometric exercise conditions

3.9 Discussion

The results of this study suggest that a single session of bilateral-leg isometric exercise is not sufficient to have caused acute responses to either SBP, DBP, or MAP, 4 hours after the exercise stimulus. Further analysis of BP data showed that all resting SBP, DBP, and MAP measures remained unchanged throughout the entire 1 week study, providing further evidence that acute sessions of bilateral-leg isometric exercise do not affect resting BP from day-to-day. This data thereby shows no change in resting BP at 48 and 96 hours post-exercise. These are typical time periods between final training sessions and post-training resting BP measures in intervention studies (Howden *et al*. 2002; Wiles *et al*. 2010; Devereux *et al*. 2010). Referring back to Chapter 2, page 47, given the sample size power calculations of SBP, DBP, and MAP, it can be confidently concluded that the study had sufficient power to declare these non-significant BP acute responses.

Recent studies (Stewart *et al*. 2007; Millar *et al*. 2009) have reported significant immediate SBP hypotensive responses to bilateral IHG exercise, at 1 minute and 5 minutes after an IHG exercise stimulus respectively. The present results suggest that any significant immediate hypotensive SBP response to bilateral-leg isometric exercise has been recovered within a 4 hour period. This can be used to justify with some confidence that the reduced BP values reported in many intervention studies (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Wiles *et al*. 2010) were indeed chronic adaptations, as long as re-tested baseline measures are not taken within 4 hours of the final training session.

The results also suggest that within this participant sample, BP was not affected by circadian rhythms in any condition, with SBP, DBP, and MAP unchanged between morning and afternoon measurements. This supports previous research using larger participant numbers, reporting no influences of circadian rhythms on BP measurement from morning to afternoon (Mancia *et al*. 1983). This apposes previously held ideas that circadian rhythms shape the 24 hour BP profile (Millar-Craig *et al*. 1979; Rowlands *et al*. 1980).

The results do suggest however, that autonomic nervous function, as reflected by relative HRV components, are affected by a single session of bilateral-leg isometric exercise. This is evidenced by significant differences in the control condition of HRV indices of HFnu, LFnu, and the LF/HF ratio, which then appear to be either suppressed or reversed by the inclusion of an isometric exercise session. These HRV resting measure differences were not present in either of the two intensities of bilateral-leg isometric exercise. Group mean percentage changes also show that these same components of HRV are significantly affected 4 hours following the high intensity isometric exercise stimulus, when compared to the control condition. Furthermore, figures 3.11, 3.12, and 3.13 appear to show that, irrespective of significant statistical results, there appears to be a trend in these HRV variables, for the 4 hour response between control conditions and the two bilateral-leg isometric exercise stimuli (according to intensity). This may be reflective of the known proportional relationship between the pressor response (BP and HR) with isometric exercise intensity (Schibye *et al*. 1981; Wiles *et al*. 2007).

A recent study conducted by Millar *et al*. (2009) reported altered autonomic function 5 minutes after an IHG exercise stimulus. It would appear that changes in autonomic state may persist for longer than previously thought, certainly for bilateral-leg isometric exercise involving larger muscle groups than IHG exercise. The magnitude of this relative change appears to depend on isometric exercise intensity. However, given the reliability data for HRV measures (presented in Chapter 2, page 49), these trends must be viewed with caution, as the inherent variance in HRV measures make it difficult to draw definite conclusions, certainly when using limited participant sample numbers. This inherent variance is again evident in the standard deviation of measurement for both absolute and relative change HRV values.

Low frequency normalised units were significantly reduced (-12.0) in control condition, where the participants experienced no isometric exercise stimulus. This reduction appears to have been suppressed in the low intensity (75% HR_{peak}) condition (-2.0), and actually reversed (+5.6), although this change did not reach significance, in the high intensity (95% HR_{peak}) condition. This pattern is reversed for the high frequency normalised units (control = +11.8, low = +0.9, and high = -6.3). Naturally elevated levels of LF activity with subsequent decreased HF activity in the morning hours compared to the afternoon have been reported previously (Sandrone *et al*. 1994). These

seemingly innate alterations to the LFnu and HFnu spectral components of HRV in the control condition (morning to afternoon) will have been the basis for the significant reduction (1.4 ± 1.1 to 0.7 ± 0.6) in the LF/HF spectral components ratio. The more magnified changes in the control condition could be due to the sedentary nature of the control protocol, in between resting measure tests.

As discussed in Chapter 2, the LFnu component of HRV is thought to represent sympathetic activation, whilst HFnu is suggested to represent parasympathetic modulation (Malliani *et al*. 1991; Pagani *et al*. 1997; Cooley *et al*. 1998). It has been reported that an increase in the LFnu component is evident during isometric exercise exertion (Iellamo *et al*. 1999b), and that parasympathetic vagal activity (HFnu) is reduced at the same time (MacDonald *et al*. 1966; Nutter *et al*. 1972). This substantiates the present findings, where increasing intensities of bilateral-leg isometric exercise have caused relative increased levels of LFnu, or sympathetic activation, to be present 4 hours after the exercise stimulus, with opposing decreases in HFnu parasympathetic activity. Previously, it has been reported that increased LF activity is associated with elevated BP (Guzzetti *et al*. 1991), as was reported in Part A of this chapter, although the acute increase in LFnu spectral component of HRV after both low and high intensity bilateral-leg isometric exercise did not coincide with alterations to SBP, DBP, or MAP in this study.

3.10 Conclusion

In conclusion, a single session of bilateral-leg isometric exercise does not result in acute responses in either SBP, DBP, or MAP 4 hours after the exercise stimulus. However, acute responses in autonomic nervous function, as represented by HRV variables are evident, although these do not seemingly affect prevailing BP.

3.11 Summary of Chapter 3

The data presented within this chapter have shown that among a group of normotensive young males there was a strong association between components of autonomic nervous function and resting blood pressure. Another supposition that can be taken from this chapter is that an acute session of bilateral-leg isometric exercise affects these same measures of autonomic nervous function for a prolonged period during recovery (4 hours), although responses in BP did not coincide with this.

Chapter 4

The Effects of Isometric Exercise Training on Resting Blood Pressure: Cardiovascular Variables

4.1 Introduction

As reviewed in Chapter 1, isometric exercise training (IET) reduces resting blood pressure (BP) (Wiley *et al*. 1992; Ray and Carrasco 2000; Howden *et al*. 2002; McGowan *et al*. 2004; Taylor *et al*. 2003; Peters *et al*. 2006; Millar *et al*. 2007; Wiles *et al*. 2010), with the duration of training commonly performed over an 8 week period (Wiley *et al*. 1992; McGowan *et al*. 2004; Wiles *et al*. 2010). Two studies have shown significant reductions in resting systolic (SBP) or diastolic (DBP) blood pressure after interim measurements made at 3 weeks of a 5 week training program (Wiley *et al*. 1992; Howden *et al*. 2002). Neither of these studies reported concomitant reductions in mean arterial pressure (MAP), which is important given that it represents the average arterial pressure during each cardiac cycle (Oblouck 1987). Measurement of MAP would be useful in understanding the rapid adaptations that occur after isometric training, if measured in concert with cardiac output (Q) and total peripheral resistance (TPR). Chapter 1 also introduced a recent 8 week training study using electromyography (EMG) determined bilateral-leg training, which indicated that most of the overall reduction in resting SBP occurred in the first 4 weeks, although it did not reach significance (Wiles *et al*. 2010).

There is evidence for rapid adaptations in cardiovascular (CV) function after IET, as introduced in Chapter 1, pages 14-17. In a 4 week study by Green *et al*. (1994) vascular adaptations to this kind of exercise training were demonstrated, including changes in peak vasodilator capacity. In another study, increased hyperemic blood flow and reduced vascular resistance were shown after 1 week of isometric handgrip (IHG) training (Alomari and Welsch, 2007), although unfortunately no resting BP measurements were reported. There have not been any shorter IET studies that have attempted to show reductions in BP, especially MAP, in concert with measures of Q and TPR. The purpose of the present study was to investigate the effects of a 4 week bilateral-leg isometric exercise training programme, similar to that used by Wiles *et al.* (2010). In contrast to the method of Wiles *et al*. (2007) training will be performed at an EMG-determined intensity prescribed from data collected during a discontinuous incremental isometric exercise test. In addition to measurements of resting BP, other variables will be assessed pre- and post-training: baroreflex sensitivity (BRS), components of heart rate variability (HRV), Q, stroke volume (SV), and TPR. This was

to offer an explanation for any changes in resting BP, and the rationale for these CV variables was outlined in Chapter 1.

4.2 Methods

Thirteen healthy normotensive males (mean age 21.0 ± 2.4 years; body mass 78.1 ± 18.2 kg; height 177.1 ± 4.6 cm) completed the crossover design study. Fourteen participants originally volunteered to participate. One participant could not complete the study due to illness during the study. All participants were moderately physically active (8.7 hours at 7.3 multiple of BMR, per week). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed an exercise readiness questionnaire, and provided written informed consent, thereby adhering to the guidelines set by the 1964 Declaration of Helsinki. All participants conformed to the selection criteria detailed in Chapter 2, pages 24-25. All participants completed identical familiarization sessions. Crossover between exercise and control conditions was separated by 6 weeks, which was similar to that previously used (Howden *et al*. 2002). Figure 4.1 provides a schematic diagram of the study design.

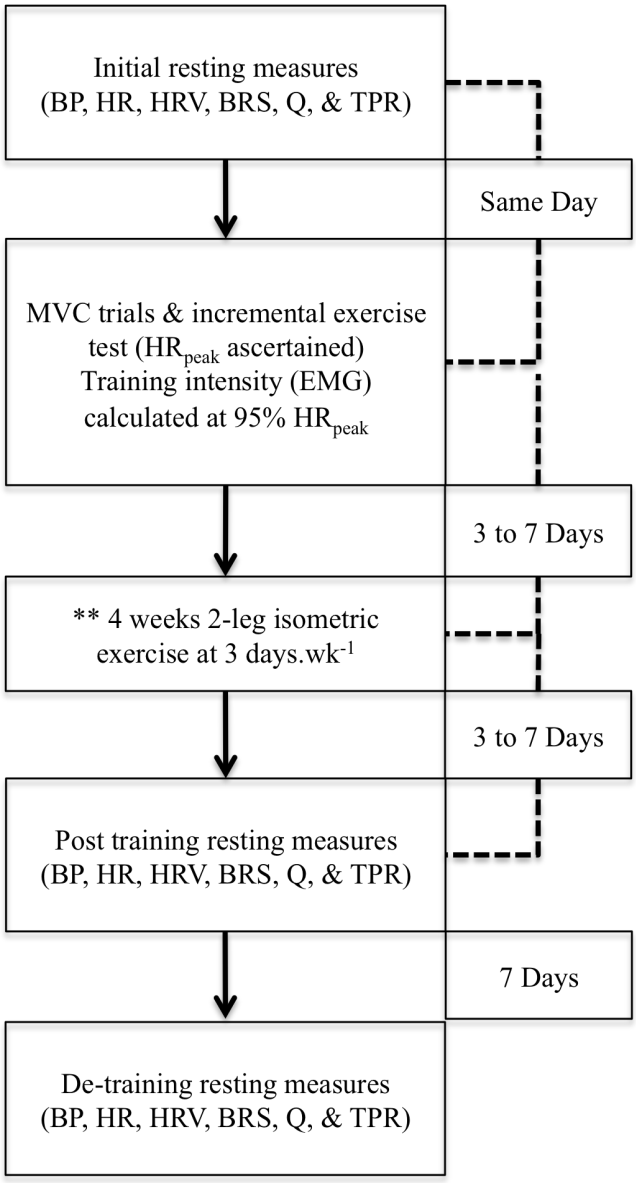

Figure 4.1. Schematic illustration of 4-week bilateral-leg isometric exercise study design. ** Participants, when in the control condition did not partake in this part of the study. Crossover between conditions was separated by 6 weeks

4.2.1 Equipment

Isometric exercise: All tests were conducted using a Biodex System 3 Pro, isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY), which is described in detail in Chapter 2, pages 25-26. The Biodex was fitted with a modified hip attachment that was inserted into the standard knee attachment, to allow for bilateral-leg extension exercise to be performed. Participants sat in the dynamometer in an upright position, with 90 degrees of flexion at the hip and with the thighs supported.

Electromyography recording: A dual bio-amplifier was used to enable surface EMG measurement from both vastus lateralis', to give a combined average, along with the 16-channel chart recorder. This is described in detail in Chapter 2, pages 27-29.

Exercising heart rate: Heart rate was recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments was used. Heart rate was sampled at a frequency of 1000 Hz.

Resting heart rate and heart rate variability: Was recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments was used. Heart rate was sampled at a frequency of 1000 Hz. Both were recorded after a standardized resting period of 15 minutes in a supine position in a silent, dimly lit room. HRV was measured using the parametric frequency domain method whereby power spectral density analysis demonstrates how power distributes as a function of frequency, as described in detail in Chapter 2, pages 29-34.

Arterial blood pressure: Resting BP measurements were made using an automated BP monitor (Dinamap Pro 300 Critikon, GEMedical Systems, Slough, Berks, UK). Blood pressure was measured after 15 minutes rest in a seated position. The lowest of three measures, separated by 60 seconds, was used for analysis. The process of automated BP measurement is described in detail in Chapter 2, pages 34-36.

Cardiac Output, Stroke Volume, and Total Peripheral Resistance: The Innocor non-invasive Q monitor (Innovision A/S, Odense S, Denmark) was used to measure SV and Q at rest via inert gas re-breathing. The relative levels of two inhaled inert gases of differing solubility in blood are measured, over four to five respirations. The average value of two measures was used for analysis. Measures were separated by 5 minutes to ensure removal of previously inhaled gases. Participants sat in an upright position to prevent any interference with normal breathing (Damgaard and Norsk 2005). Cardiac

output and SV measurement processes are discussed in detail in Chapter 2, pages 39-44. TPR was subsequently calculated using MAP from Dinamap BP readings, and Q from the Innocor device: TPR = MAP/Q (Turner *et al*. 1996; Walker *et al*. 1992).

Baroreflex Sensitivity: Resting BRS was measured non-invasively by correlating BP variance with HR variance, using a Finometer (Finapres, TNO Instruments, Amsterdam, Netherlands). The Finometer allows for measurement of constant beat-to-beat and inter-beat BP changes. Resting BRS is calculated accurately (Gizdulich *et al*. 1996), by the regression slope of the beat-to-beat and inter-beat variables. In order for phase shift calculations of BRS within Finometer software to be made, three consecutive R-R intervals in the same direction are necessary. As BRS varies with time, a recording of ten minutes is advised (Chesterton *et al*. 2005), and so was administered in this study. BRS measurement processes are discussed in detail in Chapter 2, pages 36-39.

4.2.2 Procedures

Maximal voluntary contraction and EMG_{peak}: Were determined prior to each discontinuous incremental test. Participants performed three maximal effort isometric bilateral-leg extensions against the fixed dynamometer arm. Each MVC was terminated after 2 seconds, and each was interspersed by a 120 second rest period. The isometric leg extension exercise was performed at a knee angle of 90 degrees (180 degrees corresponds to full knee extension) on the isokinetic dynamometer (Alkner *et al*. 2000). EMG_{peak} was determined from the MVC producing the highest torque and was established from the mean of the EMG activity recorded 0.25 seconds immediately prior to maximum torque (Wiles *et al*. 2007). The EMG_{peak} was then used to create $\%EMG_{peak}$ 'targets' for the subsequent incremental exercise test.

Discontinuous incremental isometric exercise test: Participants underwent a discontinuous incremental isometric exercise tests, after familiarization, both pre- and post-training, immediately after the recording of resting measures. Participants began bilateral-leg isometric exercise at 10% EMG_{peak} for 2 minutes. Thereafter, the intensity increased in 5% increments, interspersed by 5 minute rest periods, to volitional fatigue (or failure to maintain EMG signal within +/- 5% of the 'target' value). EMG was

monitored and recorded. Average values (HR) for the final 60 seconds of each increment were used for analysis.

Training Sessions: Participants began training at a participant-specific EMG 'target' that equated to 95% HR_{peak}, interpolated from the regression line of HR versus EMG ascertained during the initial incremental test. Participants trained 3 $days.wk^{-1}$ for 4 weeks. Training sessions were separated by at least 24 hours. Training intensity (EMG *mV*) was updated during training if mean of session HR deviated from the target HR (95% HR_{peak}) by more than ±5%, after each training session if necessary. Participants performed four bouts involving 2 minutes of isometric exercise separated by 3 minute rest periods. Electromyography, HR, and torque were measured and recorded continuously throughout all 12 training sessions. Participants were instructed to breathe at a normal rhythm and depth at all times to avoid Valsalva manoeuvres. Training intensity set and maintained at constant EMG allows for a relatively stable HR response to isometric exercise, whereas constant torque results in HR drift during an exercise bout.

Resting Measures: Resting SBP, DBP, MAP, HR, HRV, Q, SV, TPR, and BRS were measured both prior to and following the 4 week training period. Participants fasted for 4 hours, and abstained from caffeine and alcohol for at least 12 hours prior to testing procedures (Jáuregui-Renaud *et al*. 2001), and were instructed to maintain physical and dietary activity behaviours prior to each visit. All testing procedures were conducted prior to actual data collection to allow for participant familiarization. A final de-training resting measures session was administered 7 days following the post-training measures to investigate the effects of a 1 week de-training period.

4.2.3 Data Analyses

All data were assessed for conformity with parametric assumptions (Field 2000). Changes in BP have been associated with initial values (Millar *et al*. 2007), so analysis of covariance (ANCOVA) was used to assess whether change scores were influenced by initial baseline values. Pre- post BP differences, and differences in BP changes between the experimental and control condition were assessed using 2-way ANOVA within the ANCOVA test. An alpha level of < 0.05 was set as the threshold for statistical

significance, and the Bonferroni post-hoc procedure was used to explore any significant differences detected. Pearson product moment correlation was administered to assess for linear dependence between changes in MAP with Q and TPR, irrespective of significant differences pre- to post-intervention.

4.3 Results

Resting blood pressure: Four weeks of bilateral-leg isometric exercise resulted in a reduction in resting SBP (-4.9 ± 5.8 mmHg, $p = 0.01$), with no differences in the control data ($P = 0.92$), with ten participants out of thirteen showing reduced SBP after training. Resting DBP dropped (-2.8 ± 3.2 mmHg, $p = 0.01$), with no differences in the control data ($p = 0.13$), with ten participants out of thirteen showing reduced DBP after training (nine of which also showed reduced SBP). Resting MAP was reduced pre to post training (-2.8 ± 2.2, $p = 0.001$), with no differences in the control data ($p = 0.59$), with twelve participants out of thirteen showing reduced MAP after training. ANOVA showed that SBP, DBP and MAP changes were significantly different between experimental and control conditions ($p = 0.04$, 0.007, and 0.009 respectively). Table 4.1 demonstrates group mean values at the start and end of the isometric training. There was no change in body mass over the duration of the study (78.1 ± 18.2 to 78.1 ± 18.1 kg, $p = 0.80$).

Table 4.1. Group mean values for systolic (SBP), diastolic (DBP) and mean arterial (MAP) pressure pre- and post-training and pre- and post-control.

Condition	BP component	Pre (mmHg)	Post (mmHg)
Training	SBP	119.9 ± 11.6	115.0 ± 11.5*
	DBP	69.0 ± 4.4	66.2 ± 5.0*
	MAP	89.2 ± 4.7	86.4 ± 5.3*
Control	SBP	119.5 ± 11.8	119.4 ± 12.3
	DBP	66.4 ± 5.3	67.7 ± 3.8
	MAP	87.9 ± 5.4	88.3 ± 6.1

Group mean values (± SD)

* = significant difference to pre scores, at $p < 0.05$

De-Training: Follow-up resting BP measures (SBP = 119.0 ± 11.2 mmHg, DBP = 67.9 ± 4.9 mmHg, and MAP = 88.4 ± 5.4 mmHg), 1 week after post-intervention measures, were significantly higher than the reduced resting SBP (+4.0, $p = 0.03$), DBP (+1.9, $p = 0.01$) and MAP (+2.0, $p = 0.02$) evident after the training intervention period. The de-training values were not significantly different to initial pre-training baseline BP levels (SBP = 119.9 ± 11.6 vs. 119.0 ± 11.2 mmHg, $p = 0.64$; DBP = 69.0 ± 4.4 vs. 67.9 ± 4.9 mmHg, $p = 0.28$; and MAP = 89.2 ± 4.7 vs. 88.4 ± 5.6 mmHg, $p = 0.37$). Figure 4.2 illustrates the SBP, DBP, and MAP changes between pre-intervention, post-intervention, and de-training measures.

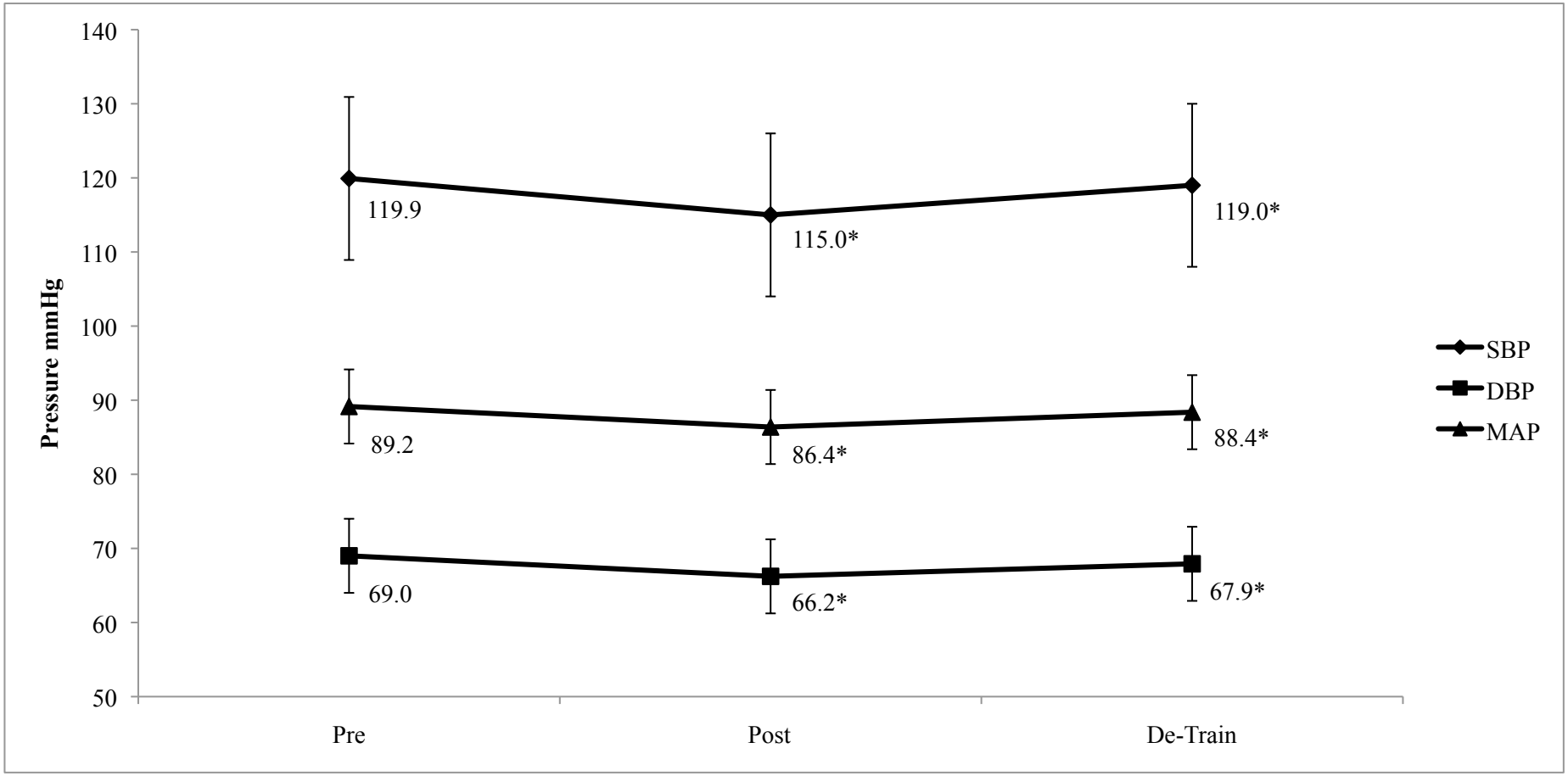

Figure 4.2. Group mean systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures pre-training, post 4 week isometric training intervention, and 1 after week de-training. * = significant ($p < 0.05$) difference to preceding group mean value

The covariate of initial baseline values did not influence change scores for SBP ($p = 0.35$) or MAP ($p = 0.87$). However, baseline DBP values predicted DBP change scores ($p = 0.02$).

Resting Measures: There was a reduction in resting HR (65 ± 11 $b.min^{-1}$, to 58 ± 6 $b.min^{-1}$, $p = 0.02$), and this was also evident in control data (62 ± 6 $b.min^{-1}$, to 58 ± 4 $b.min^{-1}$, $p = 0.005$). No significant differences were observed in any other measured variable after 4 weeks of training. See Table 4.2 for group data. Changes in MAP were not correlated with insignificant changes in Q ($r = 0.29$, $p = 0.33$) or TPR ($r = -0.04$, $p = 0.91$). See figures 4.3 and 4.4 for scatter plots of these relationships.

Table 4.2. Group mean values for cardiac output (Q), stroke volume (SV), total peripheral resistance (TPR) and baroreflex sensitivity (BRS) and relative (low frequency normalized units = LFnu, high frequency normalized units = HFnu, and low to high frequency ratio = LF/HF) and absolute (total power = TP, low frequency power = LF, and high frequency power = HF) components of heart rate variability (HRV).

	Pre (mean ± S.D)	Post (mean ± S.D)	Significance, p =
HFnu	57.25 ± 18.37	58.59 ± 18.07	0.69
LFnu	38.60 ± 18.76	38.96 ± 18.41	0.92
LF/HF	0.91 ± 0.93	0.83 ± 0.60	0.76
TP (ms^2)	4694 ± 3908	8243 ± 12090	0.32
LF (ms^2)	961 ± 805	2058 ± 3334	0.27
HF (ms^2)	2288 ± 2838	2926 ± 3662	0.40
Q ($l.min^{-1}$)	6.94 ± 1.84	6.73 ± 1.38	0.43
SV (ml)	107.27 ± 37.05	107.08 ± 30.56	0.97
TPR	13.68 ± 3.70	13.35 ± 2.79	0.57
BRS (ms/mmHg)	13.49 ± 6.52	15.22 ± 6.06	0.26

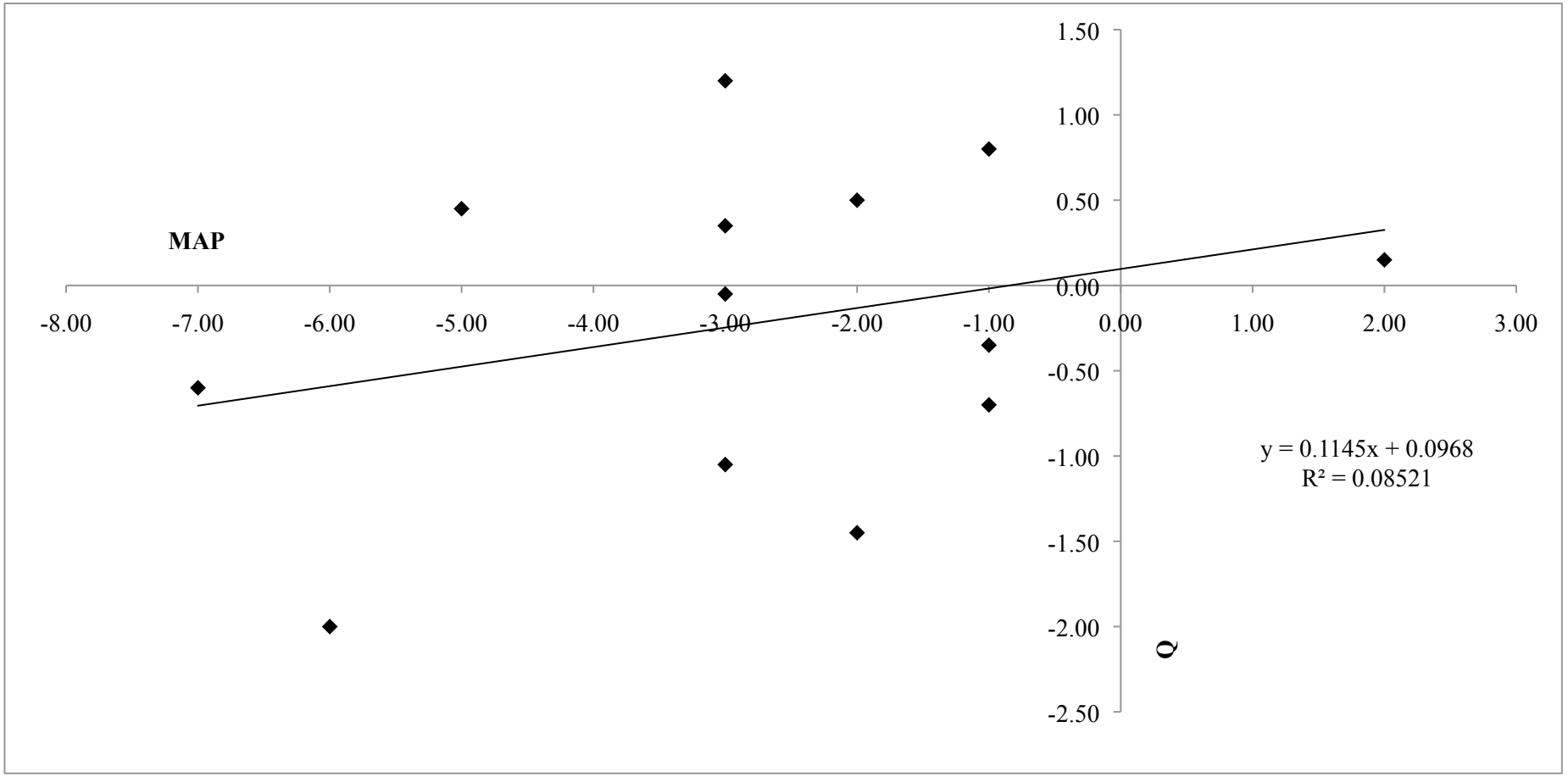

Figure 4.3. Scatter plot of individual pre to post changes in mean arterial pressure (MAP) versus changes in cardiac output (Q) in the isometric training group

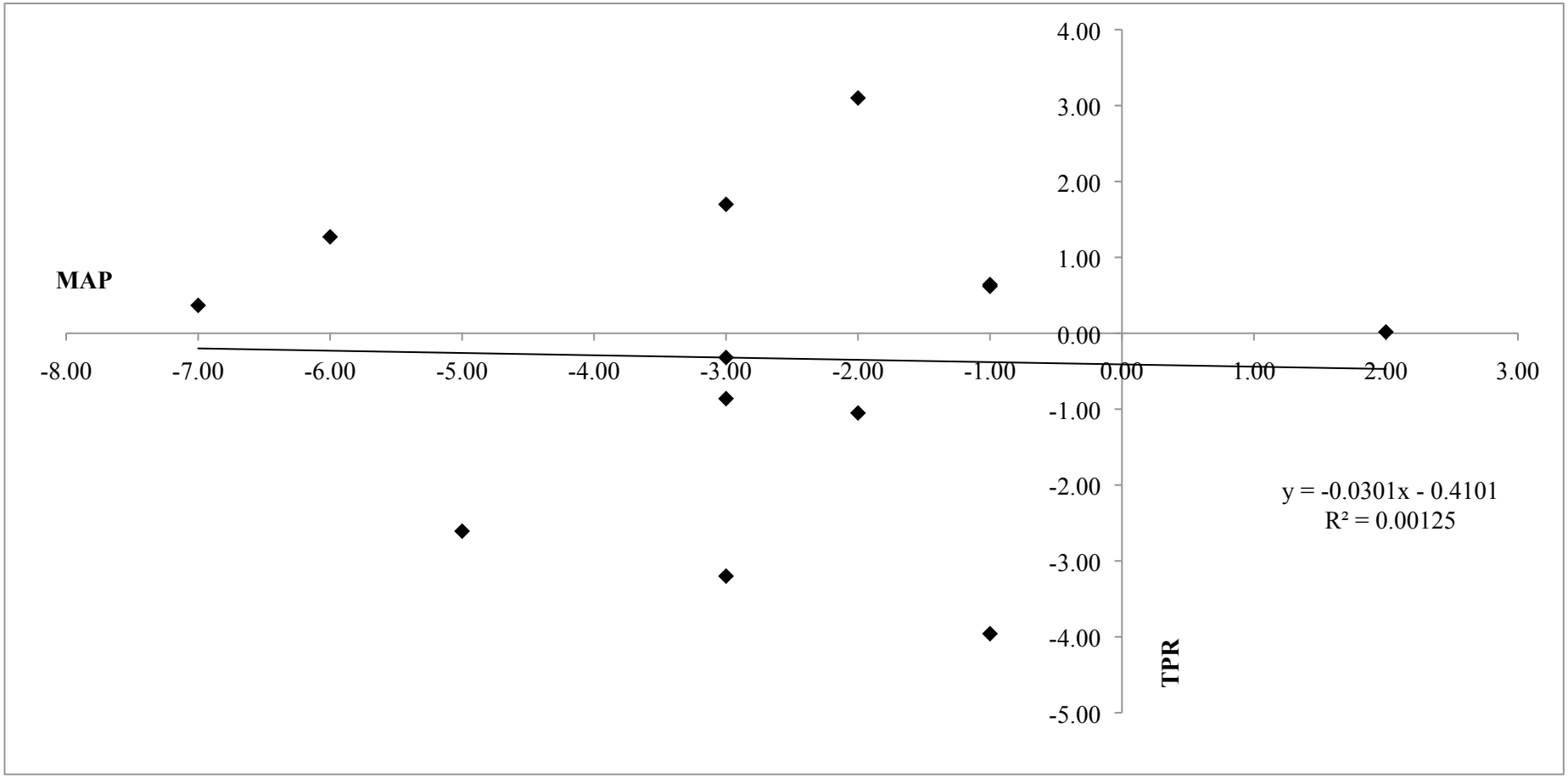

Figure 4.4**.** Scatter plot of individual pre to post changes in mean arterial pressure (MAP) versus changes in total peripheral resistance (TPR) in the isometric training group

4.4 Discussion

This study showed that 4 weeks of bilateral-leg isometric exercise for 3 days.wk^{-1}, performed at an EMG equating to 95% HR_{peak}, caused reductions in resting SBP (-4.9 mmHg), DBP (-2.8 mmHg), and MAP (-2.8 mmHg). This is the shortest bilateral-leg isometric exercise training study to have shown reductions in all resting BP components, SBP, DBP and MAP. Given the reliability data presented for SBP, DBP, and MAP in Chapter 2, page 47, it can be concluded with reasonable confidence that the BP reductions reported here were indeed physiological. This is also the case for the subsequent BP increases seen after the 7 day detraining period. For instance, with 13 participants in this two-way crossover study, the probability is 80% that the study would detect a SBP difference (at p = 0.05 significance level), if the true difference between SBP measures was 2.21 mmHg. This is based on the assumption of the unbiased typical error of the SBP variable is as presented in reliability testing Chapter 2, pages 45-47.

Decreases in SBP (up to -10.0 mmHg) have been shown at interim points during longer training programmes (after 3 weeks of a 5 week programme) using bilateral-leg (Howden *et al*. 2002), and handgrip exercise (Wiley *et al*. 1992) at a higher relative intensity (50% MVC) and frequency (5 days.wk^{-1}). Wiles *et al*. (2010) showed that bilateral-leg isometric exercise can cause reductions in SBP, DBP, and MAP after 8 weeks of training. This was similar to the changes reported in studies that have utilized IHG or forearm exercise for 8 weeks (Wiley *et al*. 1992; McGowan *et al*. 2004) and 10 weeks (Taylor *et al*. 2003). Ray and Carrasco (2000) reported reductions in DBP and MAP, but not SBP, after 5 weeks of IHG. However, that study used a training frequency of 4 days.wk^{-1}.

In the present study and that of Wiles *et al*. (2010), exercise intensity was prescribed as an EMG 'target' value, which related to a percentage of the highest HR achieved in the initial incremental exercise test (HR_{peak}). Howden *et al*. (2002) used percentage of maximal voluntary contraction (% MVC). That study used an exercise intensity of 20% MVC, and reported reductions in SBP after 3 weeks. By averaging torque produced in all training sessions for all participants against initial MVC trials in J.D Wiles' study (personal communication), the exercise intensity was 22% MVC. Wiles *et al*. (2010)

reported small but insignificant reductions after 4 weeks, but significant reductions in SBP, DBP, and MAP after 8 weeks. If torque values are averaged for all training sessions in the present study, the comparative exercise intensity would be 24% MVC.

Therefore, although the present study and that of Wiles *et al*. (2010) both prescribed training at 95% HR_{peak}, there appears to be a difference in training intensity when it is expressed as average torque (22 vs. 24% MVC). These differences are probably due to the different incremental test formats that were used. It is likely that the inclusion of rest periods between exercise increments in the discontinuous test, allowed participants to produce moderately more torque for a given HR. This would mean that, when EMG 'target' values were prescribed according to $\%HR_{peak}$, participants would be exercising at higher torques than those expected from a continuous incremental exercise test.

The exercise intensities of bilateral-leg studies (20% to 24% MVC) appear to be lower than IHG studies (30% to 50% MVC). Differences in training frequency are also often evident (3 $days.wk^{-1}$ bilateral-leg, and up to 5 $days.wk^{-1}$ for IHG). The results of these studies and the present results, when taken together, appear to suggest that the rate of reduction of resting BP after isometric training may also be related to the mode of training. Muscle mass is an important factor in the magnitude of the pressor response to isometric exercise (Iellamo *et al*. 1999a; McCloskey and Mitchell, 1972; Mitchell *et al*. 1980). Seals *et al*. (1983) and Seals (1989) reported an increase in muscle sympathetic nerve activity (MSNA) with corresponding increases in active muscle mass. Increased MSNA is reported to contribute to the increases in BP and HR during exercise (Mitchell, 1990; Mitchell and Schmidt, 1983; Rowell and O'Leary, 1990) and thus to the pressor response. For a given duration of isometric exercise, an individual performing bilateral-leg isometric exercise is likely to experience either a greater or accelerated pressor response during training than an individual who performs IHG, given the increased MSNA arising from the respective muscle masses of each mode of exercise (Seals *et al*. 1983; Seals, 1989).

The present data has not offered any explanations for the underlying physiological mechanisms responsible for the adaptations to resting BP after 4 weeks of bilateral-leg isometric exercise. There were no significant alterations to any of BRS, HRV, Q, SV, or

TPR. Of course, it is understood that in order for MAP to change, Q or TPR, (or both) must change (Walker *et al*. 1992; Turner *et al*. 1996). The only explanation for the finding that neither changed significantly, is that the measurement of variables such as Q may be hampered by high variability (Damgaard and Norsk, 2005; Pemberton *et al*. 2005). Measurement of Q by re-breathing is indirect and can exhibit higher variability when performed at rest compared to during exercise (Vanhees *et al*. 2000). Furthermore, the measure of TPR was derived from this indirect measure of Q using the formula MAP/Q.

In addition, HRV (Eckberg, 1997) and BRS (Lipman *et al*. 2003) are known to have measurement limitations. Therefore, the absence of corresponding changes in Q or TPR could be a reflection of their lack of sensitivity in detecting small changes after IET. It can also be seen in Chapter 2, page 47, that the reliability data for BRS, HRV, Q, SV, and TPR were inherently more variant than SBP, DBP, or MAP. The associations between autonomic nervous system indicators of relative HRV components with BP (Chapter 3, Part A) and differences following a single session of isometric exercise (Chapter 3, Part B) are not implicated in this study, which may be due to the vastly increased time period between recorded resting measures (Sandercock *et al*. 2005).

The present data also shows that the reductions in resting BP were reversed to pre-training baseline levels within 7 days of the post-training reduced measures. This time course of adaptation reversal is shorter than previously reported IET studies. Previously, Wiley *et al*. (1992) showed that reduced SBP (-12.5 mmHg) and DBP (-14.9 mmHg) following 5 weeks of IHG training gradually returned to pre-training baseline levels over a 5 week period. Wiley *et al*. (1992) did not report significantly increased detraining BP values, compared to reduced post-training measures, until 14 days after the post-training reductions.

More recently, Howden *et al*. (2002) reported SBP reductions (-10.0 mmHg) following 5 weeks of bilateral-leg IET had disappeared within 10 days of post-training reduced SBP measures. However, it should be noted that the SBP and DBP reductions in those studies were far greater than those reported here (-4.9 and -2.8 mmHg respectively). Therefore, given the differences in magnitude of BP changes, perhaps the most prudent

conclusion to draw from the present data is that BP reduction losses are swift, and that reversal to pre-intervention values may be dependant on the extent to which BP was reduced by the IET intervention.

4.5 Conclusion

The data presented in this chapter have shown that SBP, DBP, and MAP adaptations are evident after 4 weeks of bilateral-leg isometric exercise. There are three inferences from the data in this chapter: (1) cardiovascular variables such as Q, SV, TPR, BRS, and HRV do not play a role in the observed BP adaptations to IET, or (2) that there is an inherent lack of sensitivity, using the available methods, in detecting small changes in the measurement of these variables after IET. In addition, the transient changes in HRV measures seen after a single session of isometric exercise (Chapter 3, Part B) are not evident at rest after 4 weeks of training. Finally, (3) the evident reductions in SBP, DBP, and MAP after 4 weeks of bilateral leg IET are reversed to pre-baseline measures within 7 days of detraining, and that the time course for BP reduction losses are dependant on the magnitude of BP change.

Chapter 5

The Effects of Isometric Exercise Training on Resting Blood Pressure:

(A). Neuromuscular Variables

(B). Metabolic Variables

(A) Relationships between markers of isometric training intensity and reductions in resting blood pressure

5.1 Introduction

Chapter 4 has demonstrated that 4 weeks of bilateral-leg isometric exercise training (IET) is sufficient to bring about significant reductions in resting blood pressure (BP). However, the role of IET intensity in the reduction of resting BP after training is unclear. As previously introduced in Chapter 1, the effects of different intensities of isometric exercise on the training-induced reduction in resting BP have been explored (Wiles *et al*. 2010). This was done using a novel method for prescribing intensity, based upon a training heart rate (HR) that is relative to an initial incremental test peak HR (%HR_{peak}). Their findings were that training at intensities equivalent to either higher or lower relative heart rates caused similar reductions in resting BP after 8 weeks of training. However, in the higher intensity group the reductions had occurred mostly by mid-point measurements at 4 weeks. Given these findings, it appears that intensity, as reflected in the HR during IET, might be important in the training-induced reductions in resting BP.

Previously, IET intensity has been determined by isometric torque relative to maximum voluntary contraction (%MVC), without reporting heart rates. In addition to these two previously used markers of intensity (%HR_{peak} and %MVC), another method which involves an initial incremental isometric exercise test to volitional fatigue (Wiles *et al*. 2007), offers other markers that may help to elucidate the role that IET intensity plays in reducing resting BP. Average 2min-torque (N·m) can be calculated for each stage of this incremental test and the highest or peak mean 2min-torque (2min-$torque_{peak}$) can be identified for each individual. Since this peak is determined at volitional exhaustion and is the highest torque that can be sustained for 2 minutes, it is arguably a marker of local muscle fatigue (Start and Holmes, 1963; Fallentin *et al*. 1993; Garland *et al*. 1994; Westgaard and De Luca, 1999; Carpentier *et al*. 2001), and is determined in a similar way to markers such as maximal running speed, commonly identified during incremental tests using other types of exercise (Hambrecht *et al*. 1995; Weston *et al*. 1997).

Unlike most other IET studies, the incremental test prescription based method (Wiles *et al*. 2007; Wiles *et al*. 2010) offers a fourth marker of training intensity because it uses electromyographic (EMG) activity as the target against which participants attempt to maintain the exercise intensity during training. Changes in EMG activity have been used as an index of neuromuscular (NM) fatigue, being characterised during exercise by spectral compression of the EMG signal frequency to lower frequencies (Viitasalo and Komi, 1977; Petrofsky, 1979; Petrofsky *et al*. 1982) and by increased EMG signal amplitude (Moritani *et al*. 1986; Petrofsky, 1979; Petrofsky *et al*. 1982).

The increased EMG signal amplitude evident at the onset of fatigue is suggested to occur from either increased spatial or temporal motor unit recruitment (Vredenbregt and Rau, 1973; Moritani *et al*. 1982), or increased spatial-temporal motor unit recruitment in concert with increased motor unit synchronisation (Person and Mishin 1964; Person and Kudina 1968). The decreased EMG signal frequency at the onset of fatigue (spectral compression) is said to arise principally from decreased conduction velocities of action potentials (Bigland-Ritchie *et al*. 1981; Sadoyama *et al*. 1983). At the point where NM fatigue begins and then increases, force output typically reduces due to the less efficient excitation-contraction coupling process (Moritani *et al*. 1986; Moritani *et al*. 1993; St Clair Gibson *et al*. 2001). Therefore, changes in EMG amplitude and frequency could be used as markers of intensity during IET, by reflecting how much local muscle fatigue is induced, and could be used to investigate the role of fatigue in the training-induced BP reductions.

There are no published studies that have explored the relationship between markers of IET intensity and reductions in resting BP after training. The purpose of the present study was to explore the relationships between training intensity, expressed as $\%HR_{peak}$, $\%EMG_{peak}$, , $\%2min\ torque_{peak}$, %MVC, ΔEMG_{amp} or ΔEMG_{freq} and reductions in resting systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressure after 4 weeks of bilateral-leg IET. This study aimed to identify the markers that best reflect the association between IET intensity and reductions in resting BP.

5.2 Methods

The same thirteen healthy normotensive males as in Chapter 4 (mean age 21.0 ± 2.4 years; body mass 78.1 ± 18.2 kg; height 177.1 ± 4.6 cm) volunteered to participate in a crossover design study. All participants were moderately physically active (8.7 hours at 7.3 multiple of BMR, per week). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed an exercise readiness questionnaire, and provided written informed consent, thereby adhering to the guidelines set by the 1964 Declaration of Helsinki. All participants conformed to the selection criteria outlined in Chapter 2, pages 24-25. Participants fasted for 4 hours, and abstained from caffeine and alcohol for at least 12 hours prior to testing procedures (Jáuregui-Renaud *et al*. 2001), and were instructed to maintain physical and dietary activity behaviours prior to each visit. Crossover between exercise and control conditions was separated by 6 weeks, as previously used (Howden *et al*. 2002). All participants completed identical familiarization sessions.

5.2.1 Equipment

Isokinetic Dynamometer: All tests were conducted using a Biodex System 3 Pro, isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY), which is described in detail in Chapter 2, pages 25-26. The Biodex was fitted with a modified hip attachment that was inserted into the standard knee attachment, to allow for bilateral-leg extension exercise to be performed. Participants sat in the dynamometer in an upright position, with 90 degrees of flexion at the hip and with the thighs supported.

Electromyography recording: A dual bio-amplifier was used to enable surface EMG measurement from both vastus lateralis', to give a combined average, along with the 16-channel chart recorder. This is described in detail in Chapter 2, pages 27-29.

Exercising heart rate: Heart rate was recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu

Inc., U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments was used. Heart rate was sampled at a frequency of 1000 Hz.

Arterial blood pressure: Resting BP measurements were made using an automated BP monitor (Dinamap Pro 300 Critikon, GEMedical Systems, Slough, Berks, UK). Blood pressure was measured after 15 minutes rest in a seated position. The lowest of three measures, separated by 60 seconds, was used for analysis. The process of automated BP measurement is described in detail in Chapter 2, pages 34-36.

5.2.2 Procedures

Maximal voluntary contraction and EMG_{peak}: Were determined prior to each discontinuous incremental test. Participants performed three maximal effort isometric bilateral-leg extensions against the fixed dynamometer arm. Each MVC was terminated after 2 seconds, and each was interspersed by a 120 second rest period. The isometric leg extension exercise was performed at a knee angle of 90 degrees (180 degrees corresponds to full knee extension) on the isokinetic dynamometer (Alkner *et al*. 2000). EMG_{peak} was determined from the MVC producing the highest torque and was established from the mean of the EMG activity recorded 0.25 seconds immediately prior to maximum torque (Wiles *et al*. 2007). The EMG_{peak} was then used to create $\%EMG_{peak}$ 'targets' for the subsequent incremental exercise test.

Participants underwent a discontinuous incremental isometric exercise tests both pre- and post-training (after familiarization), immediately after the recording of resting BP. Briefly, participants began bilateral-leg isometric exercise at 10% EMG_{peak} for 2 minutes and increased this by 5% for each subsequent stage. These were interspersed by 5 minute rest periods and participants continued until volitional fatigue (or failure to maintain EMG signal within ±5% of the 'target' value). Each stage provided a 2 minute mean torque value and the final 2 minute stage that was completed prior to fatigue, was recorded as peak 2 minute torque ($2min\text{-}torque_{peak}$). Average HR for the final 60 seconds of each stage was used for determination of peak HR (HR_{peak}). Participants were instructed to avoid performing Valsalva manoeuvres during isometric exercise.

Training Sessions: Participants trained 3 days.wk^{-1} for 4 weeks at a specific EMG 'target' that equated to 95% HR_{peak}. Training intensity (EMG mV) was adjusted if mean of session HR deviated from the target HR (95% HR_{peak}) by more than ±5%. Participants performed four bouts involving 2 minutes of isometric exercise separated by 3 minute rest periods. Electromyography, HR, and torque were measured and recorded continuously throughout all 12 training sessions. Mean training torque (Train-$torque_{mean}$) was calculated as the average torque for all 48 bouts (12 sessions x 4 repetitions) of isometric training. Crossover between exercise and control conditions was separated by 6 weeks. Figure 5.1 provides a schematic diagram of the study design.

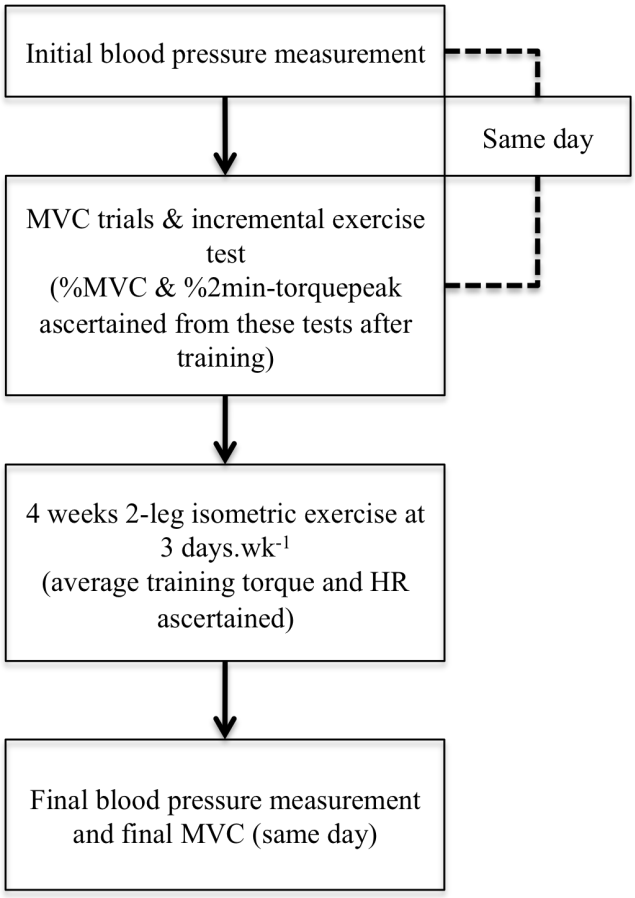

Figure 5.1. Schematic illustration of 4 week bilateral-leg isometric exercise training study

Derivation of training intensity markers: Absolute heart rates recorded during training (last 60 seconds of each repetition) were averaged to give HR_{train}. Percentage of maximal voluntary contraction was calculated by expressing Train-$torque_{mean}$ relative to MVC,

and %2min-torque$_{peak}$ was calculated by expressing Train-torque$_{mean}$ as a percentage of 2min-Torque$_{peak}$ from the initial incremental test. ΔEMG_{amp} and ΔEMG_{freq} were determined by calculating the mean difference between EMG_{amp} and EMG_{freq} in the last 60 seconds of each exercise bout in training versus that in the first 60 seconds. This difference was then expressed relative to the EMG in the first 60 seconds ((mean(0-60s)) – (mean(60-120s))/(mean(0-60s))*100). All 48 repetitions were analysed in this way for each participant, and then a mean ΔEMG_{amp} and ΔEMG_{freq} was derived for each participant from those 48 values.

5.2.3 Data Analyses

All data were assessed for conformity with parametric assumptions (Field, 2000). Changes in BP have been associated with initial values (Millar *et al*. 2007), so analysis of covariance (ANCOVA) was used to assess whether changes scores were influenced by initial baseline values. Pre- post BP differences, and differences in BP changes between the experimental and control condition were assessed using 2-way ANOVA within the ANCOVA test. Maximal voluntary contraction and body mass were also assessed pre- to post-training. An alpha level of < 0.05 was set as the threshold for statistical significance, and the Bonferroni post-hoc procedure was used to explore any significant differences detected. Pearson product moment correlation was used to explore relationships between blood pressure changes and HR_{train}, %MVC, %2min-torque$_{peak}$, ΔEMG_{amp} and ΔEMG_{freq}.

5.3 Results

Resting blood pressure: Four weeks of bilateral-leg isometric exercise resulted in a reduction in resting SBP (-4.9 ± 5.8 mmHg, $p = 0.01$), with no differences in the control data ($p = 0.92$). Resting DBP was also reduced (-2.8 ± 3.2 mmHg, $p = 0.01$), with no differences in the control data ($p = 0.13$). MAP was also reduced pre- to post-training (-2.7 ± 2.4, $p = 0.001$), with no differences in the control data ($p = 0.59$). ANOVA showed that SBP, DBP and MAP changes were significantly different between experimental and control conditions ($p = 0.04$, 0.007, and 0.009 respectively). There was no change in body mass over the duration of the study (78.1 ± 18.2 to 78.1 ± 18.1 kg, $p = 0.80$).

Maximal voluntary contractions: Group mean MVC significantly increased pre- to post-training (484 ± 116 Nm to 537 ± 112 Nm) measured immediately prior to and on completion of 4 week training intervention. Twelve out of thirteen participants increased MVC. Training intensity, expressed as %MVC also increased, comparing first and final training sessions (22.93 ± 3.8 % MVC vs. 26.68 ± 5.14 % MVC, $p < 0.01$).

Pearson Product- Moment correlations demonstrated significant relationships between ΔSBP and $\%2\text{min-torque}_{peak}$ ($r = -0.65$, $p = 0.02$; Figure 5.2) and between ΔMAP and $\%2\text{min-torque}_{peak}$ ($r = -0.59$, $p = 0.03$; Figure 5.3). In relation to EMG fatigue measures during training, there were significant correlations between ΔSBP and ΔEMG_{amp} ($r = 0.66$, $p = 0.14$; see Figure 5.4), ΔMAP and ΔEMG_{amp} ($r = 0.59$, $p = 0.03$; Figure 5.5), ΔSBP and ΔEMG_{freq} ($r = -0.67$, $p = 0.01$; Figure 5.6) and ΔMAP and ΔEMG_{freq} ($r = -0.64$, $p = 0.02$; Figure 5.7). HR_{train} was significantly correlated with ΔDBP ($r = -0.82$, $p = 0.001$; Figure 5.8) but no other changes in resting blood pressure values (SBP or MAP). Table 5.1 presents all correlation results.

Table 5.1. Correlations between training intensity markers and systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressure adaptations.

	Average training HR	Training torque as % of MVC	Training torque as % of 2 min peak	ΔEMG amplitude	ΔEMG frequency
ΔSBP	-.25	-.24	-.65*	.66*	-.67*
ΔDBP	-.82*	-.09	-.21	.12	-.21
ΔMAP	-.26	-.06	-.59*	.59*	-.64*

Pearson Correlations, * = significant at $p < 0.05$

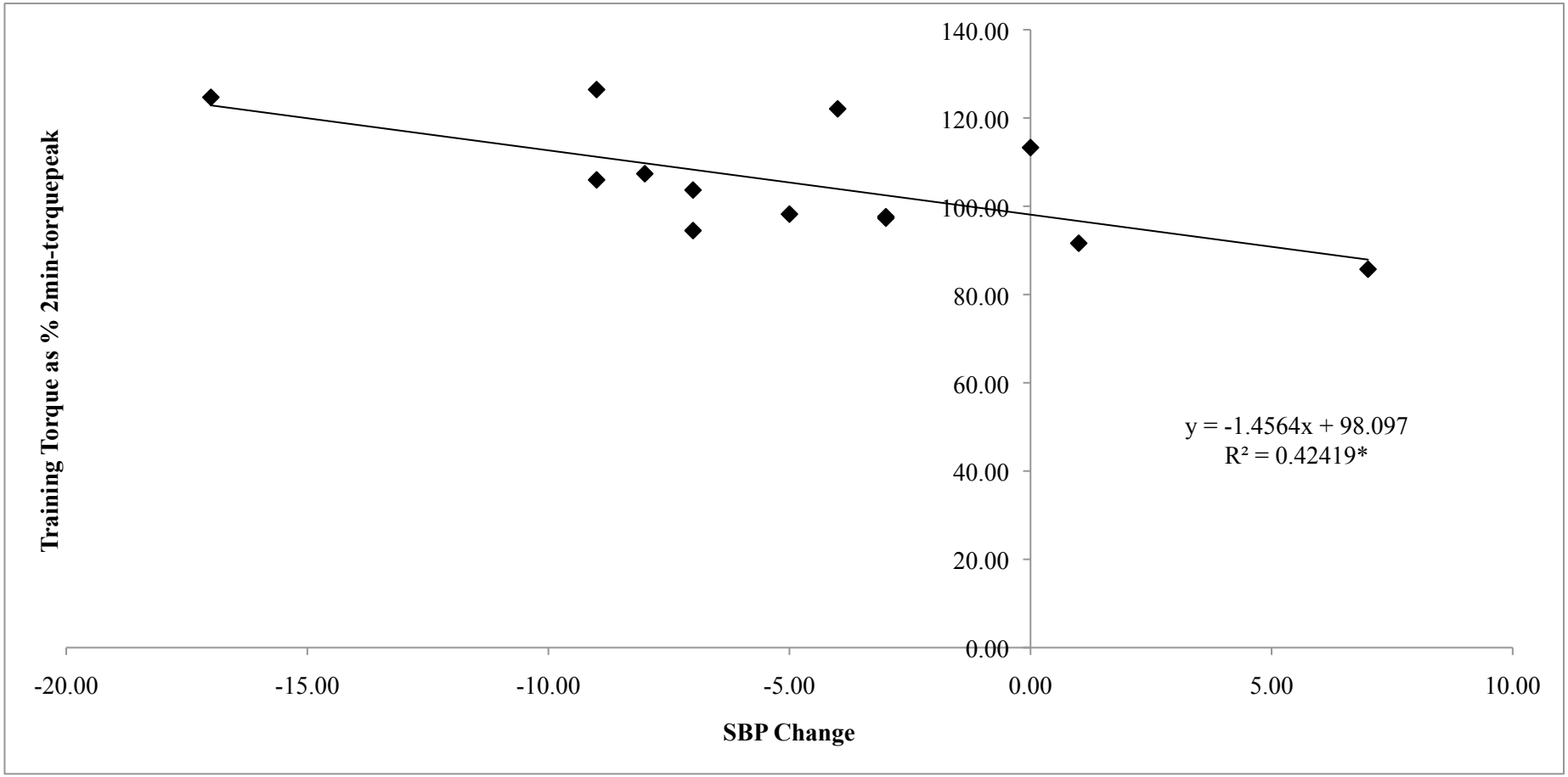

Figure 5.2. Changes in systolic blood pressure (ΔSBP) and average training torque expressed as a percentage of initial peak torque (%2min-torque$_{peak}$). * = significant at $p < 0.05$

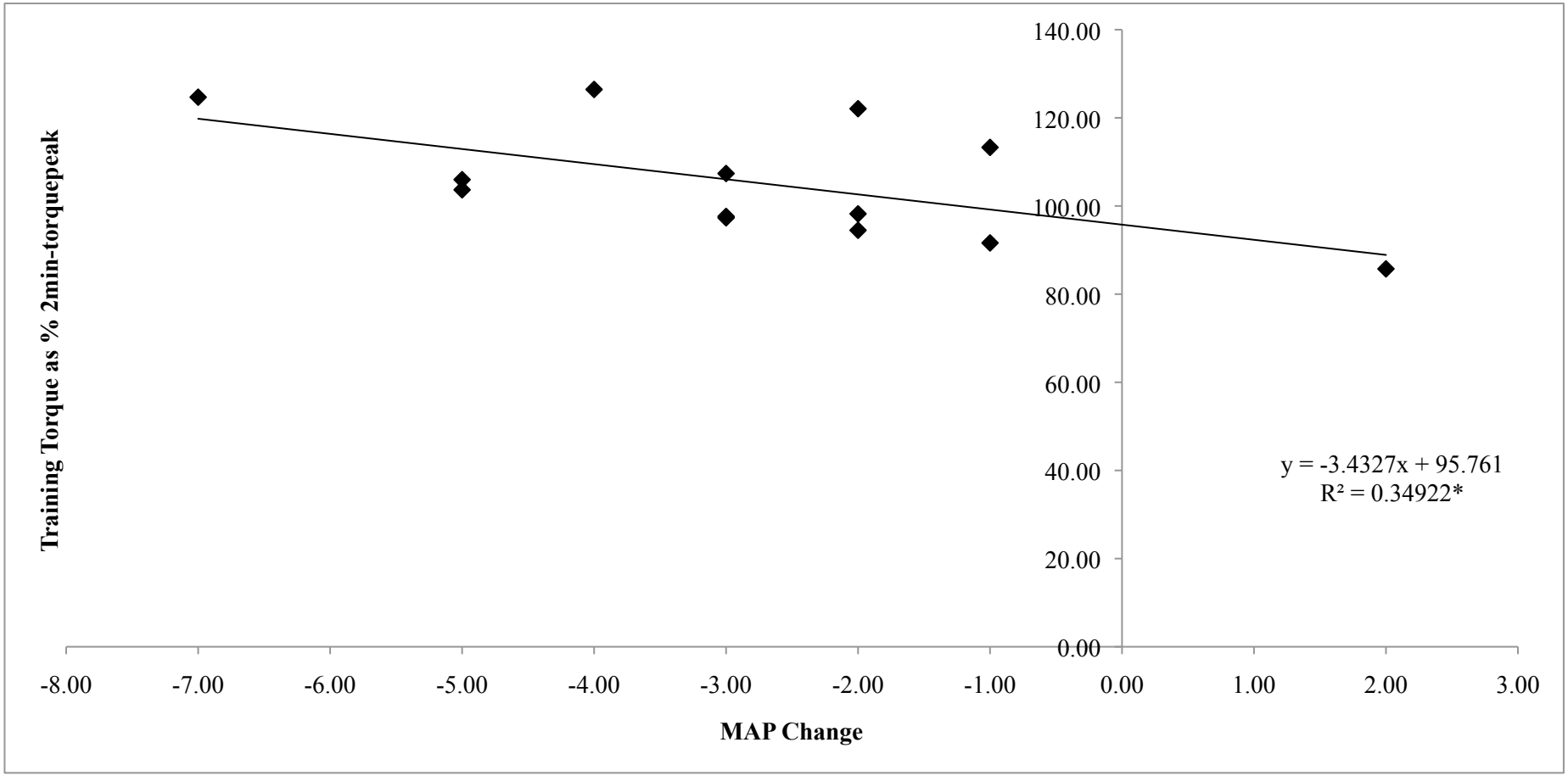

Figure 5.3. Changes in mean arterial blood pressure (ΔMAP) and average training torque expressed as a percentage of initial peak torque (%2min-torque$_{peak}$).

* = significant at $p < 0.05$

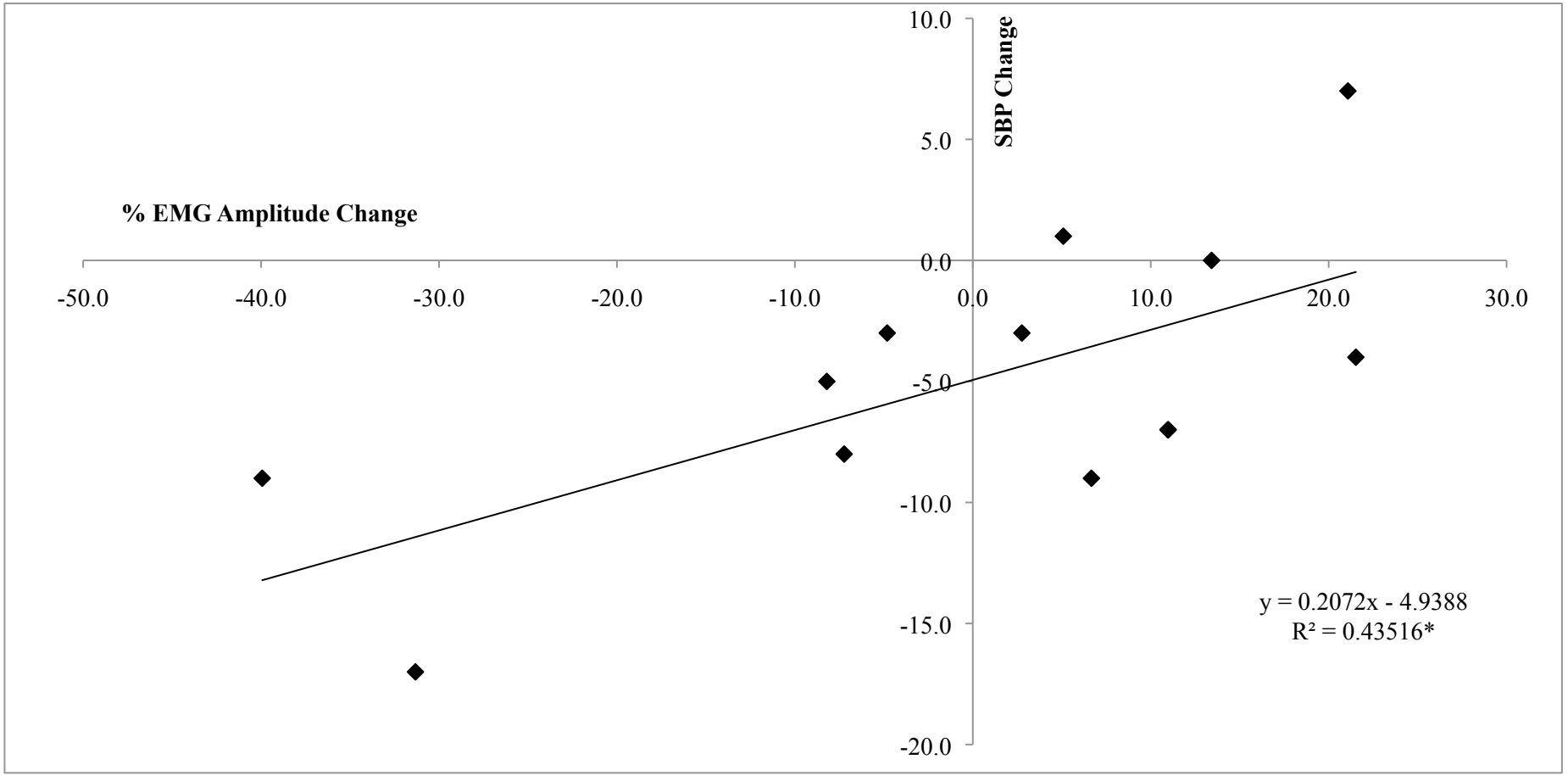

Figure 5.4. Changes in systolic blood pressure (ΔSBP) and levels of fatigue experienced during training expressed as changes in electromyography signal amplitude (ΔEMG_{amp}). * = significant at $p < 0.05$

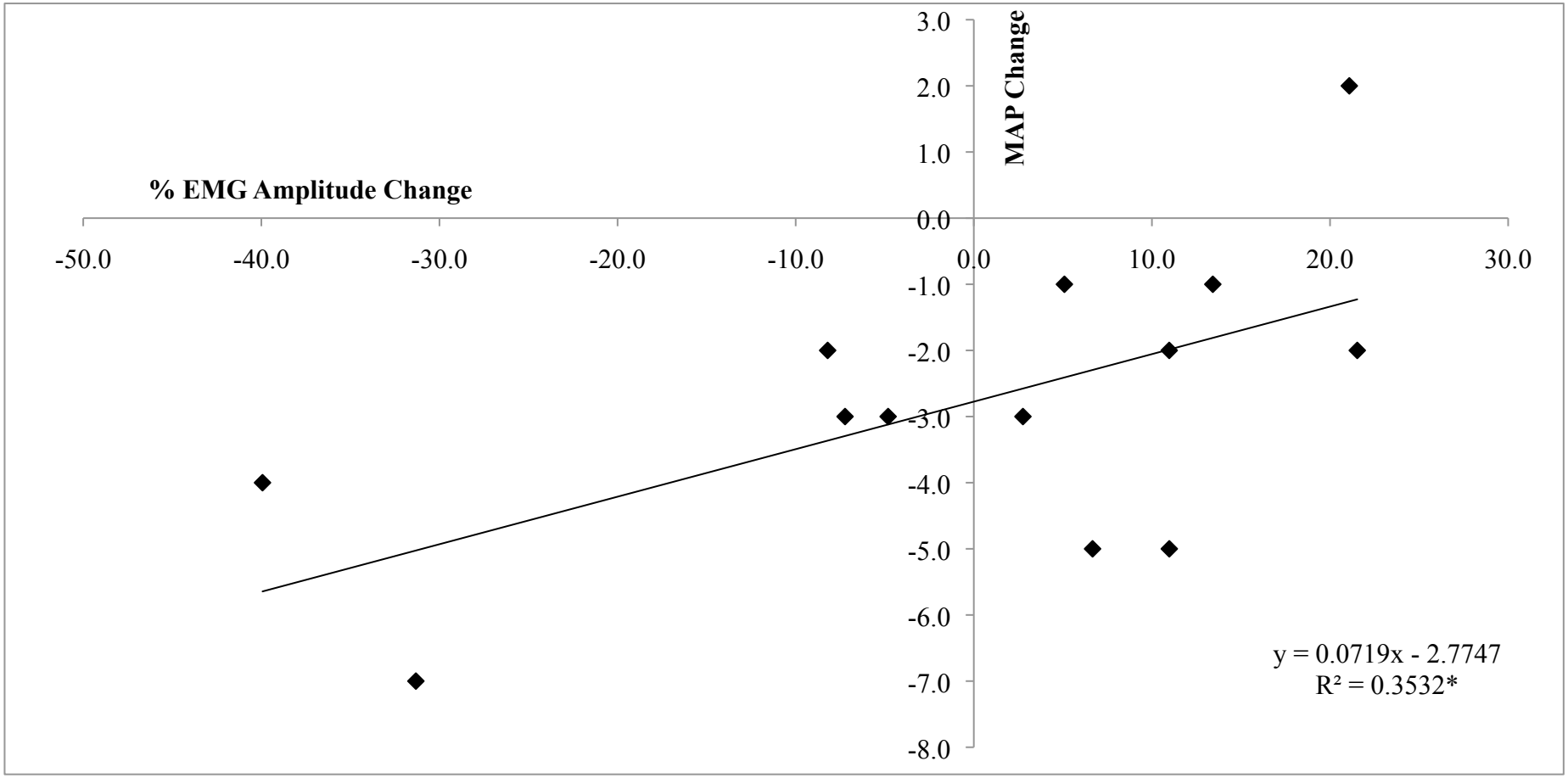

Figure 5.5. Changes in mean arterial blood pressure (ΔMAP) and levels of fatigue experienced during training expressed as changes in electromyography signal amplitude (ΔEMG_{amp}). * = significant at $p < 0.05$

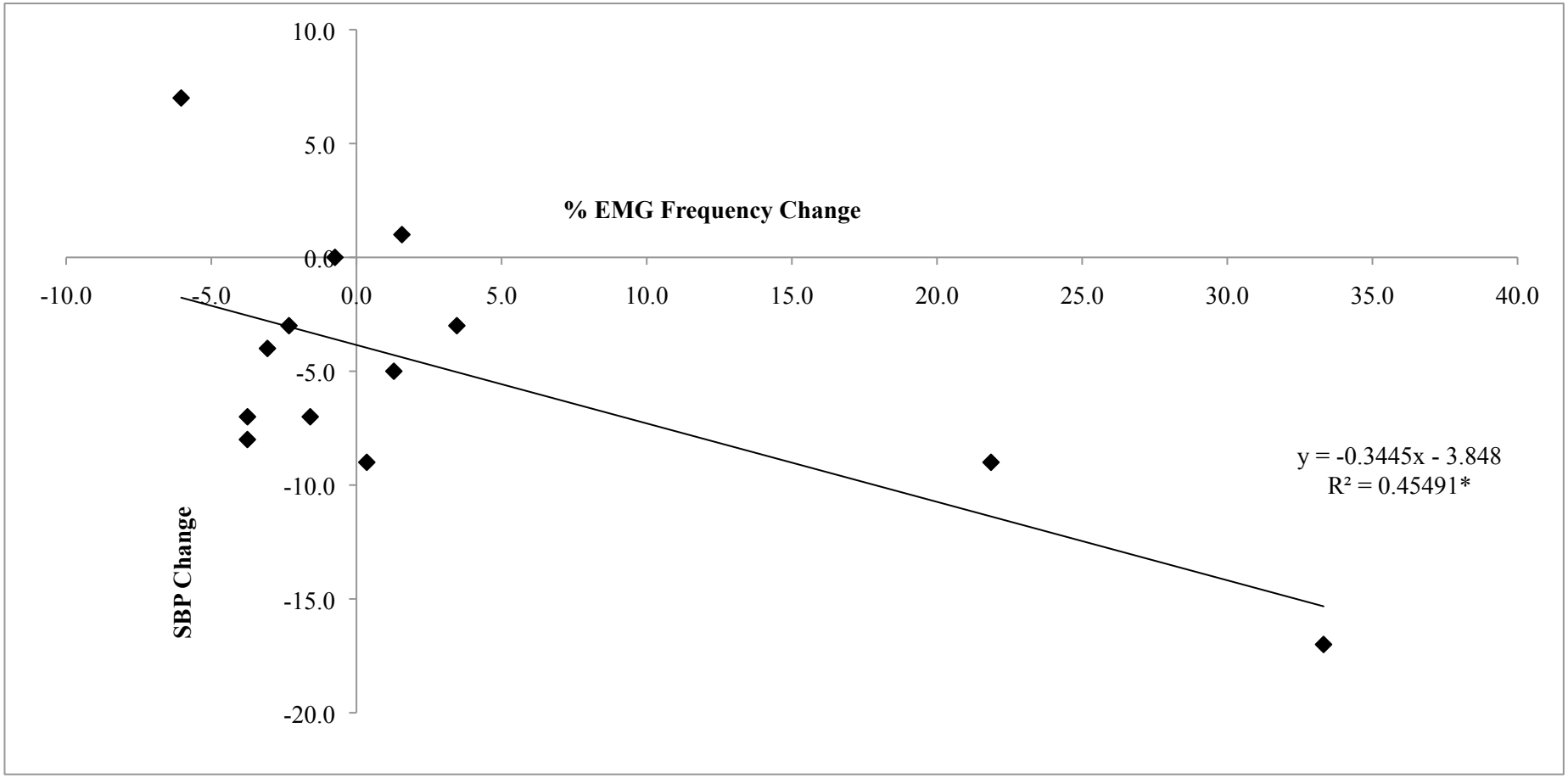

Figure 5.6. Changes in systolic blood pressure (ΔSBP) and levels of fatigue experienced during training expressed as changes in electromyography signal frequency (ΔEMG_{freq}). * = significant at $p < 0.05$

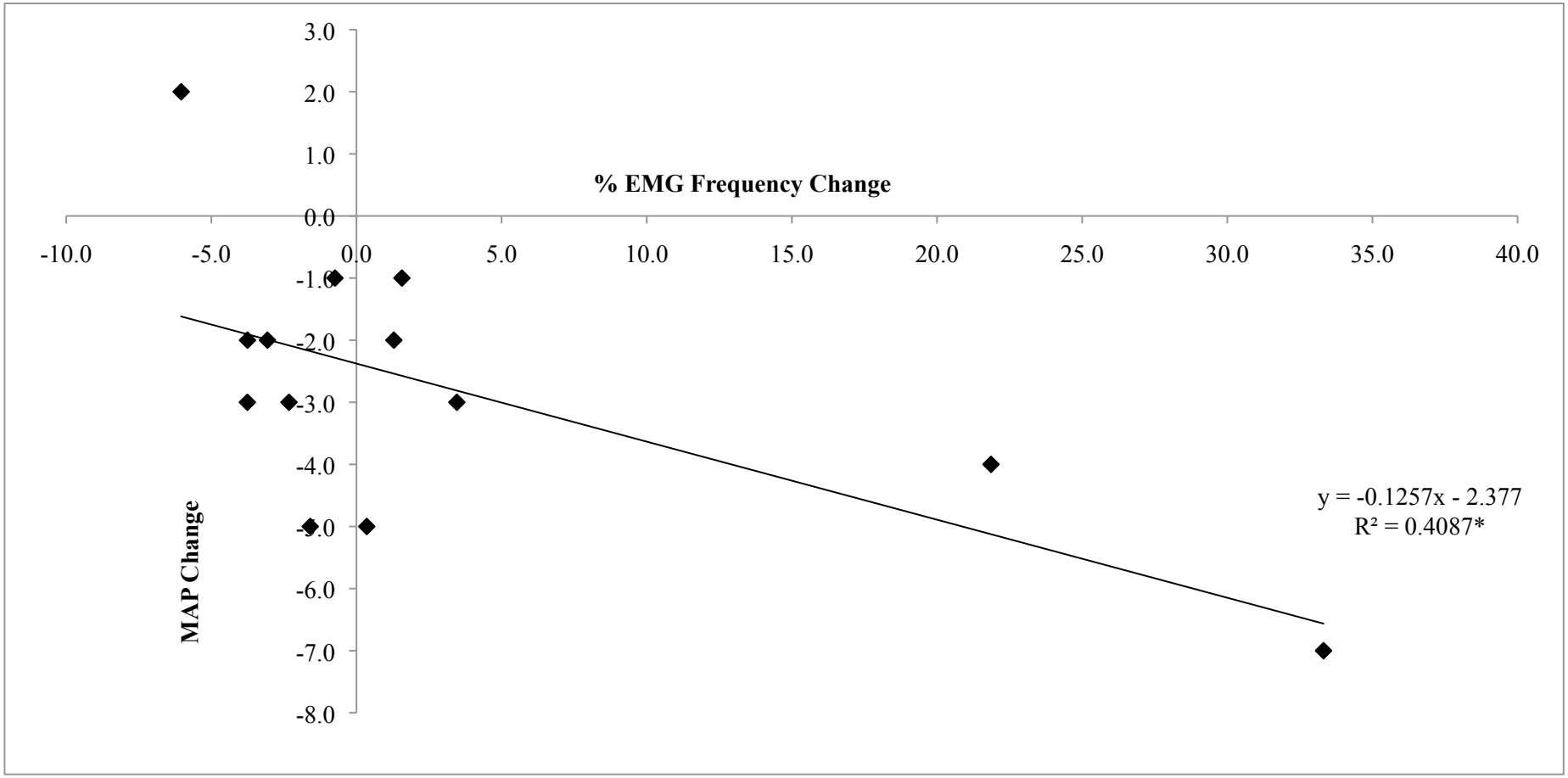

Figure 5.7. Changes in mean arterial blood pressure (ΔMAP) and levels of fatigue experienced during training expressed as changes in electromyography signal frequency (ΔEMG_{freq}). * = significant at $p < 0.05$

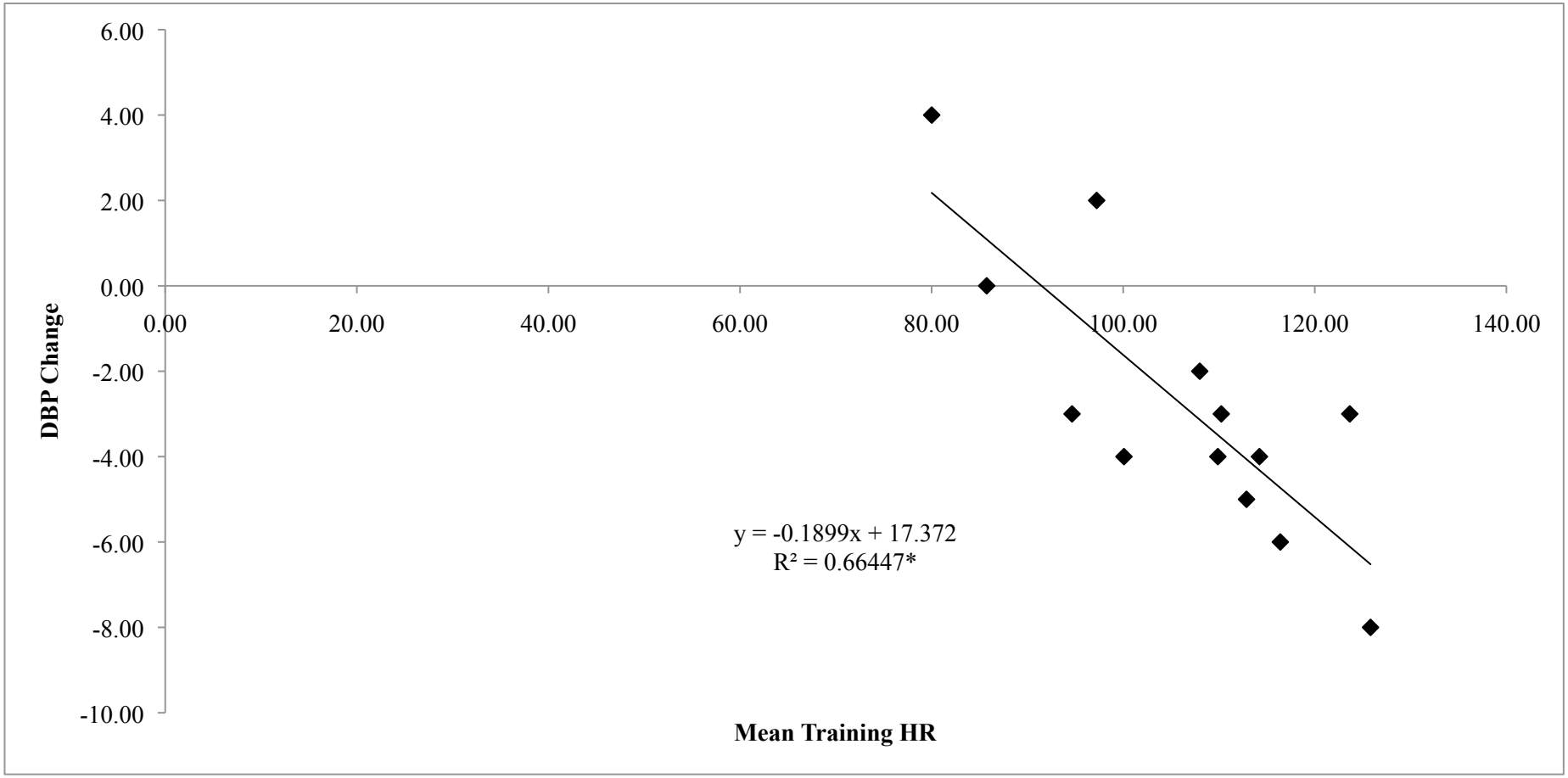

Figure 5.8. Changes in diastolic blood pressure (ΔDBP) and individual mean training heart rates (HR_{train}). * = significant at $p < 0.05$

The covariate of initial baseline values did not influence change scores for SBP ($p = 0.35$) or MAP ($p = 0.87$). However, baseline DBP values predicted DBP change scores ($p = 0.02$).

5.4 Discussion

In the previous chapter the significant reductions in resting SBP, DBP, and MAP after 4 weeks of bilateral-leg isometric exercise training were reported. In part A of this chapter, it can be seen that SBP and MAP reductions were significantly correlated with training intensity when expressed relative to the peak 2 minute torque achieved in the pre-training incremental test. Reductions in SBP were also correlated with training intensity when it was expressed relative to peak EMG (determined during the initial incremental test; $\%EMG_{peak}$). Reductions in resting BP were not related to training intensity when it was expressed relative to maximal voluntary contraction (%MVC), or reductions in SBP or MAP with training HR (HR_{train}). However, there were reductions in resting DBP after training and these reductions were significantly correlated with HR_{train}. But since the training-induced reductions in DBP were largely accounted for by initial resting DBP, the relationship between training HR and reductions in DBP after training should be viewed with caution. This type and intensity of training also caused increases in MVC and increases in the highest mean torque that could be sustained for 2 minutes ($2min\text{-}torque_{peak}$).

These findings appear to suggest that the best way to set intensity for bilateral-leg IET (when reductions in resting BP are required) is to relate training torque to the highest torque that can be sustained for 2 minutes, rather than relating it to MVC. They might also suggest that the stimulus for the reduction in resting BP in this type of training is more closely related to the action of sustaining torque, rather than the magnitude of torque relative to MVC. This is evidenced by the absence of a correlation between any change in resting BP and IET intensity, expressed as %MVC. This is surprising because %MVC has been used successfully in previous studies to dictate IET intensity (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004), interestingly with no evidenced based justification for the intensities commonly used.

The absence of a relationship between reductions in resting BP and %MVC cannot be explained by inadequate progressive overload in training torque during the training programme, because both MVC and training intensity (%MVC) increased pre- to post-training. The findings of the present study suggest that it is the maintenance of torque at a sufficient level to cause some fatigue during training which is most important in bringing about reductions in SBP and MAP when using isometric leg-training. The correlation of $\%EMG_{peak}$ with ΔSBP suggests that EMG is a valid means of exercise intensity prescription, where SBP adaptation is desired. Intensity prescription based on EMG also allows the experimenter to carefully control the CV response to isometric exercise during each training session (Schibye *et al*. 1981; Wiles *et al*. 2007).

The regression equation for the relationship between $\%2min\text{-}torque_{peak}$ and reductions in BP suggests that those individuals who trained in excess of their initial $2min\text{-}torque_{peak}$ saw the greatest reductions in resting BP. Indeed, in order to ensure at least a 5 mmHg reduction in SBP after 4 weeks of bilateral-leg training, participants would have to train at 105.4 % $2min\text{-}torque_{peak}$ ($y = -1.4564 * SBP\ reduction + 98.097$). It remains to be seen whether training in this way (at $\%2min\text{-}torque_{peak}$) can be used to ensure significant reductions in resting BP. Also, the correlation between both ΔSBP and ΔMAP with $\%2min\text{-}Torque_{peak}$ suggests that when training is conducted in 2 minute bouts, the best training-related marker of the reductions in resting BP corresponds to the stage duration of the incremental test (2 minutes) and not to MVC (which involves approximately 2-3 seconds of isometric exercise). Therefore, the implication is that the best marker from the initial incremental test is that which simply matches the training bout duration. However, it also implies that sustaining torque might be a more important stimulus in the mechanism whereby resting BP is reduced, than previously thought. This concept was initially suggested by Buck and Donner (1985) in a study that related work-based isometric activity to the incidence of hypertension.

The apparent difference in training stimulus required for reductions in DBP (which were related to HR_{train}), compared with SBP and MAP (which related to $\%2min\text{-}torque_{peak}$, ΔEMG_{amp}, ΔEMG_{freq} and $\%EMG_{peak}$) is perhaps a result of the distinct physiological aspects of the cardiovascular system that these BP measures represent (Sesso *et al*. 2000). It has been hypothesized that the controlling factor of DBP maintenance is blood flow related (Nichols and O'Rourke, 1998), so perhaps the higher the heart rates during this training study, the more directly-mediated were blood flow dynamics. Given that

training heart rates were only correlated with ΔDBP, it might be suggested that using a $\%HR_{peak}$ to prescribe bilateral-leg isometric exercise intensity is not the most prudent method when using this type of training to lower resting BP. However, it should be remembered that heart rates provide a simple indication of overall CV stress, and could be used to avoid 'over exertion' during isometric exercise (Smolander *et al*. 1998).

In relation to changes in EMG frequency and amplitude after training and their correlation with reductions in resting BP, it appears from figures 5.6 and 5.7 that outliers might have influenced correlations involving EMG frequency markers. Therefore, until greater numbers of participants have been assessed, this correlation should be viewed with caution. Nevertheless, EMG signal amplitude increases and frequency decreases are thought to represent NM fatigue at the muscle site. The acute effects of local muscle fatigue are thought to be caused by a number of factors including impairment of excitation-contraction coupling (Eberstein and Sandow, 1963; Edwards *et al*. 1977), sarcoplasmic reticulum calcium release (Tupling, 2004) and degradation of Na^{2+}/K^{+} ATPase activity. These impairments are thought to be caused by muscle chemical environment changes (Sinoway *et al*. 1989; Mostoufi-Moab *et al*. 1998). Chief amongst these are pH changes (hydrogen ion accumulation) and such changes have been suggested to play a role in the pressor response to exercise.

Several previous studies have associated local muscle fatigue and CV control during exercise with afferent input from mechanical (mechanoreceptors) and metabolic (metaboreceptors) stimulation (Coote *et al*. 1971; McCloskey and Mitchell, 1972; Kniffki *et al*. 1978; Kaufman *et al*. 1983; Mense and Stahnke, 1983). Fisher and White (2004) suggest that both metaboreceptors and mechanoreceptors may act collectively, and increased metabolite accumulation may lead to sensitisation of mechanically sensitive muscle afferents. However, how these acute effects of local muscle fatigue cause reductions in resting BP after a period of training is not clear. It is possible that the reductions in resting BP seen after the bilateral-leg IET were a direct result of local muscle fatigue, which mediated its effects on the pressor response via metaboreceptor stimulation.

5.5 Conclusion

The markers which best reflect the association between isometric training intensity and the reductions in resting BP are %2min-torque$_{peak}$, ΔEMG_{amp}, ΔEMG_{freq} and $\%EMG_{peak}$. This implies that sustaining torque might be a more important stimulus in the mechanism whereby resting BP is reduced than previously thought. It could also be argued that some of these intensity markers reflect the extent to which local muscle fatigue was induced and this appears to have been of utmost importance in the reductions in resting BP observed after bilateral-leg IET.

(B) Change in lactate metabolism after isometric exercise training and its relationship with reduced resting blood pressure

5.6 Introduction

In the previous section of this chapter it was shown that, during isometric exercise training (IET), it appears to be important to induce 'local muscle fatigue' (as measured using changes in EMG amplitude and frequency). When this finding is added to the suggestions in the literature (outlined in Chapter 1, page 13) that metaboreceptors play a role in regulation of blood pressure (BP) during isometric exercise, it seems worthwhile to study changes in metabolite accumulation during IET and then to explore whether these changes relate to the reductions in resting BP. A discontinuous protocol has been established in Chapter 2 pages 50-60, which lends itself to measurement of blood-borne metabolite accumulation.

The role of metabolite accumulation during isometric exercise (Ahlborg *et al*. 1972; Edwards *et al*. 1972; Funderburk *et al*. 1972; Karlsson and Ollander, 1972; Karlsson *et al*. 1975; Tesch and Karlsson, 1979) and the resulting potent metaboreflex stimulus (McCloskey and Mitchell; 1972; Freund *et al*. 1979; Kaufman *et al*. 1983; Mitchell *et al*. 1989; Iellamo *et al*. 1999b; Ichinose *et al*. 2006; Fisher *et al*. 2008) is well established. One of the possible metaboreceptor stimulants is lactate (Rotto and Kaufman, 1988). The only previous study to explore the possible role of lactate as a metabolite associated with the metaboreflex during IET seems to be that of Mostoufi-Moab *et al*. (1998). They investigated the effects of 4 weeks of rhythmic isometric handgrip (IHG) training, using 30 bouts of 1 second contractions/minute, at 25% of maximal voluntary contraction (%MVC) under ischemic conditions on MAP, venous lactate and pH. They found that IET decreased lactate accumulation during ischemic exercise and also observed an attenuated pressor response during this type of exercise. They suggested that training attenuated muscle metaboreceptor responses, and that this was probably due to the increased ability of trained muscle to maintain aerobic metabolism, which decreases the reliance on anaerobic metabolism and lowers the accumulation of metabolites, causing less stimulation of metaboreceptors.

In dynamic exercise training studies 'lactate curves' have been identified pre- and post-training to demonstrate how lactate metabolism changes as a result of training (Sjödin *et al*. 1982), and such changes have been shown to correlate well with the effects of training on exercise performance. The purpose of this study was to quantify the shifts in lactate curves pre- to post- IET and to correlate these changes with reductions in resting BP in individuals.

5.7 Methods

Eleven healthy normotensive males (mean age 21.6 ± 2.6 years; body mass 80.0 ± 19.2 kg; height 177.2 ± 5.3 cm) volunteered to participate in a crossover design study. All participants were moderately physically active (8.2 hours at 7.0 multiple of BMR, per week). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed an exercise readiness questionnaire, and provided written informed consent, thereby adhering to the guidelines set by the 1964 Declaration of Helsinki. All participants conformed to the selection criteria outlined in Chapter 2, pages 24-25. All participants completed identical familiarization sessions. Participants fasted for 4 hours, and abstained from caffeine and alcohol for at least 12 hours prior to testing procedures (Jáuregui-Renaud *et al*. 2001), and were instructed to maintain physical and dietary activity behaviours prior to each visit. Crossover between exercise and control conditions was separated by 6 weeks, as previously used (Howden *et al*. 2002). Figure 5.9 provides a schematic diagram of the study design.

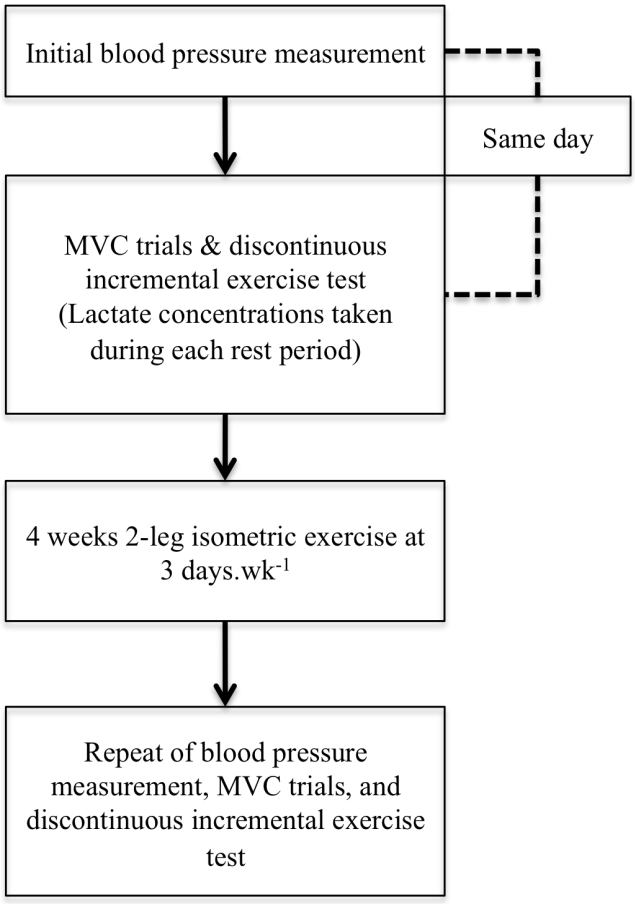

Figure 5.9. A schematic illustration of the overall study design

5.7.1 Equipment

Isometric exercise: All tests were conducted using a Biodex System 3 Pro, isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY), which is described in detail in Chapter 2, pages 25-26. The Biodex was fitted with a modified hip attachment that was inserted into the standard knee attachment, to allow for bilateral-leg extension exercise to be performed. Participants sat in the dynamometer in an upright position, with 90 degrees of flexion at the hip and with the thighs supported.

Electromyography recording: A dual bio-amplifier was used to enable surface EMG measurement from both vastus lateralis', to give a combined average, along with the 16-channel chart recorder. This is described in detail in Chapter 2, pages 27-29.

Exercising heart rate: Heart rate was recorded via ECG using the 16-channel chart recorder. Participants were fitted with three blue sensor R ECG electrode pads (Ambu Inc., U.S.A.), as a standard three lead bipolar ECG arrangement, as recommended by ADInstruments was used. Heart rate was sampled at a frequency of 1000 Hz.

Arterial blood pressure: Resting BP measurements were made using an automated BP monitor (Dinamap Pro 300 Critikon, GEMedical Systems, Slough, Berks, UK). Blood pressure was measured after 15 minutes rest in a seated position. The lowest of three measures, separated by 60 seconds, was used for analysis. The process of automated BP measurement is described in detail in Chapter 2, pages 34-36.

Blood lactate: Capillary blood samples for the determination of lactate concentration were collected from both the left thumb, index, and middle (long) fingers to prevent overuse of one sample site, and to prevent unnecessary discomfort for the participants. This protocol has been used previously in finger-prick lactate reliability studies (Buckley *et al*. 2003). Furthermore, lactate sampling from different body sites has been shown to provide stable values during an incremental dynamic exercise test (Armstrong and Kirby, 1992), so using alternate thumb and finger sites was not considered to be problematic. A 20 µl sample was immediately mixed with 200 µl of reagent solution for subsequent lactate determination. Lactate was determined using a Biosen 5030 lactate analyzer (EKF Diagnostic, Barleben/Magdeburg).

Lactate samples were collected at 60 second intervals, during the 5 minute rest periods of the discontinuous incremental test, from the point of immediate cessation of the preceding increment to the commencement of the subsequent increment (six samples for each rest period reached during the incremental test). From these 6 samples lactate peak (La_{peak}) was determined for each rest period, and, using this highest value for each rest period, an exponential lactate response curve to the entire incremental test was plotted (La vs. EMG). These curves were used to interpolate the exercise intensity (EMG) at 4, 3 and 2 $mmol.L^{-1}$.

5.7.2 Procedures

Maximal voluntary contraction and EMG_{peak}: These measures were determined prior to each discontinuous incremental test. Participants performed three maximal effort isometric bilateral-leg extensions against the fixed dynamometer arm. Each MVC was terminated after 2 seconds, and each was interspersed by a 120 second rest period. The isometric leg extension exercise was performed at a knee angle of 90 degrees (180 degrees corresponds to full knee extension) on the isokinetic dynamometer (Alkner *et al*. 2000). EMG_{peak} was determined from the MVC producing the highest torque and was established from the mean of the EMG activity recorded 0.25 seconds immediately prior to maximum torque (Wiles *et al*. 2007). The EMG_{peak} was then used to create $\%EMG_{peak}$ 'targets' for the subsequent incremental exercise test.

Discontinuous incremental isometric exercise test: Participants underwent a discontinuous incremental isometric exercise tests both pre- and post-training (after familiarization), immediately after the recording of resting measures. Participants began bilateral-leg isometric exercise at 10% EMG_{peak} for 2 minutes. Thereafter, the intensity increased in 5% increments, interspersed by 5 minute rest periods, to volitional fatigue (or failure to maintain EMG signal within +/- 5% of the 'target' value). Lactate concentrations were collected during the rest periods, as aforementioned. EMG was monitored and recorded. Average values (HR) for the final 60 seconds of each increment were used for analysis. Participants were instructed to breathe at a normal rhythm and depth at all times to avoid Valsalva manoeuvres.

Training Sessions: Participants began training at a participant-specific EMG 'target' that equated to 95% HR_{peak}, interpolated from the regression line of HR versus EMG from the initial incremental test. Participants trained 3 $days.wk^{-1}$ for 4 weeks. Training sessions were separated by at least 24 hours. Training intensity (EMG *mV*) was updated during training if mean of session HR deviated from the target HR (95% HR_{peak}) by more than ±5%. Participants performed four bouts involving 2 minutes of isometric exercise separated by 3 minute rest periods. Electromyography, HR, and torque were measured and recorded continuously throughout all 12 training sessions. Training intensity was set and maintained at constant EMG (at an EMG which equated to $95\%HR_{peak}$; EMG_{train}) and this training intensity equated to 92.6 ± 9.3 $\%EMG_{peak}$. A measure of training intensity was also derived by interpolating the lactate value (La_{train}),

which equated to EMG_{train} on the individual La vs. EMG curves. This La_{train} value was then expressed relative to the peak lactate achieved in the incremental test: ($\%La_{peak}$ = La_{train}/La_{peak}) x 100.

5.7.3 Data Analyses

All data were assessed for conformity with parametric assumptions (Field, 2000). Changes in BP have been associated with initial values (Millar *et al*. 2007), so analysis of covariance (ANCOVA) was used to assess whether changes scores were influenced by initial baseline values. Pre- post BP differences, and differences in BP changes between the experimental and control condition were assessed using 2-way ANOVA within the ANCOVA test. Pre- and post-training lactate values and body mass were also assessed. An alpha level of < 0.05 was set as the threshold for statistical significance, and the Bonferroni post-hoc procedure was used to explore any significant differences detected. Pearson Product-Moment correlation was used to analyse BP adaptations with relative training lactate levels. Non-linear regression was used (GraphPad Prism 5, Graphpad Software, Inc.) to assess differences between pre- and post-training exponential group lactate response curves, based on % of EMG_{peak}.

5.8 Results

Resting blood pressure: Four weeks of bilateral-leg IET resulted in a reduction in resting SBP (-4.9 ± 6.3 mmHg, $p = 0.01$), with no differences in the control data (+0.5 ± 3.0 mmHg, $p = 0.63$), with eight participants out of eleven showing reduced SBP after training. Resting DBP dropped (-2.6 ± 3.0 mmHg, $p = 0.01$), with no differences in the control data (+0.9 ± 2.3 mmHg, $p = 0.23$), with nine participants out of eleven showing reduced DBP after training. Resting MAP was reduced pre to post training (-2.6 ± 2.3, $p = 0.001$), with no differences in the control data (+1.0 ± 2.9 mmHg, $p = 0.28$), with ten participants out of eleven showing reduced MAP after training. ANOVA showed that SBP, DBP and MAP changes were significantly different between experimental and control conditions ($p = 0.04$, 0.007, and 0.009 respectively). Table 5.2 demonstrates group mean values at the start and end of the isometric training. There was no change in body mass over the duration of the study (79.3 ± 19.6 to 79.2 ± 19.6 kg, $p = 0.45$).

Table 5.2. Group mean values for systolic (SBP), diastolic (DBP) and mean arterial (MAP) pressure pre- and post-training and pre- and post-control.

Condition	BP component	Pre (mmHg)	Post (mmHg)
Training	SBP	121.0 ± 11.7	116.1 ± 11.9*
	DBP	69.06± 3.4	67.0 ± 4.9*
	MAP	89.6 ± 4.6	87.0 ± 5.2*
Control	SBP	120.2 ± 12.5	120.6 ± 12.1
	DBP	66.4 ± 5.7	67.3 ± 4.8
	MAP	88.0 ± 5.7	89.0 ± 6.3

Group mean values (± SD)

* = significant at $p < 0.05$

The covariate of initial baseline values did not influence change scores for SBP ($p = 0.35$) or MAP ($p = 0.87$). However, baseline DBP values again predicted DBP change scores ($p = 0.02$).

Lactate: Figure 5.10 illustrates group mean lactate points at % of EMG_{peak} during the pre-training and post-training discontinuous incremental tests. Non-linear regression showed post-training lactate as a percentage of EMG_{peak} to be statistically different to pre-training values. No differences were observed using non-linear regression with control data ($p > 0.05$). Figures 5.11 a, b, and c illustrate how lactate peak (La_{peak}) and estimated training lactate levels (La_{train}) were determined against absolute EMG from the pre-training discontinuous incremental test, using individual participant examples of differing La_{peak} responses. Data points on these figures represent La_{peak} ascertained from the highest value of each 5 minute rest period of the incremental test reached before volitional fatigue. Figures 5.12 a, b, and c illustrate individual participant examples of lactate shifts in comparison to absolute EMG values during the discontinuous incremental test, pre and post training intervention. Figures 5.13 and 5.14 illustrate the group mean EMG values at 2, 3, and 4 $mmol.L^{-1}$ lactate concentrations for control (Figure 5.13) and training (Figure 5.14) conditions.

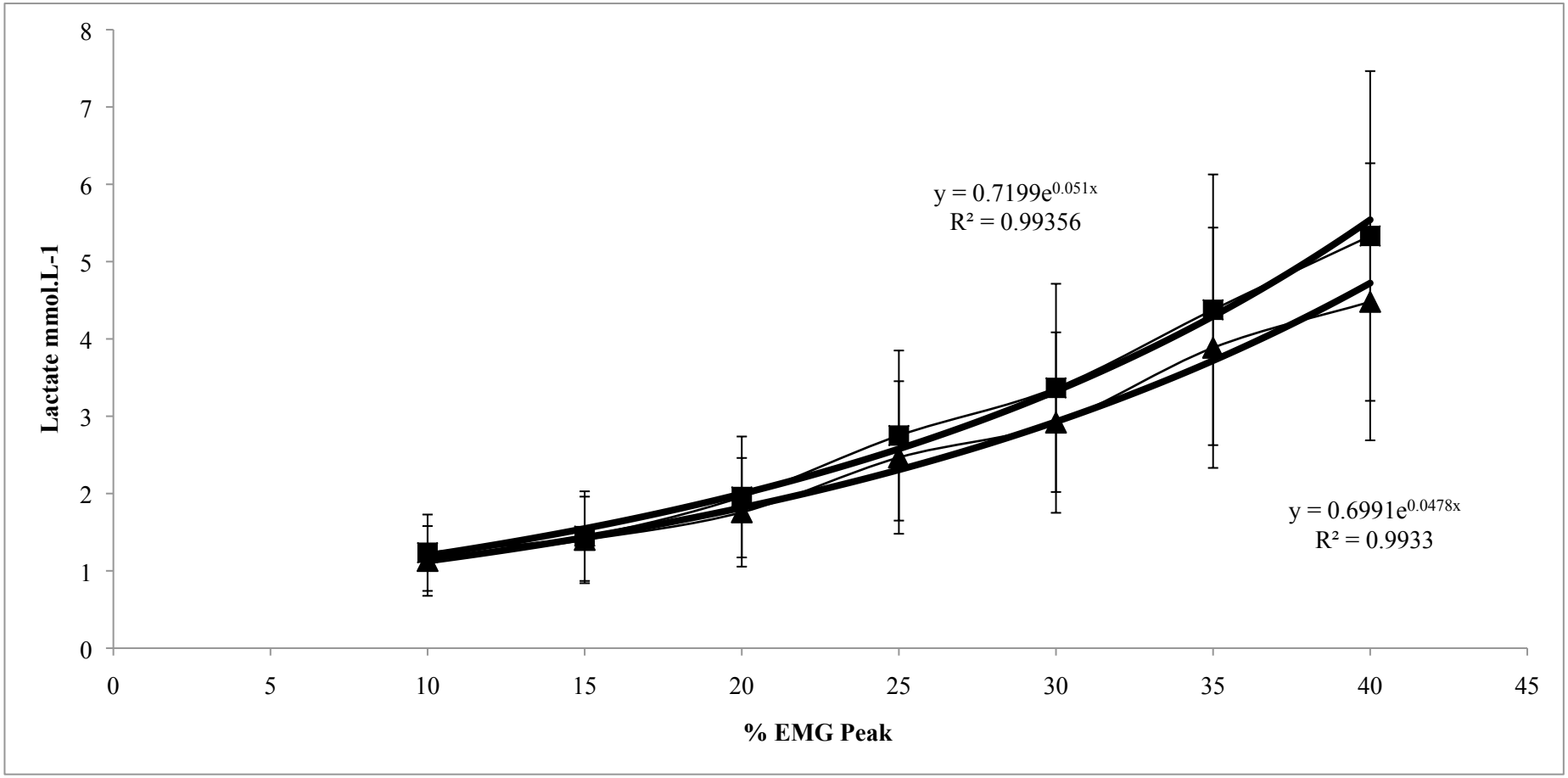

Figure 5.10. Group mean lactate points at % of EMG_{peak} determined from the pre-training (▲) and post-training (■) discontinuous incremental isometric exercise tests

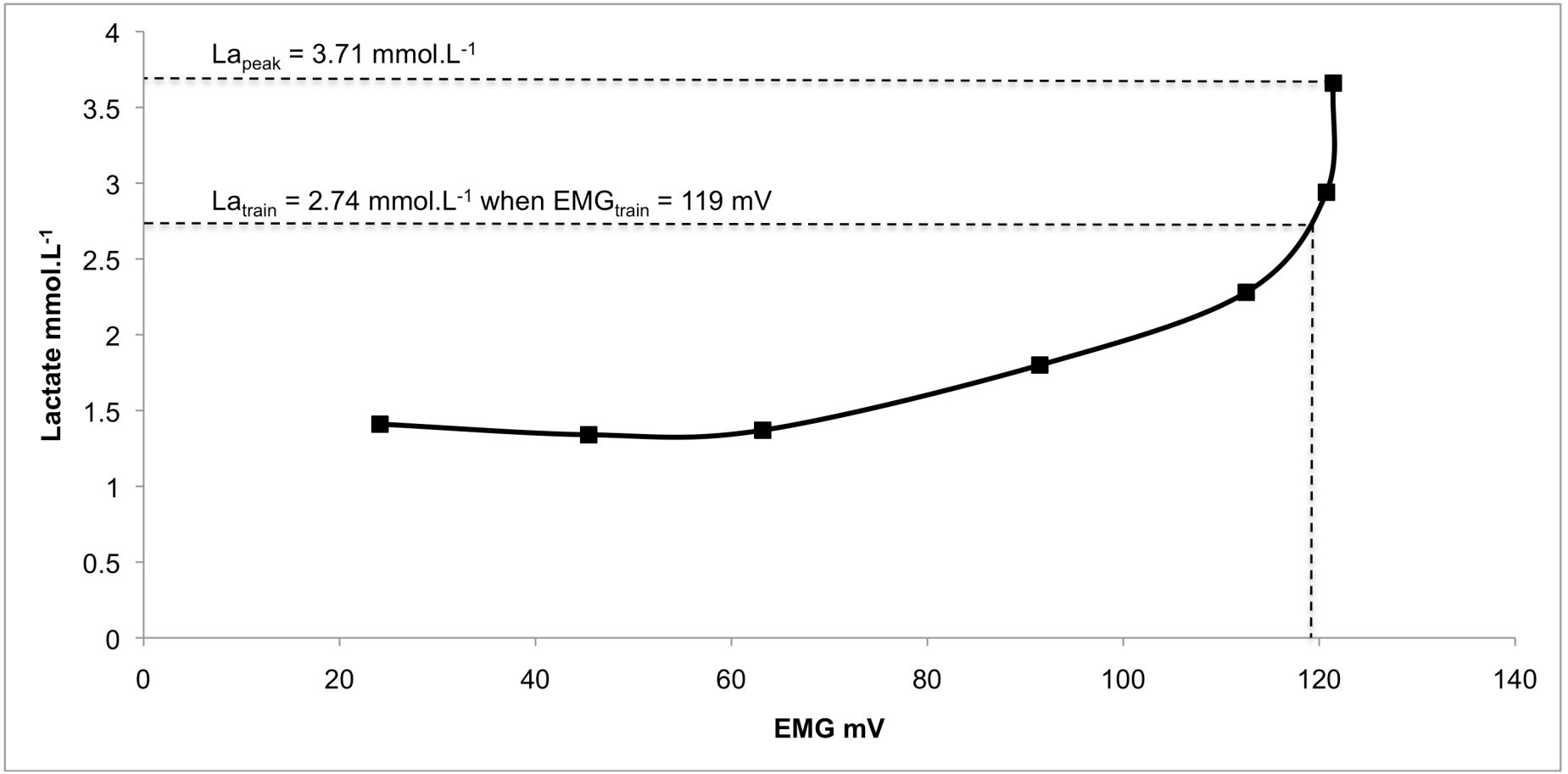

Figure 5.11a. Participant example of lactate peak and estimated training lactate level determined from the initial discontinuous incremental isometric exercise test

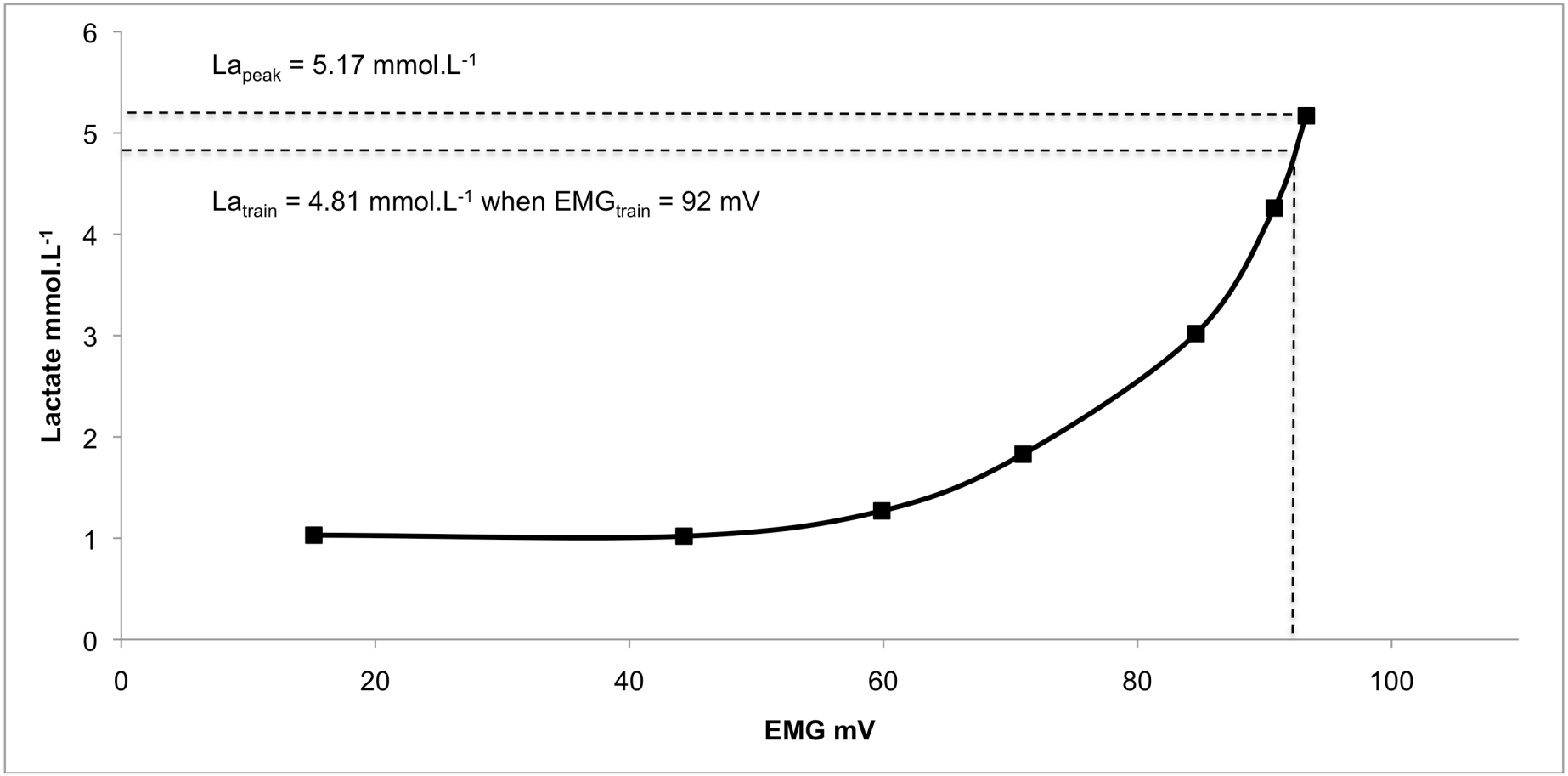

Figure 5.11b. Participant example of lactate peak and estimated training lactate level determined from the initial discontinuous incremental isometric exercise test

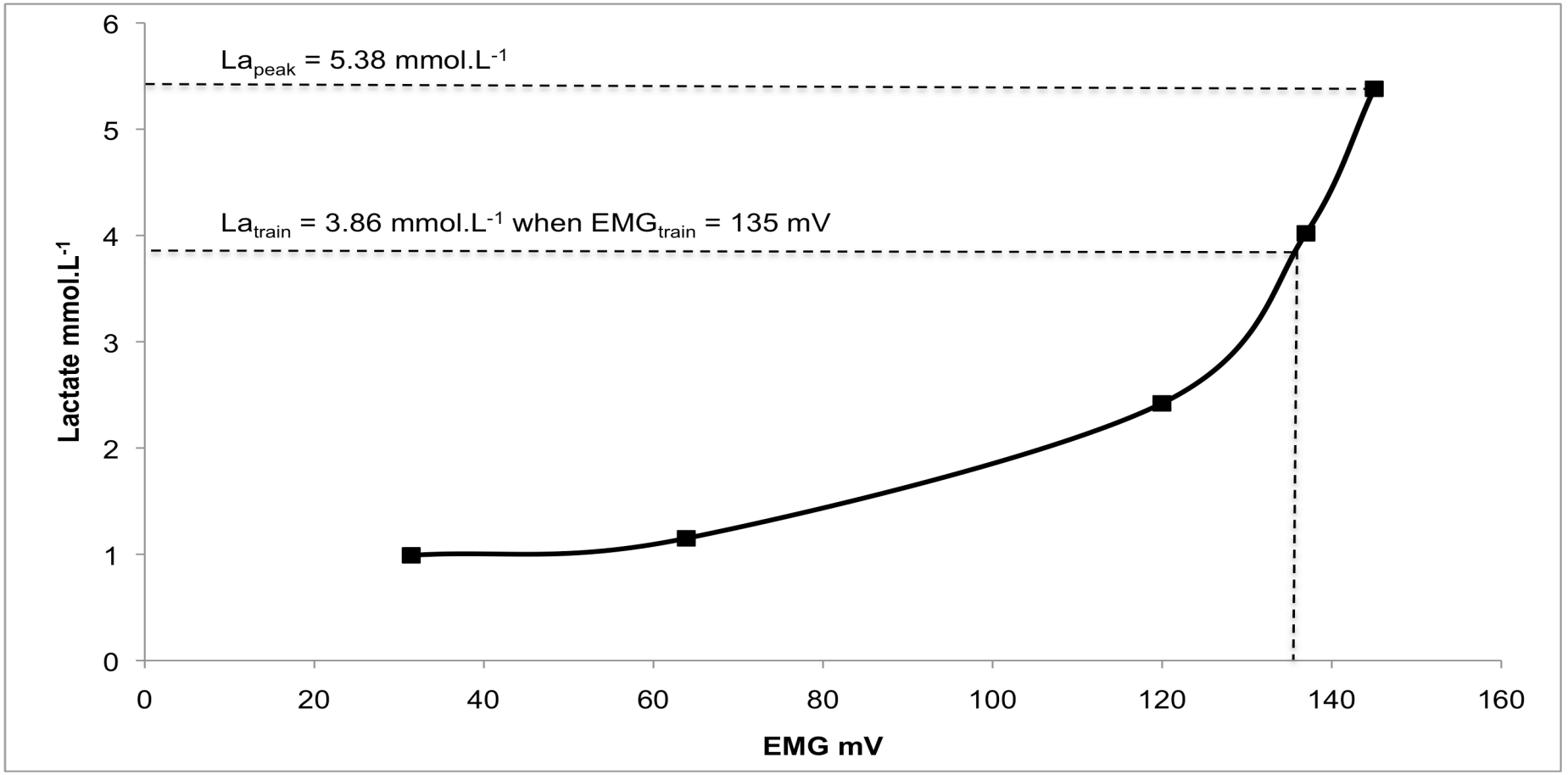

Figure 5.11c. Participant example of lactate peak and estimated training lactate level determined from the initial discontinuous incremental isometric exercise test

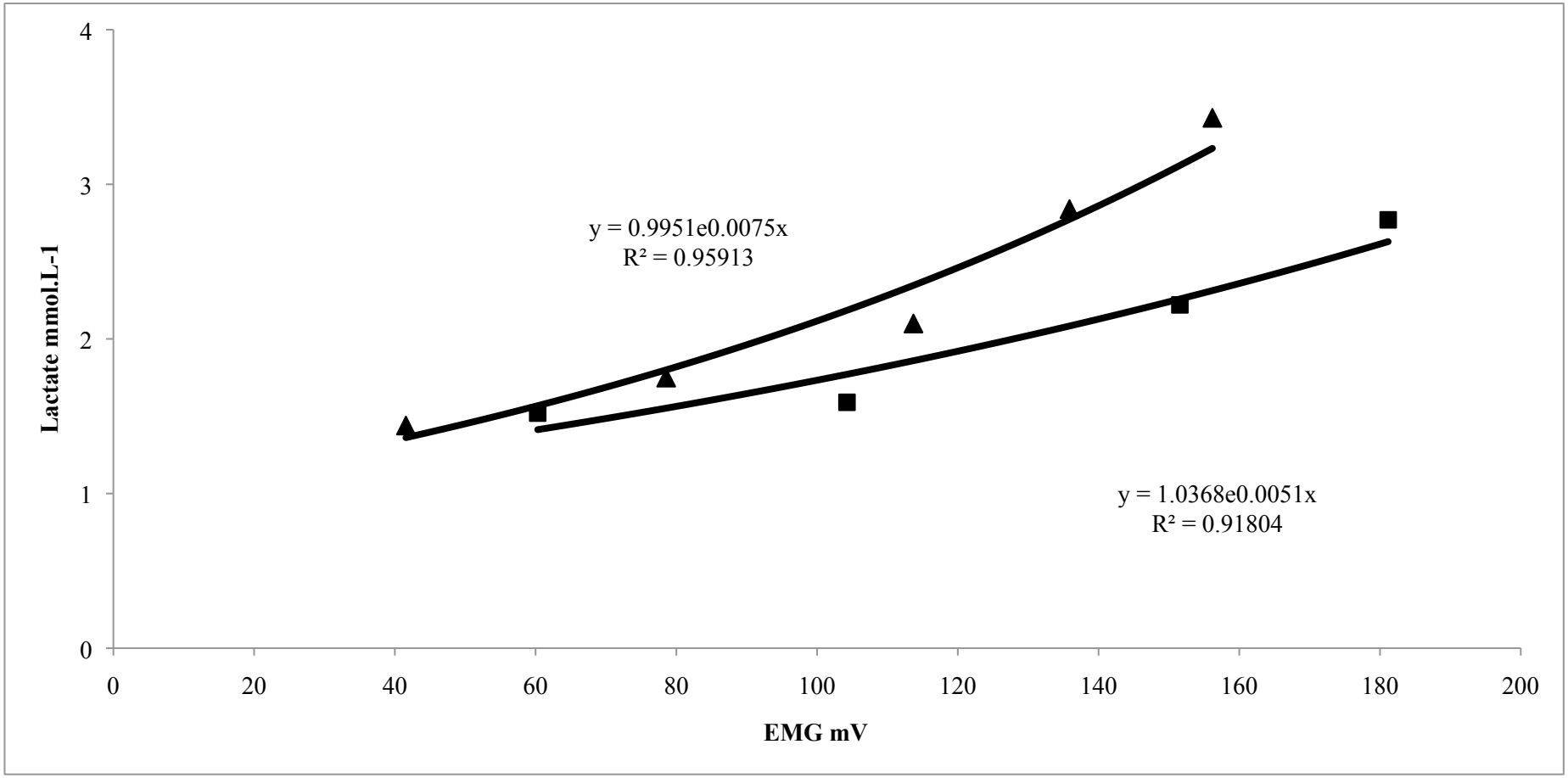

Figure 5.12a. Individual participant example of lactate shifts during discontinuous incremental isometric exercise tests, pre (▲), and post (■) 4 week training intervention

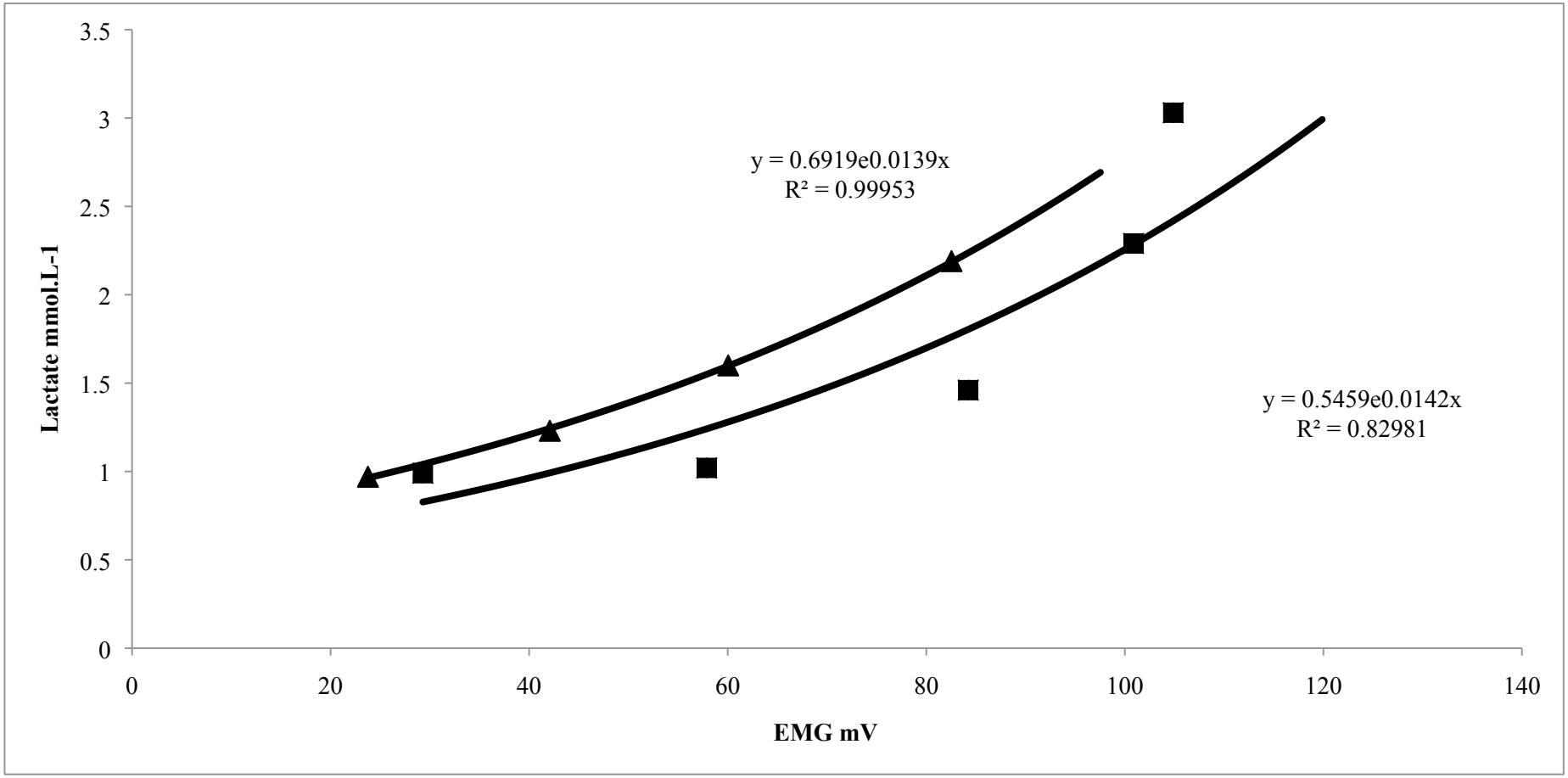

Figure 5.12b. Individual participant example of lactate shifts during discontinuous incremental isometric exercise tests, pre (▲), and post (■) 4 week training intervention

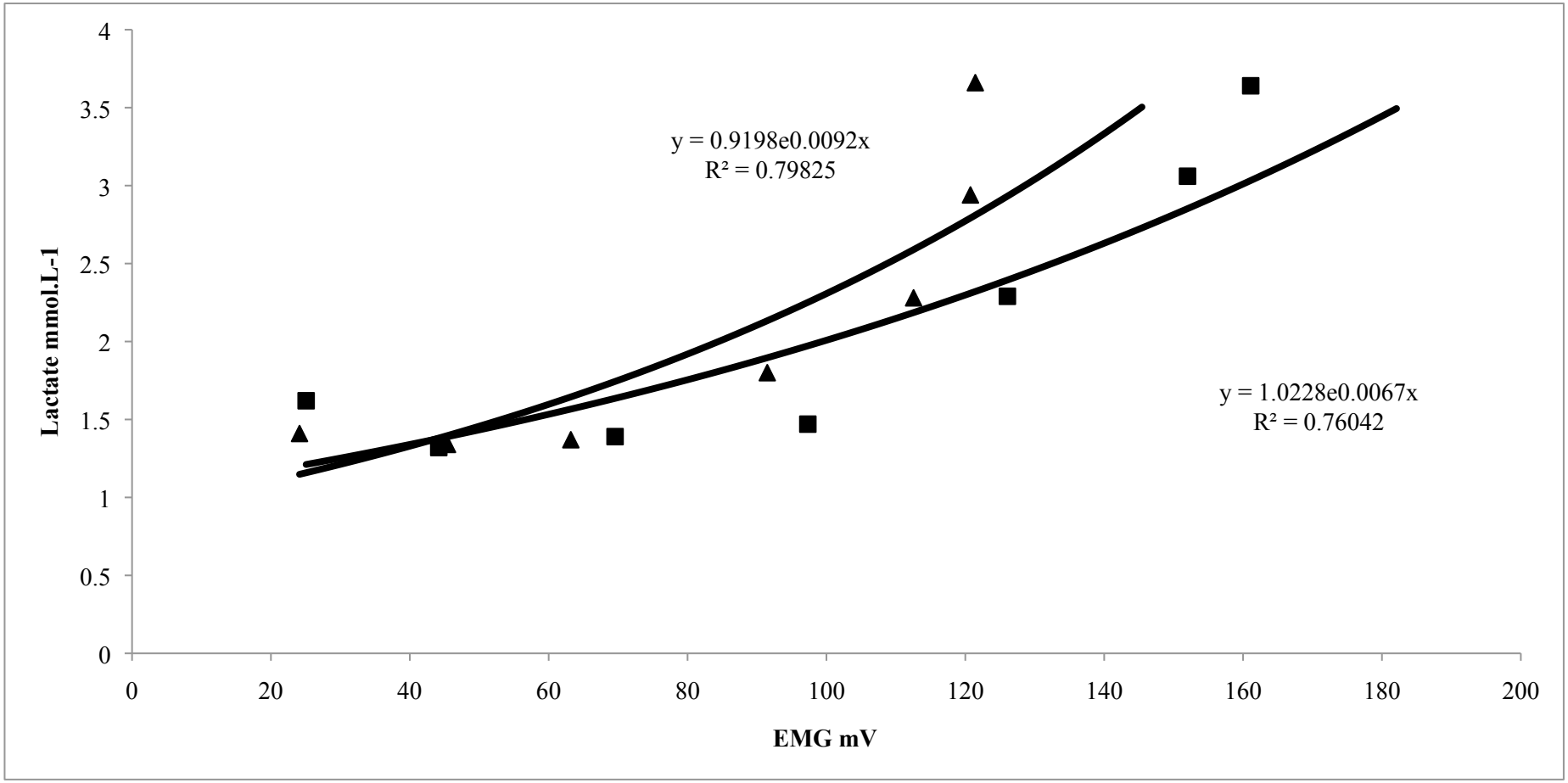

Figure 5.12c. Individual participant example of lactate shifts during discontinuous incremental isometric exercise tests, pre (▲), and post (■) 4 week training intervention

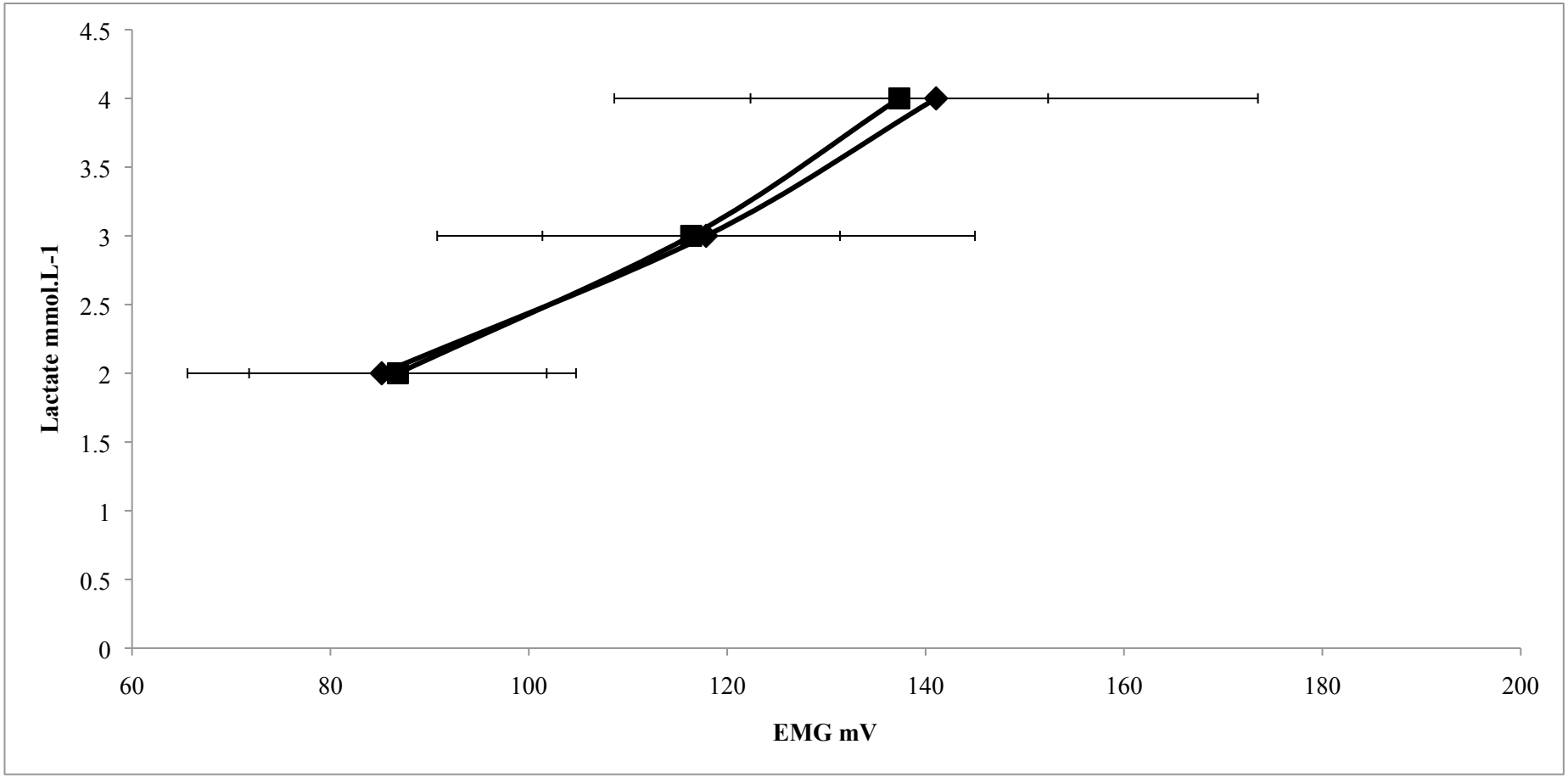

Figure 5.13. Group mean EMG values at 2, 3, and 4 mmol.L^{-1} pre (◆) and post (■) control condition

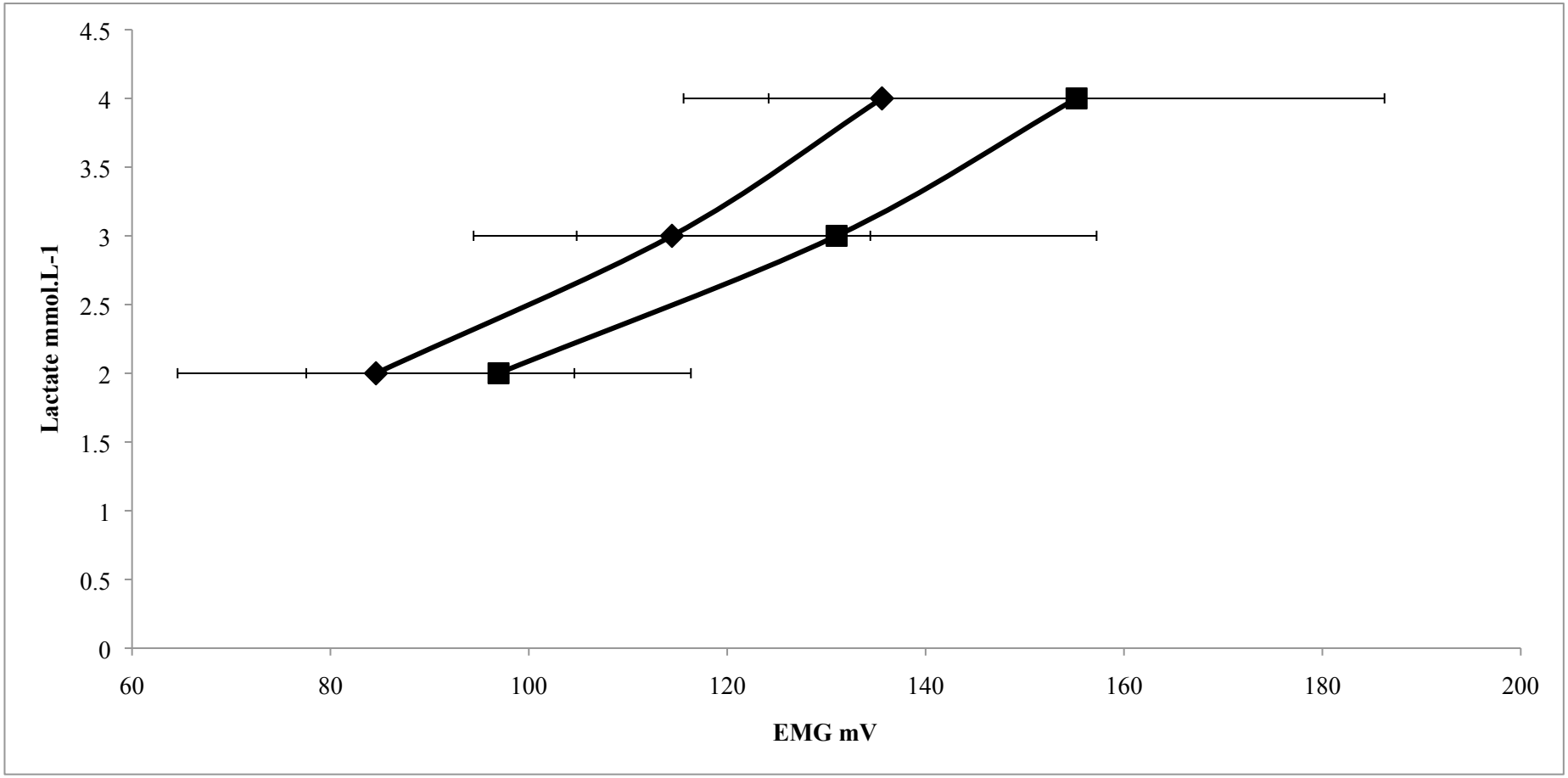

Figure 5.14. Group mean EMG values at 2, 3, and 4 mmol.L^{-1} pre (◆) and post (■) 4 weeks of bilateral-leg isometric exercise training

From the incremental test, the peak lactates that were achieved were 4.28 ± 1.63 mmol.L^{-1} and mean predicted La_{train} was 3.64 ± 1.49 mmol.L^{-1}. This gave a mean predicted training intensity (%La_{peak}) of 0.89 ± 0.16. EMG values at 2, 3 and 4 mmol.L^{-1} pre- and post-training are given in Table 5.3. These values demonstrated significant shifts pre- to post-training at 4 mmol.L^{-1} and 3 mmol.L^{-1}, but not at 2 mmol.L^{-1}. Predicted training %La_{peak} was strongly correlated with reductions in resting SBP ($r = 0.78$, $p = 0.003$, see Figure 5.15) and MAP ($r = 0.67$, $p = 0.02$, see Figure 5.16) in individuals, but not with reductions in DBP ($r = 0.43$, $p > 0.05$). Individual lactate curve shifts pre-to post-training did not correlate with individual changes after training in SBP ($r \leq 0.57$, $p > 0.05$), DBP ($r \leq 0.57$, $p > 0.05$) or MAP ($r \leq 0.47$, $p > 0.05$). Training EMG (EMG_{train}) and %EMG_{peak} did not correlate with changes in resting BP.

Table 5.3. Group mean values for electromyographic (EMG) activity at 4, 3, and 2 mmol.L^{-1} lactate, pre- and post-training and pre- and post-control.

Condition	Lactate (mmol.L^{-1})	EMG Pre (mV)	EMG Post (mV)
Training	4	135.60 ± 25.27	155.22 ± 33.55*
	3	114.43 ± 21.70	131.03 ± 27.07*
	2	84.59 ± 19.64	96.95 ± 19.96
Control	4	141.06 ± 39.20	137.35 ± 17.39
	3	117.87 ± 31.26	116.37 ± 15.18
	2	85.17 ± 20.53	86.60 ± 13.83

Group mean values (± SD) * = significant at $p < 0.05$

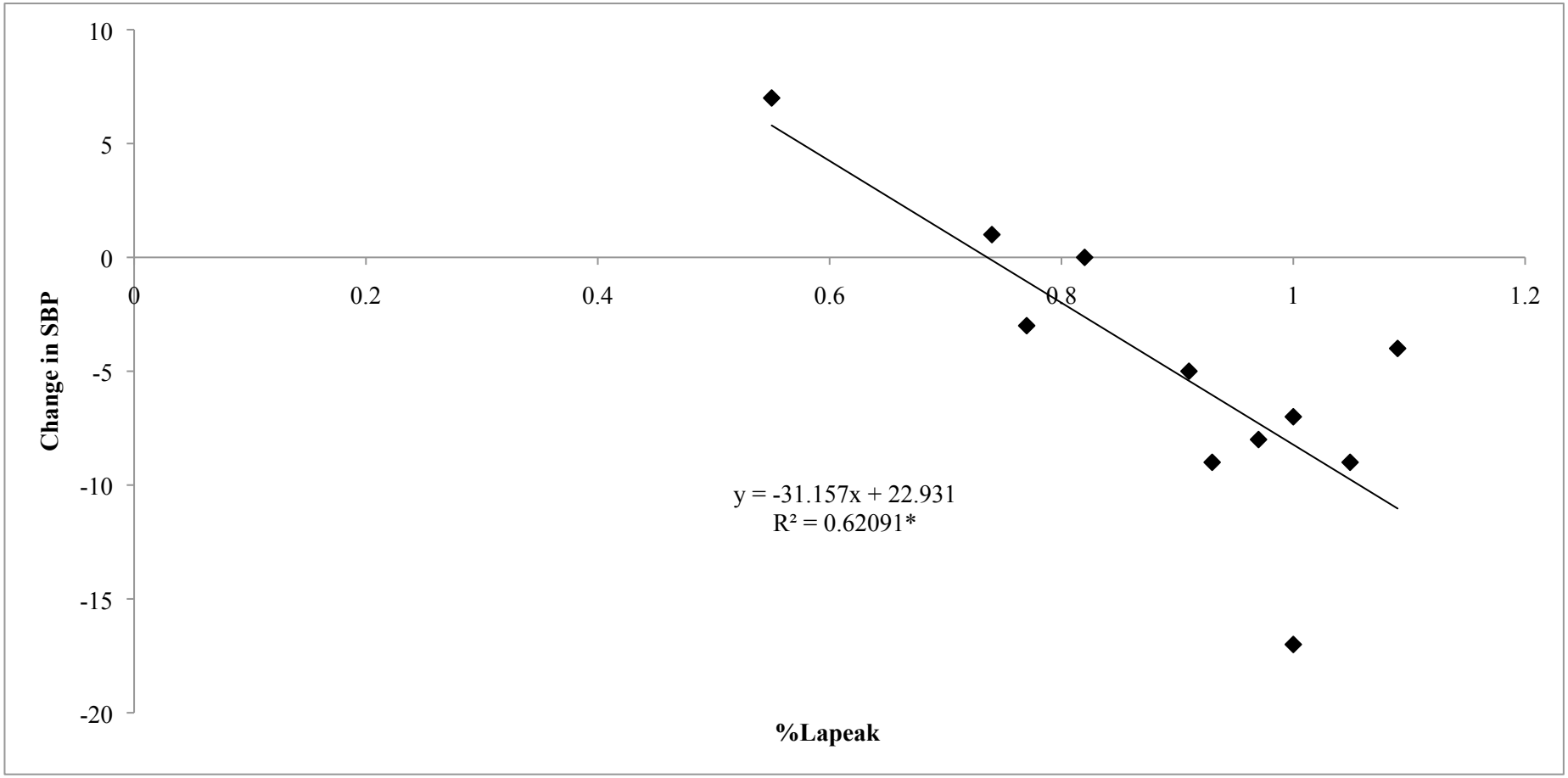

Figure 5.15. Relationship between change in resting systolic blood pressure (SBP) and predicted training intensities expressed as a mean percentage of initial lactate peak values ascertained prior to training ($\%La_{peak}$). * = significant at $p < 0.05$

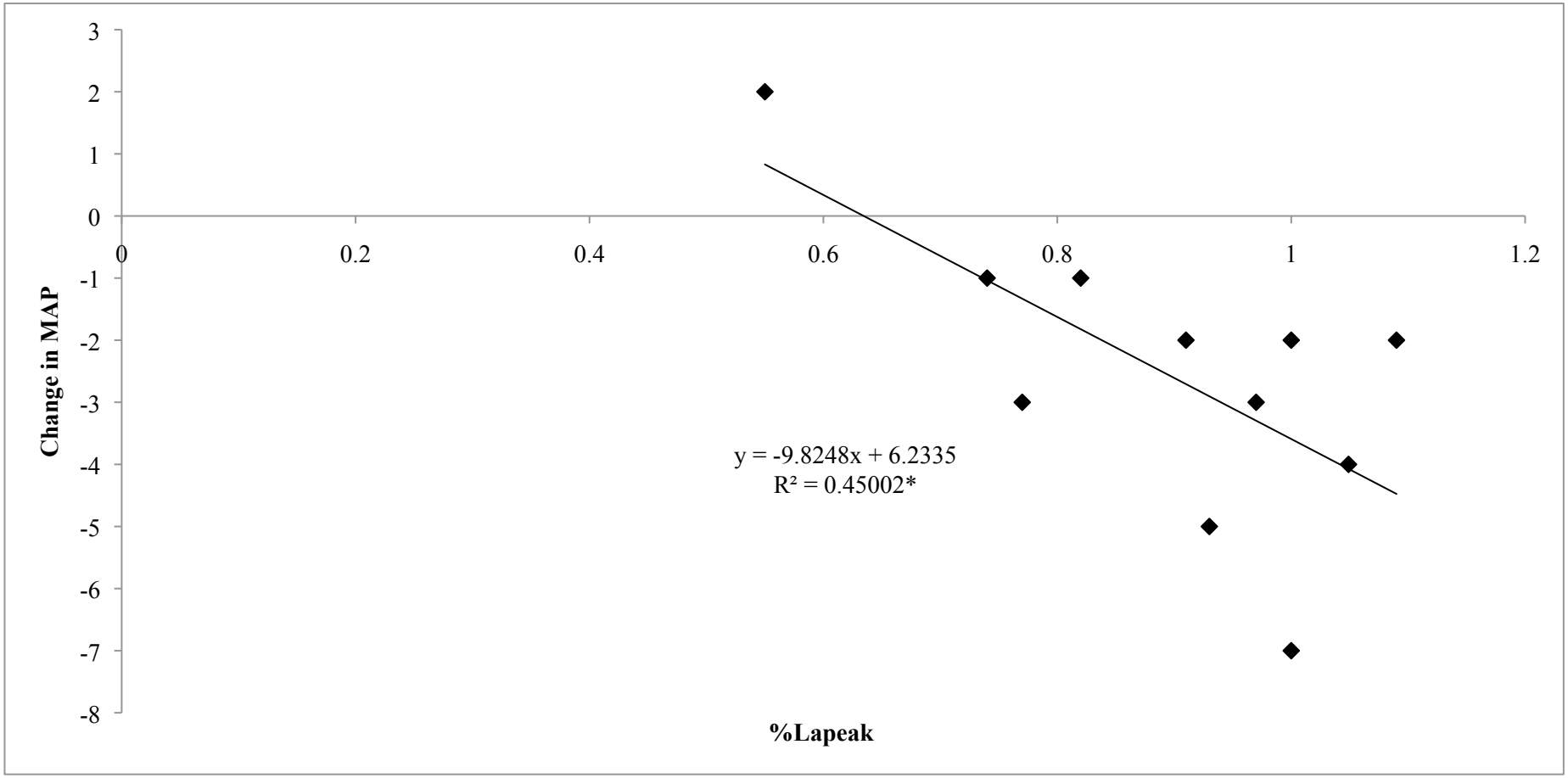

Figure 5.16. Relationship between changes in resting mean arterial blood pressure (MAP) and predicted training intensities expressed as a mean percentage of initial lactate peak values ascertained prior to training ($\%La_{peak}$). * = significant at $p < 0.05$

5.9 Discussion

The results from this study showed that 4 weeks of bilateral-leg IET caused shifts in the lactate curves when plotted against EMG. These shifts were not correlated, in individuals, with the evident reductions in resting BP. Instead, predicted training intensity, when expressed relative to peak lactate from the incremental exercise test performed before training, was correlated strongly with reductions in SBP and MAP. The peak lactates, which were recorded after incremental isometric exercise to volitional exhaustion, were lower than those seen in dynamic exercise performed for a similar duration (Klausen *et al*. 1972; Sahlin *et al*. 1976). It has been previously suggested that isometric exercise typically causes lower blood lactate levels than dynamic exercise (Harris *et al*. 1977). This is said to be due to isometric exercise inducing respiratory alkalaemia, caused by ventilatory stimulation when exercise metabolites are confined to the occluded muscle site, leading to immediate post exercise excess breathing rates and reduced blood carbon dioxide content (Poole *et al*. 1988).

The shifts in lactate metabolism observed after training, at 3 and 4 $mmol.l^{-1}$, represented 15% and 14% respectively, and these types of changes are similar to those seen after dynamic exercise training, at similar lactate concentrations (Sjödin *et al*. 1982; Denis *et al*. 1984). The statistically significant differences in group mean exponential lactate curves, pre- to post-training (Figure 5.10), were likely caused by significant increases in EMG_{peak} (388.7 ± 125.0 vs. 483.1 ± 163.1 mV, p = 0.01), which were used to formulate the incremental test procedure.

The novel finding in this study is that individuals who exercised at an intensity which represented a higher proportion of the peak lactate that was achieved in the incremental test, experienced the greatest reductions in resting SBP and MAP. The within group differences in predicted La_{train} arise from both the individual participant specific lactate response to the isometric exercise stimulus, and the participant specific slope of the HR and EMG relationship from which training intensity is prescribed. In the individuals who did not experience reduced SBP and MAP, their predicted exercise intensities represented 82%, 74% and 55% of the peak lactates achieved in the incremental test. Indeed, using the regression equation for change in SBP vs. $\%La_{peak}$, only those

individuals who train at 90% (SEE = 0.20%) of their peak lactate or above, would achieve a 5 mmHg reduction in resting SBP after 4 weeks, when using this bilateral-leg training programme. This would equate to 3.9 mmol.L^{-1} of blood lactate, on average, for the young healthy men used in this study.

The explanation for such a strong correlation between isometric exercise intensity, when expressed as a blood lactate level, and reductions in resting SBP and MAP after training may lie in the role that this metabolite has during bilateral-leg isometric exercise. It is known that metaboreceptor stimulation, during isometric exercise produces a powerful pressor response (McCloskey and Mitchell; 1972; Freund *et al*. 1979; Kaufman *et al*. 1983; Mitchell *et al*. 1989; Iellamo *et al*. 1999b; Ichinose *et al*. 2006; Fisher *et al*. 2008). However, the precise metabolite that is responsible for this response is not yet known. Several possible metaboreceptor stimulants have been proposed and these include both lactate and pH (Sheriff *et al*. 1987; Victor *et al*. 1988; Rotto *et al*. 1989; Sinoway *et al*. 1989a).

It is generally accepted that in exercising muscle under ischemic conditions, the formation of acid salt sodium lactate will increase to a point where cellular buffering capacity is surpassed, and at this point and beyond there is a subsequent decrease in cellular pH (Robergs *et al*. 2004). It is known that changes in muscle and blood hydrogen ion concentration (H^+) have a profound effect on the local vasculature, causing vasodilatation. When this is combined with the powerful pressor response that is known to be induced, the combined effects of these two responses might be the mechanism for the training-induced reduction in resting SBP and MAP. However, how this combination of local pH-mediated vasodilatation and powerful pressor response during each exercise bout in training is translated into a reduction in resting BP following training is not known.

It has previously been suggested that repeated blood acidosis enhances certain enzymes' activity, including endothelial nitric oxide synthase (Murphy, 1999), which plays an integral role in regulating vascular function (Ignarro *et al*. 1987; Palmer *et al*. 1988). Interestingly, regular dynamic exercise for a period of 4 weeks (the same duration used for IET in this study) has been shown to result in increased expression of endothelial

nitric oxide synthase (Hambrecht *et al*. 2003). However, the changes reported in that study were associated with levels of vascular shear stress caused during training, rather than investigating relationships between blood acidosis and endothelial nitric oxide synthase. The extent of vascular shear stress generated during bilateral-leg isometric exercise is not currently known, so theoretical connections between such studies remains speculative for now.

The blood lactate changes associated with increasing intensities of rhythmic IHG exercise have been related to post-exercise hyperaemia (Taylor *et al*. 1988). These workers argued that the lactate level represented an increasing metaboreceptor stimulus, and showed that post-exercise hyperaemia correlated strongly with increasing lactate levels. Therefore, the explanation for the present training-induced reductions in resting BP might lie in the adaptations made to the post-exercise hyperaemia and the challenges it presents.

5.10 Conclusion

Isometric training intensity, when expressed relative to peak lactate from the incremental exercise test performed before training, was correlated strongly with reductions in BP. Individuals who exercised at an exercise intensity which represented a higher proportion of the peak lactate that was achieved in the incremental test, experienced the greatest reductions in resting SBP and MAP.

5.11 Summary of Chapter 5

The inference from the data of this chapter is that 'local muscle fatigue' plays a role in the training stimulus responsible for the reduced BP after bilateral-leg training. Furthermore, this fatigue is reflected by blood metabolite production (lactate). Therefore, the stimulus for the training-induced reductions in BP may be local muscle fatigue-related and possibly related to lactate production. This 'fatigue' and lactate production probably mediates its effect via metaboreceptor stimulation and the resulting

powerful pressor response. It would be reasonable to assume that this powerful pressor response involves the autonomic nervous system and that repeated stimulation in this way may cause concomitant changes in resting autonomic nervous system function (as manifest in changes in HRV – Chapter 3, Part B).

Chapter 6

General Discussion

6.1 General discussion

In this thesis, five main studies reporting data associated with different aspects of isometric exercise were arranged into Chapters 3, 4 and 5. They were; (1) The relationship between selected cardiovascular (CV) variables and blood pressure (BP) at rest in normotensive healthy young males; (2) The acute CV responses to a single session of isometric exercise; (3) The CV adaptations following a 4 week course of bilateral-leg isometric exercise training (IET), and; (4) The relationship between adaptations in neuromuscular (NM) markers of training intensity (EMG_{freq} and EMG_{amp}) and reductions in resting BP following 4 weeks of bilateral-leg IET, and (5) The relationship between adaptations in metabolic markers of training intensity (blood lactate) and reductions in resting BP following 4 weeks of bilateral-leg IET.

The main findings of these studies included in this thesis were:

1. Among a group of normotensive young males there was a strong association between components of autonomic nervous system function and resting BP, but none of the other selected CV variables.
2. A single session of bilateral-leg isometric exercise affects these same measures of autonomic nervous system function for a prolonged period during recovery (4 hours), although significant responses in BP did not coincide with this.
3. Significant reductions in systolic (SBP), diastolic (DBP), and mean arterial (MAP) pressure were evident after 4 weeks of bilateral-leg IET, but these were not reflected in concomitant changes in any of the selected CV variables. Thus, the transient changes seen after a single session of isometric exercise (Chapter 3, Part B) are not evident at rest after 4 weeks of training, suggesting that either:
    - CV variables such as cardiac output (Q), stroke volume (SV), total peripheral resistance (TPR), baroreflex sensitivity (BRS), and components of heart rate variability (HRV) do not play a role in these observed BP adaptations to IET,

      Or:
    - There is an inherent lack of sensitivity, using the methodologies within this thesis, in detecting small changes in the measurement of these variables after IET.

4. Reductions in resting BP following IET correlate strongly with training intensity and thereby the extent to which fatigue is induced during each training session (as measured using changes in EMG amplitude or frequency). Furthermore:
    - Increases in 2min-$Torque_{peak}$ are evident in those individuals who experienced significant BP adaptation, and the ability to maintain torque for 2 minutes is important in eliciting BP reductions.
    - The traditional method of prescribing IET intensity as a percentage of maximal voluntary contraction appears to be poorly associated with reductions in resting BP.
5. Isometric training intensity, when expressed relative to peak lactate from the incremental exercise test performed before training, was correlated strongly with reductions in BP after training, suggesting:
    - Local muscle fatigue plays a role in the training stimulus responsible for the reduced BP after bilateral-leg training, and is reflected by blood metabolite production (lactate).
    - The stimulus for the training-induced reductions in BP may be local muscle fatigue-related and may be related to lactate production.

6.2 According to the findings of Chapter 3 (Part A), are there any non-invasive, resting cardiovascular markers that correlate strongly with resting blood pressure, amongst normotensive young males?

None of the CV variables selected for this thesis, as possible candidates in the mechanism responsible for reduced resting BP after IET, were related to the BP changes reported in Chapter 4. However, components of autonomic nervous system function (manifest by HRV parameters) seem to be important in the absolute resting BP in these individuals. As reviewed in Chapter 1, HRV has been investigated previously as an explanatory variable for reduced resting BP after IET (Taylor *et al*. 2003). However, they also reported significant changes in BP variability. Therefore, the finding in this thesis, of a correlation between resting BP and HRV alone should probably be interpreted with caution. It has been suggested that evaluating the interactions of BP variability, HRV, and respiratory activity in concert might offer a more comprehensive analysis of CV regulation (Parati *et al*. 1995).

Also, in Chapter 1, it was suggested that the evidence in support of changes in resting Q, SV and TPR is somewhat equivocal. Some studies have suggested that altered peripheral vascular resistance is predominant (McGowan *et al*. 2004; Cornelissen and Fagard, 2005) whereas others have suggested that the arterial baroreflex is in fact responsible (Coats *et al.* 1992; Grassi *et al.* 1994). Furthermore, the measurement of markers such as TPR has been hampered by the difficulties associated with its derivation from non-invasive measures of Q and MAP. Therefore, it is perhaps not surprising to show poor correlations between resting BP and measures such as these. Finally, cross-sectional data, although useful in establishing relationships between particular variables, is considered weak for 'causal inference' purposes (Bauman *et al*. 2002). In such studies far greater numbers are required to establish meaningful relationships.

6.3 According to the findings of Chapter 3 (Part B), does bilateral-leg isometric exercise elicit an acute response in blood pressure, and/or any other cardiovascular variables?

Results presented in Chapter 3, pages 84-93, show that a single session of bilateral-leg isometric exercise did not cause an acute response in SBP, DBP, or MAP, 4 hours after the exercise stimulus. However, the data did show significant differences in measures of autonomic nervous balance following a single session of bilateral-leg isometric exercise, both pre and post measures, compared to the control condition. These were the same variables reported to be correlated with resting BP in a group of normotensive individuals (Chapter 3, pages 65-74). Normalised units of low frequency (LFnu) activity were increased 4 hours after a single session of bilateral leg isometric exercise, compared to control data. Given the assumptions made about normalised units of HRV data (Malliani *et al*. 1991; Pagani *et al*. 1997; Cooley *et al*. 1998), it can be interpreted from these results that 4 hours after the exercise stimulus, there is still an increased level of sympathetic autonomic activity (sympathoexcitation), likely a protracted effect of excess levels during exercise (Iellamo *et al*. 1999b).

These findings are in accordance with a previous study, showing increased sympathetic activity 5 minutes after a single resistance exercise stimulus (Heffernan *et al*. 2008), but contrary to increased vagal activity reported 5 minutes after a single IHG exercise

stimulus (Millar *et al*. 2009). However, according to the present data, this increased sympathetic modulation has not been sufficient to elicit BP responses.

In terms of other CV variable acute responses, for normotensive participant groups and dynamic modes of exercise it is frequently reported that increased Q is evident due to increased HR (Coats, 1989; Hara and Floras, 1992; Halliwill *et al*. 1996; West *et al*. 1998), and SV (Floras *et al*. 1989; Cleroux *et al*. 1992; Kulics *et al*. 1999). It would appear that isometric exercise does not have the same impact on the acute CV responses to that of dynamic modes of exercise for normotensive individuals. Both local and peripheral vascular resistance have generally been reported to decrease during a post-dynamic exercise hypotensive period (Coats *et al*. 1989; Cleroux *et al*. 1992), whereas the results reported in this thesis (Chapter 3, pages 84-93) show no significant effect. Again, this has only previously been reported in an exclusively hypertensive participant group (Hagberg *et al*. 1987), again suggesting that the physiological occurrences following isometric exercise perhaps differ from those evident following dynamic modes of exercise.

Given the significant differences reported in HRV data (Chapter 3, pages 84-93), it might have been expected to observe changes in measures of TPR. Resting HRV derived indices of sympathetic activity have been reported to be elevated during a period of post exercise hypotension (Piepoli *et al*. 1993; MacDonald *et al*. 2001; MacDonald *et al*. 2002), perhaps in an attempt to return BP to homeostatic levels. The prolonged increased efferent sympathetic activity during rest has been directly related to the specific location that was initially the cause of the sympathoexcitation (Kenney and Morgan, 1993). Therefore, it is plausible to suggest that the increased indices of sympathetic efferent activity post exercise, reported in Chapter 3, Part B, were caused by the muscles that were most active during the bilateral-leg isometric exercise session.

Increased vasoconstrictor sympathetic activity during exercise is countered by local metabolite accumulation which causes peripheral vasodilatation of the active capillary beds (Davies, 1995; Davis and Hill, 1999; Cristensen and Mulvany, 2001; Thomas and Segal, 2004). Therefore the protracted post-exercise elevations of sympathetic activity could have been as a result of increased metabolite-induced capillary perfusion in the

leg, after the occlusion during isometric exertion. Increased capillary perfusion will cause blood to pool, elevating the resistance of flow through these areas (Cristensen and Mulvany, 2001). The absence of significant change in TPR within the data may be due to the inherent variance in measurement sensitivity (see Chapter 2, pages 46-49). Of course, temporary increased perfusion in peripheral capillary beds would also likely result in Q, SV, and HR changes (Coats, 1989; Floras *et al*. 1989; Cleroux *et al*. 1992; Hara and Floras, 1992; Halliwill *et al*. 1996; West *et al*. 1998; Kulics *et al*. 1999), although measurement sensitivity (Chapter 2, pages 46-49) may have played a role in detecting significant change.

Finally, the data from this study allowed analysis of the day-to-day changes in resting BP during subsequent isometric exercise testing (on 3 occasions within a week). It showed that BP remained stable throughout the 3 testing sessions. This allows, with some degree of confidence, confirmation of the suggestion that the changes observed after training (Chapter 4, pages 105-110), in which reduced BP is presented, are indeed adaptations to the training and not a 'prolonged acute' response to the final training session. It was necessary to confirm this supposition, since isometric exercise has previously been shown to elicit post-exercise hypotension (Stewart *et al*. 2007; Millar *et al*. 2009). A time-course for reversion to initial baseline values had not been established. These previously published post-exercise hypotension responses are typically measured for 5 minutes post-exercise. The data in Chapter 3, Part B of this thesis has provided evidence to suggest that these previously reported responses are relatively short-term hypotensive responses that disappear within 48 hours. For these reasons the post-training (4 weeks) data within this thesis was collected at least 72 hours after the final training session.

6.4 According to the findings of Chapter 4, does blood pressure undergo adaptation following 4 weeks of bilateral-leg isometric exercise training?

The findings (initially presented in Chapter 4, pages 105-110) show that 4 weeks of bilateral-leg isometric exercise is sufficient to result in significantly reduced resting BP (SBP = -5 mmHg, DBP = -3 mmHg, and MAP = -3 mmHg). The fact that BP was reduced is in agreement with previously published studies (Wiley *et al*. 1992; Ray and

Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Millar *et al*. 2007; Wiles *et al*. 2010). However, the present findings of statistically significant reductions across all three BP components (SBP, DBP, and MAP), and over such a short duration (4 weeks) have not been reported previously.

Given the short duration of the study presented in Chapter 4, and that the average isometric training intensity for that study (approximately 24% MVC) is substantially lower than that commonly used in IHG training studies (30% to 50% MVC), there appears to be a difference in the training stimulus between bilateral-leg and alternating IHG training. This is likely to be linked to the amount of muscle mass used during IET. Previous studies have reported that when isometric exercise is performed over a relatively short time, before the point of fatigue, the amount of muscle mass utilised strongly influences the CV response, including the time taken to reach maximal BP, HR, and other CV values (Mitchell *et al*. 1980). Given that the bilateral-leg isometric exercise bouts used within this thesis were just 2 minutes long, as were the majority of previous IHG studies (Wiley *et al*. 1992; Taylor *et al*. 2003; McGowan *et al*. 2004), this notion is given added credence.

Therefore, it is likely that the 'training stimulus' is greater for a bilateral-leg protocol compared to an IHG protocol, even if training duration and frequency are identical. These points would appear to suggest that the rate of CV adaptation following IET is somehow linked to the exposure to an elevated pressor response and this in turn is dictated by the total training volume (time x intensity x muscle mass).

It is unlikely that any methodological issues confounded the findings of reduced SBP, DBP, and MAP after 4 weeks of bilateral-leg isometric exercise. Firstly, it should be noted that the coefficient of variance of measurement for SBP (1.8%), DBP (2.6%), and MAP (2.1%), were smaller than the reported changes in SBP (4.0%), DBP (2.7%), and MAP (2.6%) following the exercise intervention, compared to the control condition. Secondly, BP was measured using an automated device that removed observer error and/or bias from the procedure (Coe and Houghton, 2002). And finally, the findings from the acute response study (Chapter 3, pages 84-93) showed no acute BP responses 4 hours after an exercise stimulus, identical to the protocol used in the intervention study.

In this thesis, post intervention BP measures, following the 4 week training period, were taken at least 72 hours after the final training session. Therefore it can be inferred with reasonable confidence that the reported adaptations of resting SBP, DBP, and MAP were indeed physiological. The data presented in Chapter 4, pages 105-110 also shows that the proposed physiological BP reductions evident after 4 weeks of bilateral-leg isometric exercise were reversed after a de-training period of 1 week. Taking the de-training findings in Chapter 4 together with previously published results of IET studies using differing protocols, intensities, training durations, and re-test time periods (Wiley *et al*. 1992; Howden *et al*. 2002), it is likely that BP reductions are reversed to pre-intervention baseline values in a time frame that is associated with the magnitude of the BP reductions caused by the IET.

6.5 According to these findings, what mechanisms are responsible for blood pressure adaptation following isometric exercise training?

Neither SBP, DBP, or MAP reductions were explained by any of the measured CV variables, presented in Chapter 4, after 4 weeks of bilateral-leg IET. Blood pressure is the net product of Q (itself the product of HR and SV) and TPR. Not only were these CV variables not associated with BP adaptations, but they also did not present any significant changes independently after 4 weeks of bilateral-leg isometric exercise. Previous studies have made a number of suggestions to explain the reductions in resting BP commonly observed after IET. Given the fundamental principles of BP, these previous studies frequently state that either Q and/or TPR must be involved (Wiley *et al*. 1992).

The apparent lack of any change in the selected CV variables could support the notion that after just 4 weeks of IET, the resultant BP adaptations are not predominantly 'centrally' mediated. It suggests instead that peripheral physiological mechanisms are more dominant as a means of reducing resting BP, as is suggested by Peters *et al*. (2006). This can be substantiated by both studies detailed in Chapter 5, where local muscle fatigue and metabolite production were strongly associated with the resultant BP adaptations. It appears that modified peripheral conditions present during the isometric training stimulus may well play an important role in mediating the BP changes. This

would of course have been typically supported by changes in TPR, but aforementioned measurement sensitivity issues of Q used in TPR calculations may have impeded the detection of any slight change following this IET.

Another possible explanation for this is that the measurement of variables such as Q may be hampered by high variability (Damgaard and Norsk, 2005; Pemberton *et al*. 2005). Also, the method by which TPR was calculated was probably lacking in sensitivity. Measurement of Q by re-breathing is indirect and can exhibit higher variability when performed at rest compared to during exercise (Vanhees *et al*. 2000). Furthermore, the measure of TPR used within this thesis is further derived from this indirect measure of Q. In addition, HRV (Eckberg, 1997) and BRS (Lipman *et al*. 2003) are known to have measurement limitations, as can be seen from the high coefficient of measurement variations presented for each in Chapter 2, pages 46-49. It is also worth noting that due to apparatus protocols, simultaneous measurement of Q and MAP were not possible. Therefore, the apparent absence of changes in these measures could instead be a reflection of their lack of sensitivity in detecting small changes, rather than an absence of physiological change.

A common theory mooted about isometric training-induced BP reductions is that chronic exposure to an isometric exercise stimulus could result in an altered state of autonomic nervous balance (investigated through the use of HRV in this thesis). The suppositions state that sympathetic modulation is likely reduced over time, and that importantly, it also offers a practicable solution as to how Q and/or TPR may also undergo adaptation (Ray and Carrasco, 2000; Taylor *et al*. 2003). In a similar vein to the findings presented within this thesis (Chapter 4, pages 105-110), Ray and Carrasco (2000) found that BP reductions after 5 weeks of IHG exercise were not accompanied by changes in sympathoexcitation, measured by efferent muscle sympathetic nerve activity (MSNA), which they used as their index of central sympathetic activity. This lack of association between BP and MSNA changes has also been reported following intervention periods of other modes of exercise (Carter *et al*. 2003).

The issue with using MSNA as an index of sympathetic activity however, is that it does not reflect cardiac autonomic activity as clearly and accurately as power spectral

analysis of HRV does (Kamath and Fallen, 1993). Whilst it was reported as a statistically non-significant finding, Taylor *et al*. (2003) did show a trend toward a reduction in the LF/HF ratio component of HRV, compared to control data, after 10 weeks of IHG exercise. This finding, whilst non-significant, was given extra gravitas by the fact that the same authors reported a significant reduction in SBP variability for LF/HF ratios in the same training group. This taken together suggests that there could be a decrease in the sympathetic modulation of the autonomic nervous system, which indirectly leads to reductions in BP following a course of isometric exercise. Whilst the results of Chapter 4, pages 105-110, together with previous work (Wiles *et al*. 2010), cannot support this notion, it is likely that measurement variability of power spectral analysis HRV could be masking physiological occurrences (Eckberg, 1997; Sandercock *et al*. 2005), as previously mentioned.

What this thesis has shown, in Chapter 3, pages 84-93, is that relative spectral components of HRV are significantly affected by a single session of bilateral-leg isometric exercise, so the association between the stimulus and physiological mechanism does appear to exist. It seems likely that measurement limitations, or rather measurement considerations, may be the limiting factor when transferring the idea to an intervention study. In terms of a measurement consideration, it may be pertinent to measure HRV far more frequently than simply pre and post intervention, to allow for a more comprehensive understanding of trend shifts and detection of measurement anomalies (Sandercock *et al*. 2005), which may otherwise corrupt statistical conclusions. Another consideration is the use of nonlinear methodologies, including HR complexity (Richman and Moorman, 2000) and detrended fluctuation analysis (Peng *et al*. 1995), which are based on chaos and dynamical systems theory. These nonlinear methods are purported by some researchers to be more sensitive to smaller modulations in HR than power spectral HRV measures (Huikuri *et al*. 2000; Tulppo *et al*. 2003; Heffernan *et al*. 2007), and so could be suited to isometric exercise studies.

Whilst results contained within this thesis do not offer conclusive centrally mediated mechanistic changes, the findings presented in Chapter 5, relating to fatigue and metaboreceptor stimulation perhaps offer an insight into peripherally modulated adaptations following IET that may lead to a modified BP state. In Chapter 5, pages 139-152, it can be seen that the relative levels of lactate accumulation during training had a profound effect on the subsequent SBP and MAP adaptations. It has previously

been proposed that the direct affects of repeated pH changes (as would occur with the elevated muscle and blood lactate levels) may cause localized peripheral vascular adaptation, which through altering vascular resistance might then lead to BP change (Fisher and White, 1999).

When looking at previous work conducted by Fisher and White (1999), it is possible to draw on interesting lines of thought that relate to the chronic adaptation data presented in both Chapters 4 and 5 of this thesis. Fisher and White (1999) propose that the sympathoexcitation, resulting from the stimulation of the aforementioned mechanically and metabolically sensitive receptor cells, and muscle afferent activity during exercise, can alter vascular resistance and, as such, TPR. The same authors also claim that DBP change is reflective of TPR change. So, whilst TPR did not show evidence of alterations after 4 weeks of isometric exercise in this thesis, it may be that as DBP did provide evidence of significant adaptation (together with the sensitivity issues of TPR calculation previously discussed), TPR could have actually played a role in the training caused reductions in resting BP. Interestingly, Fisher and White (1999) also propose that pH is the likely metaboreceptor stimulus that can lead to excess sympathoexcitation, as has been implicated in the lactate findings, presented in Chapter 5, Part B.

Furthermore, the fact that de-training results presented in Chapter 4 showed reductions in resting BP were reversed within 7 days of cessation of training substantiates the notion of peripherally instigated adaptations to IET. It is incredibly unlikely that any centrally mediated, structural changes could be reversed within such a short time frame. Total peripheral resistance to blood flow may be reduced by exercise within such a short time period, due to endothelial reaction to vasodilator chemicals, especially nitric oxide (Green *et al*. 2004). Specifically, it has been reported that rhythmic handgrip training results in up-regulation of nitric oxide bioavailability, via the shear stress blood exerts on the endothelium during exercise (Tinken *et al*. 2009; Tinken *et al*. 2010). This is reported to be due to significant antegrade blood flow during handgrip exercise, resulting in increased shear rates on the vascular wall, leading to changes in endothelial vasodilator activity (Green *et al*. 2002).

In a recent study, Tinken *et al*. (2010) provided evidence to suggest that the increased antegrade blood flow and shear rates during rhythmic handgrip exercise were principally responsible for subsequent changes in endothelial vasodilator function, measured by flow mediated dilatation. Interestingly, the study conducted by Tinken *et al*. (2010), also found that the same changes in endothelial vasodilator function were not evident in a rhythmically exercised arm that had shear stress attenuated by the use of external cuff inflation on the exercising limb. Therefore, in line with the work of Tinken *et al*. (2010), it could well be important to discover whether blood flow dynamics during non-rhythmic, continuous isometric exercise (where occlusion to blood flow is believed to occur) are similar to those of the cuffed limb of the aforementioned study, or if significant levels of antegrade blood flow and shear stress rates are present as they were in the uncuffed limb.

If indeed blood flow dynamics are similar to those of the uncuffed limb, this could offer an explanation as to how resting BP may be reduced within a short intervention period of IET, and then rapidly reversed to pre-intervention levels following cessation of training. An earlier study has also declared the significance of nitric oxide in the vasodilator response to prolonged handgrip exercise at a range of intensities (Gilligan *et al*. 1994) The greater the availability of nitric oxide, the more dominant vasodilatation activity is, thus reducing peripheral resistance to blood flow and so BP (Rees *et al*. 1989). In addition, changes in nitric oxide derived endothelial vasodilator function have been reported in the brachial artery of the arm, following lower limb exercise training (Green *et al*. 2004; Maiorana *et al*. 2001), which suggests that adaptation may not be isolated to the exercised muscle group.

If the work of Tinken *et al*. (2008; 2009; 2010) is to be implemented in the field of IET, then it will be necessary to measure blood flow dynamics during an isometric exercise session similar to those previously used (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Peters *et al*. 2006; Millar *et al*. 2007; Wiles *et al*. 2010; Devereux *et al*. 2010). This will involve the use of ultrasound technology and artery lumen edge detection software to provide indices of flow mediated dilatation, with synchronised pulse wave velocity waveform detection software for blood flow and shear rate measures. The technologies needed to provide such measures were not available during the work of this thesis, as they remain expensive to procure. However, it now appears to be of utmost important to include

these technologies in subsequent IET studies. The lack of this technology, together with the inherent variance of measured CV variables discussed in Chapter 2 is a limitation of this thesis.

Early work investigating the effects of isometric exercise on the CV system suggest that TPR may be also be reduced by either the growth of capillary vessels (Kiveloff and Huber, 1971) similar to adaptations evident after endurance and resistance trained muscle (Brown and Hudlicka, 2003), or increases in the size of venous vessels improving blood flow dynamics (Lind and McNicol, 1967). In relation to new capillary networks being formed, it is suggested that chemical and mechanical stimuli cause the activation of endothelial cells to reproduce and produce new capillaries from existing networks (Prior *et al*. 2004). Importantly, a state of muscle hypoxia, which is strongly related to isometric exercise intensity and duration (Rowell, 1993), is said to be a key mediator in the activity of the hormone responsible for endothelial growth regulation (Shweiki *et al*. 1992).

With regards to increased venous vessel size, a previous study utilising IHG exercise for a period of 6 weeks, at an intensity of 25% to 35% MVC, reported a significant increase in lumen size of the cephalic vein, located in the exercised limb (Leaf *et al*. 2003). However, BP measurements were not recorded during this study, which would have afforded a direct assessment of the implications of increased venous size on BP state. It is worth noting that training duration (6 weeks) and intensity (25 to 35% MVC) as used by Leaf *et al*. (2003) are similar to both those used in this thesis, and those in some previous isometric exercise studies.

The role that the arterial baroreflex system plays in the BP adaptations evident in Chapter 4 appears to be limited, or non-existent, given the lack of significant change in BRS, and any association or trend with SBP, DBP, and/or MAP changes. This supports previous research indicating that isometric exercise is an improbable means of baroreceptor resetting (O'Leary, 1996; Kamiya *et al*. 2001; Raven *et al*. 2002), and contrasts previous suggestions made by Barrett and Malpas (2005), that baroreflex function is a plausible mechanism for not only BP modification, but also autonomic nervous function change, in concert with other physiological mechanisms. However, the

inherent 'noise' in BRS measurement (Chapter 2, page 49) must be considered when interpreting results, or more specifically, reporting no significant change. Isometric exercise specifically has been said to stimulate the baroreflex system, both during and immediately after a repeated exercise stimulus, and leads to subsequent BP reductions (Kiveloff and Huber, 1971), although this theoretical standing now appears to have been superseded. It is also worth noting that a detailed explanation for the physiological occurrences responsible for BRS resetting and subsequent BP change has never been provided.

6.6 According to the findings of Chapter 5, what are the factors of isometric exercise that appear to be crucial in eliciting blood pressure adaptation?

Previously, it has been reported that BP, at the point of fatigue, reaches the same level at three different intensities of isometric exercise (20%, 40%, and 60% MVC), and that the only difference in the BP response is the rate at which it reaches that peak level (Funderburk *et al*. 1974). There is a similar finding reported by Seals and Enoka (1989), whereby the same intensity of isometric exercise (30% MVC) is repeated, with the duration of 'contraction' reduced with each repetition. Again, irrespective of duration of isometric exertion, BP levels were consistent at the point of fatigue. This led Rowell (1993) to propose that fatigue is an important factor in determining the CV response to an isometric exercise stimulus, but that it was not a quantifiable one. The findings within this thesis however (Chapter 5, Part A), have in fact provided quantifiable measures of fatigue during bilateral-leg isometric exercise, through the use of surface EMG signal amplitude and frequency analysis, previously used during dynamic modes of exercise (Person and Mishin 1964; Person and Kudina 1968; Vredenbregt and Rau, 1973; Moritani *et al*. 1982).

The data in Chapter 5, pages 121-129, shows that training torque did increase as the training progressed, but it did not relate to the ensuing BP reductions. This suggests that simply trying to generate torque is not enough to cause a reduction in BP. Furthermore, trying to generate torque at a proportion of an individual's MVC, as commonly performed previously (Wiley *et al*. 1992; Ray and Carrasco, 2000; Howden *et al*. 2002; Taylor *et al*. 2003; McGowan *et al*. 2004; Millar *et al*. 2007) is not the crucial factor.

What seems to be the deciding factor is torque has to be generated at a level that induces fatigue. To identify that level, the best way is to identify the highest torque that can be sustained for the equivalent time period that is subsequently used in training sessions (2 minutes in this thesis).

The reason for this causal link between 'fatiguing torque' and BP has previously been attributed to the chemical environment around the muscle and specifically the local muscle pH (Ahlborg *et al*. 1972; Edwards *et al*. 1972; Funderburk *et al*. 1972; Karlsson and Ollander, 1972; Karlsson *et al*. 1975; Tesch and Karlsson, 1979). The pH changes (acidosis) are likely caused by anaerobic by-products such as lactate (Harris *et al*. 1977), as was measured in Chapter 5. It can be seen in Chapter 5, pages 139-152 that training blood lactate levels correlated significantly with subsequent reductions in resting SBP and MAP, following 4 weeks of bilateral-leg IET. Any change in pH stimulates an excess pressor response (an upward adjustment in the pressor response), which maintains the local peripheral vascular stimulus for adaptation (McCloskey and Mitchell; 1972; Freund *et al*. 1979; Kaufman *et al*. 1983; Mitchell *et al*. 1989; Iellamo *et al*. 1999b; Ichinose *et al*. 2006). This metabolically derived elevated CV pressor response would theoretically act in concert with the mechanically (increased muscle mass) derived CV pressor response stimulus, as previously discussed, that together may elicit the chronic adaptations evident after such a short intervention period as presented in this thesis.

In relation to absolute levels of lactate recorded during the discontinuous incremental test, it appears that individual responses are varied, perhaps due to differences in inherent lactate production capabilities, as has been previously discovered (Katz and Sahlin, 1988). So, if looking at absolute levels, it might be largely influenced by an individual's 'habitual' lactate levels, which has previously been linked to muscle fibre type and anaerobic training history and current status (Komi *et al*. 1978; Svendhal and MacIntosh, 2003). The selection criteria for participant recruitment used within this thesis (Chapter 2, pages 24-25) did not distinguish between aerobic and anaerobic modes of habitual physical activity. However, relating estimated training lactate levels to participants' incremental test peak values removes any confounding issues of individual lactate production idiosyncrasies. The only limitation is that the present data infers lactate from EMG training levels. Although it is suggested that the presentation of a stable lactate and EMG relationship reported in Chapter 2 (pages 50-60) alleviates any

real doubt about the stability and reliability of EMG training based lactate level predictions.

So, the key principles of IET found in this thesis is that fatigue must be induced, and that resultant BP adaptation appears to be linked to the stimulation of the aforementioned metaboreceptors. This is supported by the significant correlations presented in Chapter 5, pages 139-152, between SBP reductions and relative training lactate ($r = 0.78$, $p = 0.003$), and MAP reductions and relative training lactate ($r = 0.67$, $p = 0.02$) after 4 weeks of bilateral-leg IET. Stimulating metaboreceptors in this way does provide a potent additional pressor response, as previously mentioned, which counters the arterial baroreflex response during exercise (Crisafulli *et al*. 2006). Of course, it is known that changeable chemical environments affect vascular tone, and the vasodilatory and vasoconstriction function of the vasculature (Fisher and White, 1999). But, exactly why repeatedly creating an additional, or excess CV pressor response in this way, results in reduced resting BP is not known, and the data contained within this thesis cannot offer an explanation.

There have been several recent studies that have investigated changes in vascular conductance following isometric exercise. This has been based on the theory that vascular resistance may be reduced via improved blood flow to skeletal muscle (Gleser, 1973, Klausen *et al*. 1982). The theory asserts that through changing shear rates of the vasculature during isometric muscular activity, vasodilators including nitric oxide, prostaglandins, and adenosine, stimulate vascular endothelial growth factors. It is suggested that vascular endothelial function may be enhanced within days of initiating an exercise regimen (McAllister and Laughlin, 1997). Endothelial function has been shown to improve after 4 weeks of pharmacological intervention (O'Driscoll *et al*. 1997), but after 4 weeks of IHG training, there was no improvement in endothelium-dependent and independent vasodilatation (Green *et al*. 1994).

In that IHG study, peak vasodilator capacity significantly increased after 4 weeks of IHG training in the trained limb, but not the untrained limb, leading Green *et al*., (1994) to conclude that 4 weeks of IHG exercise augments peak vasodilator capacity exclusive of stimulated activity of the nitric oxide dilator system. However, Green *et al*. (1994)

acknowledged that implications drawn from their study are limited due to the nature of the training stimulus used, and that a different mode, intensity, or duration of exercise may have resulted in different findings. Indeed, more recently significant increases in hyperemic blood flow and decreased vascular resistance after just 1 week of IHG training have been reported (Alomari and Welsch, 2007). It should be stated however, that both the frequency (5 days.wk^{-1}) and intensity (60% MVC) were much higher than usually seen. Alomari and Welsch (2007) found no changes in the untrained contra-lateral arm, together with no changes in measures of autonomic balance, which supports the findings of this thesis, and which led them to hypothesize that adaptations in hyperemic blood flow were locally modulated.

Previously (Silber *et al*. 1991), a 50% improvement in forearm blood flow with reciprocal reductions in vascular resistance after 4 weeks of cycle ergometer exercise has been reported. The idea of rapid adaptations in the vasculature is supported by a recent study (Tinken *et al*. 2008) that reported flow mediated dilatation (FMD) changed after as little as 2 weeks of dynamic treadmill exercise. This change was followed by subsequent improvements in conduit artery dilator capacity after 4 and 8 weeks. Even though this study used a dynamic mode of exercise, it could be argued that it used similarly large muscle mass to the isometric exercise currently used and thereby offers a possible explanation for the rapid adaptations in resting BP seen in this investigation.

6.7 Summary of findings in relation to the ideas presented in Figure 1 of Chapter 1

Previously, Figure 1 (in Chapter 1, page 18) was included to provide an overview of the sequence of some of the events arising from an isometric exercise stimulus. By uniting the findings of this thesis, it is possible to add information to that initial schematic, and create a new proposal of sequence of events, as now illustrated by Figure 6.

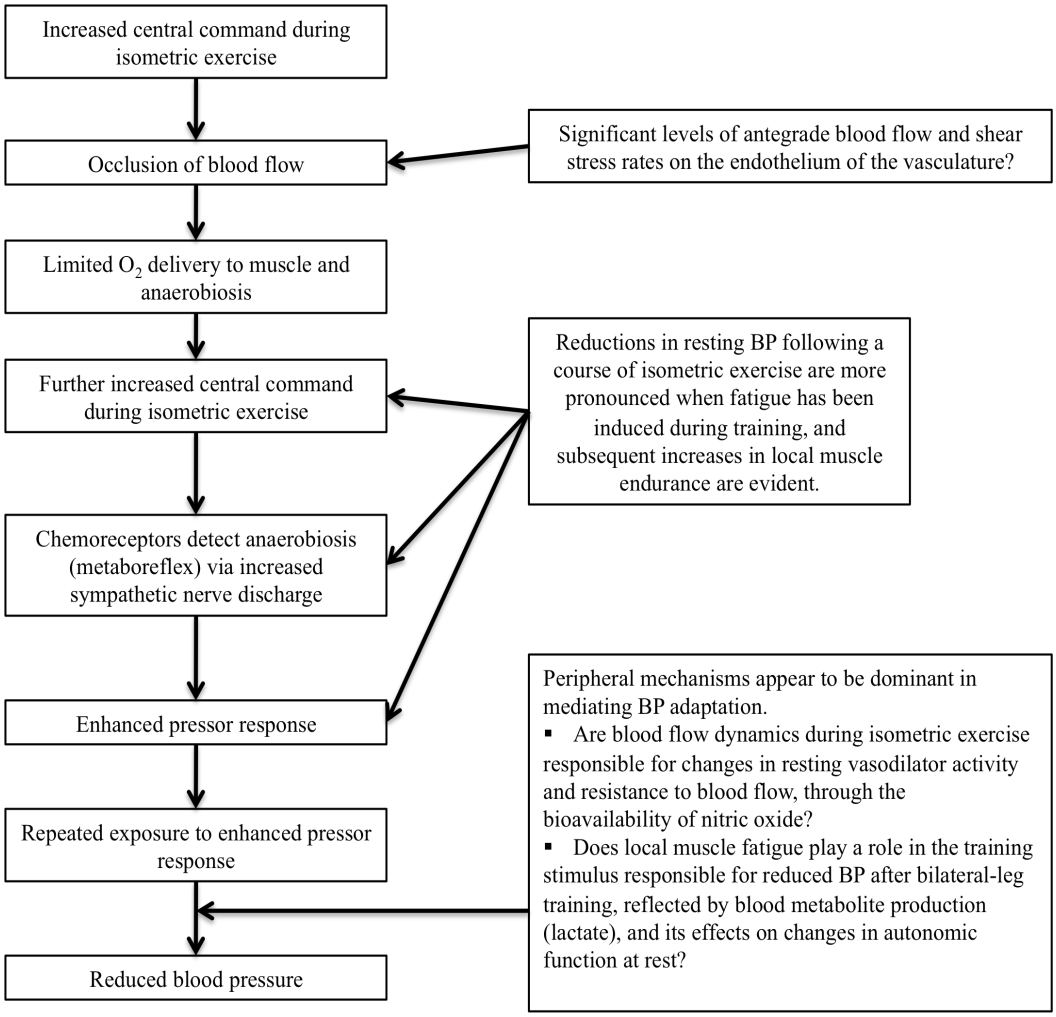

Figure 6. New proposal for sequence of events resulting from exposure to an isometric exercise stimulus

6.8 Future directions

In terms of the variables discussed within this thesis, it appears that certain centrally mediated CV variables would need to be adapted in how they are measured if used in future intervention studies. For instance, HRV might need to be measured more frequently in order to accommodate the inherent measurement variance seen within this thesis. The apparent significant aspects of autonomic nervous system function in the cross-sectional and acute response data (Chapter 3) might not have carried forward to the 4 week adaptation study (Chapter 4) due to subtle changes being lost in the measurement 'noise' over an extended test-retest time period. This same principle could also be applied to indirect measures of Q and SV. It could be argued that the use of BRS

might be better utilised in studies where either normotensive groups are compared to hypertensive groups, or where BP reductions are large enough so that the categorisation of BP levels can be altered.

Furthermore, the findings of this thesis appear to suggest that short-term IET induced BP adaptations are likely to peripherally mediated, and so detailed analysis of central CV variables during a relatively short intervention period might be unnecessary. The local metaboreceptor implications of this thesis mean that future studies, if investigating short-term intervention periods (≈ 4 weeks) could perhaps focus solely on the local vasculature of the muscle used in the IET. This could include measures of endothelial function and local blood flow parameters, in order that the seemingly rapid adaptations to the CV system evidenced in this thesis can be further understood. Such measures may also allow more detailed investigations into changes in systemic vascular resistance, than the Q and MAP derived TPR afforded within this thesis.

In terms of the acute effects of isometric exercise on BP and associated CV, NM, and metabolic variables, it may be prudent to attempt to plot the actual time course of acute variable response, rather than a singular post exercise measure. Perhaps the use of continuous post-exercise BP and HRV measurement could contribute to a more detailed understanding of their interactions following an isometric exercise stimulus.

This thesis can aid future studies by proposing specific components of isometric exercise that must be present to ensure physiological BP changes, and perhaps base the prescription of future IET studies on these factors. This could include training based either on the principle of increased torque production (%2min $torque_{peak}$) over the course of the training period (Chapter 5, Part A), the principle of lactate production during training (%La_{peak}; Chapter 5, Part B), or the inducement of fatigue as indicated by EMG signal changes (Chapter 5, Part A). The results of this thesis suggest that training based on these principles should result in significant BP reductions following bilateral-leg IET.

Finally, it was initially discussed in Chapter 1 that hypertension is one of the most prevalent and powerful risk factors for the development of CV disease, and affects a

significant proportion of not only the population of the UK, but all developed Western societies. The work of this thesis has increased the evidence supporting IET as a viable means to reducing resting BP. It is important that any advancement in the knowledge of IET and the physiological mechanisms associated with BP reductions be transferrable to more accessible exercise methodologies, so that hypertensive individuals may observe the CV benefits of IET using home-based exercise.

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Appendix

PARTICIPANT INFORMATION

Chronic Adaptations to 4 Weeks (12 Sessions) of Isometric Exercise

With De-Training Analysis

A research study is being conducted at Canterbury Christ Church University as part of a PhD thesis, by Gavin Devereux. Dr Ian Swaine, Reader in Exercise Physiology at and Dr Jonathon Wiles, Senior Lecturer in Sport and Exercise Physiology are co-researchers.

Background

It is generally acknowledged that exercise, including isometric exercise, can cause a reduction in resting blood pressure. However, how quickly reductions occur, and the mechanisms responsible for this adaptation are not fully understood. Blood pressure can be affected by many factors, and it is the aim of this research to examine which, if any, of a number of these factors may be linked and/or responsible for blood pressure alterations.

The purpose of this study is to compare a number of measures which theory suggests may have an important role in the maintenance of blood pressure, both before and after four weeks (twelve sessions) of isometric leg exercise. After the four weeks of exercise, there will be a further week of resting measures to investigate what happens when you stop exercising.

What Would You Be Required To Do?

If you decide to participate you will complete a crossover design study, whereby one term you will perform the training sessions, and the other term you will not (as a control participant). The isometric leg exercise involves you pushing against an immoveable object that is secured above your ankles with your lower legs whilst seated (imagine a leg extension machine in a gym, although your legs will not be able to move). Even if you agree to participate, you may still withdraw from the study at any time. Your involvement is entirely voluntary.

Protocol

Assessments will take place at the Sports Science Research Laboratory at CCCU, North Holmes Road (room Ag50).

Initially you will complete a set of resting measures and undertake an isometric incremental leg exercise session. This incremental test will establish exercise intensity for subsequent training sessions. Incremental means that required effort increases as time progresses.

You will then complete 12 exercise sessions spread over 4 weeks (3 per week) on days and times that suit you. These will differ from the incremental isometric test, in that your exertion will be at a steady state (exercise for 2 minutes, a total of 4 times each separated by a 3 minute rest). After completing the 4 weeks of exercise, a visit will be required for a second set of resting measures (to see if anything has altered), and a final isometric incremental test. After these tests there is then 1 further visit. This visit will involve resting measures and an incremental test only. For the second term you will only need to complete the resting measures and incremental tests (no training). For some this may happen in the first term with training in the second term.

Pre-assessment requirements for the all visits are:

- No alcohol within 12 hours before assessment
- No caffeine (tea, coffee, fizzy drinks, chocolate) within 12 hours before assessment
- No heavy physical exercise within 48 hours before assessment
- No food within 4 hours before assessment
- Not to be taking any medication that may affect cardiovascular function

Isometric exercise sessions will involve:

- Either, an incremental test (one-off)
- Or, 4 x 2 minute repetitions (12 further tests)

Listed below are measures that will be recorded during the resting assessment. Some of the terms may be new to you. They are all resting measures (you will be in a rested state either lying down or seated).

Resting measures will involve:

- ECG (measurement of your heart's electrical activity)
- Blood pressure measurement
- Baroreflex Sensitivity (a measure of the relation between your heart beats and blood pressure)
- Resting cardiac output and stroke volume measured (heart function through a ventilation (breathing) test

What to Wear
Light, comfortable clothing should be worn. For example, T-shirt with sweatshirt over the top. You will need to bring/wear shorts and training shoes.

Feedback
After your involvement in the study is completed, you will receive feedback on your results.

Confidentiality
All measurements (data) and personal information will be stored securely within CCCU premises. Data can only be accessed by Gavin Devereux, Dr Ian Swaine, and Dr Jonathon Wiles. After completion of the study all data will be made anonymous (i.e. all personal information associated with the data will be removed).

Deciding Whether to Participate
If you have any questions or concerns about the nature, procedures or requirements for participation do not hesitate to contact me on the e-mail address listed below. You should take a few days to read and digest the information in this document. Participation in this study is entirely voluntary, and you may withdraw at any time if you initially agree to participate.

Please contact Gavin Devereux: E-mail: gd45@canterbury.ac.uk

INFORMED CONSENT

STUDY 3: CROSSOVER INTERVENTION

Chronic Adaptations of Resting BP to 4 Weeks (12 Sessions) of Isometric Exercise, with 1 Week of De-Training Analysis.

The full details of the above study have been explained to me. I am clear about what will be involved and I am aware of the purpose of the assessments.

I understand that all assessments are non-medical and are not for diagnosis of any medical condition.

I know that I am not obliged to complete the assessments. I am free to stop the assessments at any point and for any reason, without explanation.

I know that I can withdraw from the study at any point and for any reason, without explanation.

I am aware of no medical condition that might put me at increased risk during my participation in the exercise protocols as described to me.

Signature of Subject: ……...

Printed Name of Subject: ……

Date: ……..

Signature of Investigator: ……