THE EFFECTS OF MUSCLE MECHANOREFLEX STIMULATION VIA PASSIVE MUSCLE STRETCH ON BAROREFLEX FUNCTION IN HUMANS

by

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ABSTRACT

Human cardiovascular control during exercise is regulated by central command, muscle mechanoreflex stimulation and muscle metaboreflex activation. The muscle mechanoreflex can be stimulated by passive muscle stretch, which causes cardiovascular responses. However, the influence of passive stretch-induced muscle mechanoreflex stimulation on the baroreflex is unknown. Therefore, this thesis investigated the effects of muscle mechanoreflex stimulation via passive calf muscle stretch on baroreflex function in humans. Firstly, spontaneous baroreflex sensitivity decreases progressively during isometric exercise of increasing intensity. A concomitant rightward resetting of the baroreflex occurs, which shifts further rightward as exercise intensity increases. Secondly, muscle mechanoreflex stimulation by passive calf muscle stretch decreases spontaneous baroreflex sensitivity at rest, and during graded levels of local metabolite accumulation following isometric exercise of increasing intensity. Thirdly, muscle mechanoreflex stimulation by passive calf muscle stretch during concurrent local metabolite accumulation decreases the maximal gain of the function curve for carotid baroreflex control of heart rate, but not blood pressure. Overall, these findings suggest that muscle mechanoreflex stimulation via passive muscle stretch decreases baroreflex sensitivity via cardiac vagal inhibition, likely by modulating inputs at central integration sites. Also, metabolite sensitisation of stretch-sensitive muscle mechanoreceptive afferents is implied, which augments this cardiac vagal inhibition.

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Abstracts:-

- Drew, R. C., Bell, M. P. D., & White, M. J. (2005). Progressive baroreflex desensitisation during incremental isometric exercise in man. *The Journal of Physiology Online, 567P,* PC34.
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ABBREVIATIONS

α,β-MeATP = α,β-methylene adenosine triphosphate

ANOVA = analysis of variance

ASIC = acid-sensing ion channel

ATP = adenosine triphosphate

BP = blood pressure

BRS = baroreflex sensitivity

CBR = carotid baroreflex

CBR-HR = carotid baroreflex-heart rate

CBR-MAP = carotid baroreflex-mean arterial pressure

CCV = common coefficient of variance

CHF = chronic heart failure

CO = circulatory occlusion

DBP = diastolic blood pressure

DCM = dilated cardiomyopathy

ECG = electrocardiogram

ECSP = estimated carotid sinus pressure

GABA = γ-aminobutyric acid

H^+ = hydrogen ion

$H_2PO_4^-$ = diprotonated phosphate

HR = heart rate

ICL = ischaemic control left

ICR = ischaemic control right

IEL = ischaemic exercise left

IER = ischaemic exercise right

MAP = mean arterial pressure

MAST = medical anti-shock trousers

MLR = mesencephalic locomotor region

MSNA = muscle sympathetic nerve activity

MVC = maximal voluntary contraction

NP = neck pressure

NS = neck suction

NTS = nucleus tractus solitarius

OP = operating point

P1 = purinergic 1

P2X = purinergic 2X

P2Y = purinergic 2Y

PAG = periaqueductal grey area

PECO = post-exercise circulatory occlusion

PPADS = pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid

rCBF = regional cerebral blood flow

RMSSD = root mean square of successive differences

RRI = R-R interval

RTX = resiniferatoxin

SBP = systolic blood pressure

SBRS = spontaneous baroreflex sensitivity

SEM = standard error of the mean

STR-CO = stretch with concurrent circulatory occlusion

UTP = uridine triphosphate

VR1 = vanilloid receptor 1

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CHAPTER 1: LITERATURE REVIEW

During exercise, the human cardiovascular system is controlled by several mechanisms, illustrated in Figure 1. Literature supporting the roles of central command, muscle afferent feedback and the arterial baroreflex will be reviewed in this chapter with a main focus on the effects of muscle afferent activation, in particular the muscle mechanoreflex, on cardiovascular control and its interaction with the baroreflex.

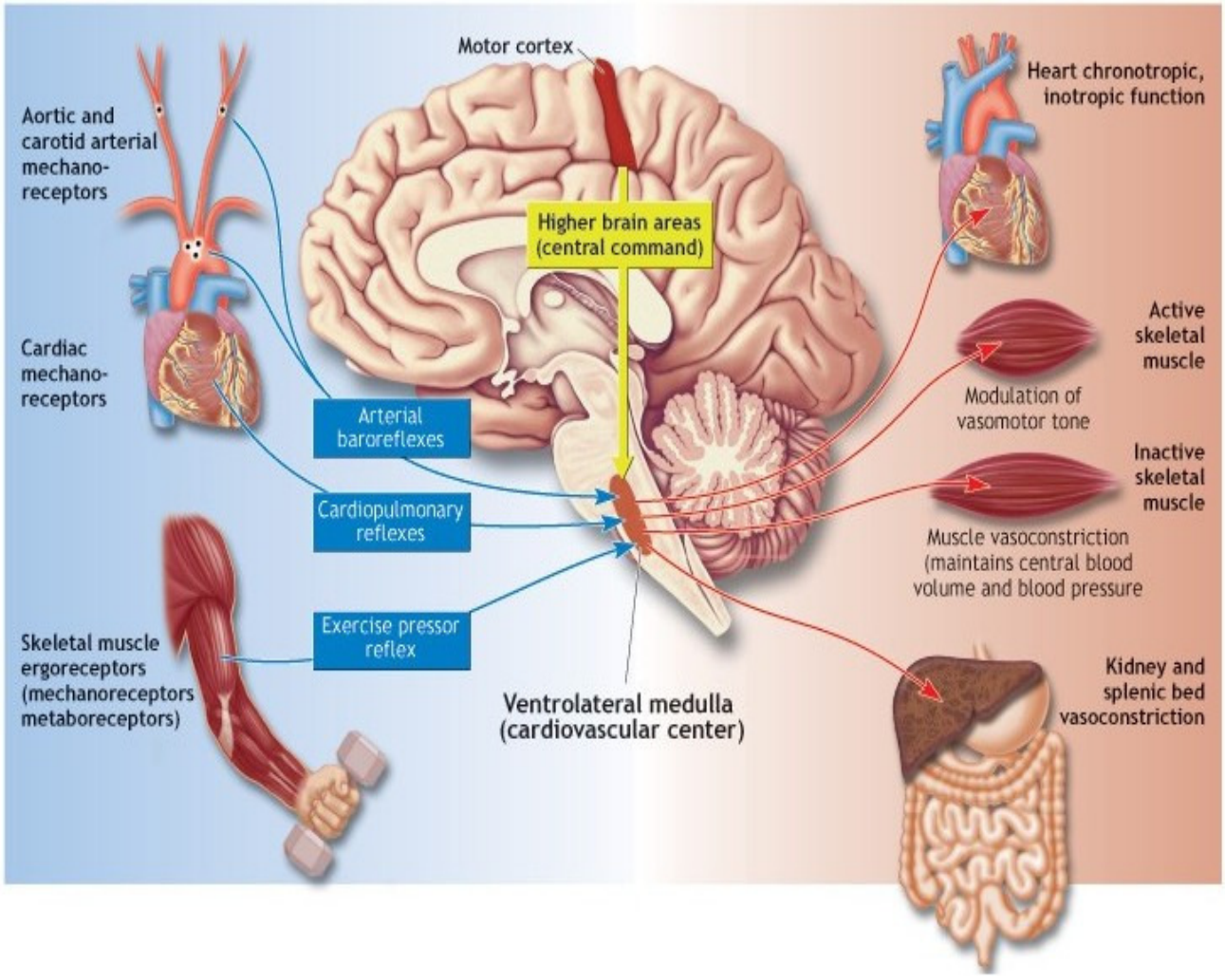

Figure 1.1: Neural control of the cardiovascular system during exercise (from McArdle *et al.*, 2001).

1.1: Central command contributions to cardiovascular control

Central command is a feedforward mechanism, which consists of descending signals from higher brain centres that concomitantly activate motor control areas as well as cardiovascular control areas of the brain. Krogh and Lindhard (1913) were the first to perform experiments in humans that indicated the initial increase in heart rate during voluntary rhythmic leg exercise was due to central "irradiation", as the increase was delayed during involuntary rhythmic leg exercise when no central "irradiation" was present. The term "central command" was coined by Goodwin *et al.* (1972), who showed that cardiovascular and respiratory responses during isometric exercise could be altered by manipulating the levels of central command present. During mild isometric exercise of the biceps brachii or triceps brachii, tendon vibration was applied to either the agonist or antagonist in order to decrease or increase central command, respectively. Goodwin *et al.* (1972) observed that during exercise, heart rate, blood pressure and ventilation all increased less when central command was reduced, and increased more when central command was enhanced. Importantly, the mechanical and metabolic conditions within the experiments were constant, as the same muscle tension was achieved during exercise with and without tendon vibration. Therefore, the observed responses were due to changes in central command activation.

In attempts to examine how central command alters cardiovascular control, experiments have focused on its effect on the carotid baroreflex. The carotid baroreflex consists of stretch receptors situated on the walls of the carotid sinus that sense changes in pressure, which are termed baroreceptors. These baroreceptors feedback information to the nucleus tractus solitarii (NTS), located in the medulla oblongata in the brainstem. The NTS has a primary role in the integration of reflex inputs to the brain that are concerned with autonomic function. Output from the NTS then brings about corrective alterations in heart rate and vasomotor tone to normalise blood pressure (Spyer, 1994).

During mild isometric knee extension and flexion with and without patellar tendon vibration, central command was required for resetting carotid baroreflex control of heart rate to higher heart rates (Ogoh *et al.*, 2002) (see 'Baroreflex control of the cardiovascular system during exercise' for further detail). Alternately, either central command or the exercise pressor reflex was required for the resetting

of carotid baroreflex control of blood pressure to higher blood pressures (Ogoh *et al.*, 2002). Partial neuromuscular blockade has been used to increase central command, as muscular force production is reduced so greater activation of central command is required to maintain the desired force level. Enhancing central command using partial neuromuscular blockade during mild isometric knee extension and dynamic cycling exercise has been shown to reset carotid baroreflex control of blood pressure and heart rate compared to control exercise (Gallagher *et al.*, 2001a). Central command seems to contribute more towards carotid baroreflex resetting of heart rate than blood pressure, as seen during mild isometric and dynamic handgrip with partial neuromuscular blockade (Querry *et al.*, 2001). However, neuromuscular blockade may have activated other central pathways associated with increased anxiety, which may have affected carotid baroreflex function.

Medical anti-shock trousers (MAST) can be used to stimulate muscle mechanoreceptors by applying external compression to the legs when inflated. Gallagher *et al.* (2006) combined MAST inflated to 100mmHg with partial neuromuscular blockade to manipulate levels of muscle afferent feedback and central command, respectively. During mild isometric knee extension, carotid baroreflex resetting of both heart rate and blood pressure was augmented during partial neuromuscular blockade compared to control exercise (Gallagher *et al.*, 2006), similar to Gallagher *et al.* (2001a). Additionally, carotid baroreflex resetting of both heart rate and blood pressure was increased further when partial neuromuscular blockade was applied with MAST inflation (Gallagher *et al.*, 2006). This suggests that inputs from central command and muscle afferents interact in order to facilitate each other and enhance carotid baroreflex resetting during exercise. The neural pathway by which central command alters baroreflex function has been investigated in decerebrate paralysed cats by electrically stimulating the mesencephalic locomotor region (MLR) in the brain (Degtyarenko and Kaufman, 2005; Degtyarenko and Kaufman, 2006). The MLR is a region of the brainstem where central motor commands originate and when stimulated, mimics the activation of central command. Stimulation of the MLR was found to inhibit the discharge of barosensory cells in the NTS (Degtyarenko and Kaufman, 2005; Degtyarenko and Kaufman, 2006). This implies that central command can influence the baroreflex at the level of the NTS. Also, these findings support evidence that the NTS acts as a central integrator and modulator of cardiovascular control.

The ‘defence reaction’ is characterised by increases in heart rate and blood pressure that prepare the human or animal for immediate high intensity physical activity. Stimulation of the hypothalamic defence area of the brain in anaesthetised cats has been shown to inhibit neurones in the NTS receiving inputs from carotid baroreceptors (Mifflin *et al.*, 1988). This finding provided evidence that the cardiovascular responses observed in the ‘defence reaction’ occur via central suppression of the baroreflex, and that the NTS is involved in mediating this response.

The periaqueductal grey area (PAG) of the midbrain is also known to be involved in modulating cardiovascular changes associated with behavioural ‘defence’ reactions, such as the ‘fight or flight’ response. With innovative technology, it has been possible to directly record neural activity in the PAG in humans. Some patient groups have electrodes chronically implanted in sub-cortical structures of the brain for the treatment of movement disorders such as Parkinson’s disease. These electrodes allow direct recordings of neural activity in ‘deep’ brain nuclei under experimental conditions in humans. Recordings have shown that neural activity in the PAG increased in anticipation of very mild cycling exercise, and was augmented further during actual exercise (Green *et al.*, 2007). These changes correlated with increases in heart rate, blood pressure and ventilation, which are known to be influenced by central command (Goodwin *et al.*, 1972). This finding suggests that the PAG is involved in the central command response to exercise, and also the response in anticipation of exercise.

Regional cerebral blood flow (rCBF) can be measured using single-photon-emission computed tomography. A study by Williamson *et al.* (2003) using this approach has shown that during moderate intensity isometric handgrip in humans, rCBF was increased in the insular and anterior cingulate regions of the cerebral cortex. These increases were not observed during local blood flow occlusion following exercise, which maintained the elevated blood pressure produced during exercise. This suggests that the insular and anterior cingulate cortex areas of the brain are also involved in the central command response to exercise, and are independent of muscle metaboreflex activation and blood pressure elevation. However, the influence of the muscle mechanoreflex stimulation on rCBF in this study was unknown.

1.2: Muscle afferent contributions to cardiovascular control

Muscle afferents are sensory nerve fibres originating in skeletal muscle that have free endings located around blood vessels and muscle fibres. These fibres provide information to the brainstem concerning conditions within the muscle. Early observations suggested their presence and importance in cardiovascular control during exercise. For example, rhythmic calf exercise was performed with cuffs around the thighs inflated to supra-systolic blood pressure to occlude the local circulation (Alam and Smirk, 1937). This occlusion continued after cessation of exercise in order to trap metabolites produced during exercise within the exercised muscles. It was found that blood pressure increased during exercise and fell slightly at the end of exercise but was maintained above resting levels for as long as occlusion was sustained. At a time when no exercise was being performed but local occlusion continued, it was concluded that a reflex originating from the muscles was maintaining the elevated blood pressure. It was argued that this would serve to increase the blood supply to the muscles where waste products were trapped and needed to be washed out. Similar reflex elevations in blood pressure were observed during mild and moderate handgrip exercise performed isometrically (Lind *et al.*, 1964). Larger blood pressure increases occurred as exercise intensity became more intense. This would be due to the greater mechanical compression of blood vessels by the muscles themselves during the isometric contraction. This would reduce and eventually occlude blood flow, which would lead to the accumulation of metabolites.

The nature of this reflex response from skeletal muscle has been intensely investigated. Inducing contractions of hindlimb muscles via electrical stimulation of ventral roots in the spinal cord stimulates the muscles without activating central command, so the effect of muscle afferent activation can be more closely examined. This technique was used by Coote *et al.* (1971) and McCloskey and Mitchell (1972) in anaesthetised cats, who observed a rise in blood pressure as well as smaller increases in heart rate and respiration. This response was abolished by neuromuscular blockade and also when the sensory input from the contracting muscles to the spinal cord was cut, indicating that this was a reflex response originating from the exercising muscle (Coote *et al.*, 1971). Coote *et al.* (1971) suggested that the accumulation of metabolites within the contracting muscles stimulated the free endings of group III and IV muscle afferents, small myelinated and unmyelinated nerve fibres, which

caused the pressor response. Group I and II muscle afferents, faster-conducting sensory nerve fibres, were not thought to contribute towards this increase in blood pressure. Unlike group III and IV afferents, stimulation of group I and II muscle afferents did not increase sympathetic nerve activity in anaesthetised cats (Coote and Perez-Gonzalez, 1970). Subsequently, McCloskey and Mitchell (1972) used electrical and pharmacological neural blockade techniques to confirm that group III and IV afferents mediated this reflex response.

Investigating the afferent impulse responses of group III and IV muscle afferents to muscle contractions has provided great insight into how these afferent fibres are stimulated. In anaesthetised cats, electrically-evoked triceps surae contractions that caused a pressor response stimulated both group III and IV afferents during isometric (Kaufman *et al.*, 1983) and rhythmic (Kaufman *et al.*, 1984a) contractions. Interestingly, the two afferent groups produced very different discharge patterns. Group III afferents were stimulated more by the mechanical distortion of the muscle during contraction, while group IV afferents were stimulated more by the metabolites produced during contraction. When electrically-evoked isometric triceps surae contractions were induced with the local circulation occluded, group IV afferents responded significantly more than group III afferents (Kaufman *et al.*, 1984b). With occlusion causing metabolite accumulation within the contracting muscle, this implied that metabolites produced during muscle contraction could stimulate a population of group III but mostly group IV afferents and generate a pressor response.

The discharge properties of group III and IV afferents in response to mechanical and metabolic stimuli were subsequently examined in anaesthetised cats and dogs (Kaufman and Rybicki, 1987). Group III afferents were found to respond more to mechanical stimuli such as distortion of the muscle or tendon stretch than group IV afferents. Conversely, group IV afferents were more responsive to electrically-evoked isometric triceps surae contractions under ischaemic conditions than group III afferents. In terms of functional roles within the central nervous system, it was suggested that the role of group III afferents might be to signal the force of muscular contraction in order to make appropriate cardiovascular responses. Group IV afferents may signal that there is an inadequate blood supply to the contracting muscles and greater blood flow needs to be delivered.

The metabolite(s) responsible for stimulating group III and IV afferents and causing a pressor response has been, and still is, an area of intense investigation. Kaufman and Rybicki (1987) found that both group III and IV afferents responded to potassium injected into the gracilis muscles' arterial supply in anaesthetised cats and dogs. However, this response adapted quickly, even though interstitial potassium concentration remained elevated, therefore making it unlikely to be the "ischaemic metabolite". Intra-arterial injection of lactic acid (Rotto and Kaufman, 1988) and bradykinin (Mense and Meyer, 1988) in anaesthetised cats has been shown to increase the discharge of group III and IV afferents with endings in the triceps surae. Arachidonic acid also augmented group III and IV afferent activity at rest (Rotto and Kaufman, 1988), as well as increasing group III afferent responses during electrically-evoked isometric triceps surae contraction in anaesthetised cats (Rotto *et al.*, 1990a). Conversely, group IV afferent responses to electrically-evoked isometric triceps surae contraction in anaesthetised cats were not increased by arachidonic acid (Rotto *et al.*, 1990b). This implied that the metabolites normally produced during muscle contraction were sufficient to sensitise group IV but not group III afferents.

Indomethacin is an inhibitor of the cyclooxygenase enzyme that normally converts arachidonic acid to prostaglandins and thromboxanes. Intra-arterial injection of indomethacin attenuated these responses to arachidonic acid (Rotto *et al.*, 1990a; Rotto *et al.*, 1990b). Local prostaglandin production can be blocked by infusing ketorolac tromethamine into muscle. In a study by Momen *et al.* (2008), passive forearm stretch was applied during PECO following moderate isometric handgrip exercise after local prostaglandin blockade. During passive forearm stretch, renal vascular resistance was attenuated compared to both control and before local prostaglandin blockade (Momen *et al.*, 2008). These findings indicate that cyclooxygenase products, such as prostaglandins, mediate the group IV afferent response and sensitise group III afferents to muscle contraction.

Although muscle contraction induced by electrical stimulation of ventral roots has enhanced our understanding of muscle afferent properties, this technique recruits the largest α-motoneurones with the fastest conduction velocities first, whereas voluntary exercise recruits these last (Henneman *et al.*, 1965). Since these motor neurones predominantly innervate fast twitch fibres, recruiting the largest α-motoneurones first may produce a different force generation profile or cause abnormal metabolite

accumulation compared to recruitment patterns that occur in voluntary exercise. Therefore, electrical stimulation of the MLR region of the brain to induce “fictive” mild rhythmic contractions in decerebrate cats has been used to stimulate muscle contractions in a way more closely linked to voluntary exercise. Exercise induced using this technique stimulated both group III and IV afferents (Adreani *et al.*, 1997), and this response was augmented during local circulatory occlusion (Adreani and Kaufman, 1998). Group III and IV afferents were equally sensitised by the metabolites that accumulated within the contracting muscles due to the occlusion and consequently responded more to contraction. However, the specific metabolites responsible for this sensitisation were not investigated. When the same exercise was performed in the presence of indomethacin, group III and IV afferent responses were attenuated when the circulation was freely perfused and more so when the circulation was occluded (Hayes *et al.*, 2006). This supports the idea that cyclooxygenase products sensitise group III and IV afferents during exercise. This occurs either directly, or indirectly by sensitising them to other metabolites such as lactic acid, bradykinin or adenosine triphosphate (ATP). Post-exercise circulatory occlusion (PECO) traps metabolites produced during exercise within the muscle and this manoeuvre is used to assess the contribution of muscle metaboreflex activation. It was observed that group IV, but not group III, afferent responses were increased during PECO compared to rest (Hayes *et al.*, 2006). This response was also attenuated with indomethacin, implying that cyclooxygenase products stimulate group IV afferents during metabolite accumulation following exercise.

In humans, it is technically very difficult to record afferent activity from muscle. However, the effect of afferent activation can be seen when recording efferent activity from muscle sympathetic nerves. Efferent muscle sympathetic nerve activity (MSNA) in humans is measured by inserting a very thin needle, usually into the peroneal or ulnar nerve, and recording sympathetic discharge to skeletal muscle. A study in humans has shown that the MSNA increase observed during mild rhythmic handgrip exercise was abolished in the presence of indomethacin (cyclooxygenase inhibitor) but not aminophylline (adenosine inhibitor) (Middlekauff and Chiu, 2004). This finding implies sensitisation of muscle mechanoreceptors by cyclooxygenase products during exercise in humans.

1.3: Muscle mechanoreflex contributions to cardiovascular control

When examining the effects of muscle afferent activation on cardiovascular responses, the techniques of external muscle compression and passive stretch of a muscle or tendon have been used. This is in an attempt to selectively stimulate muscle mechanoreceptors, and therefore the muscle mechanoreflex, in order to assess its relative contribution to cardiovascular control. External muscle compression increases interstitial pressure and stimulates mechanically-sensitive muscle afferents, and has been shown to increase blood pressure in anaesthetised cats (Stebbins *et al.*, 1988) and in humans (Williamson *et al.*, 1994; McClain *et al.*, 1994; Bell and White, 2005). These increases were abolished with section of the sciatic nerve in cats (Stebbins *et al.*, 1988) and with epidural anaesthesia in humans (Williamson *et al.*, 1994), demonstrating the reflex nature of the response. However, in the study by Williamson *et al.* (1994), local occlusion during MAST application could have trapped metabolites within the leg muscles and concurrently activated muscle metaboreceptors, which would explain the increase in blood pressure. Also, cardiovascular data was only measured every 30 seconds so initial cardiovascular responses to muscle mechanoreceptor stimulation could have been missed. External forearm compression of 110mmHg in humans augmented the blood pressure and MSNA responses to moderate ischaemic isometric handgrip exercise (McClain *et al.*, 1994). This implies that compression sensitised muscle mechanoreceptors and caused greater sympathoexcitation during exercise. Compression did not affect responses during PECO, suggesting that greater muscle metaboreceptor activation was not responsible. However, it is possible that compression activated a pool of mechanically- and metabolically-sensitive muscle afferents during exercise that were not involved in the augmented MSNA response. In a study by Bell and White (2005), compression was applied when blood pressure was already progressively elevated by muscle metaboreflex activation during PECO following increasing intensities of isometric calf exercise. Concurrent external calf compression caused greater increases in blood pressure when higher exercise intensities had been performed. This suggests that muscle mechanoreceptors activated by compression were increasingly sensitised by larger amounts of metabolites accumulated within the muscle, and produced greater blood pressure responses. However, MSNA was not measured in this study so no conclusions could be made concerning the levels of sympathetic activity when compression was applied during graded muscle metaboreflex activation.

Passive stretch of a muscle or tendon stimulates mechanically-sensitive muscle afferents, and has been shown to activate mostly group III and some group IV afferents. Greater afferent discharge occurs when stretch is sustained rather than repeated in conscious cats (Mense and Stahnke, 1983). In this study, the proportion of group III afferent receptors responding to stretch was also greater than group IV afferent receptors. Passive muscle stretch has been found to increase heart rate (Stebbins *et al.*, 1988; Gladwell and Coote, 2002; Gladwell *et al.*, 2005; Fisher *et al.*, 2005; Cui *et al.*, 2006) and blood pressure (Stebbins *et al.*, 1988; Murata and Matsukawa, 2001; Fisher *et al.*, 2005; Cui *et al.*, 2006; Cui *et al.*, 2008) in animal and human studies. The rise in blood pressure with passive triceps surae stretch has been shown to be proportional to the increase in passive tension generated in the muscle being stretched in anaesthetised cats (Stebbins *et al.*, 1988). In decerebrate cats, it has been shown that cardiac vagal efferent nerve activity is decreased throughout and cardiac sympathetic efferent nerve activity is increased at the onset of passive triceps surae stretch (Murata and Matsukawa, 2001). This suggests the differential influence of muscle mechanoreflex stimulation on parasympathetic and sympathetic efferent activity. Although decreases in cardiac vagal activity during passive stretch have been observed in both humans and animals, the increase in sympathetic activity seen in animals has not been comprehensively shown in humans.

In animal studies, passive calf muscle stretch is achieved by cutting the Achilles tendon and attaching it to a force transducer to measure the developed tension while the triceps surae is stretched. Although human studies cannot employ such invasive procedures, meaning the range of stretch that can be investigated is smaller, they can provide insight into the effects of muscle mechanoreflex stimulation on cardiovascular function. Sustained passive calf muscle stretch has been shown to cause an immediate (within the first three respiratory cycles) and maintained increase in heart rate in humans (Gladwell and Coote, 2002; Gladwell *et al.*, 2005). Rapid rhythmic passive stretch did not induce the same response (Gladwell and Coote, 2002). Cardiac vagal tone cannot be measured directly in humans as the Vagus nerve is inaccessible, so mathematical indices based on heart rate change are commonly used to indirectly assess cardiac vagal activity. The standard deviation of successive differences in R-R interval, an index of cardiac vagal tone, was decreased during passive stretch (Gladwell and Coote, 2002), implying that muscle mechanoreceptor stimulation inhibits cardiac vagal activity. This was confirmed by Gladwell *et al.* (2005), who showed that the response was abolished by cardiac vagal

blockade with glycopyrrolate administration. This demonstrated that the increase in heart rate during stretch was vagally-mediated. The stretch-induced heart rate rise was also abolished when stretch was applied during very mild rhythmic handgrip exercise (Gladwell *et al.*, 2005). Central command would have already withdrawn vagal tone during exercise, allowing heart rate to increase, so a further reduction in vagal tone could not occur with application of passive stretch. This implies that the effects of both central command activation and muscle mechanoreflex stimulation on cardiac vagal activity occur via a common neural pathway.

Contrary to Gladwell and Coote (2002) and Gladwell *et al.* (2005) who analysed the effects of short periods of passive calf muscle stretch (10-15 seconds), Fisher *et al.* (2005) observed increases in blood pressure as well as heart rate during stretch of a prolonged period (1 minute). Stretch was applied when blood pressure was already elevated at graded levels during PECO following isometric exercise of increasing intensities. The stretch-induced blood pressure increase was of a similar magnitude, irrespective of the preceding exercise intensity and consequent metabolite accumulation. This is in contrast to Bell and White (2005), who found the blood pressure increase due to external calf compression was greater during PECO following higher intensities of isometric exercise. This disparity is likely due to the different modes of mechanical stimulation activating pools of polymodal muscle afferents that have differing sensitivities towards both mechanical and metabolic stimuli. Fisher *et al.* (2005) also observed a rise in heart rate at the onset of passive stretch, even within the first three respiratory cycles of the application of stretch. This was irrespective of the prevailing heart rate and blood pressure during PECO.

Brief (5 seconds) passive calf stretch (Cui *et al.*, 2006) and forearm stretch (Cui *et al.*, 2008) have been reported to cause a transient increase in MSNA. When sustained (2 minutes) passive forearm stretch was applied during PECO following moderate handgrip exercise, MSNA was shown to increase when the previous exercise was performed to fatigue but not when the exercise was non-fatiguing (Cui *et al.*, 2008). This finding implies sensitisation of muscle mechanoreceptors when metabolites are accumulated above a certain threshold. However, in order to observe these small levels of sympathetic activation, repeated stretches and signal averaging analysis techniques were needed. The authors themselves conceded that “the haemodynamic consequences using this protocol may be limited”.

1.4: Muscle afferent receptor sub-types

The sensitivity of muscle afferent fibres to metabolic and mechanical stimuli appears to depend on the type and density of receptor sub-types on afferents' free nerve endings. One of the earliest studies to investigate this was by Mense and Stahnke (1983), who found that group III afferents had a greater proportion of "contraction-sensitive units with presumably mechanical mechanism of activation" than group IV afferents in cats. More recent research has been able to identify these receptors, summarised in Table 1, and examine the cardiovascular responses to metabolic and mechanical stimuli when they are stimulated or blocked.

Table 1.1: Muscle afferent receptor sub-types, and their agonists and antagonists.

Receptor	Full name	Agonist	Antagonist
ASIC	Acid-sensing ion channel	• Hydrogen ion (H^+) • Diprotonated phosphate ($H_2PO_4^-$)	• Amiloride
P1	Purinergic 1	• Adenosine	• CGS-15943 • 8-(p-sulfophenyl)-theophylline
P2X	Purinergic 2X	• ATP • α,β-methylene ATP (α,β-MeATP)	• pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS)
P2Y	Purinergic 2Y	• ATP • Uridine triphosphate (UTP)	• Reactive blue 2
VR1	Vanilloid receptor 1	• Capsaicin • Diprotonated phosphate ($H_2PO_4^-$)	• Capsazepine

Purinergic 2X (P2X) receptors have been found mostly on group III afferents, and can be stimulated by the selective agonist α,β-methylene ATP (α,β-MeATP). When α,β-MeATP was injected into the blood supply of the triceps surae in decerebrate cats, blood pressure increased (Li and Sinoway, 2002). A dose-dependent response was observed, as larger blood pressure rises occurred following injection of greater concentrations of α,β-MeATP (Li and Sinoway, 2002). P2X receptors can be blocked by the antagonist pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS). The pressor response induced by α,β-MeATP was attenuated by PPADS (Li and Sinoway, 2002). Purinergic 2Y (P2Y) receptors can be blocked by the antagonist reactive blue 2. However, reactive blue 2 did not attenuate the α,β-MeATP -induced increase in blood pressure (Li and Sinoway, 2002).

Vanilloid receptor 1 (VR1) has been found mostly on group IV afferents. These receptors can be stimulated by the selective agonist capsaicin, and blocked by the antagonist capsazepine. Capsaicin induced a pressor response in anaesthetised rats, which was attenuated by capsazepine (Li *et al.*, 2004). Diprotonated phosphate ($H_2PO_4^-$) and hydrogen ion (H^+) are metabolites produced during muscle contraction. Blood pressure was increased by $H_2PO_4^-$ in decerebrated rats (Gao *et al.*, 2006) and H^+ in anaesthetised rats (Li *et al.*, 2004). The $H_2PO_4^-$-induced pressor response was attenuated by capsazepine (Gao *et al.*, 2006). Pre-treating rats with resiniferatoxin (RTX) destroys muscle afferent fibres containing VR1 receptors. When this occurred in anaesthetised rats, pressor responses to both capsaicin and H^+ were reduced (Li *et al.*, 2004).

Acid-sensing ion channels (ASIC) can be blocked by the antagonist amiloride. Amiloride reduced the pressor responses to both $H_2PO_4^-$ in decerebrated rats (Gao *et al.*, 2006) and H^+ in anaesthetised rats (Li *et al.*, 2004). The pressor response to $H_2PO_4^-$ was attenuated the most when both amiloride and capsazepine were administered (Gao *et al.*, 2006). Capsazepine did not affect the H^+-induced pressor response in anaesthetised rats (Li *et al.*, 2004). This implies that H^+ does not directly stimulate VR1 receptors. However, in RTX-treated rats (no VR1 receptors), the H^+-induced pressor response was attenuated (Li *et al.*, 2004). These findings suggest that H^+ stimulates ASICs, which are likely to be found mostly on afferents containing VR1 receptors. It was therefore postulated that there is co-localisation of ASICs and VR1 receptors on muscle afferent fibres. Overall, it seems likely that

P2X, VR1 and ASICs are all involved in mediating a blood pressure increase in response to stimulation by metabolites such as ATP, $H_2PO_4^-$ and H^+.

P2X receptor blockade by PPADS has been shown to attenuate the pressor response to electrically-evoked isometric triceps surae contraction and PECO following muscle contraction in decerebrate cats (Hanna and Kaufman, 2003). Purinergic 1 (P1) receptors can be blocked by the antagonist CGS-15943. However, CGS-15943 had no effect on the pressor response during this contraction (Hanna and Kaufman, 2003). PPADS also reduced the discharge rate of both group III and group IV afferents during electrically-evoked isometric triceps surae contraction in decerebrate cats (Kindig *et al.*, 2006).

P2X receptor stimulation by α,β-MeATP enhanced the blood pressure increase caused by passive triceps surae stretch (Li and Sinoway, 2002). Again, this was attenuated by P2X receptor blockade by PPADS (Li and Sinoway, 2002; Hanna and Kaufman, 2003). However, P1 receptor blockade by either 8-(p-sulfophenyl)-theophylline (Li and Sinoway, 2002) or CGS-15943 (Hanna and Kaufman, 2003) did not attenuate this α,β-MeATP -induced pressor response. PPADS was also found to reduce the discharge rate of group III but not group IV afferents during passive triceps surae stretch (Kindig *et al.*, 2006). Additionally, it has been observed that PPADS attenuated the renal sympathetic response to electrically-evoked isometric triceps surae contraction within 2 seconds in decerebrate cats (Kindig *et al.*, 2007). Finally, PPADS attenuated the renal sympathetic response to passive triceps surae stretch within 10 seconds in decerebrate cats (Kindig *et al.*, 2007). Overall, these findings show that stimulation of ATP-sensitive P2X receptors can evoke the pressor responses induced by muscle contraction and passive stretch. With respect to the disparity seen between onset latencies during muscle contraction and passive muscle stretch, it is likely due to the sensitivities of P2X receptors on different pools of group III afferents towards ATP released during contraction and stretch. In a study by Hayes *et al.* (2005) in decerebrate cats, 18 group III afferents responded to electrically-evoked isometric triceps surae contraction, and 14 group III afferents responded to passive triceps surae stretch. Interestingly, 7 group III afferents responded to both stimuli (Hayes *et al.*, 2005). This illustrates that populations of muscle afferents have differing sensitivities towards mechanical stimuli, with some overlap in responses to contraction and stretch.

The muscle mechanosensitive receptors capable of evoking a pressor response have been postulated to be partly located at or near the myotendinous junction of the Achilles tendon. Mechanosensitive channels can be blocked by gadolinium, and group III and IV afferents can be blocked by lidocaine. When both gadolinium and lidocaine were injected into the myotendinous junction of the triceps surae in anaesthetised rats, the passive stretch-induced increases in heart rate and blood pressure were attenuated (Nakamoto and Matsukawa, 2007). However, cutting the Achilles tendon had no effect on these responses (Nakamoto and Matsukawa, 2007). This finding suggests that muscle mechanosensitive receptors could be located in the myotendinous junction of the Achilles tendon to monitor changes in muscle tension rather than muscle length in order to produce appropriate cardiovascular responses to muscular activity.

1.5: Muscle afferent contributions to cardiovascular control in heart failure

Muscle afferent feedback is known to contribute towards cardiovascular responses to exercise via increasing sympathetic activation and parasympathetic inhibition. In disease states such as chronic heart failure (CHF), over-activation of the sympathetic nervous system occurs, which may contribute to exercise intolerance. It has been postulated that abnormal muscle afferent feedback is responsible for this response. However, there is intense debate concerning whether this is mediated by an exaggerated muscle mechanoreflex, muscle metaboreflex, or both of these reflexes.

Compared to controls, Sterns *et al.* (1991) showed that MSNA was elevated at rest in CHF patients. Also, MSNA increased similarly during mild isometric handgrip exercise, but was greatly attenuated during local occlusion following exercise in CHF patients compared to controls (Sterns *et al.*, 1991). These findings suggest that in CHF, there is sympathetic over-activation at rest, and that the muscle metaboreflex is attenuated. This implies that the muscle mechanoreflex or central command mediate the exercise-induced increase in MSNA. Conversely, Piepoli *et al.* (1996) found blood pressure, respiration and vascular resistance to be augmented during PECO following moderate rhythmic handgrip in CHF patients compared to controls. This implies an increased contribution from the muscle metaboreflex to cardiovascular responses during exercise in CHF. Following six weeks of

forearm training, this exaggerated response was attenuated more in CHF patients than controls. This demonstrates that the augmented muscle metaboreflex in CHF can be reduced with training, allowing improved tolerance to exercise.

Alternatively, Carrington *et al.* (2001) observed a lower blood pressure response during mild electrically-evoked isometric calf exercise in CHF patients compared to controls, with similar blood pressure responses during PECO in both groups. These findings imply that the muscle mechanoreflex is desensitised, but the muscle metaboreflex is unchanged in CHF. A separate study by Carrington *et al.* (2004) showed that diastolic blood pressure responses were smaller during mild voluntary isometric exercise of the calf than of the forearm in CHF patients and controls. This implies that due to the weight-bearing role of the calf muscles, which would act as a training stimulus, the muscle metaboreflex generated by the calf is attenuated. Taking these studies together, the disparity between these results is likely due to differences in muscle group used (forearm v. calf), training status of muscle (trained vs. untrained) and stage of CHF (early vs. late) (reviewed by Fisher and White, 2004).

Evidence from animal models of CHF suggests that the sympathetic over-activation observed during exercise in CHF is due to an exaggerated muscle mechanoreflex. Dilated cardiomyopathy (DCM) and hypertension are both causes of CHF. Compared to control, the increase in blood pressure and heart rate due to electrically-evoked isometric triceps surae contraction is greater in rats with DCM (Smith *et al.*, 2003a) and hypertension (Smith *et al.*, 2006). Group IV muscle afferent fibres can be selectively ablated by treating neonatal rats with capsaicin. When this occurs, the augmented blood pressure and heart rate responses to exercise are still present (Smith *et al.*, 2005a). Alternately, when mechanically-sensitive muscle afferents are blocked with gadolinium, this increased response is attenuated (Smith *et al.*, 2005b). When VR1 receptors are stimulated by capsaicin, a decreased blood pressure response compared to controls is exhibited when rats have DCM, ablated group IV afferents or heart failure due to large myocardial infarctions (Li *et al.*, 2004; Smith *et al.*, 2005a). Stimulation of the muscle mechanoreflex via passive hindlimb stretch induces similar blood pressure and heart rate increases to exercise pressor reflex activation by electrical muscle contraction in rats with DCM (Smith *et al.*, 2003a). When P2X receptors are stimulated by α,β-MeATP, the pressor response to passive stretch is augmented more in rats with heart failure than controls (Li *et al.*, 2004). Overall, these

findings suggest that the role of the muscle metaboreflex in mediating cardiovascular responses to exercise is reduced in animal models of CHF, which leads to an augmentation of the contribution of the muscle mechanoreflex to compensate for this. This could be due to attenuated stimulation or sensitivity of VR1 receptors (muscle metaboreflex), causing an increased stimulation or sensitivity of P2X receptors (muscle mechanoreflex) (Sinoway and Li, 2005). However, the animal models used induce *acute* conditions similar to CHF, usually over only several weeks. Therefore, findings from these studies cannot be directly extrapolated to humans with *chronic* heart failure.

Some studies in humans have also provided evidence for an exaggerated muscle mechanoreflex in CHF. MSNA increased progressively during mild rhythmic handgrip exercise in both CHF patients and controls (Middlekauff *et al.*, 2004). This increase occurred earlier in CHF patients than controls (1st minute vs. 3rd minute) (Middlekauff *et al.*, 2004). Also, this progressive increase during exercise is greatly attenuated by indomethacin (cyclooxygenase inhibitor), but not aminophylline (adenosine inhibitor) in CHF patients (Middlekauff *et al.*, 2008). Following exercise, MSNA returned to baseline levels during PECO in both CHF patients and controls (Middlekauff *et al.*, 2004). This demonstrates that the muscle metaboreflex was not responsible for the augmented MSNA in CHF. Additionally, passive forearm stretch increased MSNA in CHF patients, but not in controls (Middlekauff *et al.*, 2004). However, these changes in sympathetic activation were relatively small (12-15%), and the techniques used to stimulate the muscle mechanoreflex were relatively crude. When passive stretch is applied by an experimenter (Middlekauff *et al.*, 2004; Middlekauff *et al.*, 2008), the force and velocity of the stretch can be very variable. This makes it difficult to control the mechanical stimulus applied to the muscle. Overall, these findings are consistent with increased baseline muscle mechanoreceptor sensitivity and metabolite sensitisation of muscle mechanoreceptors. Although sympathetic responses to muscle mechanoreflex stimulation in CHF have received much attention (reviewed by Sinoway and Li, 2005), the observed effects are small. Parasympathetic responses to muscle mechanoreflex stimulation in CHF remain unclear.

1.6: Baroreflex control of the cardiovascular system during exercise

The baroreflex is the major neural mechanism for regulating blood pressure. Since heart rate and blood pressure are well known to increase during exercise, it was once thought that the baroreflex was 'switched off' during exercise to allow these cardiovascular adjustments to occur. Part of the confusion over this issue was due to the different methods employed to assess baroreflex function. In animals, invasive procedures included surgical isolation (Melcher and Donald, 1981) or denervation (Walgenbach and Donald, 1983) of baroreceptors. In humans, the modified Oxford technique was used, which involved pharmacological manipulation of blood pressure (Ebert and Cowley, 1992). Infusion of sodium nitroprusside was used to decrease blood pressure, and was followed by infusion of phenylephrine HCl to increase blood pressure. Due to the invasive and pharmacological nature of these methodologies making it unethical or difficult to perform in humans, the neck pressure manipulation technique was consequently developed. This approach was non-invasive, did not involve drug infusions and importantly, could be used during exercise protocols in humans (reviewed by Fadel *et al.*, 2003).

The neck pressure manipulation technique involves applying positive and negative pressures externally to the neck to alter stimulation of the carotid baroreceptors. Positive pressures unload receptors, which acts as a hypotensive stimulus and causes a baroreflex-mediated increase in heart rate and blood pressure. Conversely, negative pressures load receptors, which acts as a hypertensive stimulus and causes a baroreflex-mediated decrease in heart rate and blood pressure. Full stimulus-response regression curves are constructed from these heart rate and blood pressure responses, from which specific parameters of the curves for carotid baroreflex control of heart rate and blood pressure can be calculated. These include the operating point (the point at which the baroreflex currently operates, i.e. prevailing heart rate and mean arterial blood pressure), operating point gain (the gain (or sensitivity) at the operating point) and maximal gain (the gain at the centring (middle) point of the curve) (see Methods for further description).

Ebert (1986) was the first to show that during mild isometric handgrip exercise, curves for carotid baroreflex control of heart rate and blood pressure were reset rightward to higher pressures compared to control, with no change in maximal gain. This provided evidence that the baroreflex still

operated during exercise, and was simply 'reset' to function around the prevailing higher heart rates and blood pressures (reviewed by Raven *et al.*, 2006). Similar findings were observed during dynamic cycling at mild (Potts *et al.*, 1993; Ogoh *et al.*, 2005), moderate (Potts *et al.*, 1993; Norton *et al.*, 1999; Ogoh *et al.*, 2005) and high (Norton *et al.*, 1999; Ogoh *et al.*, 2005) intensities. It was shown that curves for carotid baroreflex control of heart rate and blood pressure were reset upward and rightward, increasingly with greater exercise intensity, with no changes in maximal gain. The threshold, another calculated parameter, is the minimum carotid sinus pressure that elicits a reflex response in heart rate or blood pressure. These studies also showed that the operating points for carotid baroreflex control of heart rate and blood pressure were progressively relocated away from the centring point and towards threshold as exercise intensity increased. Potts and Mitchell (1998) found that electrically-evoked isometric calf contraction increased the threshold pressures for heart rate and blood pressure in anaesthetised dogs. These responses were prevented by neuromuscular blockade (Potts and Mitchell, 1998). This illustrates that muscle afferent activation can reset carotid baroreflex control of heart rate and blood pressure independently of central command. However, full stimulus-response function curve analysis was not performed in this study, so conclusions concerning the overall effects of muscle afferent activation on the carotid baroreflex (e.g. effects on operating point, operating point gain, maximal gain) could not be made.

Brief moderate intensity isometric handgrip exercise has been shown to alter vagal and sympathetic responses to changes in carotid baroreceptor activity (Eckberg and Wallin, 1987). Specifically, exercise did not affect the neck pressure-induced tachycardia, but reduced the neck suction-induced bradycardia (Eckberg and Wallin, 1987). Also, exercise attenuated the neck pressure-induced increases in MSNA, and augmented decreases in MSNA (Eckberg and Wallin, 1987). Overall, the nature of these responses shows that baroreflex function can be rapidly altered in order to facilitate heart rate and blood pressure increases necessary for the performance of exercise, even within the first few seconds of exercise. The balance between parasympathetic and sympathetic activity during exercise was also investigated by Ogoh *et al.* (2005). The progressive rightward and upward resetting of the curve for carotid baroreflex control of heart rate was largely unaffected by β-1 adrenergic (sympathetic) blockade (Ogoh *et al.*, 2005). However, this was abolished by cardiac vagal (parasympathetic) blockade (Ogoh *et al.*, 2005). These findings show that cardiac vagal withdrawal has

a greater contribution to the resetting of carotid baroreflex control of heart rate during exercise than increased sympathetic activation. Additionally, during control mild, moderate and high intensity dynamic cycling, the gain at the operating point of the carotid-cardiac curve progressively decreased as exercise intensity increased (Ogoh *et al.*, 2005). This attenuation was still observed when exercise was performed with β-1 adrenergic blockade (Ogoh *et al.*, 2005). Alternately, this operating point gain was greatly reduced at rest and during exercise with cardiac vagal blockade (Ogoh *et al.*, 2005). This demonstrates that during exercise, the decrease in operating point gain for carotid-cardiac control, and therefore arterial baroreflex sensitivity, is due to vagal withdrawal rather than increased sympathoexcitation.

1.7: Muscle afferent influence on baroreflex control

Many studies have investigated how muscle afferent stimulation influences baroreflex control of blood pressure and heart rate (reviewed by Spyer, 1994). One approach has been to assess the sensitivity of the cardiac vagal component of the baroreflex. This is achieved by comparing R-R interval duration at rest and during carotid sinus pressure elevation. Carotid sinus pressure elevation increases vagal tone, induces bradycardia and therefore prolongs R-R intervals. It was found that R-R interval prolongation was reduced compared to rest by electrical stimulation of the peroneal nerve to separately recruit group III and group IV afferents in decerebrate cats (McWilliam and Yang, 1991). This response was also evoked by electrically-evoked isometric triceps surae contraction in decerebrate cats (McWilliam *et al.*, 1991). High carotid sinus pressure increases vagal tone, whereas low carotid sinus pressure decreases vagal tone. This reduction in R-R interval prolongation is greater when electrically-evoked isometric triceps surae contraction occurs at high carotid sinus pressure compared to low carotid sinus pressure in decerebrate cats (McMahon and McWilliam, 1992). Atropine abolished any R-R interval changes due to muscle contraction (McMahon and McWilliam, 1992). This demonstrates that muscle afferent feedback can attenuate the baroreflex-induced bradycardia evoked at the onset of muscle contraction. Additionally, this inhibition is mediated by cardiac vagal withdrawal.

More specifically, electrical stimulation of the peroneal nerve to recruit group III and group IV afferents has been shown to reduce the firing of carotid sinus baroreceptor neurones in the NTS in anaesthetised cats (McMahon *et al.*, 1992). This inhibition was found to be mediated by γ-aminobutyric acid (GABA) (McMahon *et al.*, 1992), which is an inhibitory neurotransmitter in the central nervous system. In reviews by Potts (2002) and Potts (2006), a hypothetical model was proposed illustrating the central interaction between baroreceptor and somatosensory receptor afferents in the NTS. This included the presence of GABA interneurones that mediate the input of the somatosensory afferents. This hypothesis was confirmed by Potts *et al.* (2003), who observed that electrically-evoked rhythmic forelimb exercise attenuated baroreflex responsiveness in decerebrate rats. This response did not occur when GABA receptors in the NTS were blocked by bicuculline methiodide (Potts *et al.*, 2003). Therefore, this inhibition of baroreceptor signalling in the NTS by contraction-sensitive muscle afferents occurred via a GABA mechanism.

1.8: Spontaneous baroreflex sensitivity during exercise

With the baroreflex known to continue operating during exercise, many studies in humans have investigated the relative influences of central command and the muscle mechanoreflex and metaboreflex on baroreflex sensitivity during exercise using the sequence technique. This involves assessing sequences of three or more beats where systolic blood pressure and R-R interval both increase or decrease (arterial baroreflex engagement). Regression lines are calculated from these sequences, from which the slope value can be used to represent spontaneous baroreflex sensitivity. During low intensity isometric handgrip exercise, spontaneous baroreflex sensitivity was unchanged from rest, with an apparent rightward shift of the regression line to a higher pressure (Iellamo *et al.*, 1994). Similar observations were made during mild electrically-evoked isometric calf exercise (Carrington and White, 2001; Carrington *et al.*, 2003), and voluntary dynamic knee extension exercise (Iellamo *et al.*, 1997). When this dynamic knee extension exercise was electrically induced, the regression line was similarly reset rightward, but spontaneous baroreflex sensitivity was decreased (Iellamo *et al.*, 1997). Passive cycling, where only the muscle mechanoreflex is intended to be stimulated, has also been shown to decrease spontaneous baroreflex sensitivity with a rightward

resetting (Vorluni and Volianitis, 2008). However, when electrically-induced dynamic knee extension exercise was performed with concurrent local occlusion to activate the muscle metaboreflex, the regression line was reset further rightward, with spontaneous baroreflex sensitivity maintained at resting levels (Iellamo *et al.*, 1997). Overall, these findings show that during exercise of a low intensity using a relatively small muscle mass, a rightward resetting of the baroreflex to the prevailing higher blood pressure is sufficient to allow physical work to continue, without the need for a change in spontaneous baroreflex sensitivity. Also, the muscle mechanoreflex can modulate baroreflex function, as its inhibitory effect can decrease spontaneous baroreflex sensitivity during exercise.

Iellamo *et al.* (1998) showed that during heavy dynamic cycling, spontaneous baroreflex sensitivity was greatly decreased. No intercept data was provided however, so no comment could be made concerning resetting of the baroreflex. A similar observation was made when different levels of dynamic cycling exercise were performed, ranging from mild, moderate to high intensity (Ogoh *et al.*, 2005). Spontaneous baroreflex sensitivity was shown to progressively decrease with increasing exercise intensity (Ogoh *et al.*, 2005). Similar findings were illustrated in dogs, where during mild and moderate dynamic exercise, spontaneous baroreflex sensitivity decreased progressively (Sala-Mercado *et al.*, 2007). Also, regression lines were increasingly reset rightward with greater intensity of exercise (Sala-Mercado *et al.*, 2007). Collectively, these findings demonstrate that as exercise intensity increases utilising a larger muscle mass, spontaneous baroreflex sensitivity decreases and the function curve is reset further rightward to higher pressures. This modulation of baroreflex control of heart rate occurs in order to allow an increased blood supply to the working muscles.

During low intensity (30% maximal voluntary contraction) isometric leg extension exercise (Iellamo *et al.*, 1999a; Iellamo *et al.*, 1999b) but not very low intensity (15% maximal voluntary contraction) isometric leg extension exercise or low intensity isometric handgrip exercise (Iellamo *et al.*, 1999a), spontaneous baroreflex sensitivity was decreased. Again, no intercept data was provided, which did not assist in discerning if there was any resetting of the baroreflex. Different intensities of dynamic exercise cause graded alterations in baroreflex function, but it is not known how the differing levels of central command and muscle afferent activation caused by progressive increases in isometric exercise intensity affect spontaneous baroreflex sensitivity in humans.

1.9: Muscle metaboreflex influence on MSNA and baroreflex control

Much research has been conducted into the effects of local metabolite accumulation in skeletal muscles on the cardiovascular system during exercise in humans. It is well known that local metabolite accumulation increases MSNA, demonstrating that muscle metaboreflex activation causes sympathoexcitation (reviewed by Seals and Victor, 1991). One of the initial studies illustrating this was by Mark *et al.* (1985), who measured MSNA responses during PECO following mild isometric handgrip exercise. It was shown that during PECO, muscle metaboreflex activation maintained the elevated blood pressure and MSNA observed during exercise (Mark *et al.*, 1985). Heart rate rapidly returned to baseline levels at this time (Mark *et al.*, 1985).

During PECO following moderate isometric handgrip exercise, the diastolic blood pressure vs. total MSNA activity relationship was reset rightward, and its slope was increased (Ichinose *et al.*, 2004). When sodium nitroprusside decreased and phenylephrine HCl increased blood pressure (Oxford technique) during PECO following moderate isometric handgrip exercise, the diastolic blood pressure vs. total MSNA activity relationship was more negative (steeper slope) compared to control (Cui *et al.*, 2001). These findings illustrate that muscle metaboreflex activation increases the sensitivity of baroreflex control of MSNA. This would allow finer control of blood pressure during exercise. However, only a small range of low to moderate diastolic blood pressures were used, so no conclusions could be made concerning effects over a full range of pressures. Also, no comment could be made regarding whether the maximal gain of baroreflex control of MSNA was altered.

During PECO following moderate isometric handgrip exercise, Ichinose *et al.* (2002) applied positive neck pressure to unload carotid baroreceptors, and negative neck pressure to load carotid baroreceptors. Compared to control, blood pressure and MSNA responses to positive neck pressure were augmented during PECO (Ichinose *et al.*, 2002). Conversely, blood pressure and MSNA responses to negative neck pressure were attenuated during PECO compared to control (Ichinose *et al.*, 2002). No changes in heart rate responses were observed during PECO compared to control (Ichinose *et al.*, 2002). These findings demonstrate that when blood pressure is already elevated by local metabolite accumulation, baroreflex responses to further changes in blood pressure are influenced by

muscle metaboreflex activation. Augmenting responses to decreases in blood pressure and attenuating responses to increases in blood pressure serves to maintain blood pressure at this elevated level while metabolites are still accumulated.

Many human studies have used the sequence technique when investigating the effects of muscle afferent activation on the baroreflex. During local occlusion following mild isometric handgrip exercise (Iellamo *et al.*, 1994) and mild isometric leg extension exercise (Iellamo *et al.*, 1999b), muscle metaboreflex activation has been found to reset the baroreflex in a rightward direction, with no change in spontaneous baroreflex sensitivity. During local occlusion following mild electrically-evoked isometric calf exercise, muscle metaboreflex activation similarly reset the baroreflex rightward (Carrington and White, 2001; Carrington *et al.*, 2003). Spontaneous baroreflex sensitivity was increased compared to exercise, although it was not different to rest (Carrington and White, 2001; Carrington *et al.*, 2003). The inhibitory input of the muscle mechanoreflex would decrease spontaneous baroreflex sensitivity during exercise. Therefore, this apparent increase in spontaneous baroreflex sensitivity during muscle metaboreflex activation alone in PECO is likely due to the cessation of muscle mechanoreflex stimulation at the end of exercise.

When electrically-evoked dynamic knee extension exercise was performed in free-flow conditions (no concurrent occlusion), the baroreflex was reset rightward, and spontaneous baroreflex sensitivity was decreased (Iellamo *et al.*, 1997). However, when this exercise was performed with concurrent local occlusion to activate the muscle metaboreflex during exercise, the baroreflex was reset further rightward, and spontaneous baroreflex sensitivity was maintained at resting levels (Iellamo *et al.*, 1997). In a study by Sala-Mercado *et al.* (2007), mild and moderate dynamic exercise was performed with concurrent local occlusion to activate the muscle metaboreflex during exercise in dogs. In contrast to Iellamo *et al.* (1997), the baroreflex was further reset rightwards, and spontaneous baroreflex sensitivity was further decreased compared to control exercise (Sala-Mercado *et al.*, 2007). The discrepancies in these findings are likely a result of studies using different modes and intensities of exercise, which utilise various muscle masses and place differing demands on the cardiovascular system. Also, it is possible there could be activation of different populations of group III and IV muscle

afferents that may terminate at different central integrative sites in the brainstem. This could result in variations in inputs to the central control sites in the brain, such as the NTS.

During occlusion following moderate cycling exercise, Papelier *et al.* (1997) applied positive and negative neck pressures to manipulate blood pressure. The curve for carotid baroreflex control of blood pressure was reset further rightward during occlusion compared to exercise (Papelier *et al.*, 1997). At this time, the gain of the curve increased during hypotensive stimuli, and decreased during hypertensive stimuli, compared to control (Papelier *et al.*, 1997). Hence, when blood pressure is elevated during PECO, responses to hypotension are increased, and responses to hypertension are decreased. Similar to the effects of muscle metaboreflex activation on baroreflex control of MSNA, this would maintain the augmented blood pressure while metabolites are accumulated. However, the neck pressures were applied for 20seconds each, so it would have taken 2minutes 40seconds to apply all eight pressures that were used. Therefore, the metabolic stimulus is likely to have been different during the hypotensive stimuli at the start, compared to the hypertensive stimuli towards the end. This could have influenced the blood pressure responses to these neck pressure changes.

Papelier *et al.* (1997) found that heart rate responses to hypotensive and hypertensive stimuli were not altered by muscle metaboreflex activation compared to control. This was also observed by Fisher *et al.* (2008) during PECO following moderate and high intensity isometric handgrip exercise. The curve for carotid baroreflex control of heart rate was reset progressively rightward from rest with higher exercise intensity, with no changes in operating point location, operating point gain or maximal gain (Fisher *et al.*, 2008). This indicates that muscle metaboreflex activation can reset carotid baroreflex control of heart rate, without altering its responsiveness to changes in pressure. Spontaneous baroreflex sensitivity calculated using the sequence technique reflects the sensitivity at the operating point of the curve for carotid baroreflex control of heart rate (Ogoh *et al.*, 2005). However, it cannot distinguish between movements of the operating point and changes in maximal gain of the curve for carotid baroreflex control of heart rate. Full stimulus-response carotid baroreflex function curve analysis can do this, therefore the observations of Fisher *et al.* (2008) confirm and extend the findings of previous studies that used the sequence technique. These studies found the arterial baroreflex to be

reset rightward, with no change in spontaneous baroreflex sensitivity, during muscle metaboreflex activation following exercise.

Studies investigating the effects of muscle metaboreflex activation during or following exercise on baroreflex control of heart rate are likely to find differing results. This is due to differences in the balance between sympathetic and parasympathetic neural outflow to the heart at these times. During voluntary exercise, central command and the muscle mechanoreflex and metaboreflex are all activated. This causes parasympathetic withdrawal and sympathetic activation. These neural changes decrease cardiac baroreflex sensitivity, and increase heart rate and blood pressure. During PECO following exercise, central command and muscle mechanoreflex stimulation cease. The removal of their inhibitory inputs results in a rapid restoration of parasympathetic activity. Blood pressure and therefore sympathetic activation remain elevated during PECO. Together with the cessation of central command and muscle mechanoreflex inputs, this causes a powerful baroreflex-mediated increase in parasympathetic outflow. This is in an attempt to correct the increased blood pressure. Therefore, this parasympathetic activity overpowers the sympathetic outflow. This results in spontaneous baroreflex sensitivity and heart rate returning to resting levels, while blood pressure remains elevated. However, the effects of graded levels of muscle metaboreflex activation following isometric exercise on spontaneous baroreflex sensitivity in humans are unknown.

1.10: Muscle mechanoreflex influence on baroreflex control

The effects of muscle mechanoreflex stimulation on baroreflex function during exercise are less well known than the influences of muscle metaboreflex activation and central command. This is because it is more difficult to isolate activation of the muscle mechanoreflex. Partial blockade of muscle afferents can be achieved by administering epidural anaesthesia. When this occurred during mild dynamic cycling and mild isometric one-legged knee extension exercise, the rightward and upward resetting of carotid baroreflex control of blood pressure was attenuated in both exercise conditions compared to control exercise (Smith *et al.*, 2003b). Only the rightward resetting of carotid

baroreflex control of heart rate was attenuated during isometric exercise with epidural anaesthesia compared to control exercise (Smith *et al.*, 2003b).

Gallagher *et al.* (2001b) and Gallagher *et al.* (2006) used MAST to stimulate muscle mechanoreceptors during mild isometric one-legged knee extension exercise and mild dynamic cycling. Compared to control exercise, carotid baroreflex control of blood pressure was reset further rightward and upward during mild isometric one-legged knee extension exercise with MAST (Gallagher *et al.*, 2001b; Gallagher *et al.*, 2006). Carotid baroreflex control of blood pressure was greatly reset further rightward and upward during mild dynamic cycling with MAST compared to control exercise (Gallagher *et al.*, 2001b). Carotid baroreflex control of heart rate was reset slightly further rightward and upward during both mild dynamic cycling (Gallagher *et al.*, 2001b) and mild isometric one-legged knee extension exercise (Gallagher *et al.*, 2001b). Gallagher *et al.* (2006) found no resetting during mild isometric one-legged knee extension exercise with MAST. Together, the studies of Smith *et al.* (2003b), Gallagher *et al.* (2001b) and Gallagher *et al.* (2006) argue that muscle afferent activation modifies baroreflex control of blood pressure more than heart rate. However, epidural anaesthesia would block both muscle mechanoreceptor and metaboreceptor feedback (Smith *et al.*, 2003b). Additionally, MAST could inadvertently activate muscle metaboreceptors as well as mechanoreceptors via external compression (Gallagher *et al.*, 2001b; Gallagher *et al.*, 2006). Therefore, no conclusions can be made about the selective influence of the muscle mechanoreflex on carotid baroreflex function.

The most reliable method of stimulating the muscle mechanoreflex has been by using passive muscle stretch. Passive triceps surae stretch decreased cardiac vagal efferent nerve activity throughout and increased cardiac sympathetic efferent nerve activity at the onset in decerebrate cats (Murata and Matsukawa, 2001). This was also observed after partial sinoaortic denervation (Murata and Matsukawa, 2001). This indicates that these responses were evoked by stimulation of muscle mechanoreceptors rather than arterial baroreceptors. Additionally, the sympathetic response was augmented following partial sinoaortic denervation. This suggests that the stretch-induced sympathetic activation was restrained by intact arterial baroreceptor input. Conversely, the vagal response occurred independently of baroreceptor input. Potts and Mitchell (1998) used passive hindlimb stretch to increase hindlimb tension to similar levels to electrically-evoked hindlimb contraction. Passive

hindlimb stretch increased the threshold pressure for carotid baroreflex control of blood pressure, and to a lesser extent heart rate, in dogs (Potts and Mitchell, 1998). However, the influence of muscle mechanoreflex stimulation via passive muscle stretch on spontaneous baroreflex sensitivity is unknown. Also, it is not known how muscle mechanoreflex stimulation via passive muscle stretch during graded levels of concurrent muscle metaboreflex activation affects spontaneous baroreflex sensitivity. Furthermore, full stimulus-response function curve analysis has not been performed during passive muscle stretch, at rest or during concurrent muscle metaboreflex activation. Therefore, the overall effects of muscle mechanoreflex stimulation on the carotid baroreflex also require investigation.

1.11: Proposed studies

Based on the previous literature and their own studies, Carrington *et al.* (2003) proposed a simple model that summarised the interaction between the muscle mechanoreflex, muscle metaboreflex and central command with the baroreflex at the NTS (Figure 1.2). The intention of the work in this thesis is to use this theoretical model to control the respective inputs to the NTS, and examine their influence on cardiac vagal activity. Each input will be manipulated or eliminated in turn, and the resulting effects on baroreflex function will be assessed.

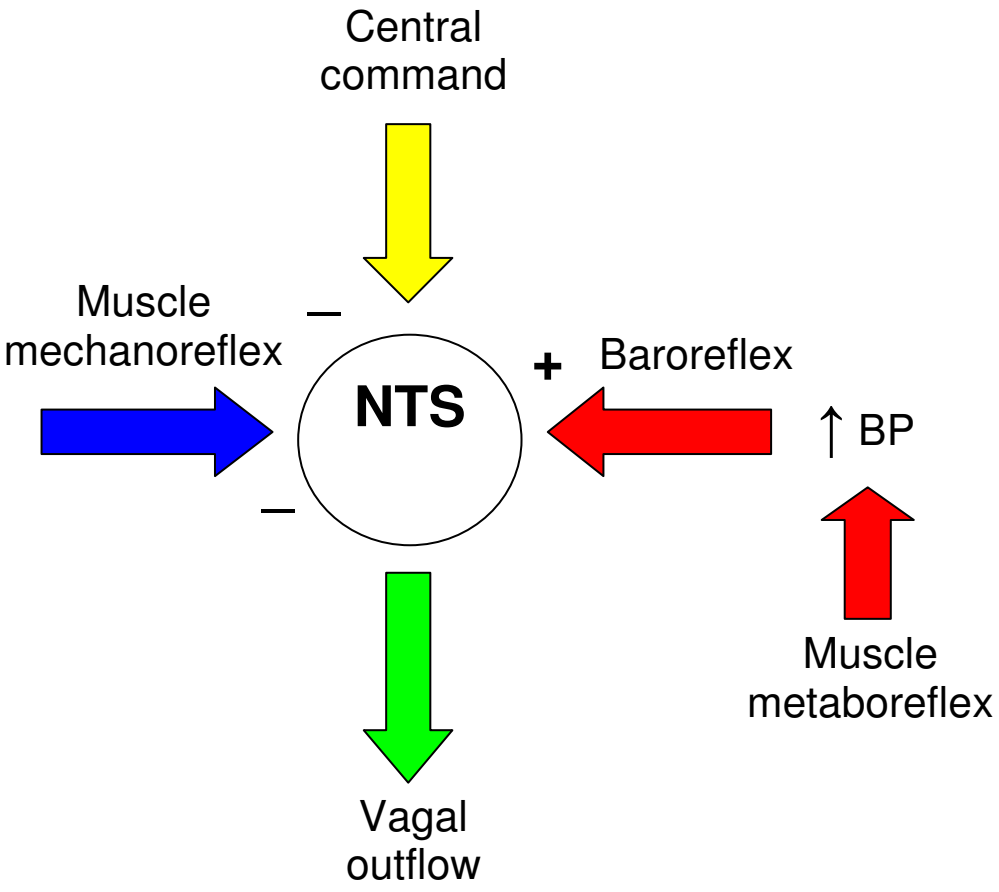

Figure 1.2: A simplified diagram illustrating the integration of muscle mechanoreflex, muscle metaboreflex, central command and baroreflex inputs at the nucleus tractus solitarii (NTS). BP, blood pressure.

In this thesis, the following studies in humans will:-

- Examine how the differing levels of central command and muscle afferent activation caused by progressive increases in isometric exercise intensity affect spontaneous baroreflex sensitivity
- Investigate the effects of graded levels of muscle metaboreflex activation following isometric exercise on spontaneous baroreflex sensitivity
- Examine the influence of muscle mechanoreflex stimulation via passive muscle stretch on spontaneous baroreflex sensitivity
- Investigate how muscle mechanoreflex stimulation via passive muscle stretch during graded levels of concurrent muscle metaboreflex activation affects spontaneous baroreflex sensitivity
- Examine how muscle mechanoreflex stimulation via passive muscle stretch affects carotid baroreflex function
- Investigate how muscle mechanoreflex stimulation via passive muscle stretch during concurrent muscle metaboreflex activation affects carotid baroreflex function

CHAPTER 2: GENERAL METHODS

2.1: Isometric exercise and passive stretch

In all experiments, subjects were seated in a semi-recumbent position in a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA) (Figure 2.1). In the studies described in Chapters 3 and 4, the subjects' right knee was flexed by 30°, and the right foot strapped to the footplate so the right lower leg was horizontal to the floor. In the study described in Chapter 5, both of the subjects' knees were flexed by 30°, and both feet strapped to the footplate so both lower legs were horizontal to the floor. Velcro straps were used to fix the foot/feet, and minimise heel lift during voluntary plantarflexor exercise and passive stretch. Maximal voluntary contraction (MVC) of the calf plantarflexors were assessed by recording maximal efforts and accepting three that were within 5% of each other. In experiments where passive stretch was applied, passive range of dorsiflexion of the ankle joint was established by manually moving the footplate as far as was comfortable, prior to each trial. This information was programmed into the machine, so that the subsequent stretching movement could be performed automatically by the Biodex. Active plantarflexor torques were recorded using the Biodex. Respiratory rate was standardised for each subject across all trials by asking them to breathe in time to a metronome set at a rate which they found to be the most comfortable on first testing.

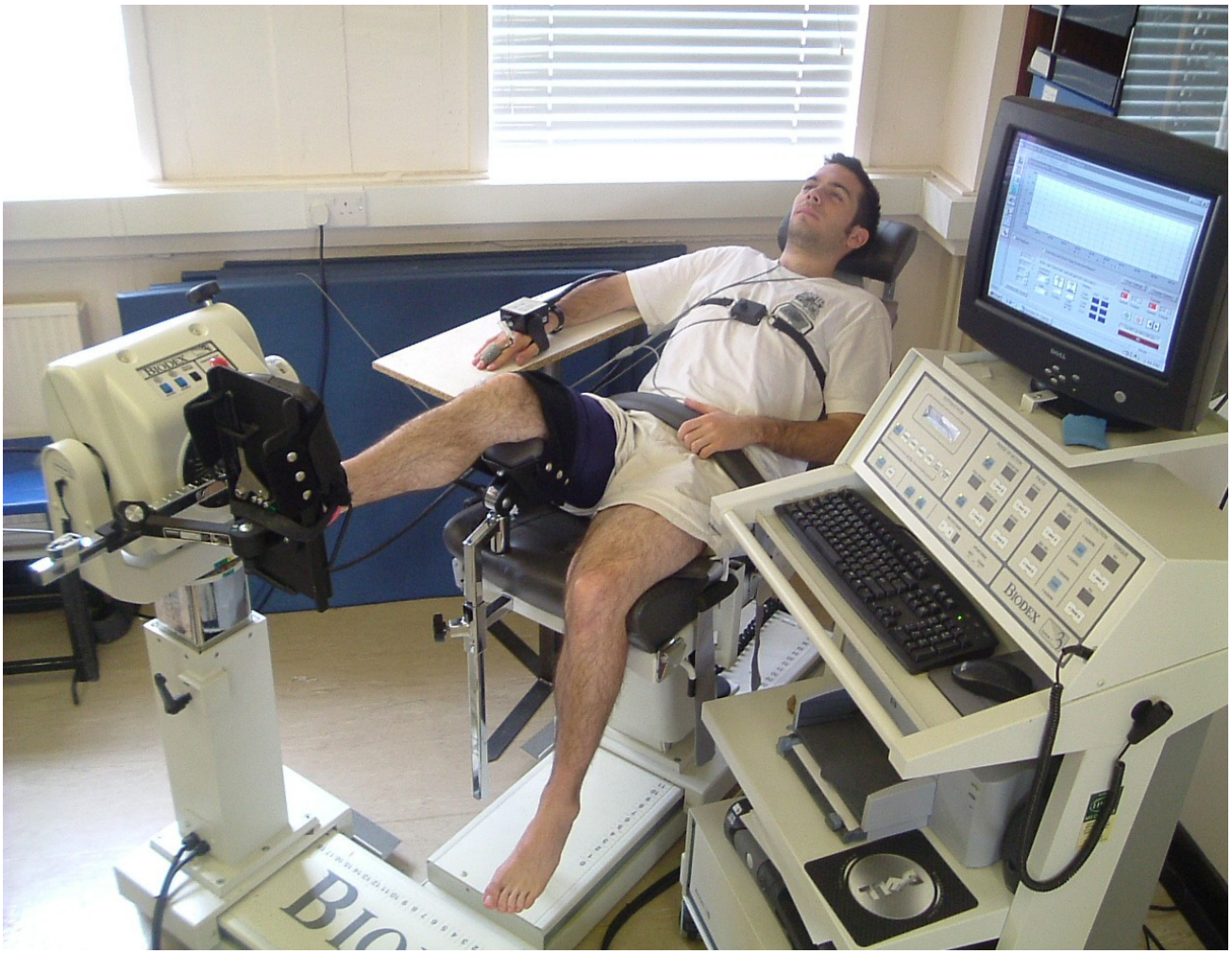

Figure 2.1: Subject in experimental setup.

2.2: Cardiovascular and respiratory variables

R-R interval (RRI) was measured using a three-lead electrocardiogram (ECG; Cardiorater CR7, Cardiac Records Ltd, London, UK) in the lead II position, from which heart rate (HR) was derived. Blood pressure (BP) was measured non-invasively using a Finapres (Ohmeda 2300, Louisville, CO, USA) on the middle finger of the right hand, which was supported at heart-level. Phase of respiratory cycle was monitored using a pneumograph, consisting of a band strapped around the subjects' chest that was attached to a strain gauge. Active plantarflexor torques were recorded using the Biodex. All signals were sampled by an analogue-to-digital converter (Cambridge Electronic Design 1401plus, CED, Cambridge, UK) at 100Hz, with the exception of the ECG signal which was sampled at 1000 Hz. Data was recorded and displayed using Spike 2 software (CED, Cambridge, UK).

2.3: Sequence analysis

Spontaneous baroreflex sensitivity (SBRS) was assessed offline using the sequence technique, which involved detecting sequences of three or more successive beats where systolic BP (SBP) and RRI were either both increasing or both decreasing. Regression equations from these sequences of SBP (X axis) and RRI (Y axis) provided slope values representative of SBRS, and intercept values. Slope values calculated from sequence analysis provide an index of cardiac vagal tone because they disappear almost completely after injection of atropine (Parati *et al.*, 2000).

2.4: Root mean square of successive differences

Root mean square of successive differences (RMSSD) assesses the variation between RRIs by calculating the square root of the mean of the sum of the squares of differences between successive RRIs. This is a recommended time-domain measure of short-term HR variability, and a sensitive index of vagal tone (Task Force, 1996).

2.5: Common coefficient of variance

Common coefficient of variance (CCV), another index of vagal tone, measures the variation between RRIs when normalising for different HRs. This removes the confounding influence of smaller RRIs (higher HRs) having naturally less variation between RRI. This was calculated using the formula:- (standard deviation of RRI/mean RRI) x 100 (Al-Ani *et al.*, 1996).

2.6: Carotid baroreflex analysis

Carotid baroreflex (CBR) control of HR and mean arterial pressure (MAP) was assessed using the neck pressure (NP)/neck suction (NS) technique. Variable pressure stimuli were applied through a rubber-lined malleable lead collar that was secured around the front and sides of the neck with a Velcro strap. This methodology was utilised by Eckberg *et al.* (1975), subsequently adapted by Pawelczyk and Raven (1989) to operate via computer control, and the equipment used in Chapter 5 in this thesis was used by Edwards *et al.* (2003). A rapid series of 12 computer-controlled pressures lasting 500ms each were produced in the collar. These were triggered off consecutive R-waves, each delayed 50ms after the R-wave. Pressures were in the order +40, +40, +40, +40, +20, +10, 0, –10, –20, –40, –60 and –80mmHg. These occurred during an end-expiratory breath-hold lasting ~10s.

HR and MAP responses to NPs and NSs were plotted against estimated carotid sinus pressure (ECSP). This was calculated as pre-stimulus MAP – neck collar pressure. This produced carotid baroreflex-heart rate (CBR-HR) and carotid baroreflex-mean arterial pressure (CBR-MAP) stimulus-response function curves, respectively (Figure 2.2).

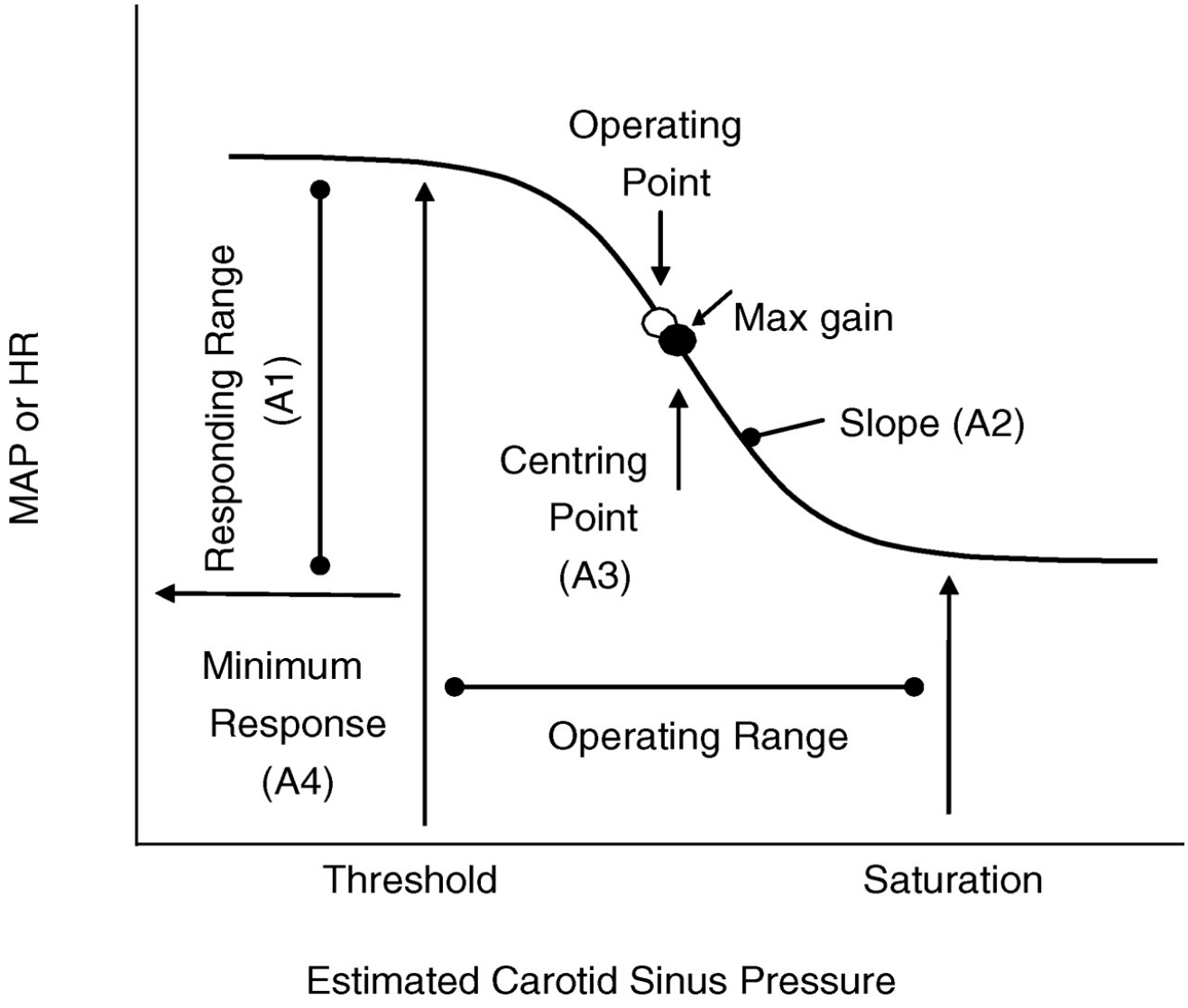

Figure 2.2: Schematic model of the carotid baroreflex stimulus-response function curve and its calculated parameters, obtained from responses to changes in neck pressure (Raven *et al.*, 2006).

Individual responses were fitted to a four-parameter logistic function model (Kent *et al.*, 1972), which incorporated the following equation:-

$$\text{HR or MAP} = A_1 \times (1 + \exp [A_2 \times \{\text{ECSP} - A_3\}])^{-1} + A_4$$

A_1 is the range of response of HR or MAP (maximum – minimum), A_2 is the gain coefficient, A_3 is the carotid sinus pressure required to elicit an equal pressor and depressor response (centring point), and A_4 is the minimum response of HR or MAP. Responses were fitted to this model by a non-linear least-squares regression. This minimised the sum of squares error to predict a curve of 'best fit' for each individual subject's response. Only curves with a correlation coefficient of $R^2 > 0.95$ were accepted. Threshold and saturation are the minimum and maximum carotid sinus pressures, respectively, that elicit a reflex response in HR or MAP. These were calculated as follows:-

$$\text{Threshold} = A_3 - 2 / A_2 \qquad \text{Saturation} = A_3 + 2 / A_2$$

These calculations are the carotid sinus pressures at which HR or MAP are within 5% of their minimum or maximum response (Chen and Chang, 1991). OP was defined as the mean pre-stimulus HR or MAP, calculated over the complete respiratory cycle which preceded the onset of neck pressure changes. OP gain is a measure of responsiveness at the OP of the function curve. This was calculated using the following equation:-

$$\text{OP gain} = -A_1 A_2 \exp (A_2 [\text{ECSP}_{OP} - A_3]) / (1 + \exp [A_2 \{\text{ECSP}_{OP} - A_3\}])^2$$

$ECSP_{OP}$ is the ECSP at the OP. Maximal gain of the stimulus-response function curves is the gain at the centring point of the curve, and is used as an index of CBR responsiveness. This was calculated using the following equation:-

$$\text{Maximal gain} = -A_1 \times A_2 / 4$$

2.7: Statistical analysis

Raw data files were analysed offline using custom-written script files to produce beat-to-beat values for RRI, HR, SBP, diastolic blood pressure (DBP) and MAP. These values were then analysed using Microsoft Excel. All values presented are mean ± the standard error of the mean (SEM).

CHAPTER 3: SPONTANEOUS BAROREFLEX SENSITIVITY DURING INCREMENTAL ISOMETRIC EXERCISE IN HUMANS

3.1: Introduction

During exercise, the baroreflex continues to function, and is 'reset' to operate around the prevailing higher BP (Potts *et al.*, 1993). Baroreflex control of BP and HR during exercise is known to be affected by levels of central command and muscle afferent feedback (reviewed by Raven *et al.*, 2006). Studies using variable neck pressure stimuli have shown that dynamic cycling resets CBR control of BP and HR rightward and upward, with no changes in maximal gain. This occurred during low (Potts *et al.*, 1993; Ogoh *et al.*, 2005), moderate (Potts *et al.*, 1993; Norton *et al.*, 1999; Ogoh *et al.*, 2005) and high (Norton *et al.*, 1999; Ogoh *et al.*, 2005) intensities, with greater resetting as exercise intensity increased. The OPs for CBR control of BP and HR were also progressively relocated away from the centring point and towards threshold, to a position of lower gain, as exercise intensity increased. SBRS, measured using the sequence technique, reflects the gain at the OP of the function curve for CBR control of HR (Ogoh *et al.*, 2005). During exercise of increasing intensity, SBRS has been shown to decrease progressively, and the baroreflex reset further rightward. This was observed during low, moderate and high intensity dynamic cycling in humans (Iellamo *et al.*, 1998; Ogoh *et al.*, 2005), and low and moderate dynamic exercise in dogs (Sala-Mercado *et al.*, 2007).

Studies using the sequence technique have attempted to assess the relative influences of central command, muscle metaboreflex activation and muscle mechanoreflex stimulation on SBRS. Low intensity isometric handgrip exercise (Iellamo *et al.*, 1994), electrically-evoked isometric calf exercise (Carrington and White, 2001; Carrington *et al.*, 2003), and voluntary dynamic knee extension exercise (Iellamo *et al.*, 1997) have been shown to cause a rightward resetting of the baroreflex, with no change in SBRS. When this dynamic knee extension exercise was electrically induced, the baroreflex was similarly reset rightward, but SBRS was decreased (Iellamo *et al.*, 1997). However, when electrically-induced dynamic knee extension exercise was performed with concurrent local occlusion to activate the muscle metaboreflex, the baroreflex was reset further rightward, with SBRS maintained at resting levels (Iellamo *et al.*, 1997). Passive cycling, where only the muscle mechanoreflex is intended to be stimulated, has also been shown to decrease SBRS with a rightward resetting (Vorluni and Volianitis, 2008). Overall, central command, muscle metaboreflex activation and muscle mechanoreflex

stimulation can each influence SBRS, with different modulations depending on the presence and intensity of each input.

During low intensity (30% MVC) isometric leg extension exercise (Iellamo *et al.*, 1999a; Iellamo *et al.*, 1999b) but not very low intensity (15% MVC) isometric leg extension exercise or low intensity isometric handgrip exercise (Iellamo *et al.*, 1999a), SBRS was decreased. However, it is not known how the differing levels of central command, muscle metaboreflex activation and muscle mechanoreflex stimulation caused by progressive increases in isometric exercise affect SBRS in humans. Therefore, this study measured SBRS, using the sequence technique, during isometric exercise of low, moderate and high intensity. It was hypothesised that as exercise intensity increased, SBRS would decrease progressively, and the baroreflex would be reset further rightward.

3.2: Methods

10 healthy subjects (7 male, 22 ± 1 yr, 71 ± 4 kg, 171 ± 3 cm) were recruited from the University of Birmingham student population. All subjects gave informed written consent and were habituated to the experimental procedures, which were approved by the School of Sport and Exercise Sciences local ethics committee and conformed to the Declaration of Helsinki (2000). Subjects were asked to refrain from consuming food and caffeine in the 2 hours preceding the trials, and performing strenuous exercise in the 24 hours preceding the trials. Each subject participated in all four experimental trials once (after completing a habituation session), with no more than one trial performed each day, and the order of all trials was randomised.

3.2.1: Experimental protocol

Subjects were seated in a semi-recumbent position in a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA) with the right knee flexed by 30° and the foot strapped to the footplate so the lower leg was horizontal to the floor. Velcro straps were used to fix

the foot and minimise heel lift during voluntary plantarflexor exercise. MVCs of the calf plantarflexors were assessed by recording maximal efforts and accepting three that were within 5% of each other. Respiratory rate was standardised for each subject across all trials by asking them to breathe in time to a metronome set at a rate which they found to be the most comfortable on first testing.

A schematic diagram of the experimental protocol is shown in Figure 3.1. After subjects were settled for 10 minutes, the protocol began with a 5 minute rest period. 10 seconds before the end of this period, a cuff placed around the right thigh was inflated to 200mmHg by a rapid cuff inflator (E20, Hokanson, Bellevue, WA, USA) and this remained inflated for a further 1.5 minutes. At the end of the rest period, subjects were instructed to either rest for a further 1.5 minutes (0% control trial), or perform ischaemic isometric plantarflexion using their right calf muscles in order to produce a torque that matched a pre-determined exercise intensity of 30, 50 or 70% MVC, for 1.5 minutes. The level of torque produced during the exercise period was displayed on a computer screen in front of the subjects for visual feedback.

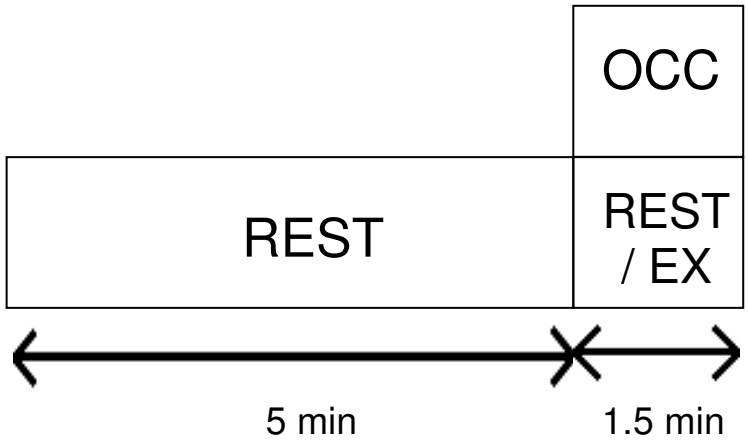

Figure 3.1: Schematic diagram of experimental protocol. EX, exercise; OCC, occlusion.

3.2.2: Measured variables

RRI was measured using a three-lead ECG (Cardiorater CR7, Cardiac Records Ltd, London, UK) in the lead II position, from which HR was derived. BP was measured non-invasively using a Finapres (Ohmeda 2300, Louisville, CO, USA) on the middle finger of the right hand, which was supported at heart-level. Phase of respiratory cycle was monitored using a pneumograph, consisting of a band strapped around the subjects' chest that was attached to a strain gauge. Active plantarflexor torques were recorded using the Biodex. All signals were sampled by an analogue-to-digital converter (Cambridge Electronic Design 1401plus, CED, Cambridge, UK) at 100Hz, with the exception of the ECG signal which was sampled at 1000 Hz. Data was recorded and displayed using Spike 2 software (CED, Cambridge, UK).

3.2.3: Spontaneous baroreflex sensitivity

SBRS at the OP of the baroreflex function curve was assessed offline using the sequence technique, which involved detecting sequences of three or more successive beats where SBP and RRI were either both increasing or both decreasing. Regression equations from these sequences of SBP (X axis) and RRI (Y axis) provided slope values representative of SBRS, and intercept values.

3.2.4: Statistical analysis

Raw data files were analysed using custom-written script files to produce beat-to-beat values for RRI, HR, SBP, DBP and MAP. 15-second averages were calculated for each subject over the whole protocol, and these were averaged across the group to produce group means during each period and each condition. SBRS was calculated using a custom-written sequence analysis program, and only baroreflex sequences with correlation coefficients greater than 0.95 were accepted. Mean SBRS values were calculated from all slope data for the rest (5min) and exercise (1.5min) phases of the protocol. All values are expressed as the mean ± SEM. Repeated measures ANOVA was used to identify significant

differences within the protocol. Statistical significance was set at $P<0.05$ and if this was reached, *post hoc* analysis using paired-samples *t*-tests with a Bonferroni correction was performed (SPSS Inc., Chicago, IL, USA).

3.3: Results

3.3.1: Contraction torques

No MVCs were measured prior to the 0% control trial, as no exercise was performed in this trial. MVCs measured before each exercise trial were not significantly different between conditions, with values of 128.8 ± 6.9, 124.9 ± 6.1 and 123.6 ± 6.8 Nm being recorded prior to the 30, 50 and 70% MVC trials, respectively. Exercise torques averaged 38.6 ± 2.1, 62.4 ± 3.1 and 86.5 ± 4.8 Nm for the 30, 50 and 70% trials, respectively.

3.3.2: Cardiovascular variables

HR, MAP and DBP values in the rest phase were not significantly different between conditions (Table 3.1). However, SBP in the rest phase during the 50 and 70% trials was significantly higher than during the 0% trial ($P<0.05$). BP increased from baseline during exercise, and the magnitude of this change was related to increasing exercise intensity. MAP, SBP and DBP changed with similar time course during the exercise phase of the protocol so for simplicity, only the MAP response to the different trials is illustrated here (Figure 3.3).

Table 3.1: Resting values for cardiovascular variables in the 0, 30, 50 and 70% trials.

Trial (%MVC)	HR (b.min^{-1})	MAP (mmHg)	SBP (mmHg)	DBP (mmHg)
0	70 ± 3	86 ± 2	125 ± 3	67 ± 2
30	68 ± 2	89 ± 3	132 ± 4	68 ± 3
50	73 ± 3	93 ± 2	138 ± 3 *	70 ± 2
70	73 ± 3	94 ± 3	139 ± 3 *	72 ± 2

Values are means ± SEM. * = significantly different from 0%. MVC, maximal voluntary contraction; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

HR and MAP changes from rest during the exercise phase in all trials are shown in Figures 3.2 and 3.3, respectively. HR and MAP rose progressively during exercise, reaching significantly higher levels with greater exercise intensity (P<0.05). By end-exercise, HR increased to 85 ± 4, 100 ± 5 and 119 ± 7 b.min^{-1} in the 30, 50 and 70% trials, respectively. MAP elevated to 109 ± 3, 126 ± 4 and 141 ± 4 mmHg by the end of exercise in the 30, 50 and 70% trials, respectively. HR and MAP remained unchanged from rest in the 0% trial.

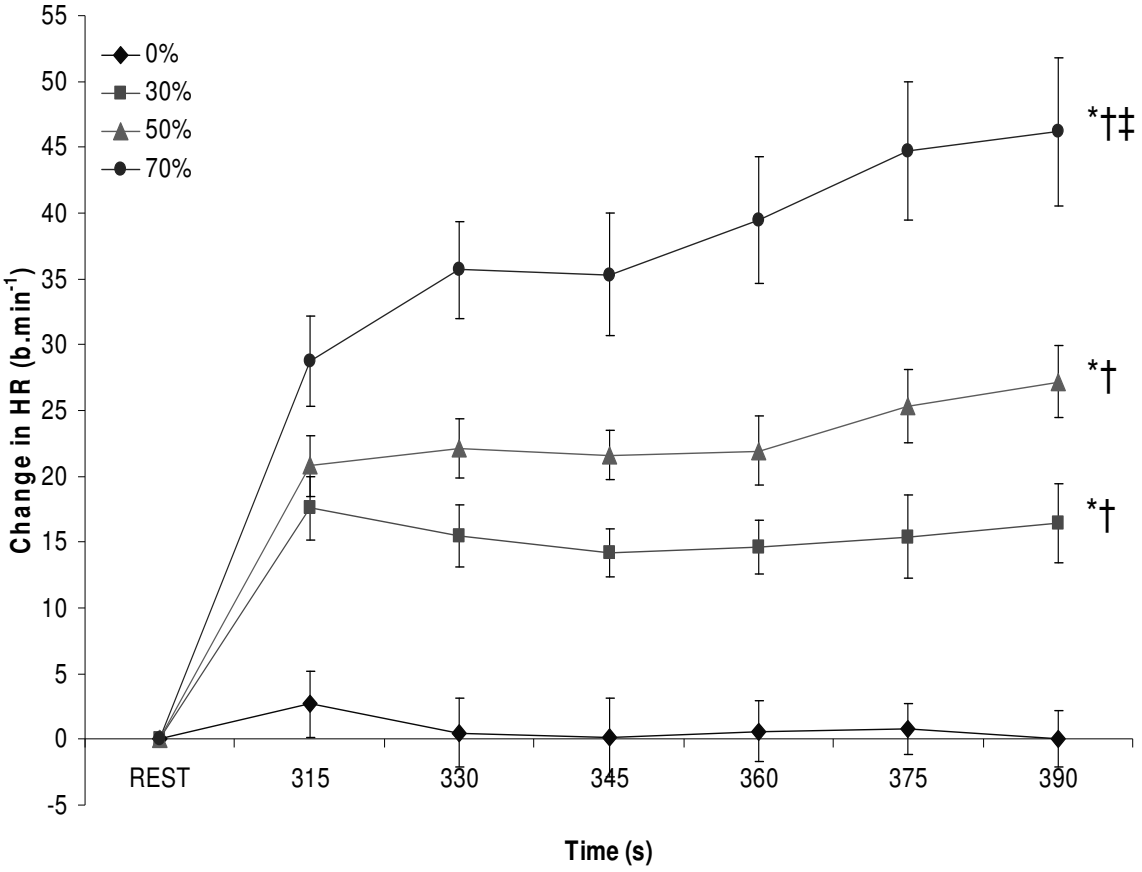

Figure 3.2: Group mean changes from rest in heart rate (HR) during exercise in the 0, 30, 50 and 70% trials. * = significantly different from rest. † = significantly different from 0%. ‡ = significantly different from 30%.

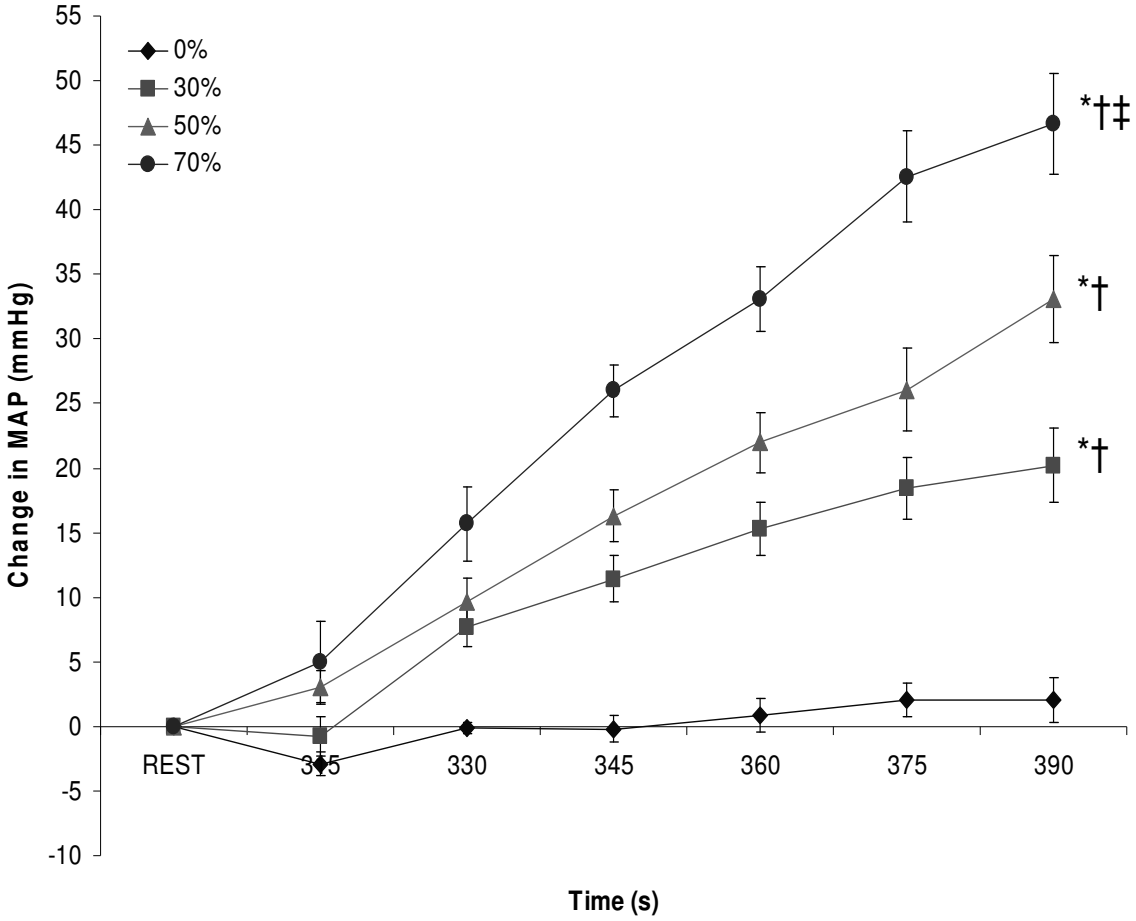

Figure 3.3: Group mean changes from rest in mean arterial pressure (MAP) during exercise in the 0, 30, 50 and 70% trials. * = significantly different from rest. † = significantly different from 0%. ‡ = significantly different from 30%.

3.3.3: Spontaneous baroreflex sensitivity

Regression lines calculated from sequence analysis during rest and exercise in all trials are shown in Figure 3.4. Lines calculated during rest in the four trials were similar, so an overall mean regression line for the rest phase is illustrated. There was no significant difference in SBRS during the rest phases of the four trials (Figure 3.5). When exercise was performed, there was a significant decrease in SBRS ($P<0.05$), with significantly greater decreases occurring during exercise of greater intensity ($P<0.05$).

There was no significant difference in the mean intercept of the regression lines, which were calculated from the sequence data, during the rest phases of all trials (Figure 3.6). When exercise was performed, there was a significant increase in intercept ($P<0.05$), with significantly greater increases occurring during exercise of greater intensity ($P<0.05$).

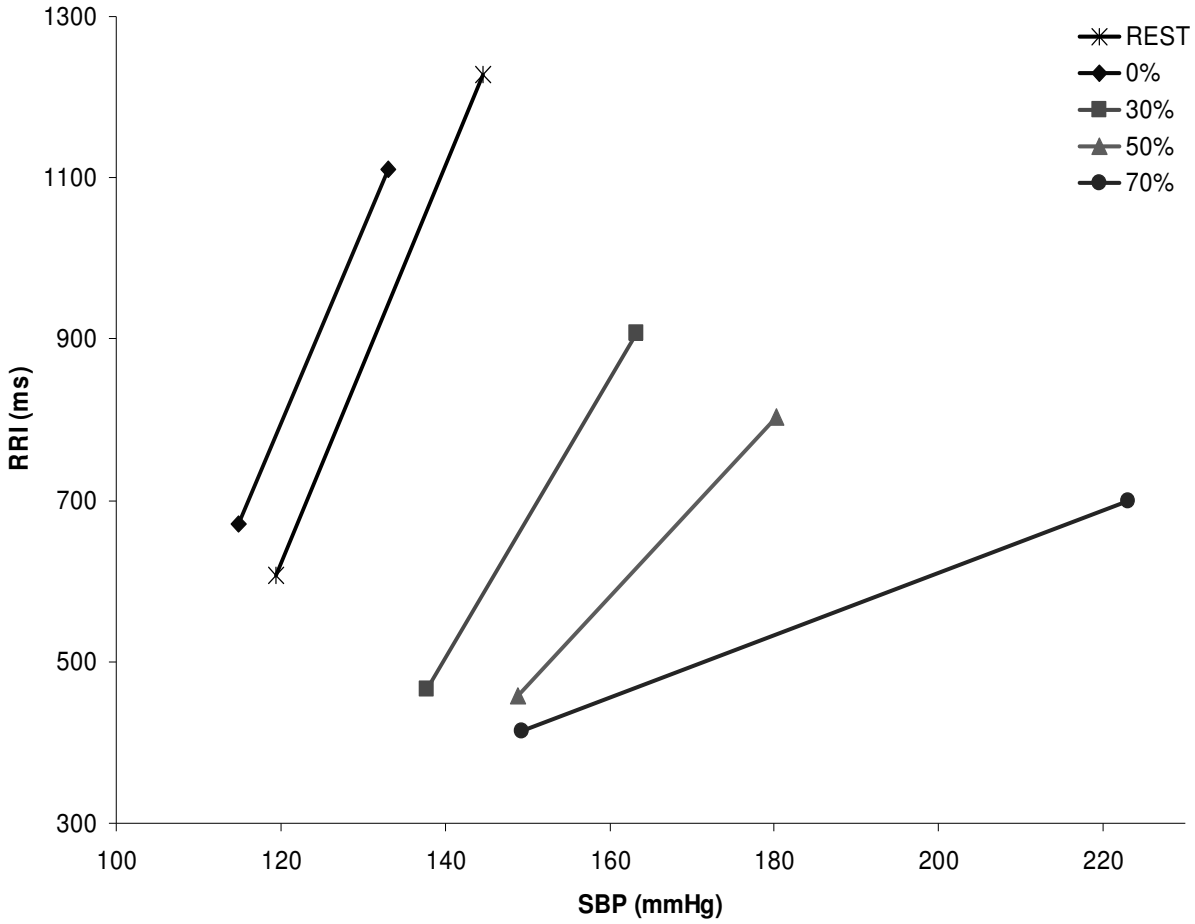

Figure 3.4: Regression lines calculated from sequence analysis during rest and exercise in the 0, 30, 50 and 70% trials. Rest line represents the overall mean regression line of the rest phases in the four trials.

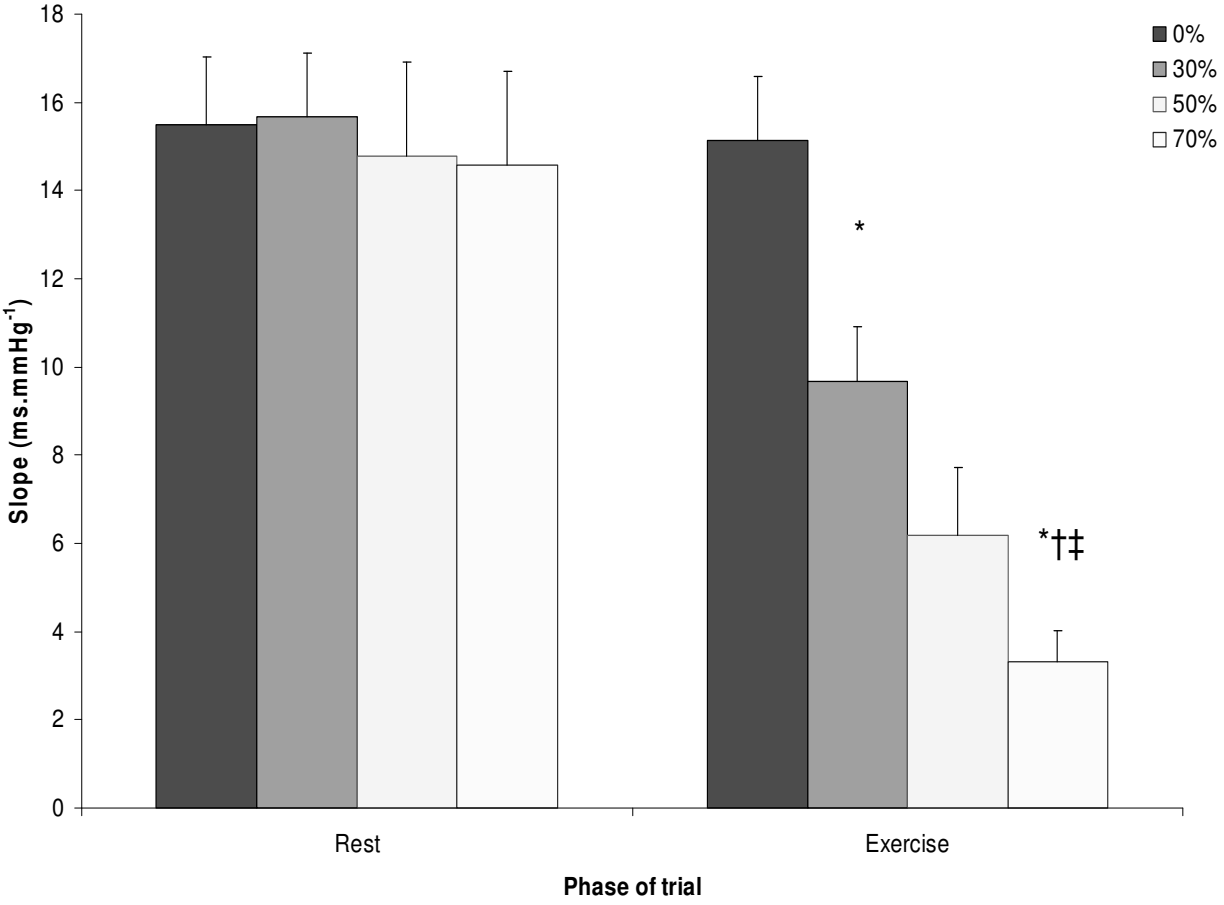

Figure 3.5: Slope values of regression lines calculated from sequence analysis during the rest and exercise phases of the 0, 30, 50 and 70% trials. * = significantly different from rest. † = significantly different from 0%. ‡ = significantly different from 30%.

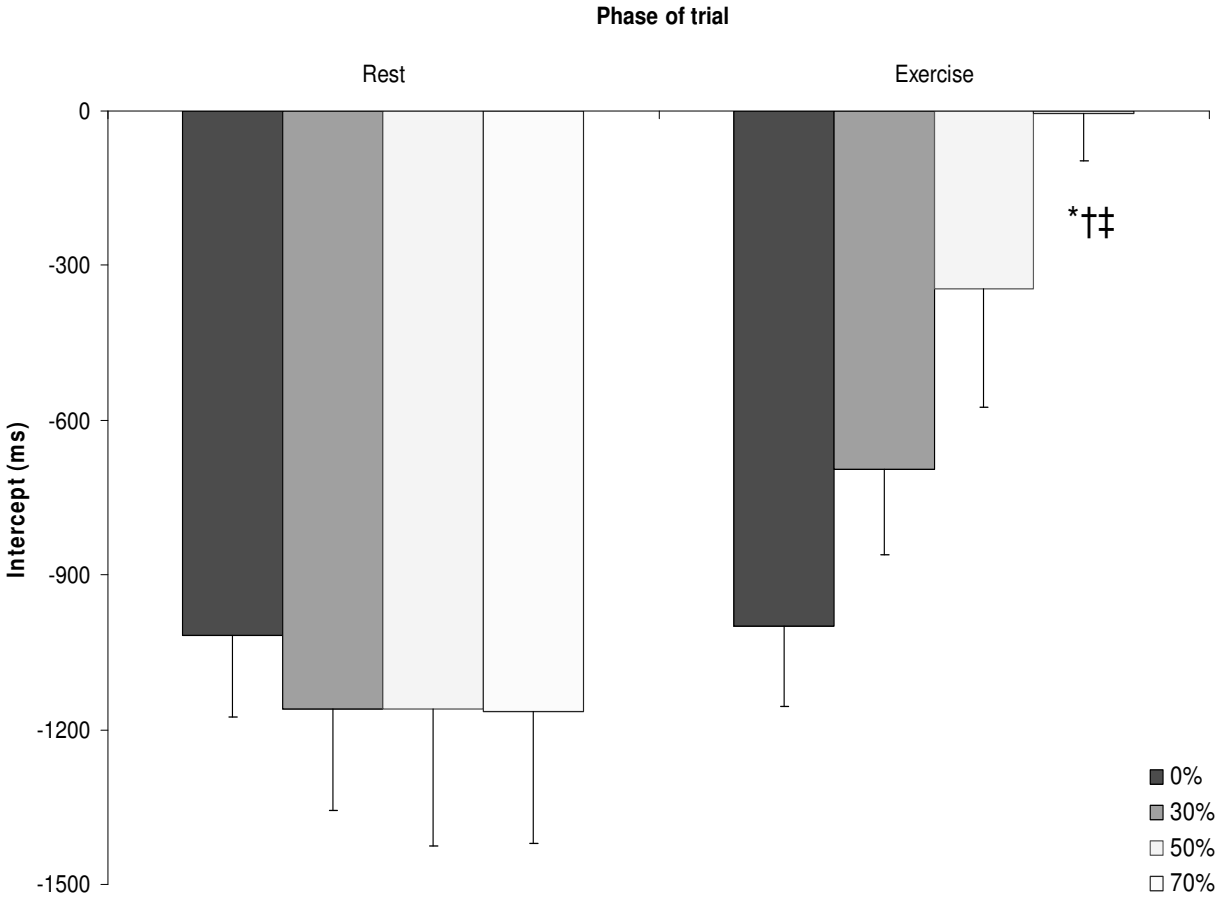

Figure 3.6: Intercept values of regression lines calculated from sequence analysis during the rest and exercise phases of the 0, 30, 50 and 70% trials. * = significantly different from rest. † = significantly different from 0%. ‡ = significantly different from 30%.

3.4: Discussion

The main finding in this study is that spontaneous cardiac baroreflex sensitivity, indicated by the slope of regression lines calculated from sequence analysis, was progressively decreased during increasing intensities of isometric exercise. Calculated regression lines were also shifted further rightward and downward as exercise intensity increased. Together, this indicates a progressive resetting of baroreflex control of heart rate and, most likely, a relocation of the operating point to a position of reduced sensitivity during greater isometric exercise intensity.

SBRS, calculated from sequence analysis, has been shown to represent the OP of the carotid-cardiac baroreflex function curve during dynamic exercise (Ogoh *et al.*, 2005). Hence, it is likely that a progressive decrease in SBRS during isometric exercise of increasing intensity reflects a decrease in OP gain, due to relocation of the OP away from the centring point towards the threshold on the carotid-cardiac baroreflex function curve. This would be in agreement with other studies examining different exercise intensities using the sequence technique (Iellamo *et al.*, 1998) and CBR function (Potts *et al.*, 1993; Norton *et al.*, 1999; Ogoh *et al.*, 2005). However, without being able to construct full stimulus-response CBR function curves, the exact location of the OP during the different exercise intensities cannot be specified. Nevertheless, the progressive resetting of the regression lines to higher BPs and lower RRIs (higher HRs; Figure 3.4) is also in agreement with the classical resetting of carotid-cardiac baroreflex function curves. Therefore, it is likely that baroreflex control of HR was increasingly reset, and the OP continually relocated to a position of reduced sensitivity, as exercise became more intense.

Ogoh *et al.* (2005) illustrated that the decreased sensitivity at the OP for baroreflex control of HR during exercise was due to vagal withdrawal. When applied to the present findings, this would suggest that the decreased SBRS (sensitivity at the OP) was due to progressive reductions in vagal tone as exercise intensity increased. Greater levels of central command are present as physical work increases (Goodwin *et al.*, 1972), and is known to alter baroreflex function (Gallagher *et al.*, 2001; Querry *et al.*, 2001; Ogoh *et al.*, 2002). This occurs possibly by opposing barosensitive cells in the NTS (Degtyarenko and Kaufman, 2005; Degtyarenko and Kaufman, 2006). Therefore, it is likely that central command caused the majority of the cardiac vagal inhibition and hence decrease in SBRS,

which occurred more so as exercise intensity increased. A contributory role in inhibiting cardiac vagal activity during exercise may originate from the muscle mechanoreflex. Stimulation of group III muscle mechanoreceptors also increases with higher exercise intensity as greater muscle forces are generated (Kaufman *et al.*, 1983). At present, the effect of muscle mechanoreflex stimulation on full baroreflex function in humans is unknown and requires investigation. However, it has been shown that muscle mechanoreceptive afferents can inhibit cardiac vagal activity in humans (Gladwell *et al.*, 2005). This occurs even when vagal tone is increased by a single negative neck pressure stimulus, which excites the baroreflex (Gladwell *et al.*, 2005). Cardiac vagal inhibition by muscle mechanoreceptive afferents could occur by opposing barosensory cells in the NTS (McMahon *et al.*, 1992; Potts *et al.*, 2003). It is possible that these are the same barosensitive cells in the NTS that central command has been shown to inhibit (Degtyarenko and Kaufman, 2006). Also, metabolite sensitisation of muscle mechanoreceptors is known to occur in animals (Kaufman *et al.*, 1984; Kaufman and Rybicki, 1987), and has been suggested in humans (Middlekauff and Chiu, 2004; Bell and White, 2005). Therefore, it is also possible that if muscle mechanoreceptors are sensitised by metabolites produced during exercise, they may increase their inhibitory input and cause further reductions in cardiac vagal activity. However, studies would be needed to investigate metabolite sensitisation of muscle mechanoreceptive afferents and their influence on baroreflex function in humans.

During exercise, metabolite accumulation from muscular work activates the muscle metaboreflex, which increases BP (Coote *et al.*, 1971; McCloskey and Mitchell, 1972). Muscle metaboreflex activation resets the baroreflex to operate at this higher BP during exercise, with no change in SBRS (Iellamo *et al.*, 1997). This resetting is maintained even after the end of exercise when blood flow to the exercising limb is occluded just before the cessation of exercise (Iellamo *et al.*, 1994; Papelier *et al.*, 1997; Iellamo *et al.*, 1999; Carrington and White, 2001). Muscle metaboreflex activation is not thought to directly decrease cardiac vagal (parasympathetic) activity during exercise, although an increase in BP would excite the baroreflex and so could influence vagal tone. Rather, muscle metaboreflex activation causes cardiac sympathetic activation to increase HR (Iellamo *et al.*, 1999). Therefore, it is unlikely that greater levels of muscle metaboreflex activation during increasing exercise intensity contributed to the decrease in SBRS in this study. However, muscle metaboreflex

activation would have been responsible for the greater resetting of the baroreflex that occurred as exercise intensity increased.

Studies have used various methods to examine the relative influences of central command and muscle afferent feedback on SBRS during isometric exercise in humans, with differing outcomes. Using electrically-evoked calf exercise, Carrington and White (2001) and Carrington *et al.* (2003) found SBRS to be unchanged from rest at low exercise intensity, with a rightward resetting of the regression line. As only the muscle mechanoreflex and muscle metaboreflex were present at this time, a combination of mechanoreflex-induced decrease and metaboreflex-induced increase in SBRS could have resulted in no overall change in SBRS. This occurred in the absence of central command, which is known to relocate the OP of the carotid-cardiac baroreflex function curve during exercise to a point of reduced gain or sensitivity (Gallagher *et al.*, 2001; Querry *et al.*, 2001). As SBRS calculated by the sequence technique represents the carotid-cardiac OP (Ogoh *et al.*, 2005), it is likely that if voluntary exercise were performed at the same intensity, SBRS would decrease as a result of central command inhibiting cardiac vagal tone. Indeed, Iellamo *et al.* (1999a; 1999b) observed a decreased SBRS during low intensity voluntary isometric leg extension. Conversely, SBRS was found to be unchanged during very low intensity isometric leg extension (Iellamo *et al.*, 1999a) and low intensity isometric handgrip exercise (Iellamo *et al.*, 1994; Iellamo *et al.*, 1999a). If exercise induces only small demands on muscular work, only minor cardiovascular adjustments will be needed. A rightward resetting of baroreflex function may be sufficient to allow very light physical activity to continue without causing changes in SBRS (Iellamo *et al.*, 1994). Hence, exercise intensity and muscle mass involved appear to play important roles in the degree of change in SBRS and overall resetting of the baroreflex. As observed in this study, during low, moderate and high levels of isometric calf exercise, SBRS progressively decreased and regression lines were further reset with increasing exercise intensity. However, the relative contributions of central command, muscle mechanoreflex and muscle metaboreflex towards these autonomic and cardiovascular changes during exercise is unknown.

In summary, spontaneous cardiac baroreflex sensitivity progressively decreases during isometric exercise of increasing intensity. Regression lines from sequence analysis were also shifted further rightward and downward with more intense exercise. This suggests a progressive resetting of

baroreflex control of heart rate, with a continual relocation of the operating point to a position of reduced sensitivity, during greater isometric exercise intensity. The relative contributions of central command, muscle mechanoreflex and muscle metaboreflex towards this resetting requires further investigation.

CHAPTER 4: MODULATION OF SPONTANEOUS BAROREFLEX CONTROL OF HEART RATE AND INDICES OF VAGAL TONE BY PASSIVE CALF MUSCLE STRETCH DURING GRADED METABOREFLEX ACTIVATION IN HUMANS

4.1: Introduction

It is now well established that mechanoreceptive muscle afferents contribute a significant proportion of the drive controlling the cardiovascular system during exercise (Adreani *et al.*, 1997; Adreani and Kaufman, 1998; Gladwell and Coote, 2002; Gladwell *et al.*, 2005). The sensitivity of afferent fibres to mechanical stimuli is believed to be linked to the expression of different receptor sub-types (Li and Sinoway, 2002; Hanna and Kaufman, 2003; Middlekauff and Chiu, 2004; Kindig *et al.*, 2006). Differences in receptor type and density are likely to explain why mechanoreflex sensitivity can in turn be linked to muscle fibre type, training status and severity of disease states, e.g. CHF (Fisher and White, 2004). Some afferent fibres may be purely mechanosensitive (Kaufman *et al.*, 1984; Adreani and Kaufman, 1998; Gladwell *et al.*, 2005), but the response of others to mechanical stimulation is influenced by the prevailing local metabolic conditions (Mense and Stahnke, 1983; Rotto and Kaufman, 1988; Rotto *et al.*, 1990; Adreani and Kaufman, 1998; Middlekauff and Chiu, 2004; Bell and White, 2005; Hayes *et al.*, 2006). A further distinction can be made between populations of afferents that respond to passive tendon stretch or active force generated by muscle contraction (Hayes *et al.*, 2005). This makes it quite difficult to develop tests of muscle mechanoreflex involvement in human cardiovascular control during exercise, and to establish whether muscle mechanoreflex sensitivity has been changed by training or disease. Further complexity is added when it is considered that during voluntary exercise, central command plays a role in the cardiovascular control system, as does activation of the muscle metaboreflex, if metabolites are accumulated in sufficient quantity. This makes it even more difficult to attribute the precise contribution made by mechanoreceptive afferents to any cardiovascular change during voluntary exercise.

A number of different approaches have been used to try to evaluate the contribution of the muscle mechanoreflex to human cardiovascular control in exercise. For example, electrically-evoked exercise has been used to remove central command (Carrington *et al.*, 2001). When this is followed by a period of circulatory occlusion (CO) to establish the contribution of the muscle metaboreflex, the muscle mechanoreflex contribution to the exercise pressor response is then revealed by subtracting the response during CO from that seen during evoked exercise (Carrington *et al.*, 2001). Alternatively, the role of muscle mechanoreceptive afferents in human cardiovascular control has been examined using

passive stretch (Gladwell and Coote, 2002; Fisher *et al.*, 2005; Gladwell *et al.*, 2005; Cui *et al.*, 2006) or external compression of muscle (McClain *et al.*, 1994; Williamson *et al.*, 1994; Bell and White, 2005) in resting subjects. There have also been attempts to examine metabolite sensitisation of the mechanoreflex by applying these techniques during CO following isometric exercise (Bell and White, 2005; Fisher *et al.*, 2005).

Abnormal muscle afferent feedback has been linked to over-activation of the sympathetic nervous system and effort intolerance in disease states such as CHF. However, the exact role of muscle mechanoreceptors here remains fiercely disputed (Piepoli *et al.*, 1996; Carrington *et al.*, 2001; Smith *et al.*, 2003; Li *et al.*, 2004; Middlekauff *et al.*, 2004). This is largely because of the methodological problems outlined above, and indeed the search for a test of muscle mechanoreflex sensitivity in humans is ongoing.

For both practical and theoretical reasons, it has proved quite difficult to establish a reliable method for examining the influence of the muscle mechanoreflex on efferent sympathetic nerve activity in humans. Muscle mechanoreceptor activation via passive stretch has an apparently small and transient effect on human muscle sympathetic nerve activity (MSNA) (Cui *et al.*, 2006), even in patients thought to possess increased muscle mechanoreflex sensitivity (Middlekauff *et al.*, 2004). This suggests that this approach is unlikely to lead to a universally useful tool with which to examine the level of muscle mechanoreceptor afferent activation. In the present study, a different approach has been taken in the search for a non-invasive tool that reveals muscle mechanoreflex involvement and sensitivity in human cardiovascular control. With this goal in mind, the intention was to examine the influence of controlled passive muscle stretch on parasympathetically-mediated changes in HR, RRI and SBRS, applied during CO at rest and following graded increases in isometric exercise intensity.

Gladwell *et al.* (2005) demonstrated that in resting humans, activation of mechanoreceptive afferents by passive calf muscle stretch decreased indices of vagal tone and caused HR to rise, by vagal inhibition. They termed the mechanoreceptive afferents responsible for this response, “tentonoreceptors”. Using different intensities of isometric calf exercise followed by CO to manipulate metabolic conditions in the muscle interstitium, Fisher *et al.* (2005) showed that cardiovascular

changes caused by a short but standard passive stretch stimulus of the same calf were not influenced by the level of preceding exercise. They argued that this indicated unchanged sensitivity of muscle mechanoreceptive afferents during stretch, irrespective of metabolite accumulation. It is known that CO following increasingly intense calf exercise results in progressively greater BP elevations (Alam and Smirk, 1937; Fisher *et al.*, 2005). Higher BP inevitably causes greater activation of baroreceptors, so increasing their afferent input to the NTS. This input is known to exert a powerful modulatory effect on cardiac vagal outflow under both resting and exercise conditions (Spyer, 1994). Against this background of increased baroreceptor activation, the effects of a standardised mechanoreceptor afferent input might therefore be expected to become less effective at modulating vagal tone and altering HR (McMahon and McWilliam, 1992; Potts, 2002). However, if metabolite sensitisation of the muscle mechanoreceptors occurs, their activation during a standard stretch stimulus is increased. It might then be hypothesised that changes in HR and indices of vagal tone would be maintained during stretch, despite elevated baroreceptor activation. This idea, though noted by Gladwell *et al.* (2005) and Fisher *et al.* (2005), has not been fully explored, especially the influence of a longer stretch period. The practical advantage of this longer data collection period is that it allows the use of the SBRS technique, a robust measure of the cardiac arm of the baroreflex and index of vagal tone (Parati *et al.*, 2000). Furthermore, knowledge of the behaviour of such measures during the present experimental conditions could be useful in the development of future tests of muscle mechanoreflex sensitivity in humans. Therefore, the aim of this study was to extend the studies of Gladwell *et al.* (2005) and Fisher *et al.* (2005), to examine whether HR, SBRS and other indices of vagal tone can be altered by passive stretch of the human calf muscle against a background of increased metabolite accumulation and BP elevation. The hypothesis was that passive calf muscle stretch would alter cardiac vagal control at rest, and will still do so during CO following isometric exercise of progressively increasing intensities, irrespective of increased BP elevation.

4.2: Methods

10 healthy subjects (7 male, 22 ± 1 yr, 71 ± 4 kg, 171 ± 3 cm) were recruited from the University of Birmingham student population. All subjects gave informed written consent and were

habituated to the experimental procedures, which were approved by the School of Sport and Exercise Sciences local ethics committee and conformed to the Declaration of Helsinki (2000). Subjects were asked to refrain from consuming food and caffeine in the 2 hours preceding the trials, and performing strenuous exercise in the 24 hours preceding the trials. Each subject participated in all four experimental trials once (after completing a habituation session), with no more than one trial performed each day, and the order of all trials was randomised.

4.2.1: Experimental protocol

Subjects were seated in a semi-recumbent position in a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA) with the right knee flexed by 30° and the foot strapped to the footplate so the lower leg was horizontal to the floor. Velcro straps were used to fix the foot and minimise heel lift during voluntary plantarflexor exercise and passive stretch. Prior to each trial, passive range of dorsiflexion of the ankle joint was established by manually moving the footplate as far as was comfortable. This information was programmed into the machine so that the subsequent stretching movement could be performed automatically by the Biodex. MVCs of the calf plantarflexors were assessed by recording maximal efforts and accepting three that were within 5% of each other. Respiratory rate was standardised for each subject across all trials by asking them to breathe in time to a metronome set at a rate which they found to be the most comfortable on first testing.

A schematic diagram of the experimental protocol is shown in Figure 4.1. After subjects were settled for 10 minutes, the protocol began with a 5 minute baseline period. 10 seconds before the end of this period, a cuff placed around the right thigh was inflated to 200mmHg by a rapid cuff inflator (E20, Hokanson, Bellevue, WA, USA) and this remained inflated for a further 9 minutes. At the end of the rest period, subjects were instructed to either rest for a further 1.5 minutes (0% control trial), or perform ischaemic isometric plantarflexion using their right calf muscles in order to produce a torque that matched a pre-determined exercise intensity of 30, 50 or 70% MVC, for 1.5 minutes. The level of torque produced during the exercise period was displayed on a computer screen in front of the subjects for visual feedback. After a further 3.5 minutes of local CO, the foot was passively dorsiflexed by the

Biodex to the preset angle at a velocity of $30°.s^{-1}$, and held there for the next 3 minutes with continued occlusion. After this stretch period, the foot was returned to its starting position and local CO continued for a further minute. The thigh cuff was then deflated to restore circulation to the lower leg, and subjects recovered for a further 2 minutes.

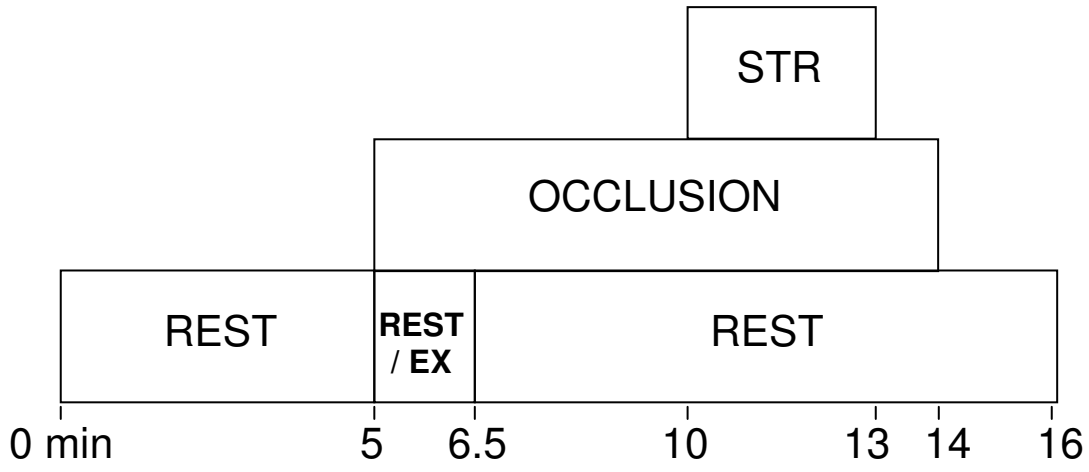

Figure 4.1: Schematic diagram of experimental protocol. EX, exercise; STR, stretch.

4.2.2: Measured variables

RRI was measured using a three-lead ECG (Cardiorater CR7, Cardiac Records Ltd, London, UK) in the lead II position, from which HR was derived. BP was measured non-invasively using a Finapres (Ohmeda 2300, Louisville, CO, USA) on the middle finger of the right hand, which was supported at heart-level. Phase of respiratory cycle was monitored using a pneumograph, consisting of a band strapped around the subjects' chest that was attached to a strain gauge. Active and passive

plantarflexor torques were recorded using the Biodex. All signals were sampled by an analogue-to-digital converter (Cambridge Electronic Design 1401plus, CED, Cambridge, UK) at 100Hz, with the exception of the ECG signal which was sampled at 1000 Hz. Data was recorded and displayed using Spike 2 software (CED, Cambridge, UK).

4.2.3: Spontaneous baroreflex sensitivity

SBRS at the OP of the baroreflex function curve was assessed offline using the sequence technique, which involved detecting sequences of three or more successive beats where SBP and RRI were either both increasing or both decreasing. Regression equations from these sequences of SBP (X axis) and RRI (Y axis) provided slope values representative of SBRS, and intercept values.

4.2.4: Heart rate variability

RMSSD assesses the variation between RRIs by calculating the square root of the mean of the sum of the squares of differences between successive RRIs. RMSSD was calculated over four 15-second periods during the minute preceding stretch, and the first two 15-second periods of stretch (the time of the greatest HR changes during stretch). A third index of vagal tone, CCV, measures the variation between RRIs when normalising for different HRs. This was calculated using the formula:- (standard deviation of RRI/mean RRI) x 100 (Al-Ani *et al.*, 1996). CCV was calculated for the three minutes of local CO before stretch, and the last two minutes of stretch with continued occlusion. This excluded the first minute of stretch in order to ensure only stable HR data was analysed.

4.2.5: Statistical analysis

Raw data files were analysed using custom-written script files to produce beat-to-beat values for RRI, HR, SBP, DBP and MAP. 15-second averages were calculated for each subject over the whole

protocol, and these were averaged across the group to produce group means during each period and each condition. The first two 15-second averages following exercise were excluded from the group means calculation, as only steady-state data was required for the CO phase. SBRS was calculated using a custom-written sequence analysis program, and only baroreflex sequences with correlation coefficients greater than 0.95 were accepted. Mean SBRS values were calculated from all slope data for the rest (5min), exercise (1.5min), CO (3min) and stretch with concurrent CO (3min) phases of the protocol. HR variability analysis was performed using Nevrokard software (Medistar, Ljubljana, Slovenia). All values are expressed as the mean ± SEM. One-way and repeated measures ANOVA or paired-samples *t*-tests were used to identify significant differences within the protocol. Statistical significance was set at $P<0.05$ and if this was reached using ANOVA, *post hoc* analysis using paired-samples *t*-tests with a Bonferroni correction was performed (SPSS Inc., Chicago, IL, USA).

4.3: Results

4.3.1: Contraction and stretch torques

No MVCs were measured prior to the 0% control trial, as no exercise was performed in this trial. MVCs measured before each exercise trial were not significantly different between conditions, with values of 128.8 ± 6.9, 124.9 ± 6.1 and 123.6 ± 6.8 Nm being recorded prior to the 30, 50 and 70% MVC trials, respectively. Exercise torques averaged 38.6 ± 2.1, 62.4 ± 3.1 and 86.5 ± 4.8 Nm for the 30, 50 and 70% trials, respectively. The range of ankle motion assessed by passive stretch was not significantly different between conditions, with an overall mean of 31 ± 1° of dorsiflexion from vertical. There was no significant difference between peak torques during stretch in the 30, 50 and 70% trials. Values of 25.2 ± 4.0, 23.8 ± 3.9 and 25.5 ± 5.4 Nm, respectively, were seen within the first 5 seconds of stretch. This corresponded to 20, 19 and 20% of the MVC assessed prior to the 30, 50 and 70% trials, respectively. By the end of the stretch period, torque had fallen significantly from initial levels in all conditions. Torque decreased to 60, 55 and 57% of the initial peak levels (14.7 ± 2.0, 12.8 ± 1.8 and 14.0 ± 2.7 Nm) by the end of stretch in the 30, 50 and 70% trials, respectively (Figure 4.2).

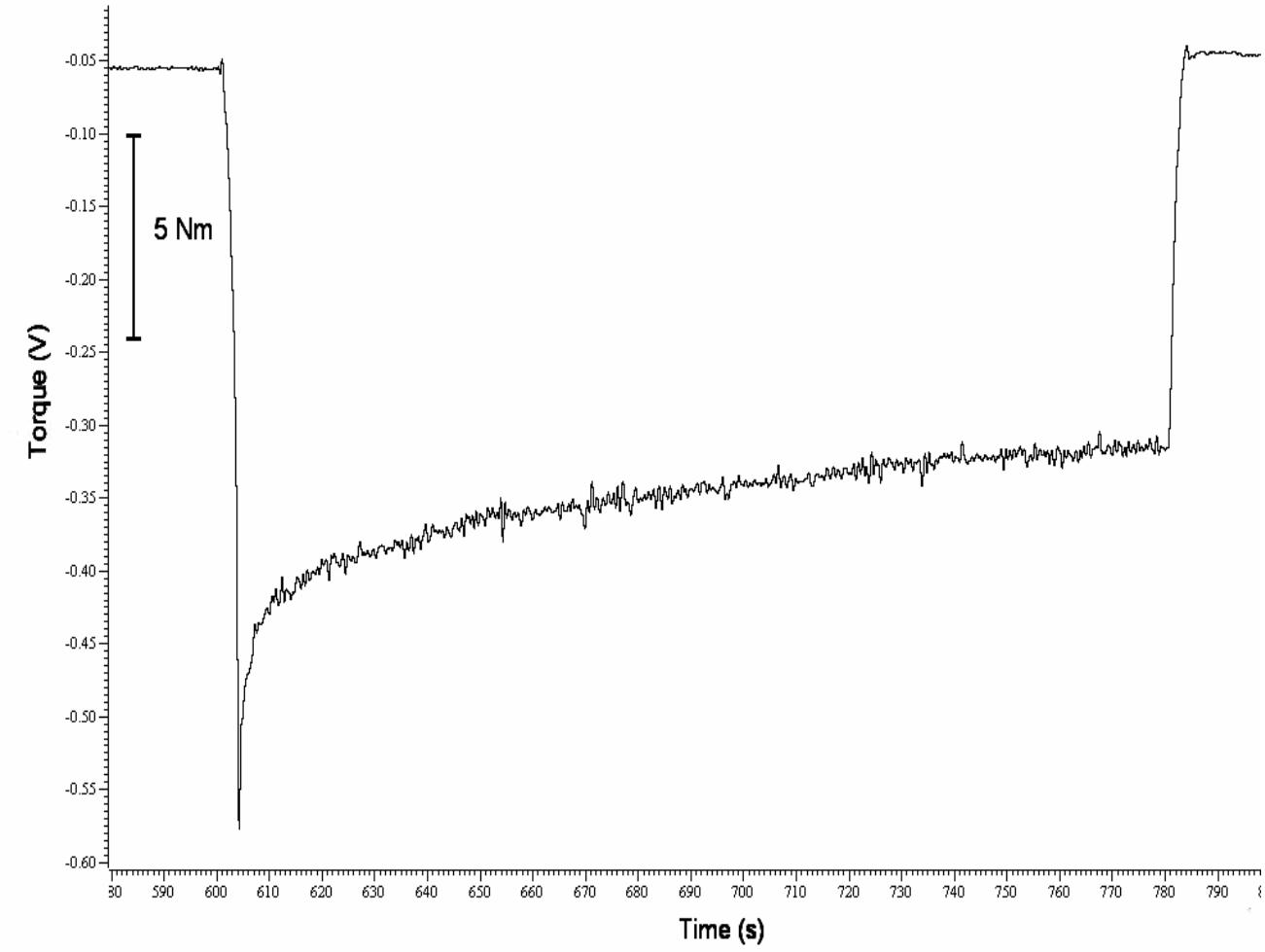

Figure 4.2: Original recording of a typical torque trace during the whole 3min stretch phase. Scale, 0.1V = 3.57Nm.

4.3.2: Cardiovascular variables

HR, MAP and DBP values in the rest phase were not significantly different between conditions (Table 3.1, Chapter 3). However, SBP in the rest phase during the 50 and 70% trials was significantly higher than during the 0% trial ($P<0.05$). BP increased from baseline during exercise, and was elevated above baseline during local occlusion following exercise. The magnitude of this change was related to increasing exercise intensity. SBP, MAP and DBP changed with similar time course during the different phases of the protocol, so for simplicity and in keeping with previous publications (Fisher *et al.*, 2005), only the DBP response to the different trials is illustrated here. An original recording of BP and HR during a 50% MVC trial is shown in Figure 4.3.

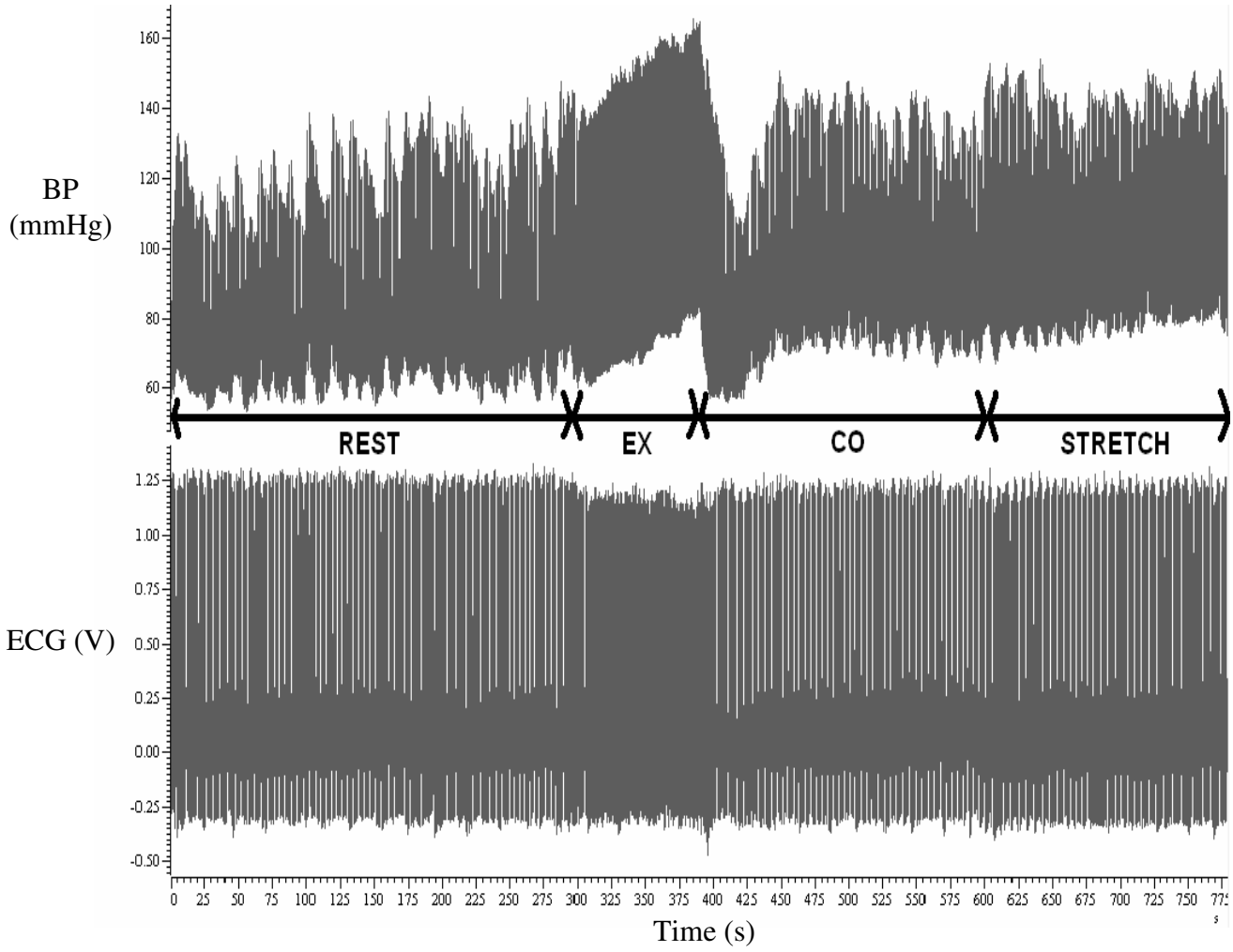

Figure 4.3: Original recordings of BP (top) and ECG (bottom) during a 50% MVC trial. EX, exercise; CO, circulatory occlusion; STRETCH, stretch with concurrent circulatory occlusion.

DBP changes from rest during the whole protocol are shown in Figure 4.4. DBP rose progressively during exercise, reaching significantly higher levels with increasing exercise intensity ($P<0.05$; Chapter 3). During CO, DBP fell from end-exercise levels, but remained significantly elevated above baseline in all exercise trials, with significantly greater elevation with increasing exercise intensity ($P<0.05$). Stretch, applied to the limb with no prior exercise and when applied during CO following progressively more intense exercise, caused a progressive increase in DBP over the three minutes ($P<0.05$). This increased DBP by an average of 4 ± 1, 4 ± 1, 4 ± 0 and 2 ± 1 mmHg during the 0, 30, 50 and 70% trials, respectively (Figure 4.5). There was no significant difference in the magnitude of the change between trials ($P=0.809$). Once stretch was removed, there was a significant fall in DBP ($P<0.05$), but it remained elevated above resting levels during the continued occlusion.

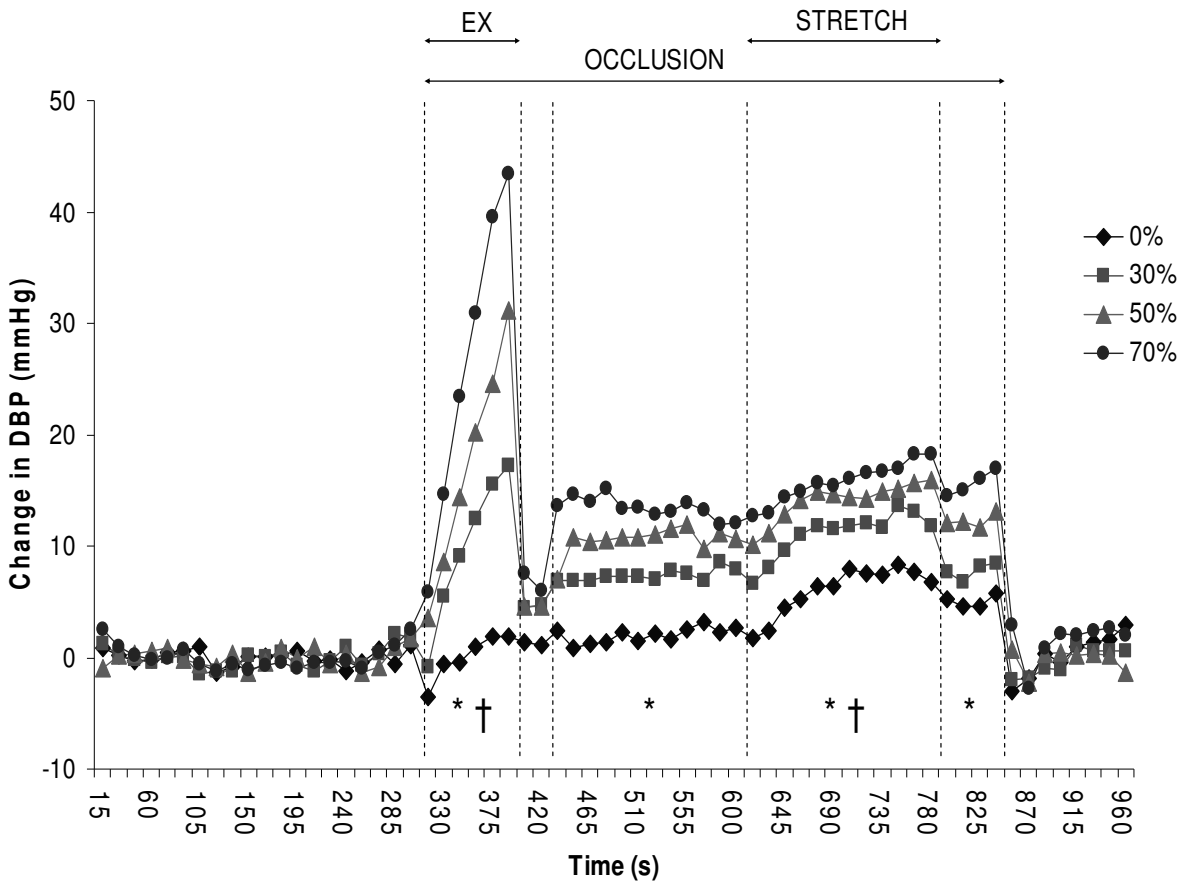

Figure 4.4: Group mean changes from rest in diastolic blood pressure (DBP) during each phase of the 0, 30, 50 and 70% trials. * = significant effect of condition. † = significant effect of time.

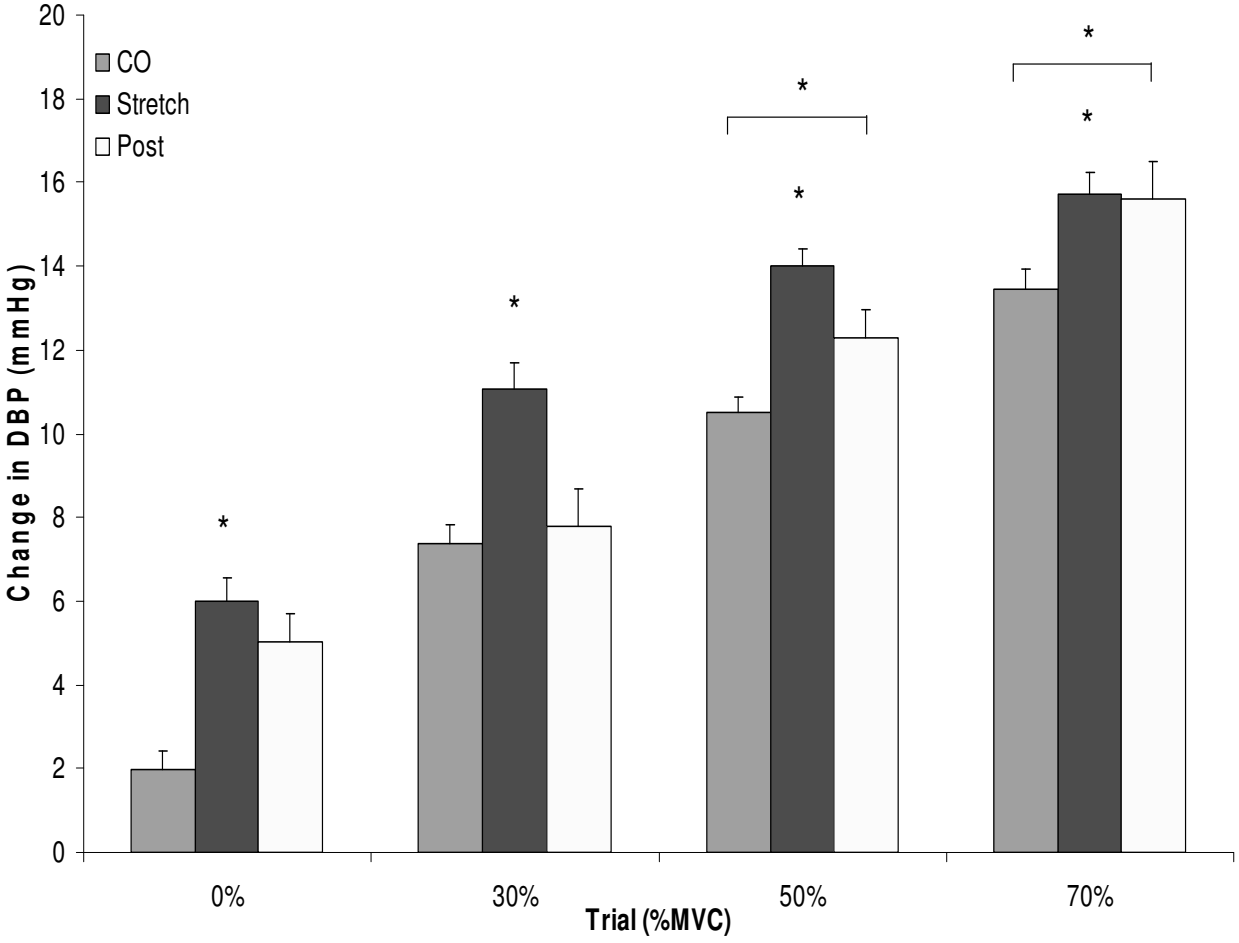

Figure 4.5: Group mean (±SEM) changes from rest in diastolic blood pressure (DBP) during the CO-alone, stretch with concurrent CO and post-stretch CO-alone phases of the 0, 30, 50 and 70% trials. * = significantly different from CO-alone and Post. * above bracket = significantly different from 0% trial.

HR changes from rest during the whole protocol are shown in Figure 4.6. HR rose progressively during exercise, reaching significantly higher levels with increasing exercise intensity ($P<0.05$; Chapter 3). After exercise, HR fell from end-exercise levels in all trials and returned to resting values, with the exception of the 70% trial where HR remained significantly elevated above rest ($P<0.05$). At the onset of stretch, there was an immediate increase in HR ($P<0.05$), which was of a similar magnitude in each trial. Stretch increased HR by 6 ± 1, 6 ± 1, 8 ± 1 and 6 ± 2 $b.min^{-1}$ during the 0, 30, 50 and 70% trials, respectively, within the first 15s of stretch. This peak value was not sustained throughout the whole stretch phase, where the HR increase averaged 3 ± 1, 4 ± 1, 3 ± 2 and 1 ± 1 $b.min^{-1}$ during the 0, 30, 50 and 70% trials, respectively ($P<0.05$; Figure 4.7). Once stretch was removed, HR fell to pre-stretch levels ($P<0.05$).

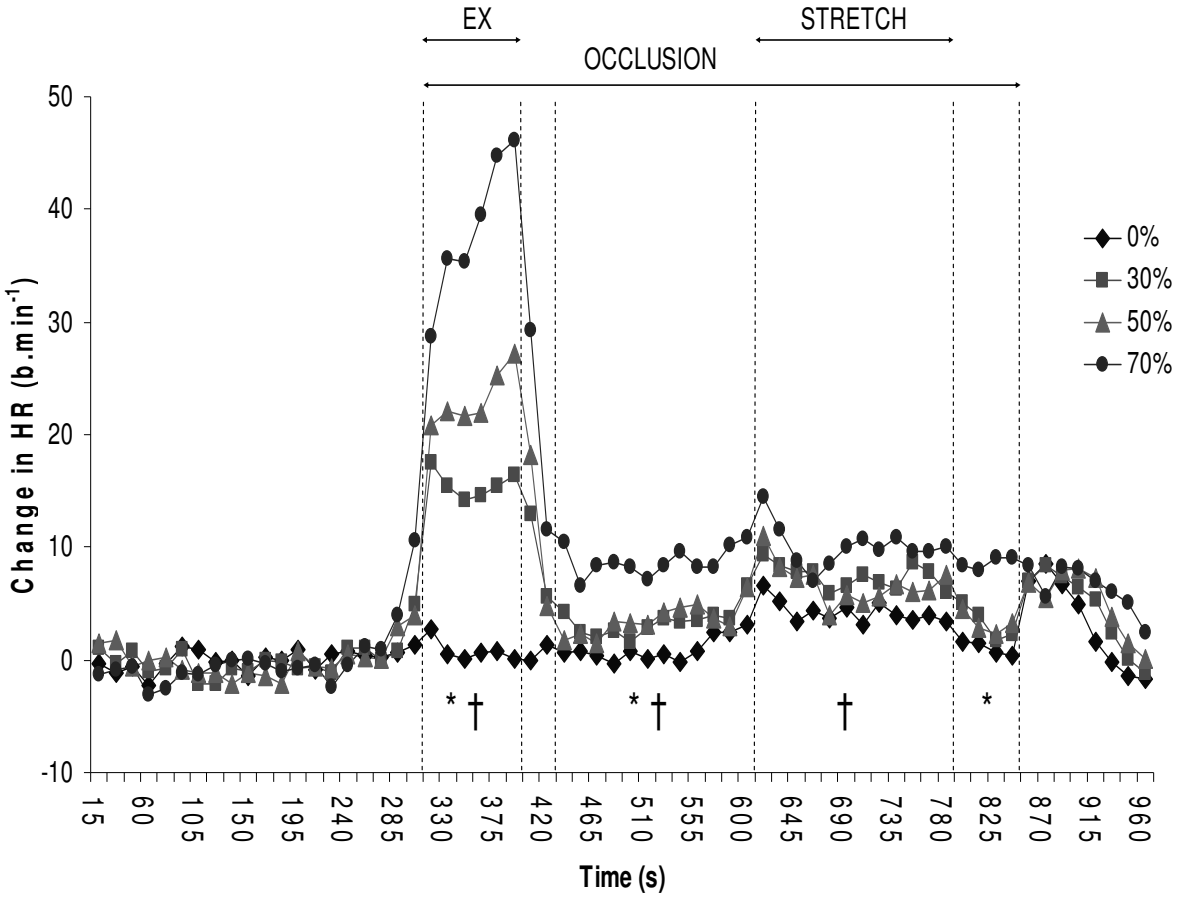

Figure 4.6: Group mean changes from rest in heart rate (HR) during each phase of the 0, 30, 50 and 70% trials. * = significant effect of condition. † = significant effect of time.

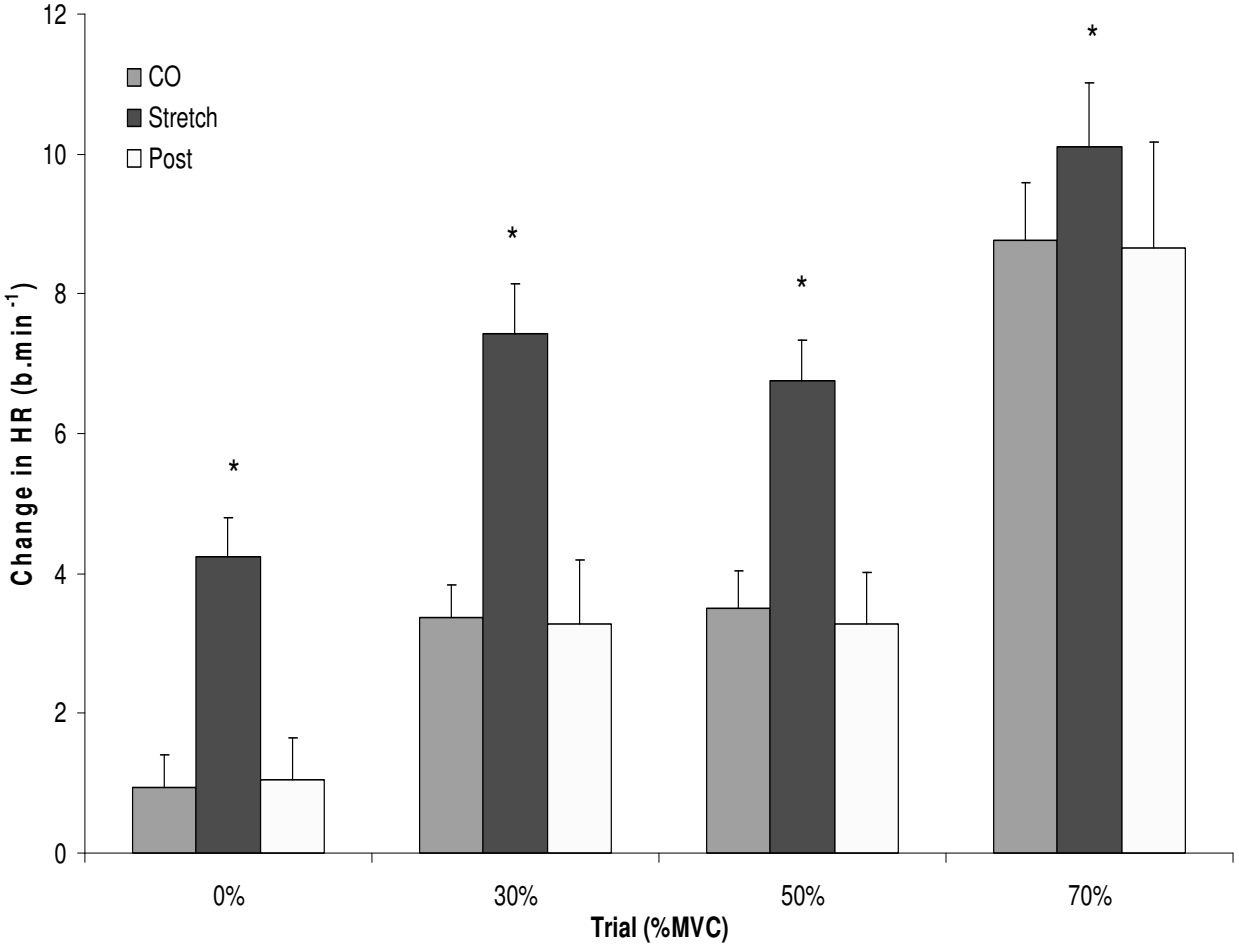

Figure 4.7: Group mean (±SEM) changes from rest in heart rate (HR) during the CO-alone, stretch with concurrent CO and post-stretch CO-alone phases of the 0, 30, 50 and 70% trials. * = significantly different from CO-alone and Post.

4.3.3: Spontaneous baroreflex sensitivity

Regression lines calculated from sequence analysis during CO and stretch with concurrent CO in all trials are shown in Figures 4.8 and 4.9, respectively. There was no significant difference in SBRS during the rest phases of the four trials (Table 4.1). When exercise was performed, there was a significant decrease in SBRS ($P<0.05$), with significantly greater decreases occurring during exercise of greater intensity ($P<0.05$; Chapter 3). During CO following rest or exercise, SBRS returned to resting levels. During stretch with concurrent CO, there was a significant decrease in SBRS ($P<0.05$), which was of a similar magnitude in each trial. From CO-alone levels, stretch decreased SBRS by 1.2 ± 0.9, 1.1 ± 0.6, 2.5 ± 0.9 and 2.0 ± 0.6 $ms.mmHg^{-1}$ in the 0, 30, 50 and 70% trials, respectively.

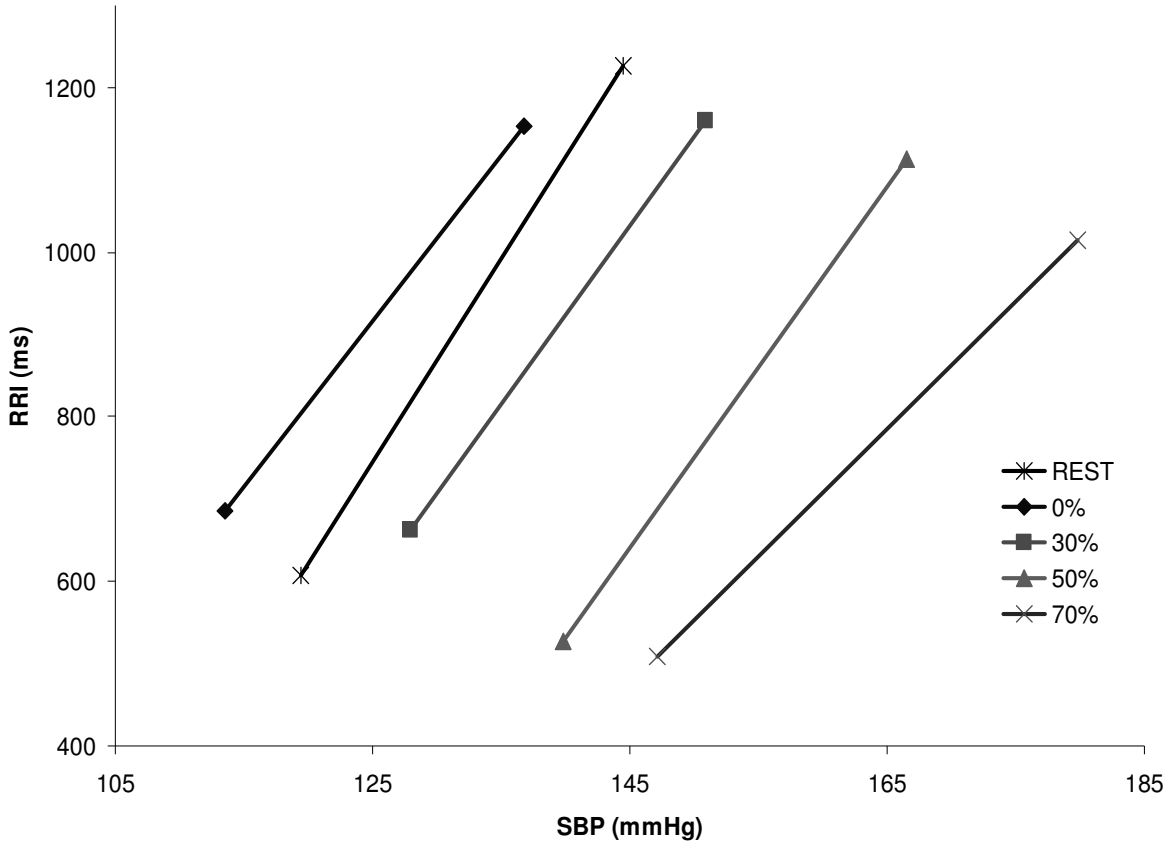

Figure 4.8: Regression lines calculated from sequence analysis during circulatory occlusion (CO) in the 0, 30, 50 and 70% trials. Rest line represents the overall mean regression line of the rest phases in the four trials.

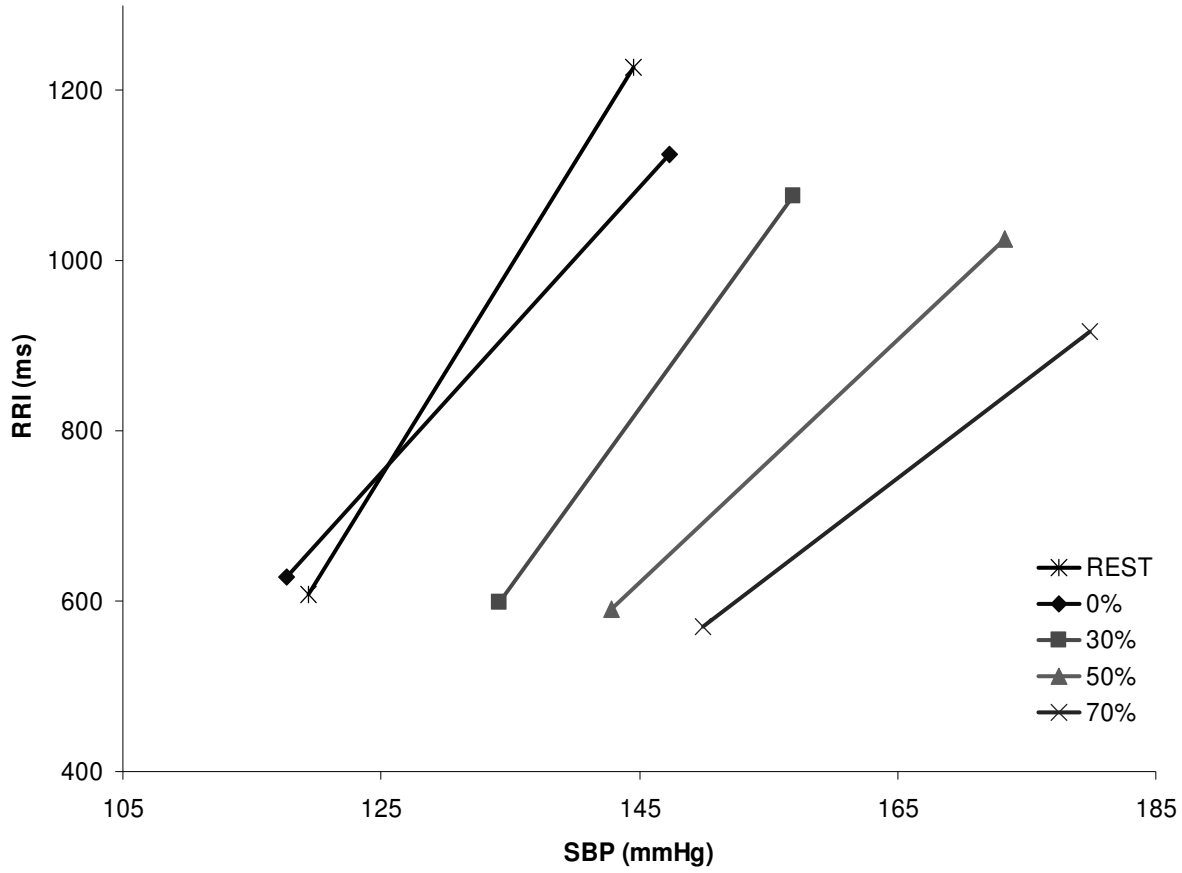

Figure 4.9: Regression lines calculated from sequence analysis during stretch with concurrent circulatory occlusion (CO) in the 0, 30, 50 and 70% trials. Rest line represents the overall mean regression line of the rest phases in the four trials.

Table 4.1: Slope values from sequence analysis, representing spontaneous baroreflex sensitivity (SBRS).

Trial (%MVC)	Rest SBRS ($ms.mmHg^{-1}$)	CO SBRS ($ms.mmHg^{-1}$)	Stretch SBRS ($ms.mmHg^{-1}$)
0	15.5 ± 1.5	14.7 ± 1.6	13.5 ± 1.7 *
30	15.7 ± 1.5	14.7 ± 1.7	13.6 ± 1.7 *
50	14.8 ± 2.1	13.6 ± 1.9	11.1 ± 1.8 *
70	14.6 ± 2.1	11.7 ± 1.2	9.7 ± 1.1 *

Values are means ± SEM. * = significantly different from both rest and CO. MVC, maximum voluntary contraction; CO, circulatory occlusion alone; Stretch, stretch with concurrent CO.

There was no significant difference in the mean intercept of the regression lines during the rest phases of all trials (Table 4.2). When exercise was performed, there was a significant increase in intercept ($P<0.05$), with significantly greater increases occurring during exercise of greater intensity ($P<0.05$; Chapter 3). During CO following rest or exercise, intercepts returned to resting levels and remained at these levels thereafter.

Table 4.2: Intercept values from sequence analysis.

Trial (%MVC)	Rest intercept (ms)	CO intercept (ms)	Stretch intercept (ms)
0	-1017 ± 158	-958 ± 172	-930 ± 193
30	-1160 ± 198	-1173 ± 216	-1157 ± 213
50	-1160 ± 265	-1235 ± 271	-946 ± 233
70	-1165 ± 256	-1108 ± 155	-837 ± 162

Values are means ± SEM. MVC, maximum voluntary contraction; CO, circulatory occlusion alone; Stretch, stretch with concurrent CO.

4.3.4: Heart rate variability

Over the phase of the experimental protocol examined with this technique (transition from CO to stretch with concurrent CO), there was no significant difference between trials in RMSSD, so the data were pooled to give mean values for each time point. CO -4, CO -3, CO -2 and CO -1 are the last four 15s periods of CO, and S +1 and S +2 are the first two 15s periods of stretch with concurrent CO (Figure 4.10). There was no significant difference in RMSSD during the CO-alone time points. At the onset of stretch, there was an immediate and significant decrease in RMSSD ($P<0.05$). From a mean CO-alone level of 42.2 ± 1.2 ms, stretch decreased RMSSD to 31.2 ± 2.3 ms, within the first 15s of stretch (coincident with the peak torque values obtained during stretch; see Figure 4.2).

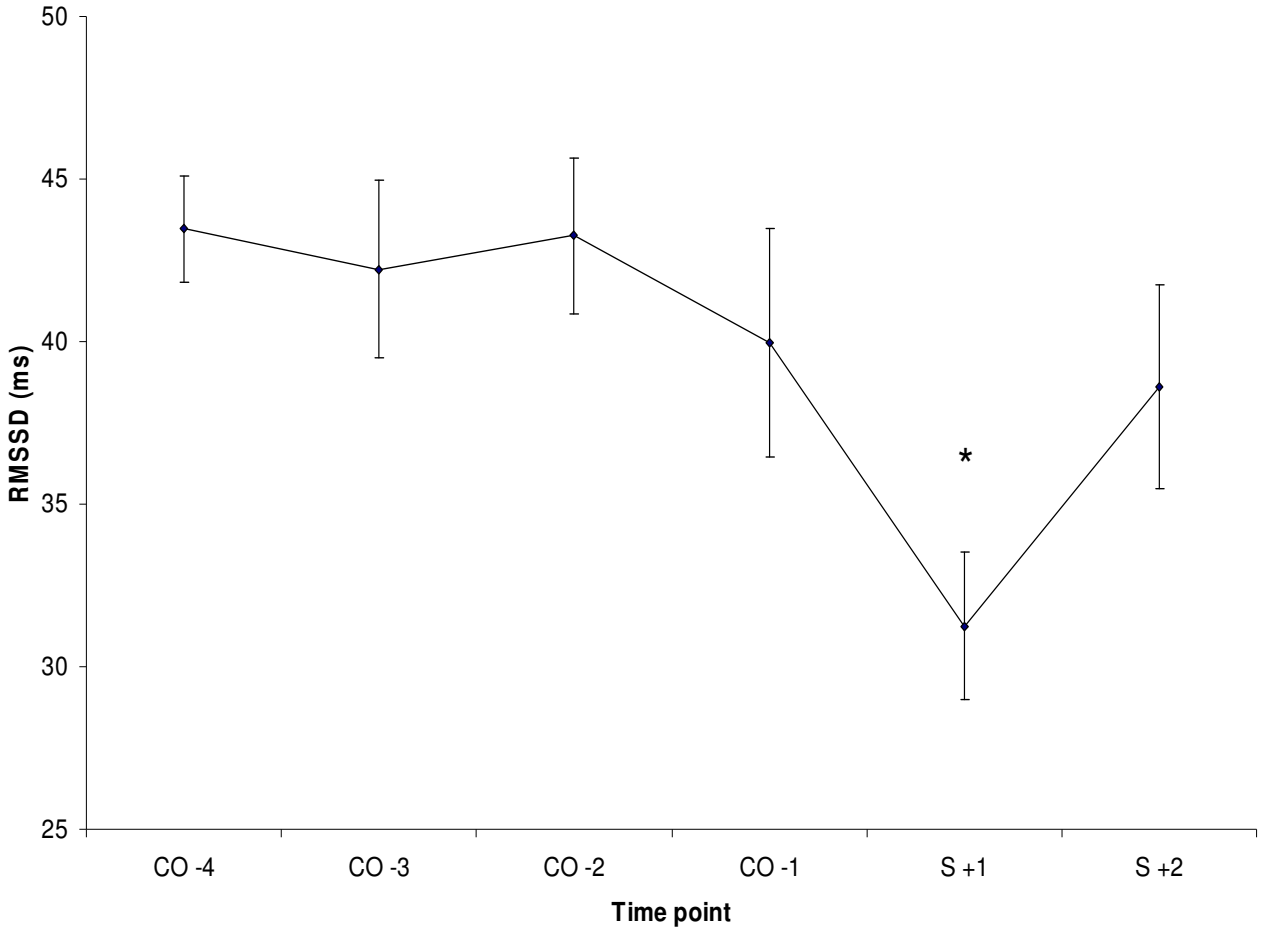

Figure 4.10: Group mean (±SEM) ensemble averages of root mean square of successive differences (RMSSD) for the last four 15s periods of CO-alone (CO -4, CO -3, CO -2 and CO -1), and the first two 15s periods of stretch with concurrent CO (S +1 and S +2). * = significantly different from CO-alone time points.

Mean CCV values during CO following rest or exercise were 3.8 ± 0.4, 3.9 ± 0.4, 4.3 ± 0.6 and 4.6 ± 0.3 % in the 0, 30, 50 and 70% trials, respectively, and this decreased to 3.7 ± 0.4, 3.4 ± 0.4, 3.5 ± 0.2 and 3.2 ± 0.2 % during the last two minutes of stretch with concurrent CO. The magnitude of this decrease became progressively greater with higher preceding exercise intensity (Figure 4.11), with CCV being significantly lower in the 70% compared to the 0% trial (P<0.05).

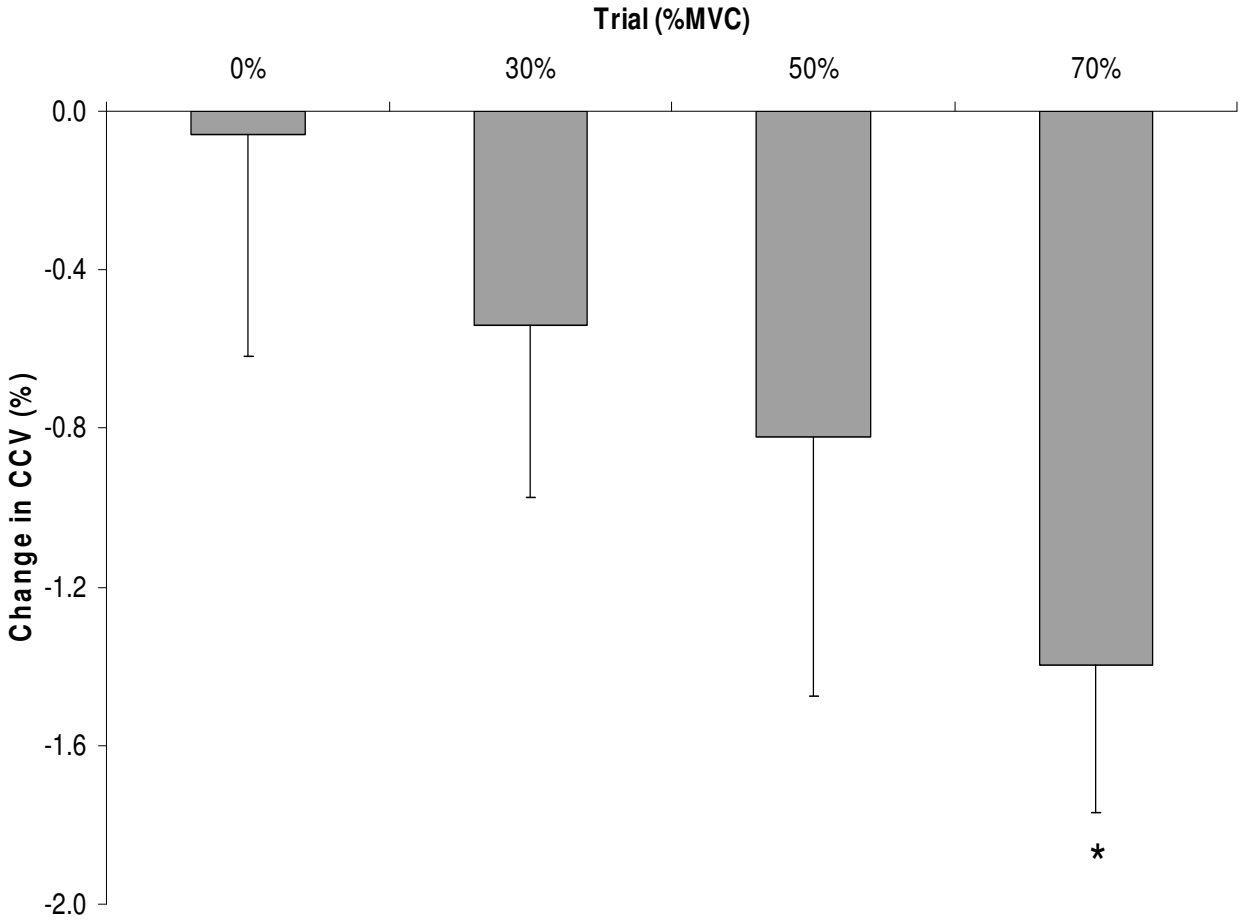

Figure 4.11: Group mean (±SEM) changes from CO-alone in common coefficient of variance (CCV) with application of concurrent stretch during the 0, 30, 50 and 70% trials. * = significantly different from 0% trial.

4.4: Discussion

The major new finding in this study is that passive stretch of the calf muscles after rest or increasingly intense isometric exercise, caused similar decreases in spontaneous cardiac baroreflex sensitivity, and other indices of vagal tone, irrespective of the level of blood pressure sustained by circulatory occlusion.

Murata and Matsukawa (2001) found that in a decerebrate cat preparation, cardiac vagal tone decreased gradually during sustained passive stretch of the hindlimb or the triceps surae. The HR rise that this caused was therefore slow in onset. However, Gladwell and Coote (2002) demonstrated that passive stretch of resting human calf muscle caused an immediate increase in HR. They suggested that this was caused by activation of a population of mechanoreceptive afferents which they termed, "tentonoreceptors". Subsequently, they provided evidence from pharmacological blockade (glycopyrrolate) and time-domain analysis (RMSSD) that the initial HR rise during stretch was mediated by immediate vagal inhibition (Gladwell *et al.*, 2005). In the present study, it was found that during the first 15s of stretch in each trial, HR rapidly increased from CO-alone levels, and RMSSD was similarly decreased from CO-alone levels. Additionally, when HR had recovered from the initial high torque transient of the stretch (see Figure 4.2) but remained stable and still elevated above CO-alone levels, CCV was decreased during the second and third minutes of each stretch. This study also shows that over the three minutes of stretch that were used to gather information on baroreflex function, in all conditions there was a sustained decrease in the slope of the regression lines, which give SBRS. The sequence technique that was used to assess SBRS, located at the OP of the cardiac function curve, is known to reflect baroreflex modulation of vagal tone (Parati *et al.*, 2000). Together with the observations of change in HR and its variability, this suggests that stretch caused a similar level of vagal inhibition in each trial. This was irrespective of the level of preceding exercise and consequent muscle metaboreflex activation, BP increase and presumably baroreceptor excitation that the CO maintained.

Since the muscle was relaxed and there was no intention by the subject to exercise during CO following exercise, the muscle mechanoreflex and central command can be ruled out as causes of the

increase in BP at this time. Therefore, the progressive increase in BP following exercise of greater intensity can only be because of greater metaboreflex activation. Increased systemic BP during CO must result in greater stimulation of baroreceptors, and thus presumably increase the level of their modulatory input to the NTS (Spyer, 1994). However, studies on animals have shown that the muscle mechanoreflex opposes baroreceptor modulation of cardiac vagal neurones (McWilliam *et al.*, 1991).Also, passive stretch of the human calf muscles has been shown to cause a decrease in vagal tone and increase in resting HR (Gladwell *et al.*, 2005). In this study, well-maintained decrements were observed in indices of vagal tone and increases in HR during passive calf stretch applied during CO. This happened despite progressive elevations in BP, and so could be taken to suggest that stretch-activated input to the NTS increased in parallel with the increased baroreceptor activation (due to the raised BP) during CO.

One possible source of this increased input could be from the stretch-sensitive muscle mechanoreceptors themselves. Since the stretch stimulus was standardised across each trial, muscle mechanoreceptors would have to be sensitised for this increased input to be supplied solely by the muscle mechanoreflex. Indeed, sensitisation of stretch-sensitive muscle mechanoreceptors by metabolites has been demonstrated in animal studies, especially those concerned with afferent sensitisation and effort intolerance in heart failure (Kaufman *et al.*, 1984; Li and Sinoway, 2002; Smith *et al.*, 2003). This mechanism would facilitate mechanoreflex-driven elevation in HR during exercise, irrespective of the metabolic conditions within the muscle.

For this appealing explanation to hold true, it would have been expected to find a graded increase in vagal tone during CO after exercise of increasing intensity, as well as HRs held below baseline. However, HR was maintained at baseline levels, RMSSD was unchanged, and there was a trend towards decreased SBRS during CO prior to stretch. Although CCV was increased during CO following greater exercise intensities, it appears that vagal tone was not elevated. The implication of this is that there is another source of modulatory input to cardiac vagal neurones during the steady-state phase of CO-alone. It is possible that the increased levels of metabolite in the muscle interstitium during CO-alone not only caused sympathoexcitation (Seals and Victor, 1991), but also caused activation of afferents which influence cardiac vagal neurones. Polymodal afferents, capable of being

activated without direct mechanical stimulation, are known to exist (Kaufman *et al.*, 1984). Their progressive activation during CO following each increment in exercise intensity might then explain the maintenance of low vagal tone, despite progressive elevations in BP. Therefore, when the standard stretch was applied to the calf, some mechanoreceptors were activated normally. In combination with the polymodal afferents, they opposed the baroreflex-mediated input to the NTS. This caused a standard rise in HR, and decreases in indices of vagal tone at the onset (RMSSD) and later phases (SBRS and CCV) of the stretch period.

During CO-alone, it was found that SBRS was not significantly altered prior to stretch. This is despite the progressive increases in BP seen after more intense exercise. This suggests a progressive resetting of the baroreflex, without obvious movement of the OP, during graded levels of muscle metaboreflex activation (Figure 4.8). As discussed earlier, this fits with the idea that the muscle metaboreflex causes sympathoexcitation and so promotes BP increase, but at the same time, some polymodal afferent activation opposes baroreceptor-driven modulation of cardiac vagal neurones. The net result is that SBRS is unchanged about a higher BP.

With each increment in preceding exercise, the 'baseline' BP was increased during CO-alone. During 3 minutes of concurrent stretch, it was found that BP gradually increased and then on cessation of stretch, fell back to the pre-stretch CO value. This agrees with the pattern of BP change during CO and 1 minute of stretch reported by Fisher *et al.* (2005), who also used the Biodex system to passively stretch the calf. However, Gladwell and Coote (2002) and Gladwell *et al.* (2005), applying the stretch to the calf manually, found that BP did not rise over 1 minute of stretch. It is possible that these differences simply reflect how well maintained the stretch stimulus was in the two different apparatus. Further, it is clear from examination of published original records that when an observer applies external force to a limb, it is much more variable than when applied by a machine (Cui *et al.*, 2006). Also, when stretch is applied to the forearm, it appears to be relatively less intense than when applied to the calf, which could further limit the BP response (Cui *et al.*, 2006). In Figure 4.2, it is shown that torque around the ankle joint declined by approximately 40% over 3 minutes of stretch applied by the Biodex. This is presumably due to tendon creep and muscle fibre re-adjustment, as the muscle-tendon complex overall must remain at a fixed length.

On the basis of the known discharge characteristics of mechanoreceptive afferent fibres (Kaufman *et al.*, 1984; Adreani and Kaufman, 1998), it is possible that some fibres may have adapted to the stretch stimulus during the sustained stretch period. This would tend to reduce the level of afferent feedback that was generated as the stretch period progressed. However, it is also likely that some reactivation of these mechanoreceptive afferents would occur after their initial burst of firing, due to metabolite sensitisation (Kaufman *et al.*, 1984; Adreani and Kaufman, 1998). This would restore their modulatory input. Attempts were made to take account of this possible variation in feedback by assessing vagal tone at the onset of the stretch period and over the longer term, using different tools with different time resolutions. The SBRS technique relies on sequences being present and this cannot be guaranteed at any given time point. Typically, sequences occur in no more than 50% of the cardiac cycles recorded. Nevertheless, SBRS is a robust index of cardiac vagal tone. Hence, it was used to explore changes in vagal tone over the whole stretch period. The RMSSD measure can be used over shorter time periods. Therefore, it was used to detect any change in vagal tone immediately after stretch onset when the probability of muscle mechanoreflex activation was highest.

The rapid fall in BP that was observed at the end of the exercise phase in each exercise trial, despite CO, could be taken to suggest that there was little activation, or indeed variation of the level, of muscle metaboreflex activation that was induced by the different exercise intensities. These well-documented initial falls in BP may relate to withdrawal of central command and loss of muscle mechanoreflex activation. Nonetheless, there is a clear increase in BP to a progressively higher, stable level with increasing exercise intensity. This suggests progressively greater muscle metabolite accumulation and muscle metaboreflex activation with each increment in exercise intensity. However, only direct measurement of afferent nerve activity and metabolite levels would confirm this.

In summary, it is shown that during circulatory occlusion at rest and following ischaemic isometric exercise of progressively increasing intensities, passive stretch of the human calf muscles can decrease spontaneous baroreflex sensitivity and other indices of vagal tone, and increase heart rate. These changes occur irrespective of the levels of blood pressure increase caused by muscle metaboreflex activation. This implies central modulation of baroreceptor input mediated by the actions of stretch-activated mechanoreceptive and/or polymodal muscle afferent fibres. Further studies are

required, but it is believed that the present study provides important new information suggesting that an approach based on the measurement of modulation of cardiac parasympathetic outflow could in the future lead to a useful tool for the non-invasive measurement of muscle mechanoreflex sensitivity in humans.

CHAPTER 5: LOCAL METABOLITE ACCUMULATION AUGMENTS PASSIVE MUSCLE STRETCH-INDUCED MODULATION OF CAROTID-CARDIAC BUT NOT CAROTID-VASOMOTOR BAROREFLEX SENSITIVITY IN HUMANS

5.1: Introduction

It is well established that the baroreflex is 'reset' to allow and to regulate the higher BPs and HRs associated with muscular work (Raven *et al.*, 2006). During voluntary dynamic exercise, the maximal gain of the CBR function curves for HR and BP appear to be well maintained. For the carotid-cardiac baroreflex function curve, the point about which BP is regulated, the OP, progressively relocates towards threshold, and so a lower gain, as exercise intensity increases. However, a similar change in OP of the carotid-vasomotor baroreflex function curve has not been consistently shown.

Various approaches have been used to identify the contributions of central command and muscle afferent feedback in modulating changes in the OP. These include blocking afferent feedback with epidural anaesthesia (Smith *et al.*, 2003a), removing central command by involuntary electrically-evoked exercise (Iellamo *et al.*, 1997; Carrington and White, 2001; Carrington *et al.*, 2003; Smith *et al.*, 2006), and activation of the muscle metaboreflex alone by occluding circulation through a previously active limb (Iellamo *et al.*, 1997; Papelier *et al.*, 1997; Iellamo *et al.*, 1999a; Carrington and White, 2001; Ichinose *et al.*, 2002; Carrington *et al.*, 2003). However, the influence of muscle mechanoreceptive afferent stimulation alone on baroreflex sensitivity (BRS) has been more difficult to describe. This is because of inadvertent activation of the muscle metaboreflex in some studies (Williamson *et al.*, 1994; Gallagher *et al.*, 2006).

The most reliable method to activate human muscle mechanoreceptive afferents is passive muscle stretch. Stretch of the human calf muscles has been shown to cause a clear inhibition of cardiac vagal activity (Gladwell and Coote, 2002; Gladwell *et al.*, 2005). This occurs even when vagal tone is increased by a single step change in neck pressure designed to excite the baroreflex (Gladwell *et al.*, 2005). Conversely, passive muscle stretch has been shown to exert only a small modulatory influence on MSNA (Middlekauff *et al.*, 2004; Cui *et al.*, 2006). In Chapter 4, it was observed that during passive calf muscle stretch, there was a decrease in SBRS using the sequence technique. However, this technique cannot distinguish between movements of the OP and change in CBR-HR maximal gain. Therefore, the first objective of the present study was to describe the effects of muscle mechanoreflex

stimulation using passive stretch of previously inactive calf muscles on full CBR-HR and CBR-MAP function curves.

The decrease in SBRS was found at rest, as well as during local CO following isometric calf exercise of increasing intensity, and hence with graded levels of metabolite accumulation in the muscle. These experimental manipulations were also associated with progressive elevations in SBP during CO. It is known that elevated SBP increases excitatory baroreceptor input to the NTS (Spyer, 1994). One interpretation of this finding of the same decrease in SBRS despite graded increases in SBP, is that the increased NTS excitation by baroreceptors was opposed by greater muscle mechanoreflex-induced inhibition of vagal outflow (McWilliam *et al.*, 1991). Since the mechanical stimulus (stretch) applied to the limb was the same in all conditions, this implied metabolite-linked sensitisation of muscle mechanoreceptive afferents. Therefore, the second objective of this study was to extend these earlier observations by measuring full CBR-HR and CBR-MAP function curves under controlled SBP conditions, and when metabolite-linked sensitisation of muscle mechanoreceptive afferents might occur. It was hypothesised that passive stretch of a previously active calf muscle, with concurrent local metabolite accumulation in that muscle, would decrease maximal gain of the CBR-HR function curve, but would not affect maximal gain of the CBR-MAP function curve.

5.2: Methods

12 healthy subjects (6 male, 20 ± 1 yr, 67 ± 3 kg, 1.7 ± 0.1 m) were recruited from the University of Birmingham student population. All subjects gave informed written consent and were habituated to the experimental procedures, which were approved by the School of Sport and Exercise Sciences local ethics committee and conformed to the Declaration of Helsinki (2000). Subjects were asked to refrain from consuming food and caffeine in the 2 hours preceding the trials, and performing strenuous exercise in the 24 hours preceding the trials. Subjects participated in no more than one trial each day, and the order of all trials was randomised.

5.2.1: Experimental protocol

Subjects were seated in a semi-recumbent position in a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA) with both knees flexed by 30° and the feet strapped to the footplate so the lower legs were horizontal to the floor. Velcro straps were used to fix the feet and minimise heel lift during voluntary plantarflexor exercise and passive stretch. Prior to each trial, with only the right foot strapped to the footplate, passive range of dorsiflexion of the right ankle joint was established by manually moving the footplate as far as was comfortable. This information was programmed into the machine so that the subsequent stretching movement could be performed automatically by the Biodex. MVCs of the calf plantarflexors that would perform exercise during the trial were assessed by recording maximum torques generated during repeated efforts, each separated by at least 1min. Three trials giving torques within 5% of each other were taken to indicate maximum effort and the highest value recorded was taken as the maximum voluntary torque. Respiratory rate was standardised for each subject across all trials by asking them to breathe in time to a metronome set at a rate which they found to be the most comfortable on first testing. The mean rate was 13 ± 1 breaths per min.

The habituation trial allowed subjects to become accustomed to the protocol, and practice breathing to the metronome at their chosen frequency. After this, each subject performed two exercise and two control trials. Each trial was performed on a separate day over a period of 1-2 weeks. A schematic diagram of the experimental protocol is shown in Figure 5.1. After subjects were settled for 10min, the protocol began with a 5-minute baseline period. 10 seconds before the end of this period, a cuff placed around either the right or left thigh was inflated to 200mmHg by a rapid cuff inflator (E20, Hokanson, Bellevue, WA, USA), and this remained inflated for a further 9 minutes. At the end of the rest period, in the exercise trials subjects were instructed to perform isometric plantarflexion using either their right or left calf muscles, in order to produce a torque that matched a pre-determined exercise intensity of 50% MVC for 1.5 minutes (two ischaemic exercise (IE) trials, IER and IEL). The level of torque produced during the exercise period was displayed on a computer screen in front of the subjects for visual feedback. The left foot was then released from the footplate and placed comfortably on a support just underneath the footplate. After 3.5 minutes of CO (CO 1), the right foot was passively

dorsiflexed by the Biodex to the preset angle at a velocity of $30°.s^{-1}$, and held there for the next 3 minutes (STR-CO). After this stretch period, the right foot was returned to its starting position, and CO continued for a further minute (CO 2). The thigh cuff was then deflated, and subjects recovered for a further 2 minutes. In the control trials, subjects followed the same protocol with the exception that instead of exercising, they rested for a further 1.5 minutes (two ischaemic control (IC) trials, ICR and ICL).

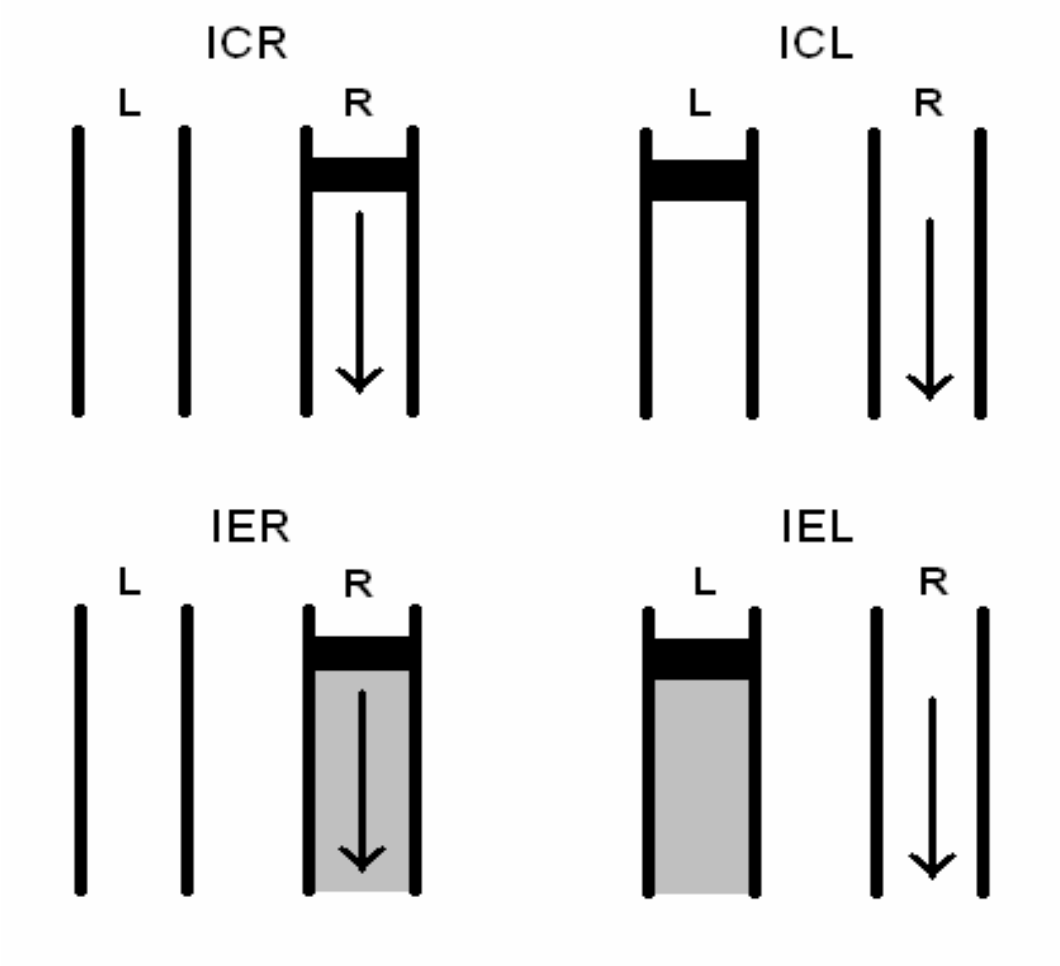

Figure 5.1: Schematic diagram of a subject's legs during the stretch phases in the exercise (IER and IEL) and control (ICR and ICL) trials. In IER, metaboreflex activation occurs in the right lower leg (grey area), due to a cuff inflated to supra-systolic pressure proximally (black area), with mechanoreflex stimulation in the same leg (arrow). In IEL, metaboreflex activation occurs in the left lower leg (grey area), due to cuff inflation to supra-systolic pressure (black area), with mechanoreflex stimulation in the contralateral right lower leg (arrow). In ICR, a cuff is inflated on the right leg as in IER but there is no metaboreflex activation as no exercise was performed, and mechanoreflex stimulation occurs in the right lower leg. In ICL, a cuff is inflated on the left leg as in IEL but there is no metaboreflex activation due to no prior exercise being performed, and mechanoreflex stimulation occurs in the contralateral right lower leg. L, left leg; R, right leg.

5.2.2: Measured variables

RRI was measured using a three-lead ECG (Cardiorater CR7, Cardiac Records Ltd, London, UK) in the lead II position, from which HR was derived. MAP was measured non-invasively using a Finapres (Ohmeda 2300, Louisville, CO, USA) on the middle finger of the right hand, which was supported at heart-level. Phase of respiratory cycle was monitored using a pneumograph, consisting of a band strapped around the subjects' chest that was attached to a strain gauge. Active and passive plantarflexor torques were recorded using the Biodex. An analogue-to-digital converter (Cambridge Electronic Design 1401plus, CED, Cambridge, UK) was used to sample the ECG (2000Hz), BP (1000Hz), torque (1000Hz) and pneumograph (100Hz) signals. Data was recorded and displayed using Spike 2 software (CED, Cambridge, UK).

5.2.3: Carotid baroreflex assessment

CBR control of HR and MAP was assessed using the NP/NS technique, where variable pressure stimuli were applied through a rubber-lined malleable lead collar that was secured around the front and sides of the neck with a Velcro strap (Eckberg *et al.*, 1975; Pawelczyk and Raven, 1989; Edwards *et al.*, 2003). NPs and NSs were applied at least once in every phase of the protocol, with at least 1min rest between each series. The timing of these was 1, 2 and 3min into the rest phase; 1min into exercise; 1 and 2min into CO 1; 15s, 1.5 and 2.5min into STR-CO; 30s into CO 2; 30s and 1.5min into recovery. The pressure generated in the neck collar was measured by a transducer fitted to the front of the collar (BTEM5P35OD, Sensortechnics, Puchheim, Germany), and sampled at 2000Hz through the analogue-to-digital converter. The air chamber used to produce these pressures has been previously described (Edwards *et al.*, 2003).

The four-parameter logistic function model (Kent *et al.*, 1972) was used to calculate the parameters A1, A2, A3 and A4. A1 is the responding range of HR or MAP, A2 is the gain, A3 is the ECSP at the centring point, and A4 is the minimum response of HR or MAP. Threshold and saturation were calculated using equations devised by Chen and Chang (1991). OP was defined as the mean pre-

stimulus HR or MAP, calculated over the complete respiratory cycle which preceded the onset of neck pressure changes. OP gain was calculated, which is a measure of responsiveness at the OP of the function curve. Maximal gain of the stimulus-response function curves, which is the gain at the centring point and is used as an index of CBR responsiveness, was also calculated.

5.2.4: Spontaneous baroreflex sensitivity

SBRS was assessed offline using the sequence technique, which involved detecting sequences of three or more successive beats where SBP and RRI were either both increasing or both decreasing. Regression equations from these sequences of SBP (X axis) and RRI (Y axis) provided slope values representative of SBRS, and intercept values.

5.2.5: Statistical analysis

Raw data files were analysed using custom-written script files to produce beat-to-beat values for RRI, HR, SBP, DBP and MAP. Group means were calculated for each phase and each trial. End-exercise values were calculated from the last 15s of exercise. The first 30s following exercise was excluded from the group means calculation, as only steady-state data was required for the CO phase. Neck collar pressures were measured directly from raw data files, and CBR parameters were calculated using Sigmaplot (SPSS Inc., Chicago, IL, USA). When more than one series of NPs and NSs were applied in a phase of the protocol, calculated CBR parameters were averaged to produce a mean for that phase for each subject. Individual subject data were then averaged to produce group means for each phase and each trial. SBRS was calculated using a custom-written sequence analysis program, and only baroreflex sequences with correlation coefficients greater than 0.95 were accepted. Due to neck pressure stimuli causing transient changes in HR and MAP, the data at these times was excluded from sequence analysis. ~30s of data was omitted around each set of pressures, so mean SBRS values were then calculated for the rest (3min15s), exercise (1min10s), CO 1 (2min) and STR-CO (1min30s) phases of the protocol. All values are expressed as the mean ± SEM. In all 12 subjects, significant differences

in cardiovascular variables within the protocol were identified using repeated measures ANOVA, with *post hoc* analysis using paired-samples *t*-tests with a Bonferroni correction. In a subset of 7 subjects, significant differences in CBR parameters within the protocol were identified using Friedman ANOVA, with *post hoc* analysis using Wilcoxon paired-samples tests. In a subset of 8 subjects, significant differences in slope and intercept within the protocol were identified using repeated measures ANOVA, with *post hoc* analysis using paired-samples *t*-tests with a Bonferroni correction. Significant differences in SBRS during the STR-CO phase were identified using paired-samples *t*-test. Statistical significance was set at $P<0.05$, and all statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA).

5.3: Results

5.3.1: Contraction and stretch torques

MVCs and hence exercise torques were significantly greater in IER trials (10 subjects right leg dominant) compared to IEL trials (2 subjects left leg dominant). For the right and left legs, MVC values were 139.3 ± 8.6 v 120.6 ± 7.1 Nm, respectively, ($P<0.05$), and exercise torques were 65.6 ± 4.0 and 56.3 ± 3.2 Nm, respectively, ($P<0.05$). The range of motion of the right ankle, assessed by passive stretch, was not significantly different between trials, with an overall mean of 30 ± 2° of dorsiflexion from vertical. Peak torques seen within the first 5s of passive stretch of the right calf were not significantly different between all trials, with values of 36.8 ± 3.8, 41.3 ± 4.9, 40.8 ± 6.0 and 36.3 ± 6.0 Nm in the ICL, IEL, ICR and IER trials, respectively. This corresponded to between 26 and 30% of the right calf MVC assessed prior to the IER trials. By the end of the stretch period, torque had fallen significantly from initial levels in all trials. Torque decreased to 68, 64, 63 and 67% of peak values (24.5 ± 2.3, 26.2 ± 3.0, 24.9 ± 3.0 and 22.9 ± 3.3 Nm) by end-stretch in the ICL, IEL, ICR and IER trials, respectively ($P<0.05$).

5.3.2: Cardiovascular variables

There were no significant differences in HR values at rest between trials. HR changes from rest during the whole protocol are shown in Figure 5.2. HR significantly increased during exercise in IEL and IER from 64 ± 1 and 65 ± 1 $b.min^{-1}$ at rest, to 80 ± 4 and 81 ± 4 $b.min^{-1}$ at end-exercise, respectively ($P<0.05$). In the control experiments, HR was unchanged from resting levels of 64 ± 1 and 62 ± 1 $b.min^{-1}$ during the no-exercise phase in ICL and ICR, respectively. In CO 1, HR fell from exercising levels, but remained significantly elevated above resting levels in IEL and IER ($P<0.05$). Application of concurrent stretch caused small but significant increases in mean HR in all trials ($P<0.05$). Mean increases from CO 1 levels across the whole stretch period were 2 ± 1, 1 ± 1, 3 ± 1 and 2 ± 1 $b.min^{-1}$ in ICL, IEL, ICR and IER, respectively. After stretch was removed in CO 2, HR fell towards but remained above CO 1 levels in ICL, ICR and IER. HR increased slightly in IEL. In recovery, the large transient cardiovascular changes associated with thigh cuff deflation resulted in mean HR values remaining above resting levels in all trials. However, HR had returned to resting levels in all trials by the end of the recovery period.

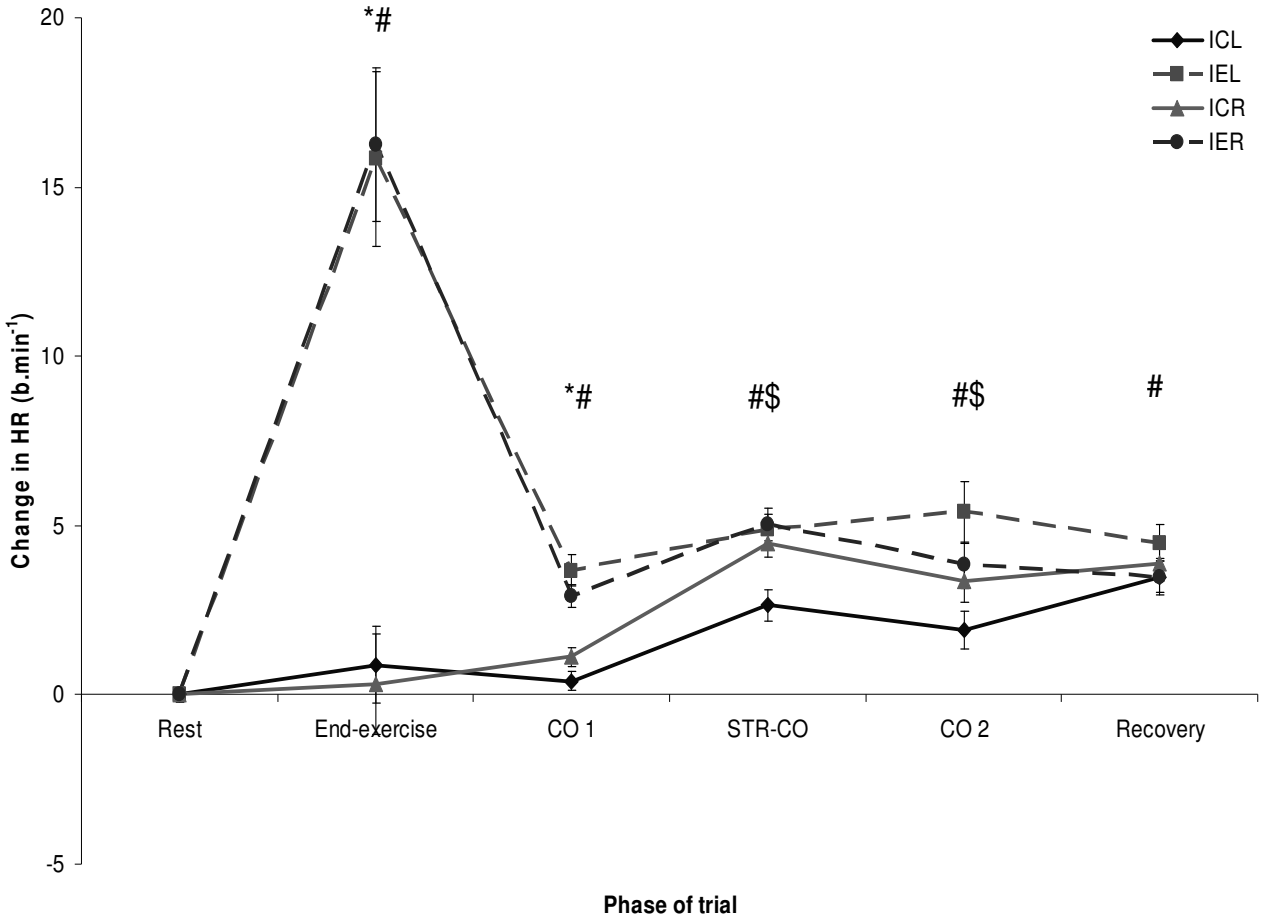

Figure 5.2: Heart rate (HR) changes from rest during each phase of the ICL, IEL, ICR and IER trials. * = significant effect of trial. # = significantly different from rest. $ = significantly different from CO 1.

There were no significant differences in MAP values at rest between trials. MAP changes from rest during the whole protocol are shown in Figure 5.3. MAP increased identically during exercise in the IEL and IER trials, from 87 ± 1 mmHg at rest to 105 ± 4 mmHg at end-exercise in both trials (P<0.05). In the control experiments, MAP was unchanged from resting levels of 86 ± 1 and 83 ± 1 mmHg during the no-exercise phase in ICL and ICR, respectively. In CO 1, MAP fell from exercising levels, but remained significantly elevated above resting levels at 92 ± 1 and 94 ± 1 mmHg in IEL and IER, respectively (P<0.05). Application of concurrent stretch significantly increased MAP in all trials (P<0.05). Increases from CO 1 levels were 2 ± 1, 2 ± 1, 6 ± 2 and 2 ± 2 mmHg in ICL, IEL, ICR and IER, respectively. After stretch was removed in CO 2, MAP fell back to CO 1 levels (P<0.05), and returned to resting levels in recovery.

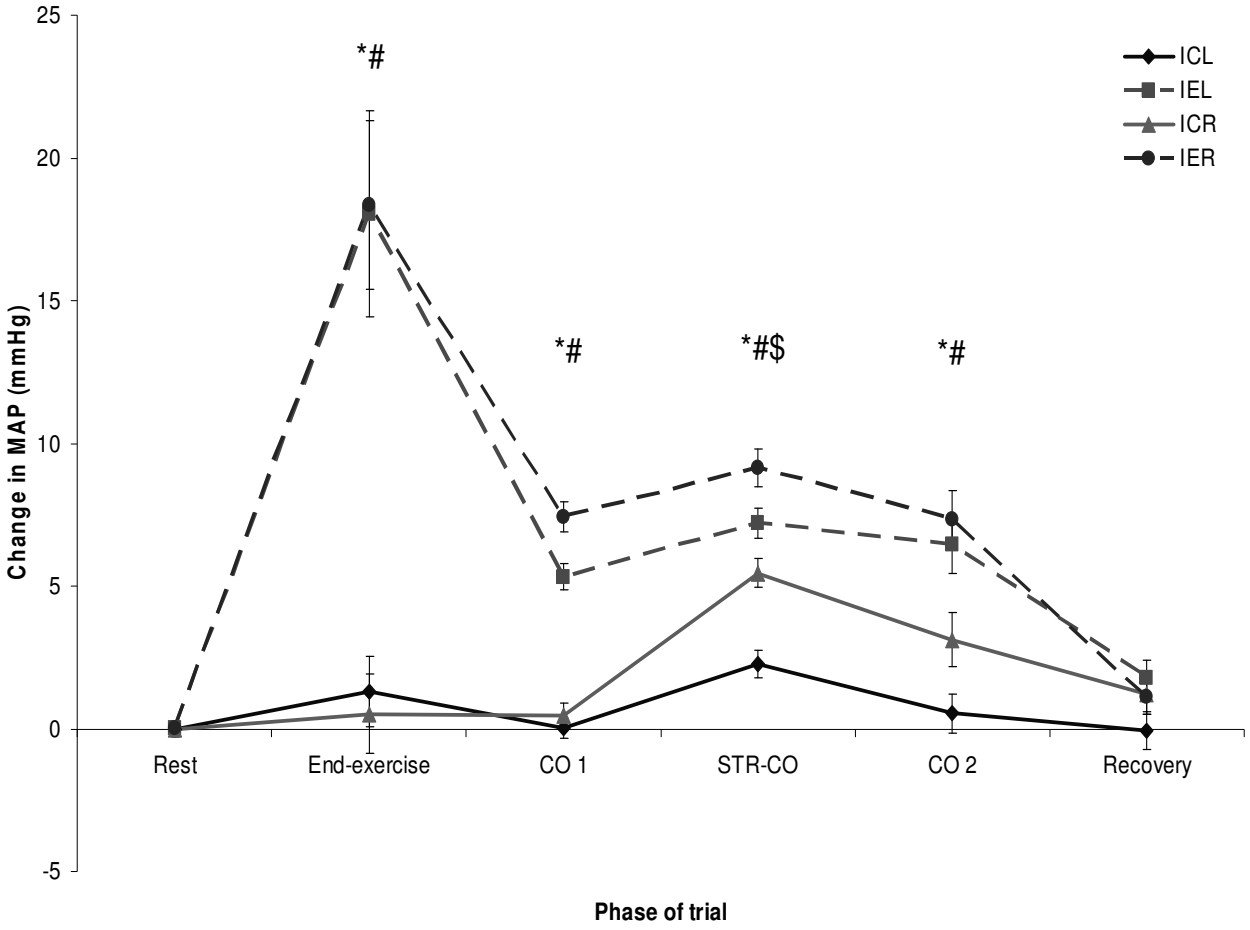

Figure 5.3: Mean arterial pressure (MAP) changes from rest during each phase of the ICL, IEL, ICR and IER trials. * = significant effect of trial. # = significantly different from rest. $ = significantly different from CO 1 and CO 2.

5.3.3: Carotid baroreflex function curves and parameters

Since each subject performed four trials, each of which had four important phases, at least 16 measurements of baroreflex function were made on each subject across all trials. However, for 5 of the subjects, it was not possible to fit data to the logistical regression model in every phase of every trial. In some cases, there was only one missing value. Nevertheless, for this reason their data were excluded from all aspects of the CBR analysis, reducing the number of subjects providing complete data sets to 7.

CBR-HR stimulus-response function curves during rest, exercise, CO 1 and STR-CO phases in the IER and IEL trials are shown in Figure 5.4 and 5.5, respectively. Logistic model parameters and derived variables describing CBR control of HR during each phase of the experiment in all trials are shown in Table 5.1 and 5.2, respectively.

In both IER and IEL, exercise caused an upward resetting of the function curve from rest. This was indicated by significant increases in minimum response (A4), and a rightward shift indicated by increases in ECSP at the centring point (A3). During CO 1, the function curves returned towards but did not fully regain their resting positions. Minimum response (A4) was not significantly different from rest, but ECSP at the centring point (A3) was significantly higher than rest in IEL, and higher in IER. Maximal gain at the centring point of the function curve during CO 1 was not significantly different between trials. In the STR-CO phase, maximal gain in IER was significantly smaller than in IEL ($P<0.05$, Figure 5.6). Maximal gain was not significantly different between CO 1 and STR-CO across all four trials but in IER, maximal gain in STR-CO was significantly reduced from CO 1 ($P=0.016$, paired *t*-test). There was minimal resetting of the function curve in the STR-CO phase in IER. In IEL, there was a further significant upward resetting from CO 1, indicated by increases in minimum response (A4).

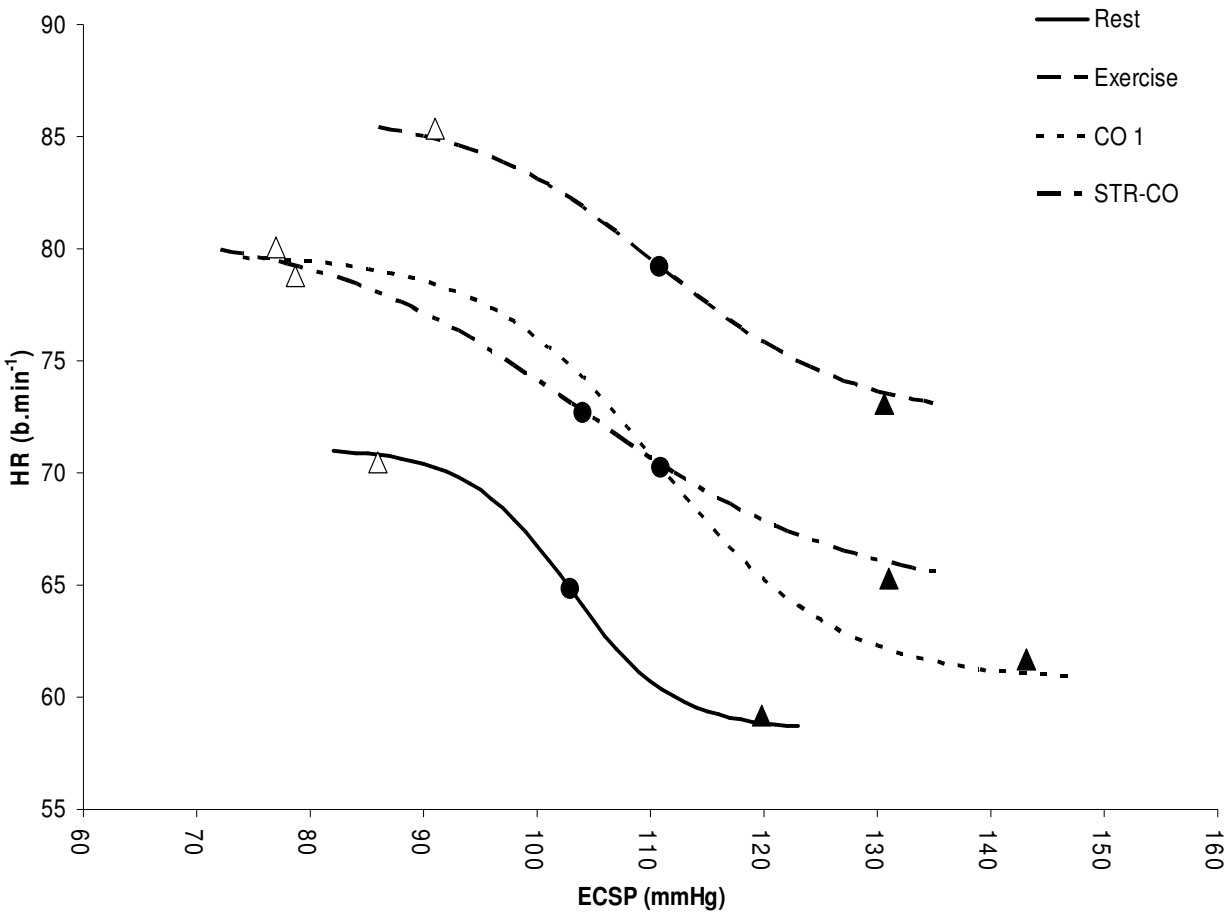

Figure 5.4: Carotid-cardiac baroreflex function curves during rest, exercise, CO 1 and STR-CO phases in the IER trial. Open triangle, threshold; closed triangle, saturation; closed circle, centring point; ECSP, estimated carotid sinus pressure.

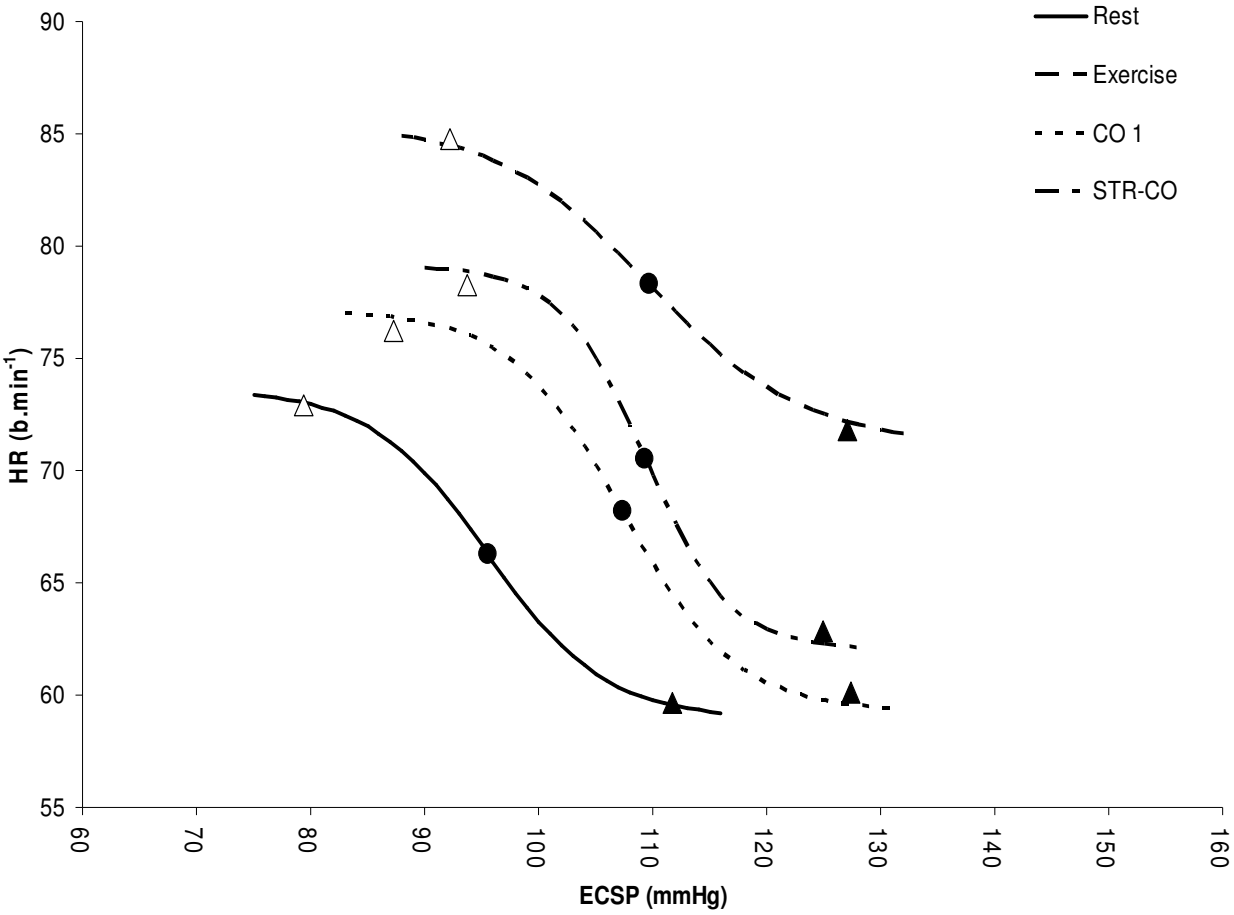

Figure 5.5: Carotid-cardiac baroreflex function curves during rest, exercise, CO 1 and STR-CO phases in the IEL trial. Open triangle, threshold; closed triangle, saturation; closed circle, centring point; ECSP, estimated carotid sinus pressure.

Table 5.1: Logistic model parameters describing carotid baroreflex control of HR during the rest, exercise, CO 1 and STR-CO phases of the ICL, IEL, ICR and IER trials. * = significantly different from rest. † = significantly different from exercise. ‡ = significantly different from CO 1. # = significantly different from ICL. $ = significantly different from IEL. & = significantly different from ICR.

		ICL	IEL	ICR	IER
A1 (b.min^{-1})	**Rest**	15 ± 3	15 ± 2	14 ± 2	13 ± 1
	Exercise	15 ± 3	14 ± 3	12 ± 2	14 ± 2
	CO 1	16 ± 2	18 ± 2	18 ± 2 †	19 ± 2 *
	STR-CO	14 ± 2	17 ± 2	18 ± 1 *†	17 ± 1 *
A2	**Rest**	0.20 ± 0.05	0.19 ± 0.05	0.24 ± 0.07	0.22 ± 0.05
	Exercise	0.21 ± 0.06	0.15 ± 0.03	0.16 ± 0.04	0.12 ± 0.02
	CO 1	0.17 ± 0.04	0.20 ± 0.05	0.16 ± 0.06	0.13 ± 0.04
	STR-CO	0.22 ± 0.06	0.27 ± 0.04	0.16 ± 0.04	0.08 ± 0.01 #$
A3 (mmHg)	**Rest**	92 ± 4	96 ± 3	95 ± 6	103 ± 4
	Exercise	92 ± 4	110 ± 2 *#&	96 ± 5	111 ± 4 #
	CO 1	94 ± 5	107 ± 7 *	95 ± 5	111 ± 4
	STR-CO	102 ± 5	109 ± 6 *	102 ± 5	104 ± 5
A4 (b.min^{-1})	**Rest**	62 ± 4	59 ± 2	57 ± 3	59 ± 3
	Exercise	58 ± 3 *	71 ± 4 *	57 ± 3	72 ± 3 *
	CO 1	61 ± 3	59 ± 4 †	57 ± 4	61 ± 3 †
	STR-CO	64 ± 4 †	62 ± 4 †‡	60 ± 3	65 ± 3 *†

Table 5.2: Derived variables describing carotid baroreflex control of HR during the rest, exercise, CO 1 and STR-CO phases of the ICL, IEL, ICR and IER trials. * = significantly different from rest. † = significantly different from exercise. ‡ = significantly different from CO 1. # = significantly different from ICL. $ = significantly different from IEL. & = significantly different from ICR.

		ICL	IEL	ICR	IER
Threshold (mmHg)	**Rest**	77 ± 6	79 ± 3	80 ± 7	86 ± 6
	Exercise	73 ± 7	92 ± 5 *#	78 ± 8	91 ± 3 #
	CO 1	75 ± 8	87 ± 7	74 ± 6	79 ± 7
	STR-CO	88 ± 6	94 ± 7 *‡	80 ± 7	77 ± 8
Saturation (mmHg)	**Rest**	108 ± 6	112 ± 6	111 ± 8	120 ± 6
	Exercise	112 ± 8	127 ± 4 *	113 ± 4	131 ± 6
	CO 1	113 ± 5	127 ± 11 *	116 ± 6	143 ± 14
	STR-CO	116 ± 5	125 ± 8 *	123 ± 8	131 ± 5
OP ($b.min^{-1}$)	**Rest**	70 ± 4	67 ± 3	64 ± 3	66 ± 3
	Exercise	69 ± 5	80 ± 5 &	66 ± 4	81 ± 5 *&
	CO 1	69 ± 5	69 ± 4	65 ± 3	69 ± 4 †
	STR-CO	70 ± 5	71 ± 4	69 ± 4	76 ± 3 *‡$&
OP gain ($b.min^{-1}.mmHg^{-1}$)	**Rest**	–0.32 ± 0.09	–0.30 ± 0.07	–0.40 ± 0.12	–0.19 ± 0.08
	Exercise	–0.23 ± 0.06	–0.29 ± 0.08	–0.13 ± 0.03	–0.29 ± 0.14
	CO 1	–0.18 ± 0.07	–0.13 ± 0.03	–0.33 ± 0.09	–0.30 ± 0.18
	STR-CO	–0.14 ± 0.06	–0.28 ± 0.11	–0.19 ± 0.06	–0.27 ± 0.07
Maximal gain ($b.min^{-1}.mmHg^{-1}$)	**Rest**	–0.68 ± 0.19	–0.63 ± 0.13	–0.68 ± 0.13	–0.61 ± 0.12
	Exercise	–0.66 ± 0.18	–0.44 ± 0.03	–0.52 ± 0.17	–0.37 ± 0.04
	CO 1	–0.66 ± 0.14	–0.83 ± 0.23	–0.62 ± 0.18	–0.59 ± 0.10
	STR-CO	–0.76 ± 0.20	–0.94 ± 0.14	–0.66 ± 0.18	–0.34 ± 0.04 $

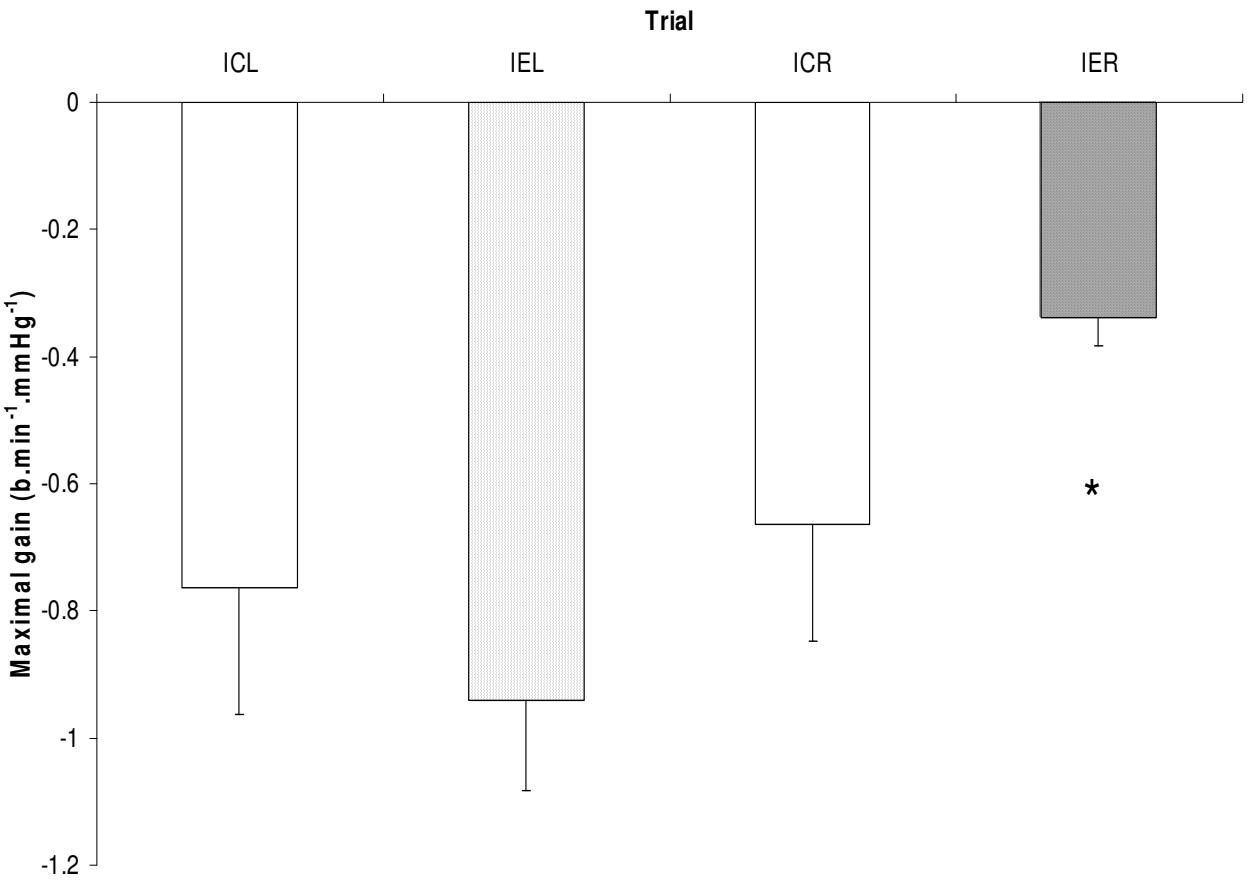

Figure 5.6: Maximal gain of carotid-cardiac baroreflex function curves during STR-CO in the ICL, IEL, ICR and IER trials. Light shading represents concurrent metaboreflex activation in the contralateral limb to the limb with mechanoreflex stimulation. Dark shading represents concurrent metaboreflex activation in the limb with mechanoreflex stimulation. * = significantly different from IEL.

CBR-MAP stimulus-response function curves during rest, exercise, CO 1 and STR-CO phases in the IER and IEL trials are shown in Figure 5.7 and 5.8, respectively. Logistic model parameters and derived variables describing CBR control of MAP during each phase of the experiment in all trials are shown in Table 5.3 and 5.4, respectively.

In both IER and IEL, exercise caused an upward and rightward resetting of the function curve from rest. This was indicated by significant increases in minimum response (A4) and ECSP at the centring point (A3), respectively. The function curves returned towards rest in CO 1 but some of the resetting was maintained, as indicated by significant alterations in minimum response (A4) and ECSP at the centring point (A3). Maximal gain at the centring point of the function curve was similar in all trials during CO 1. In STR-CO, there were no differences in maximal gain between all trials (Figure 5.9), and these values were similar to CO 1 values. During STR-CO, values for ECSP at the centring point (A3) were not significantly different from exercise values. This indicated a trend towards further rightward resetting from CO 1 during STR-CO in both IER and IEL.

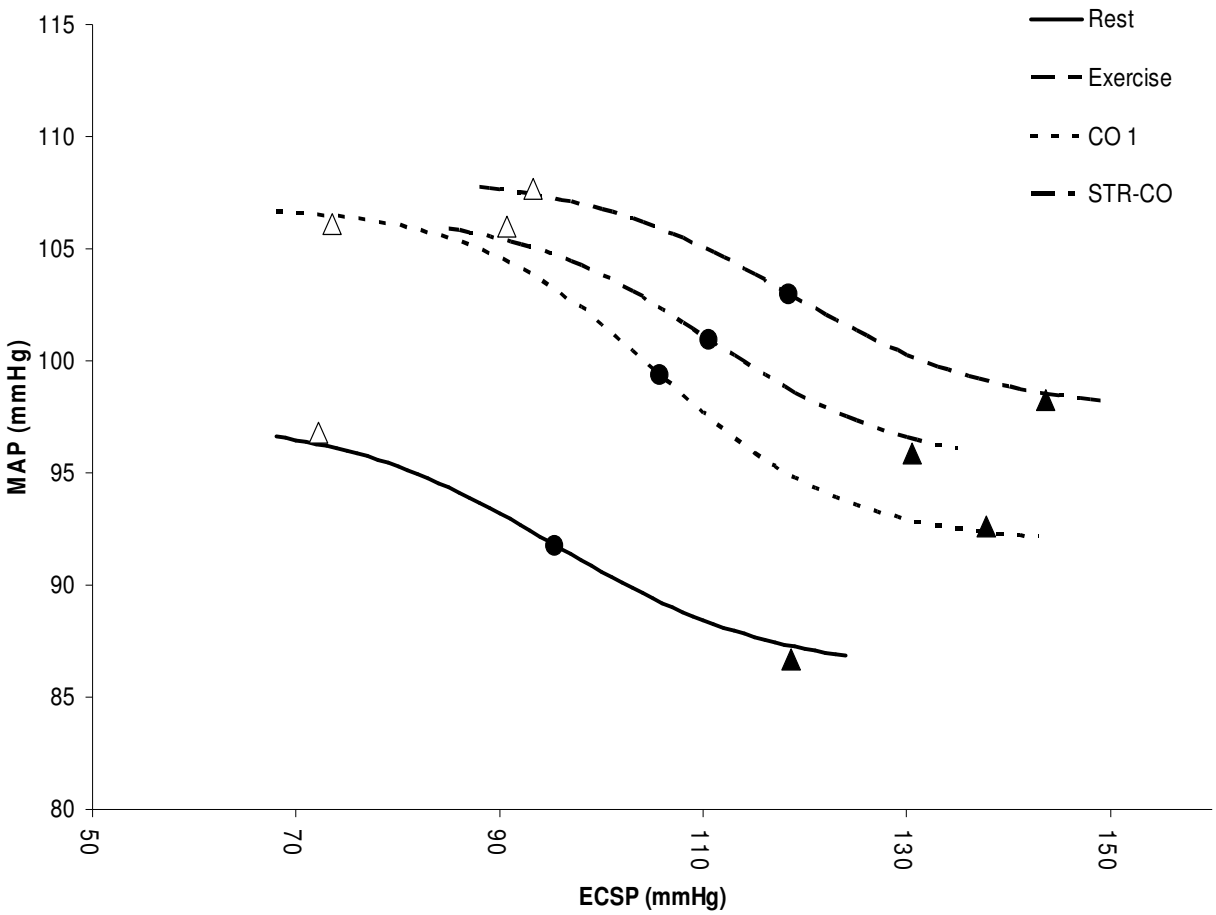

Figure 5.7: Carotid-vasomotor baroreflex function curves during rest, exercise, CO 1 and STR-CO phases in the IER trial. Open triangle, threshold; closed triangle, saturation; closed circle, centring point; ECSP, estimated carotid sinus pressure.

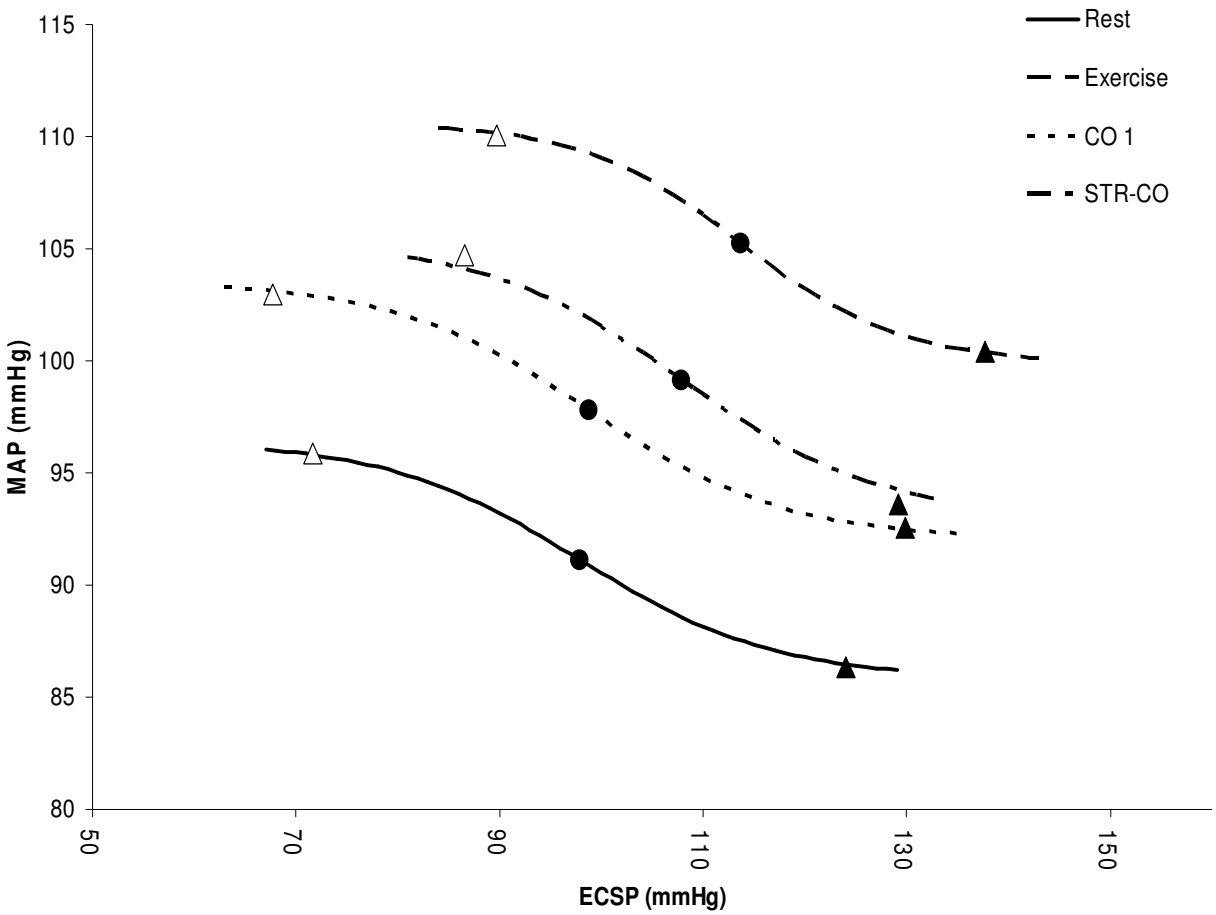

Figure 5.8: Carotid-vasomotor baroreflex function curves during rest, exercise, CO 1 and STR-CO phases in the IEL trial. Open triangle, threshold; closed triangle, saturation; closed circle, centring point; ECSP, estimated carotid sinus pressure.

Table 5.3: Logistic model parameters describing carotid baroreflex control of MAP during the rest, exercise, CO 1 and STR-CO phases of the ICL, IEL, ICR and IER trials. * = significantly different from rest. † = significantly different from exercise. ‡ = significantly different from CO 1. # = significantly different from ICL. & = significantly different from ICR.

		ICL	IEL	ICR	IER
A1 (mmHg)	**Rest**	12 ± 1	11 ± 1	12 ± 2	11 ± 1
	Exercise	11 ± 1	11 ± 1	11 ± 1	11 ± 1
	CO 1	14 ± 1	12 ± 2	12 ± 1	15 ± 2
	STR-CO	13 ± 1	12 ± 1	13 ± 1	11 ± 1
A2	**Rest**	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.09 ± 0.01
	Exercise	0.09 ± 0.02	0.13 ± 0.04	0.08 ± 0.01	0.10 ± 0.02
	CO 1	0.08 ± 0.01	0.10 ± 0.03	0.10 ± 0.03	0.11 ± 0.03
	STR-CO	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.01
A3 (mmHg)	**Rest**	94 ± 4	98 ± 7	97 ± 5	95 ± 3
	Exercise	91 ± 4	114 ± 7 *#&	91 ± 5	118 ± 3 *#&
	CO 1	92 ± 5	99 ± 6 †	93 ± 2	106 ± 5 *†
	STR-CO	102 ± 4 †	108 ± 6	104 ± 5 *†‡	111 ± 4 *
A4 (mmHg)	**Rest**	83 ± 4	86 ± 3	85 ± 2	86 ± 3
	Exercise	84 ± 4	100 ± 4 *	85 ± 2	98 ± 4 *
	CO 1	81 ± 4	92 ± 4 †	83 ± 2	92 ± 3 *
	STR-CO	88 ± 4	93 ± 4 †#&	86 ± 3	95 ± 4 *#&

Table 5.4: Derived variables describing carotid baroreflex control of MAP during the rest, exercise, CO 1 and STR-CO phases of the ICL, IEL, ICR and IER trials. * = significantly different from rest. † = significantly different from exercise. ‡ = significantly different from CO 1. # = significantly different from ICL. & = significantly different from ICR.

		ICL	IEL	ICR	IER
Threshold (mmHg)	**Rest**	75 ± 3	72 ± 8	72 ± 6	72 ± 4
	Exercise	67 ± 4 *	90 ± 10 &	65 ± 4	93 ± 6 #&
	CO 1	65 ± 5 *	68 ± 11	68 ± 4	74 ± 9
	STR-CO	80 ± 4 ‡	87 ± 5	77 ± 8	91 ± 5
Saturation (mmHg)	**Rest**	113 ± 5	124 ± 7	121 ± 7	119 ± 5
	Exercise	115 ± 6	138 ± 7 *#	117 ± 7	144 ± 6 *#&
	CO 1	119 ± 7	130 ± 9	118 ± 4	138 ± 9
	STR-CO	125 ± 5	129 ± 8	130 ± 4	131 ± 4
OP (mmHg)	**Rest**	87 ± 4	91 ± 4	90 ± 3	90 ± 4
	Exercise	87 ± 4	103 ± 4 *#&	89 ± 4	102 ± 4 *&
	CO 1	87 ± 4	96 ± 5 †	89 ± 4	98 ± 3 *
	STR-CO	90 ± 5	98 ± 4 *†	91 ± 3	99 ± 4 *
OP gain (mmHg. mmHg^{-1})	**Rest**	–0.31 ± 0.11	–0.19 ± 0.05	–0.21 ± 0.08	–0.16 ± 0.05
	Exercise	–0.21 ± 0.06	–0.12 ± 0.07	–0.20 ± 0.08	–0.05 ± 0.02 *#&
	CO 1	–0.25 ± 0.08	–0.10 ± 0.02	–0.15 ± 0.05	–0.15 ± 0.03
	STR-CO	–0.12 ± 0.04	–0.18 ± 0.05	–0.09 ± 0.03	–0.10 ± 0.03 †
Maximal gain (mmHg. mmHg^{-1})	**Rest**	–0.34 ± 0.04	–0.25 ± 0.03	–0.29 ± 0.04	–0.25 ± 0.02
	Exercise	–0.23 ± 0.02 *	–0.30 ± 0.07	–0.21 ± 0.02	–0.24 ± 0.03
	CO 1	–0.27 ± 0.02 *	–0.22 ± 0.02	–0.27 ± 0.04	–0.31 ± 0.04
	STR-CO	–0.30 ± 0.01 †	–0.30 ± 0.03	–0.29 ± 0.03	–0.29 ± 0.03

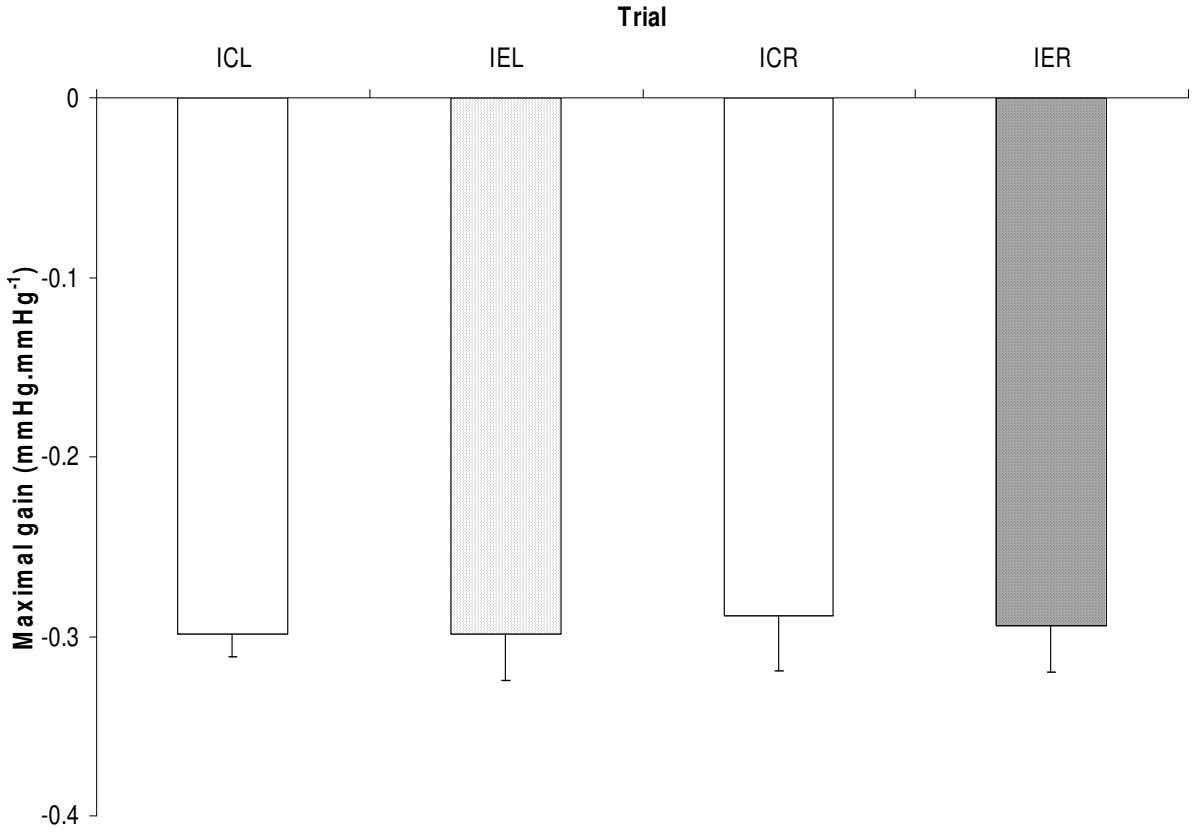

Figure 5.9: Maximal gain of carotid-vasomotor baroreflex function curves during STR-CO in the ICL, IEL, ICR and IER trials. Light shading represents concurrent metaboreflex activation in the contralateral limb to the limb with mechanoreflex stimulation. Dark shading represents concurrent metaboreflex activation in the limb with mechanoreflex stimulation.

5.3.4: Spontaneous baroreflex sensitivity

Data from 2 subjects did not include sequences in one or more of the 16 phases across all trials. Therefore, sequence analysis was performed on full data sets from 8 subjects. Regression lines calculated from sequence analysis during STR-CO in all trials are shown in Figure 5.10. There was no significant difference in SBRS during the rest phases of the four trials (Table 5.5). When exercise was performed in IER and IEL, there was a significant decrease in SBRS ($P<0.05$). During CO following exercise, SBRS returned to resting levels. When no exercise was performed in ICR and ICL, SBRS was unchanged from rest during the 'exercise' and CO 1 phases. SBRS was significantly lower in STR-CO than CO 1 ($P<0.05$), with passive stretch decreasing SBRS from CO 1 levels by 1.4 ± 2.1, 6.4 ± 3.8, 6.1 ± 3.3 and 4.8 ± 1.6 $ms.mmHg^{-1}$ in the ICL, IEL, ICR and IER trials, respectively. During STR-CO, SBRS was significantly lower in IER compared to IEL ($P<0.05$).

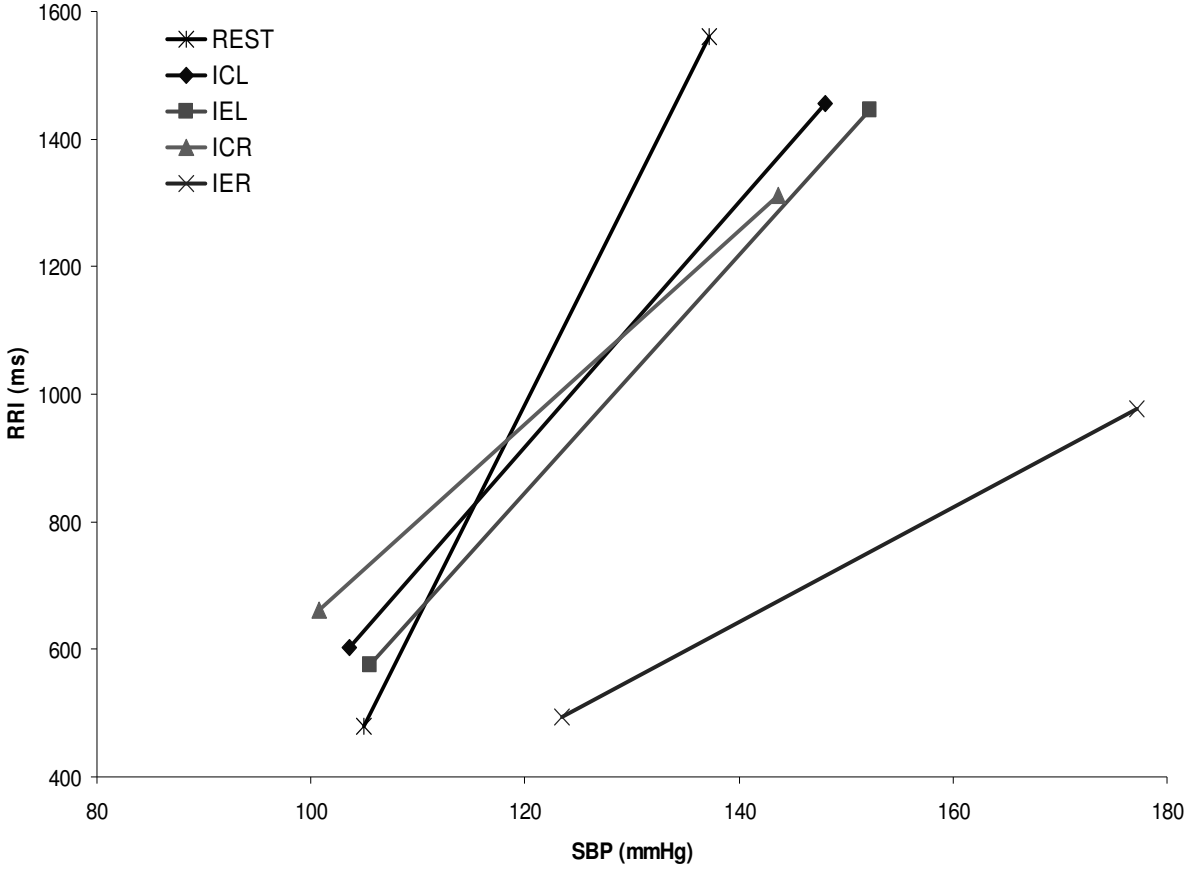

Figure 5.10: Regression lines calculated from sequence analysis during STR-CO in the ICL, IEL, ICR and IER trials. Rest line represents the overall mean regression line of the rest phases in the four trials.

Table 5.5: Slope values of regression lines calculated from sequence analysis during the rest, exercise, CO 1 and STR-CO phases in the ICL, IEL, ICR and IER trials. * = significantly different from rest. † = significantly different from CO 1. ‡ = significantly different from corresponding IC trial. # = significantly different from IEL STR-CO.

Trial	Rest slope ($ms.mmHg^{-1}$)	Exercise slope ($ms.mmHg^{-1}$)	CO 1 slope ($ms.mmHg^{-1}$)	STR-CO slope ($ms.mmHg^{-1}$)
ICL	17.5 ± 2.7	14.4 ± 1.3	15.2 ± 2.7	13.9 ± 1.1 *†
IEL	16.2 ± 2.6	6.1 ± 1.0 *‡	19.5 ± 4.4	13.1 ± 1.8 *†
ICR	21.8 ± 2.7	19.3 ± 3.9	20.1 ± 5.5	14.4 ± 3.0 *†
IER	16.1 ± 1.7	5.7 ± 1.1 *‡	14.4 ± 1.9	9.6 ± 0.9 *†#

There was no significant difference in the mean intercept of the regression lines during the rest phases of all trials (Table 5.6). When exercise was performed in IER and IEL, there was a significant increase in intercept compared to the IC trials ($P<0.05$). During CO following exercise, intercept returned to resting levels. When no exercise was performed in ICR and ICL, intercept was unchanged from rest during the 'exercise' and CO 1 phases. Intercept was significantly higher in STR-CO than CO 1 ($P<0.05$).

Table 5.6: Intercept values of regression lines calculated from sequence analysis during the rest, exercise, CO 1 and STR-CO phases in the ICL, IEL, ICR and IER trials. * = significantly different from CO 1. † = significantly different from corresponding IC trial.

Trial	Rest intercept (ms)	Exercise intercept (ms)	CO 1 intercept (ms)	STR-CO intercept (ms)
ICL	-1194 ± 268	-858 ± 122	-991 ± 284	-761 ± 119 *
IEL	-996 ± 248	-15 ± 96 †	-1581 ± 424	-800 ± 128 *
ICR	-1681 ± 258	-1360 ± 332	-1397 ± 445	-819 ± 212 *
IER	-930 ± 182	-311 ± 243 †	-931 ± 196	-580 ± 69 *

5.4: Discussion

The major finding in this study was that during local circulatory occlusion following isometric exercise, passive stretch of the previously active calf muscles markedly decreased the maximal gain of the carotid-cardiac baroreflex function curve. When the same stretch stimulus was applied to a calf muscle which had not previously exercised, and therefore did not have metabolites trapped within it, there was no change in the maximal gain of the carotid-cardiac baroreflex function curve. Since blood pressure elevation and hence the level of baroreceptor stimulation was the same in each trial, and the mechanical stimulus applied to the muscle was also standardised, this implies that metabolites produced during the prior exercise caused sensitisation of muscle mechanoreceptive afferent fibres. This is supported by the finding that spontaneous baroreflex sensitivity was at its lowest when passive stretch was applied to a previously active muscle with local metabolite accumulation. This metabolite

sensitisation of muscle mechanoreceptors modulates baroreflex control of heart rate, but not blood pressure.

In Chapter 4, it was shown that during the same standardised passive calf muscle stretch that was used in the present study, SBRS was significantly decreased both at rest, and when SBP was elevated by metabolite accumulation during CO. This technique reflects only the sensitivity at the OP of the CBR-HR function curve (Ogoh *et al.*, 2005). The present study extends this observation by showing that the maximal gain at the centring point, and therefore responsiveness of the whole CBR-HR function curve, is reduced during muscle mechanoreflex stimulation in the previously exercised limb with concurrent metaboreflex activation (IER trial). In IEL, muscle mechanoreceptor stimulation in the previously inactive limb had no effect on maximal CBR-HR gain. Since the CBR-HR function curve largely reflects vagal modulation of HR (Ogoh *et al.*, 2005), these findings further our understanding of the central integration of inputs from muscle mechanoreceptive and baroreceptive afferents, and also their combined influence on vagal outflow.

During CO in the IER and IEL trials, BP was elevated to the same extent. This indicates that the exercise performed by the right or left calf muscles resulted in equal levels of muscle metaboreflex activation, and presumably baroreceptor stimulation and input to the NTS. The differences in the CBR-HR response during stretch of the previously active or inactive calf cannot then be attributed to differences in baroreceptor input during CO prior to stretch. During stretch in IER and IEL, there were equal further increases in BP, which would have maintained similar levels of baroreceptor stimulation in both trials. In the IEL trial, this resulted in a resetting of the CBR-HR function curve without a change in maximal gain. However, in the IER trial, stretch caused a marked reduction in the maximal gain of the CBR-HR function curve (Figure 5.6), without further resetting of the curve from the CO position. Therefore, it seems that stretch applied to a previously active muscle with local metabolite accumulation, but not stretch of a previously inactive muscle, decreases maximal gain of the CBR-HR function curve. This suggests that metabolites sensitise muscle mechanoreceptive afferents, and increase their influence on cardiac vagal outflow via increased afferent input to the NTS (McMahon *et al.*, 1992; Potts, 2002; Degtyarenko and Kaufman, 2006) or possibly the nucleus ambiguus (see Gladwell *et al.*, (2005) for discussion). This results in the carotid-cardiac baroreflex being less

responsive to changes in BP. Muscle mechanoreceptor activation is known to inhibit cardiac vagal outflow in humans (Gladwell *et al.*, 2005), whilst metabolite sensitisation of stretch-sensitive muscle mechanoreceptors has been demonstrated in animal preparations (Kaufman *et al.*, 1984b; Li and Sinoway, 2002; Smith *et al.*, 2003b) and suggested in human studies (Middlekauff *et al.*, 2004; Middlekauff and Chiu, 2004).

At the time of this decreased maximal gain in the CBR-HR function curve in IER, the CBR-MAP function curve was reset further rightward from CO 1, with no change in maximal gain. Maximal gain of the CBR-MAP function curve was similar in all trials during stretch (Figure 5.9). With passive stretch of the previously inactive muscle during concurrent muscle metaboreflex activation in the contralateral limb (IEL), the CBR-HR and CBR-MAP function curves were reset slightly from CO 1 (upward and rightward, respectively), with no changes in maximal gain. This would be consistent with mechanoreflex-induced increases in cardiac vagal inhibition as explained above. This would also be consistent with some increase in MSNA (Cui *et al.*, 2006), despite the elevated SBP and baroreceptor activation due to CO.

Previous studies have examined the relative contribution of muscle mechanoreceptive and metaboreceptive afferents towards baroreflex resetting in exercise. There have been attempts to either over-activate muscle mechanoreceptors (Williamson *et al.*, 1994; Gallagher *et al.*, 2001b; Gallagher *et al.*, 2006) or partially block afferent feedback (Smith *et al.*, 2003a), in order to assess their role during exercise in humans. However, external compression would have potentially activated both mechanoreceptors and metaboreceptors, due to the mechanical stimulation and metabolite trapping by CO (Williamson *et al.*, 1994). Epidural anaesthesia would have certainly blocked both muscle afferent types (Smith *et al.*, 2003a). Hence, these methods have limited ability to differentiate the roles of muscle mechanoreceptors and metaboreceptors. Therefore, the role of muscle mechanoreflex stimulation in modulating baroreflex control of HR and BP remains unclear. The advantage of using passive muscle stretch in this study is that it should selectively stimulate muscle mechanoreceptive afferents, and so allow investigation of the input of the muscle mechanoreflex. Although it has been shown in cats that there are populations of muscle afferents that respond differentially to passive tendon stretch and muscle contraction (Hayes *et al.*, 2005), there are some afferents that respond to

both modes of stimulation (Kaufman and Rybicki, 1987). Hence, passive muscle stretch can be used as a simple, non-invasive tool with which to examine the role of muscle mechanoreflex stimulation in the modulation of baroreflex control of HR and BP in humans.

Isometric exercise caused the greatest upward and rightward resetting of both CBR-HR and CBR-MAP function curves (Figure 5.4, 5.5, 5.7 and 5.8). This has been attributed to contributions from the muscle mechanoreflex, metaboreflex and central command (Raven *et al.*, 2006). It was not the main purpose of this study to repeat earlier work which examined baroreflex function during exercise. The exercise was used to induce metabolite production, which could then be trapped in the exercised muscle by CO. Nevertheless, in agreement with other work (Smith *et al.*, 2003a), the OP of the CBR-MAP curve moved slightly away from the centring point towards threshold during isometric exercise. However, in contrast to Ogoh *et al.* (2005), there was no movement of the CBR-HR OP towards threshold. By comparing Figure 5.4, 5.5, 5.7 and 5.8, it can be seen that following exercise, both CBR-HR and CBR-MAP curves move back towards rest during CO 1, but the CBR-MAP curve maintains more of the resetting than the CBR-HR curve. Muscle metaboreflex activation is known to modulate baroreflex control of BP more than HR, via sympathetically-mediated increases in MSNA and consequent elevations in SBP (Papelier *et al.*, 1997; Cui *et al.*, 2001; Ichinose *et al.*, 2002; Ichinose *et al.*, 2004). Collectively, these curves further illustrate the extent to which the muscle metaboreflex and mechanoreflex could contribute towards baroreflex resetting during isometric exercise, with the remaining input coming from central command. Central command has a greater influence on resetting of the CBR-HR rather than CBR-MAP function curve (Figure 5.4, 5.5, 5.7 and 5.8), which has been demonstrated previously (Querry *et al.*, 2001; Ogoh *et al.*, 2002; Gallagher *et al.*, 2006).

The decrease in SBRS caused by passive calf stretch in all trials confirms the findings of Chapter 4, where passive stretch decreased SBRS at rest, as well as during concurrent local metabolite accumulation of increasing levels. SBRS was lowest when muscle metaboreflex activation occurred in the same muscle as the mechanical stimulation. This implies that metabolites sensitised muscle mechanoreceptors, which increased their afferent feedback and caused greater cardiac vagal inhibition. As discussed in Chapter 3, if only information from sequence analysis were available, it cannot be determined whether the decrease in SBRS is due to a relocation of the OP to a position of lower gain on

the CBR-HR curve, or a decrease in the maximal gain of the whole CBR-HR function curve. In this study, both sequence analysis and full CBR function curve analysis were performed, in order to compare these measures of BRS. It was found that the gain at the OP of the CBR-HR function curve was similar during stretch in all trials. At first glance, this may seem in contrast to the finding of reduced SBRS during stretch, both in this study and Chapter 4, as SBRS measured using the sequence technique reflects the sensitivity at the OP of the CBR-HR function curve (Ogoh *et al.*, 2005). The explanation for this apparent inconsistency may lie in the use of directly measured RRIs and SBP to plot simple linear regression lines for the sequence technique, compared to the use of HR to construct CBR-HR function curves. While both approaches provide robust measures of cardiac vagal tone, the sequence technique appears to be a more sensitive index of vagal activity when smaller HR changes occur at lower HRs.

This study has shown that during passive stretch of a previously exercised muscle with local metabolite accumulation, SBRS was at its lowest, and the maximal gain of the CBR-HR function curve was reduced. Therefore, SBRS calculated from sequence analysis revealed the same decrease in BRS, due to muscle mechanoreflex-induced inhibition of cardiac vagal activity, as calculating the whole CBR-HR function curve. Hence, it could be suggested that SBRS, as an index of vagal tone, could be a useful measure of muscle mechanoreflex sensitivity in humans. It is non-invasive and relies on natural baroreflex fluctuations in HR and BP during muscle mechanoreflex stimulation, with no need for external interventions to measure baroreflex function. The measurement of modulations in cardiac parasympathetic activity during muscle mechanoreflex stimulation seems more reliable than efforts focusing on changes in sympathetic activity (Middlekauff *et al.*, 2004; Cui *et al.*, 2006). This could prove valuable in assessing muscle mechanoreflex sensitivity when abnormal muscle afferent feedback occurs, such as in patients with chronic heart failure (Sinoway and Li, 2005).

In summary, it has been shown that during local circulatory occlusion following isometric exercise, passive stretch of the previously active calf muscles decreases the maximal gain of the carotid-cardiac baroreflex function curve. This requires the presence of metabolites within the muscle being mechanically stimulated, as opposed to in the contralateral limb. Additionally, spontaneous baroreflex sensitivity was at its lowest when passive stretch was applied to a previously active muscle

with local metabolite accumulation. These decrements occur irrespective of the muscle metaboreflex-driven increase in blood pressure that intramuscular metabolite accumulation causes. This implies metabolite sensitisation of stretch-sensitive muscle mechanoreceptive afferents, leading to augmented inhibitory feedback to the central integrative sites in the brainstem. This modulates baroreflex control of heart rate via the Vagus nerve, but not blood pressure.

CHAPTER 6: GENERAL CONCLUSIONS

Cardiovascular control during exercise in humans is known to be regulated by central command, muscle mechanoreflex stimulation and muscle metaboreflex activation. The muscle mechanoreflex can be stimulated by passive muscle stretch, which activates muscle mechanoreceptors that are mostly group III muscle afferent nerve fibres. Stimulation of group III afferents causes increases in heart rate and blood pressure. The baroreflex is the neural mechanism for controlling blood pressure, and continues to operate during exercise. Its function is reset to operate around the prevailing high blood pressure and heart rate present during physical work. However, the influence of muscle mechanoreflex stimulation induced by passive muscle stretch on the human baroreflex is unknown.

The first study in this thesis investigated how differing levels of central command, muscle mechanoreflex stimulation and muscle metaboreflex activation caused by progressive increases in isometric exercise intensity affect spontaneous baroreflex sensitivity. It was demonstrated that spontaneous baroreflex sensitivity decreases progressively during exercise of increasing intensity. This occurs with a concomitant rightward resetting of the baroreflex, which is reset further rightward as exercise intensity increases. This is the first time that spontaneous baroreflex sensitivity has been assessed during higher intensities of isometric exercise, and this finding is consistent with evidence from numerous studies illustrating decreased spontaneous baroreflex sensitivity during low intensity isometric exercise, and progressive decreases during increasing intensities of dynamic exercise. The decrease in spontaneous baroreflex sensitivity would allow the appropriate blood pressure and heart rate increases to occur in an attempt to provide the working muscles with a sufficient blood supply. However, a greater supply of blood cannot be delivered to the active muscles during isometric exercise, due to the high intramuscular pressure during contraction. Nonetheless, the vasoconstriction-induced pressor response is designed to protect the body from sudden decreases in blood pressure, due to large levels of vasodilation during exercise. The progressive rightward resetting of the baroreflex would allow the baroreflex to continue operating at the elevated blood pressure present during increasing intensities of exercise.

In the second study of this thesis, the effects on spontaneous baroreflex sensitivity of graded levels of muscle metaboreflex activation following isometric exercise of increasing intensity were examined. It was shown that spontaneous baroreflex sensitivity returned to resting levels during graded

levels of muscle metaboreflex activation following isometric exercise of increasing intensity. The baroreflex appeared to be progressively reset rightward as the intensity of the previous exercise increased. At the cessation of exercise, the removal of the inhibitory inputs of central command and muscle mechanoreflex stimulation would result in a rapid restoration of parasympathetic activity. The elevated blood pressure and therefore sympathetic activation caused by muscle metaboreflex activation would also increase parasympathetic outflow. Overall, this augmented level of parasympathetic activity would overpower the sympathetic activation due to the elevated blood pressure. This would return spontaneous baroreflex sensitivity and heart rate to resting levels. The progressive rightward resetting would allow the baroreflex to continue to operate at the elevated blood pressures present during graded levels of muscle metaboreflex activation.

The main objective of the second study was to investigate the influence of muscle mechanoreflex stimulation via passive calf muscle stretch on spontaneous baroreflex sensitivity. Passive calf muscle stretch was applied at rest, and during concurrent graded levels of muscle metaboreflex activation following isometric exercise of increasing intensity. Spontaneous baroreflex sensitivity was decreased by passive stretch at rest, and during graded levels of local metabolite accumulation following increasing intensities of isometric exercise. Stimulation of stretch-sensitive muscle mechanoreceptors would inhibit cardiac vagal tone, as illustrated by the decrease in the root mean square of successive differences, an index of vagal tone, at the onset of passive stretch. This would cause the decrease in spontaneous baroreflex sensitivity, as well as an increase in heart rate. During local metabolite accumulation, the elevated blood pressure and sympathetic activation would increase parasympathetic activity (see Figure 1.2 in Chapter 1). When passive stretch was applied at this time in the absence of central command, the inhibition of cardiac vagal tone was maintained, as spontaneous baroreflex sensitivity was decreased further. This occurred even with increased levels of metabolite accumulation. This implies that these metabolites sensitised stretch-sensitive muscle mechanoreceptors during stretch, and augmented their feedback. This is illustrated by the progressive reduction in the common coefficient of variance, an index of vagal tone, as previous exercise intensity and level of metabolites increased. Therefore, the high levels of cardiac vagal inhibition would overpower the increased parasympathetic activity due to the elevated blood pressure. This modulation of spontaneous baroreflex sensitivity by passive stretch-induced muscle mechanoreflex stimulation

would allow heart rate to rise in an attempt to increase the blood supply to the muscle, even though the blood supply cannot reach the active muscle during isometric exercise. Metabolite sensitisation of stretch-sensitive muscle mechanoreceptive afferents would augment the cardiac vagal inhibition during passive stretch, to enhance the modulation of spontaneous baroreflex sensitivity while these metabolites were still accumulated.

A limitation of this finding is that spontaneous baroreflex sensitivity only represents the sensitivity at the operating point of the carotid-cardiac baroreflex function curve. It cannot distinguish between movements of the operating point and changes in maximal gain of the curve for carotid baroreflex control of heart rate. This limitation is addressed in the third study of this thesis, where full stimulus-response carotid baroreflex function curve analysis is performed.

Another limitation with this study is that it cannot be confirmed whether spontaneous baroreflex sensitivity decreased due to metabolite sensitisation of stretch-sensitive muscle mechanoreceptive afferents during passive stretch, or an independent effect of passive stretch. It is likely that metabolite sensitisation occurred, as this is known to occur in animals, and has been suggested in humans. An attempt to clarify this point is made in the third study of this thesis. Future studies could inhibit the production of metabolites, for example administering indomethacin would prevent the production of cyclooxygenase products such as prostaglandins. It could then be observed if the cardiovascular and baroreflex responses to passive muscle stretch are attenuated during graded levels of local metabolite accumulation. This would indicate that metabolite sensitisation had occurred.

The third study in this thesis examined the influence of muscle mechanoreflex stimulation via passive calf muscle stretch on carotid baroreflex function. It was shown that passive calf muscle stretch reset the carotid-cardiac and carotid-vasomotor baroreflex to higher blood pressures and heart rates. This occurred with no changes in maximal gain, which is the overall sensitivity of the reflex. This simple resetting during passive stretch would allow the carotid baroreflex to continue operating at the elevated blood pressures and heart rates present during stretch.

The third study also attempted to clarify if metabolite sensitisation of stretch-sensitive muscle mechanoreceptive afferents occurs. It was demonstrated that muscle mechanoreflex stimulation by passive calf muscle stretch during concurrent local metabolite accumulation decreased the maximal gain of the function curve for carotid baroreflex control of heart rate, but not blood pressure. This decrease in carotid-cardiac maximal gain was found when metabolites were accumulated in the muscle being passively stretched, but not when trapped in the contralateral muscle or when there was no metabolite accumulation. This suggests that the presence of metabolites was necessary to sensitise stretch-sensitive muscle mechanoreceptive afferents during stretch, and augment their inhibitory feedback to central integration sites. This is indicative of metabolite sensitisation of stretch-sensitive muscle mechanoreceptors. The increased cardiac vagal inhibition would have caused the decrease in maximal gain of carotid-cardiac control, a reduction of overall sensitivity of this reflex control of heart rate. The maximal gain of cardiac-vasomotor control was unaffected in all conditions, indicating that passive stretch does not affect the overall sensitivity of this reflex control of blood pressure, in the presence or absence of metabolites. Therefore, metabolite sensitisation of stretch-sensitive muscle mechanoreceptive afferents would augment the cardiac vagal inhibition during passive stretch, to enhance the modulation of carotid baroreflex control of heart rate, but not blood pressure, while these metabolites were still accumulated.

Overall, muscle mechanoreflex stimulation via passive stretch of human calf muscle influences baroreflex function. Passive muscle stretch reduces the sensitivity of the baroreflex, likely via cardiac vagal inhibition. This occurs at rest, and during local metabolite accumulation. When metabolites are accumulated within a muscle, they appear to sensitise stretch-sensitive muscle mechanoreceptive afferents during passive stretch. This would augment the cardiac vagal inhibition caused by stretch, which modulates baroreflex control of heart rate more than blood pressure. As an index of cardiac vagal activity, spontaneous baroreflex sensitivity could be a useful measure of muscle mechanoreflex sensitivity in humans. This could prove valuable in assessing muscle mechanoreflex sensitivity when abnormal muscle afferent feedback occurs, such as in patients with chronic heart failure. Future studies could investigate the effects of muscle mechanoreflex stimulation via passive muscle stretch, at rest and during local metabolite accumulation, on baroreflex function in patients with chronic heart failure. These patients may have augmented muscle mechanoreflex sensitivity, and so could exhibit greater

responses to muscle mechanoreflex stimulation than controls. This could result in larger decrements in baroreflex function.

Additionally, muscle sympathetic nerve activity could be measured during passive calf muscle stretch, at rest and during local metabolite accumulation, in healthy controls and patients with chronic heart failure. It would be important to standardise the mechanical stimulus, as this does not appear to have been achieved in other studies so far. Applying passive stretch with a machine, such as the Biodex used in the studies presented here, would ensure a controlled and repeatable mechanical stimulus was applied every time. It is likely that passive calf muscle stretch would increase muscle sympathetic nerve activity, and this response would be augmented with greater levels of concurrent local metabolite accumulation. The 'normal' response observed in healthy controls is likely to be augmented further in patients with chronic heart failure, as these patients have been shown to have heightened sympathetic activity.

The work in this thesis has enhanced current knowledge regarding the metabolic sensitisation of mechanically-sensitive muscle afferents and their role in human cardiovascular control. It was demonstrated that the local accumulation of metabolites within a muscle that is mechanically stimulated results in decreases in baroreflex sensitivity, via augmented inhibition of cardiac vagal tone. This might suggest how muscle mechanoreflex stimulation contributes to the heart rate increase seen during exercise, with greater tachycardia when more metabolites are accumulated locally. This knowledge is of importance because by first understanding 'normal' baroreflex responses to enhanced muscle mechanoreflex stimulation via metabolite sensitisation in healthy individuals, this could help us understand the augmented mechanoreflex-mediated responses that have been reported in patients with chronic heart failure. It is hoped that the assessment of changes in spontaneous baroreflex sensitivity in response to passive muscle stretch could be developed into a simple, non-invasive clinical tool for measuring muscle mechanoreflex sensitivity in humans.

To summarise, muscle mechanoreflex stimulation via passive stretch of the human calf muscle alters baroreflex function, in order to allow cardiovascular adjustments to occur in response to muscular movement. In the future, this may provide a method by which to measure the sensitivity of the muscle mechanoreflex.

REFERENCES

Adreani, C. M., Hill, J. M., & Kaufman, M. P. (1997). Responses of group III and IV muscle afferents to dynamic exercise. *Journal of Applied Physiology, 82,* 1811-1817.

Adreani, C. M. & Kaufman, M. P. (1998). Effect of arterial occlusion on responses of group III and IV afferents to dynamic exercise. *Journal of Applied Physiology, 84,* 1827-1833.

Al-Ani, M., Munir, S. M., White, M., Townend, J., & Coote, J. H. (1996). Changes in R-R variability before and after endurance training measured by power spectral analysis and by the effect of isometric muscle contraction. *European Journal of Applied Physiology and Occupational Physiology, 74,* 397-403.

Alam, M. & Smirk, F. H. (1937). Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *The Journal of Physiology Online, 89,* 372-383.

Bell, M. P. D. & White, M. J. (2005). Cardiovascular responses to external compression of human calf muscle vary during graded metaboreflex stimulation. *Experimental Physiology, 90,* 383-391.

Carrington, C. A. & White, M. J. (2001). Exercise-induced muscle chemoreflex modulation of spontaneous baroreflex sensitivity in man. *The Journal of Physiology Online, 536,* 957-962.

Carrington, C. A., Fisher, W. J., Davies, M. K., & White, M. J. (2001). Muscle afferent and central command contributions to the cardiovascular response to isometric exercise of postural muscle in patients with mild chronic heart failure. *Clinical Science, 100,* 643-651.

Carrington, C. A., Ubolsakka, C., & White, M. J. (2003). Interaction between muscle metaboreflex and mechanoreflex modulation of arterial baroreflex sensitivity in exercise. *Journal of Applied Physiology, 95,* 43-48.

Carrington, C. A., Fisher, J. P., Davies, M. K., & White, M. J. (2004). Muscle afferent inputs to cardiovascular control during isometric exercise vary with muscle group in patients with chronic heart failure. *Clinical Science, 107,* 197-204.

Chen, H. I. & Chang, K. C. (1991). Assessment of threshold and saturation pressure in the baroreflex function curve: a new mathematical analysis. *Japanese Journal of Physiology, 41,* 861-877.

Coote, J. H. & Perez-Gonzalez, J. F. (1970). The response of some sympathetic neurones to volleys in various afferent nerves. *The Journal of Physiology Online, 208,* 261-278.

Coote, J. H., Hilton, S. M., & Perez-Gonzalez, J. F. (1971). The reflex nature of the pressor response to muscular exercise. *The Journal of Physiology Online, 215,* 789-804.

Cui, J., Wilson, T. E., Shibasaki, M., Hodges, N. A., & Crandall, C. G. (2001). Baroreflex modulation of muscle sympathetic nerve activity during posthandgrip muscle ischemia in humans. *Journal of Applied Physiology, 91,* 1679-1686.

Cui, J., Blaha, C., Moradkhan, R., Gray, K. S., & Sinoway, L. I. (2006). Muscle sympathetic nerve activity responses to dynamic passive muscle stretch in humans. *The Journal of Physiology Online, 576,* 625-634.

Cui, J., Mascarenhas, V., Moradkhan, R., Blaha, C., & Sinoway, L. I. (2008). Effects of muscle metabolites on responses of muscle sympathetic nerve activity to mechanoreceptor(s) stimulation in healthy humans. *AJP - Regulatory, Integrative and Comparative Physiology, 294,* R458-R466.

Degtyarenko, A. M. & Kaufman, M. P. (2005). MLR-induced inhibition of barosensory cells in the NTS. *AJP - Heart and Circulatory Physiology, 289,* H2575-H2584.

Degtyarenko, A. M. & Kaufman, M. P. (2006). Barosensory cells in the nucleus tractus solitarius receive convergent input from group III muscle afferents and central command. *Neuroscience, 140,* 1041-1050.

Ebert, T. J. (1986). Baroreflex responsiveness is maintained during isometric exercise in humans. *Journal of Applied Physiology, 61,* 797-803.

Ebert, T. J. & Cowley, A. W., Jr. (1992). Baroreflex modulation of sympathetic outflow during physiological increases of vasopressin in humans. *AJP - Heart and Circulatory Physiology, 262,* H1372-H1378.

Eckberg, D. L., Cavanaugh, M. S., Mark, A. L., & Abboud, F. M. (1975). A simplified neck suction device for activation of carotid baroreceptors. *Journal of Laboratory and Clinical Medicine, 85,* 167-173.

Eckberg, D. L. & Wallin, B. G. (1987). Isometric exercise modifies autonomic baroreflex responses in humans. *Journal of Applied Physiology, 63,* 2325-2330.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40,* 762-769.

Fadel, P. J., Ogoh, S., Keller, D. M., & Raven, P. B. (2003). Recent insights into carotid baroreflex function in humans using the variable pressure neck chamber. *Experimental Physiology, 88,* 671-680.

Fisher, J. P. & White, M. J. (2004). Muscle afferent contributions to the cardiovascular response to isometric exercise. *Experimental Physiology, 89,* 639-646.

Fisher, J. P., Bell, M. P. D., & White, M. J. (2005). Cardiovascular responses to human calf muscle stretch during varying levels of muscle metaboreflex activation. *Experimental Physiology, 90,* 773-781.

Fisher, J. P., Young, C. N., & Fadel, P. J. (2008). Effect of muscle metaboreflex activation on carotid-cardiac baroreflex function in humans. *AJP - Heart and Circulatory Physiology, 294,* H2296-H2304.

Gallagher, K. M., Fadel, P. J., Stromstad, M., Ide, K., Smith, S. A., Querry, R. G., Raven, P. B., & Secher, N. H. (2001a). Effects of partial neuromuscular blockade on carotid baroreflex function during exercise in humans. *The Journal of Physiology Online, 533,* 861-870.

Gallagher, K. M., Fadel, P. J., Stromstad, M., Ide, K., Smith, S. A., Querry, R. G., Raven, P. B., & Secher, N. H. (2001b). Effects of exercise pressor reflex activation on carotid baroreflex function during exercise in humans. *The Journal of Physiology Online, 533,* 871-880.

Gallagher, K. M., Fadel, P. J., Smith, S. A., Stromstad, M., Ide, K., Secher, N. H., & Raven, P. B. (2006). The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in humans. *Experimental Physiology, 91,* 79-87.

Gao, Z., Henig, O., Kehoe, V., Sinoway, L. I., & Li, J. (2006). Vanilloid type 1 receptor and the acid-sensing ion channel mediate acid phosphate activation of muscle afferent nerves in rats. *Journal of Applied Physiology, 100,* 421-426.

Gladwell, V. F. & Coote, J. H. (2002). Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *The Journal of Physiology Online, 540,* 1095-1102.

Gladwell, V. F., Fletcher, J., Patel, N., Elvidge, L. J., Lloyd, D., Chowdhary, S., & Coote, J. H. (2005). The influence of small fibre muscle mechanoreceptors on the cardiac vagus in humans. *The Journal of Physiology Online, 567,* 713-721.

Goodwin, G. M., McCloskey, D. I., & Mitchell, J. H. (1972). Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. *The Journal of Physiology Online, 226,* 173-190.

Green, A. L., Wang, S., Purvis, S., Owen, S. L. F., Bain, P. G., Stein, J. F., Guz, A., Aziz, T. Z., & Paterson, D. J. (2007). Identifying cardiorespiratory neurocircuitry involved in central command during exercise in humans. *The Journal of Physiology Online, 578,* 605-612.

Hanna, R. L. & Kaufman, M. P. (2003). Role played by purinergic receptors on muscle afferents in evoking the exercise pressor reflex. *Journal of Applied Physiology, 94,* 1437-1445.

Hayes, S. G., Kindig, A. E., & Kaufman, M. P. (2005). Comparison between the effect of static contraction and tendon stretch on the discharge of group III and IV muscle afferents. *Journal of Applied Physiology, 99,* 1891-1896.

Hayes, S. G., Kindig, A. E., & Kaufman, M. P. (2006). Cyclooxygenase blockade attenuates responses of group III and IV muscle afferents to dynamic exercise in cats. *AJP - Heart and Circulatory Physiology, 290,* H2239-H2246.

Henneman, E., Somjen, G., & Carpenter, D. O. (1965). Functional significance of cell size in spinal motoneurons. *Journal of Neurophysiology, 28,* 560-580.

Ichinose, M., Saito, M., Wada, H., Kitano, A., Kondo, N., & Nishiyasu, T. (2002). Modulation of arterial baroreflex dynamic response during muscle metaboreflex activation in humans. *The Journal of Physiology Online, 544,* 939-948.

Ichinose, M., Saito, M., Wada, H., Kitano, A., Kondo, N., & Nishiyasu, T. (2004). Modulation of arterial baroreflex control of muscle sympathetic nerve activity by muscle metaboreflex in humans. *AJP - Heart and Circulatory Physiology, 286,* H701-H707.

Iellamo, F., Hughson, R. L., Castrucci, F., Legramante, J. M., Raimondi, G., Peruzzi, G., & Tallarida, G. (1994). Evaluation of spontaneous baroreflex modulation of sinus node during isometric exercise in healthy humans. *AJP - Heart and Circulatory Physiology, 267,* H994-1001.

Iellamo, F., Legramante, J. M., Raimondi, G., & Peruzzi, G. (1997). Baroreflex control of sinus node during dynamic exercise in humans: effects of central command and muscle reflexes. *AJP - Heart and Circulatory Physiology, 272,* H1157-H1164.

Iellamo, F., Massaro, M., Legramante, J. M., Raimondi, G., Peruzzi, G., & Galante, A. (1998). Spontaneous baroreflex modulation of heart rate during incremental exercise test in humans. *FASEB Journal, 12,* A692.

Iellamo, F., Pizzinelli, P., Massaro, M., Raimondi, G., Peruzzi, G., & Legramante, J. M. (1999a). Muscle metaboreflex contribution to sinus node regulation during static exercise: insights from spectral analysis of heart rate variability. *Circulation, 100,* 27-32.

Iellamo, F., Massaro, M., Raimondi, G., Peruzzi, G., & Legramante, J. M. (1999b). Role of muscular factors in cardiorespiratory responses to static exercise: contribution of reflex mechanisms. *Journal of Applied Physiology, 86,* 174-180.

Kaufman, M. P., Longhurst, J. C., Rybicki, K. J., Wallach, J. H., & Mitchell, J. H. (1983). Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *Journal of Applied Physiology, 55,* 105-112.

Kaufman, M. P., Rybicki, K. J., Waldrop, T. G., & Mitchell, J. H. (1984a). Effect on arterial pressure of rhythmically contracting the hindlimb muscles of cats. *Journal of Applied Physiology, 56,* 1265-1271.

Kaufman, M. P., Rybicki, K. J., Waldrop, T. G., & Ordway, G. A. (1984b). Effect of ischemia on responses of group III and IV afferents to contraction. *Journal of Applied Physiology, 57,* 644-650.
Kaufman, M. P. & Rybicki, K. J. (1987). Discharge properties of group III and IV muscle afferents: their responses to mechanical and metabolic stimuli. *Circulation Research, 61,* I60-I65.

Kent, B. B., Drane, J. W., Blumenstein, B., & Manning, J. W. (1972). A mathematical model to assess changes in the baroreceptor reflex. *Cardiology, 57,* 295-310.

Kindig, A. E., Hayes, S. G., Hanna, R. L., & Kaufman, M. P. (2006). P2 antagonist PPADS attenuates responses of thin fiber afferents to static contraction and tendon stretch. *AJP - Heart and Circulatory Physiology, 290,* H1214-H1219.

Kindig, A. E., Hayes, S. G., & Kaufman, M. P. (2007). Blockade of purinergic 2 receptors attenuates the mechanoreceptor component of the exercise pressor reflex. *AJP - Heart and Circulatory Physiology, 293,* H2995-H3000.

Krogh, A. & Lindhard, J. (1913). The regulation of respiration and circulation during the initial stages of muscular work. *The Journal of Physiology Online, 47,* 112-136.

Li, J. & Sinoway, L. I. (2002). ATP stimulates chemically sensitive and sensitizes mechanically sensitive afferents. *AJP - Heart and Circulatory Physiology, 283,* H2636-H2643.

Li, J., Maile, M. D., Sinoway, A. N., & Sinoway, L. I. (2004a). Muscle pressor reflex: potential role of vanilloid type 1 receptor and acid-sensing ion channel. *Journal of Applied Physiology, 97,* 1709-1714.

Li, J., Sinoway, A. N., Gao, Z., Maile, M. D., Pu, M., & Sinoway, L. I. (2004b). Muscle mechanoreflex and metaboreflex responses after myocardial infarction in rats. *Circulation, 110,* 3049-3054.

Lind, A. R., Taylor, S. H., Humphreys, P. W., Kennelly, B. M., & Donald, K. W. (1964). The circulatory effects of sustained voluntary muscle contraction. *Clinical Science, 27,* 229-244.

Mark, A. L., Victor, R. G., Nerhed, C., & Wallin, B. G. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circulation Research, 57,* 461-469.

McArdle, W. D., Katch, F. I., & Katch, V. L. (2001). *Exercise Physiology: energy, nutrition and human performance,* 5th edition. Lippincott, Williams and Wilkins, USA.

McClain, J., Hardy, J. C., & Sinoway, L. I. (1994). Forearm compression during exercise increases sympathetic nerve traffic. *Journal of Applied Physiology, 77,* 2612-2617.

McCloskey, D. I. & Mitchell, J. H. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *The Journal of Physiology Online, 224,* 173-186.

McMahon, S. E. & McWilliam, P. N. (1992). Changes in R-R interval at the start of muscle contraction in the decerebrate cat. *The Journal of Physiology Online, 447,* 549-562.

McMahon, S. E., McWilliam, P. N., Robertson, J., & Kaye, J. C. (1992). Inhibition of carotid sinus baroreceptor neurones in the nucleus tractus solitarius of an anaesthetized cat by electrical stimulation of hindlimb afferent fibres. *The Journal of Physiology Online, 452,* 224P.

McWilliam, P. N., Yang, T., & Chen, L. X. (1991). Changes in the baroreceptor reflex at the start of muscle contraction in the decerebrate cat. *The Journal of Physiology Online, 436,* 549-558.

McWilliam, P. N. & Yang, T. (1991). Inhibition of cardiac vagal component of baroreflex by group III and IV afferents. *AJP - Heart and Circulatory Physiology, 260,* H730-H734.

Melcher, A. & Donald, D. E. (1981). Maintained ability of carotid baroreflex to regulate arterial pressure during exercise. *AJP - Heart and Circulatory Physiology, 241,* H838-H849.

Mense, S. & Stahnke, M. (1983). Responses in muscle afferent fibres of slow conduction velocity to contractions and ischaemia in the cat. *The Journal of Physiology Online, 342,* 383-397.

Mense, S. & Meyer, H. (1988). Bradykinin-induced modulation of the response behaviour of different types of feline group III and IV muscle receptors. *The Journal of Physiology Online, 398,* 49-63.

Middlekauff, H. R., Chiu, J., Hamilton, M. A., Fonarow, G. C., MacLellan, W. R., Hage, A., Moriguchi, J., & Patel, J. (2004). Muscle mechanoreceptor sensitivity in heart failure. *AJP - Heart and Circulatory Physiology, 287,* H1937-H1943.

Middlekauff, H. R. & Chiu, J. (2004). Cyclooxygenase products sensitize muscle mechanoreceptors in healthy humans. *AJP - Heart and Circulatory Physiology, 287,* H1944-H1949.

Middlekauff, H. R., Chui, J., Hamilton, M. A., Fonarow, G. C., Maclellan, W. R., Hage, A., Moriguchi, J., & Patel, J. (2008). Cyclooxygenase products sensitize muscle mechanoreceptors in humans with heart failure. *AJP - Heart and Circulatory Physiology, 294,* H1956-H1962.

Mifflin, S. W., Spyer, K. M., & Withington-Wray, D. J. (1988). Baroreceptor inputs to the nucleus tractus solitarius in the cat: modulation by the hypothalamus. *The Journal of Physiology Online, 399,* 369-387.

Momen, A., Cui, J., McQuillan, P., & Sinoway, L. I. (2008). Local prostaglandin blockade attenuates muscle mechanoreflex mediated renal vasoconstriction during muscle stretch in humans. *AJP - Heart and Circulatory Physiology, 294,* H2184-H2190.

Murata, J. & Matsukawa, K. (2001). Cardiac vagal and sympathetic efferent discharges are differentially modified by stretch of skeletal muscle. *AJP - Heart and Circulatory Physiology, 280,* H237-H245.

Nakamoto, T. & Matsukawa, K. (2007). Muscle mechanosensitive receptors close to the myotendinous junction of the Achilles tendon elicit a pressor reflex. *Journal of Applied Physiology, 102,* 2112-2120.

Norton, K. H., Gallagher, K. M., Smith, S. A., Querry, R. G., Welch-O'Connor, R. M., & Raven, P. B. (1999). Carotid baroreflex function during prolonged exercise. *Journal of Applied Physiology, 87,* 339-347.

Ogoh, S., Wasmund, W. L., Keller, D. M., Yurvati, A., Gallagher, K. M., Mitchell, J. H., & Raven, P. B. (2002). Role of central command in carotid baroreflex resetting in humans during static exercise. *The Journal of Physiology Online, 543,* 349-364.

Ogoh, S., Fisher, J. P., Dawson, E. A., White, M. J., Secher, N. H., & Raven, P. B. (2005). Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans. *The Journal of Physiology Online, 566,* 599-611.

Papelier, Y., Escourrou, P., Helloco, F., & Rowell, L. B. (1997). Muscle chemoreflex alters carotid sinus baroreflex response in humans. *Journal of Applied Physiology, 82,* 577-583.

Parati, G., Di Rienzo, M., & Mancia, G. (2000). How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life. *Journal of Hypertension, 18,* 7-19.

Pawelczyk, J. A. & Raven, P. B. (1989). Reductions in central venous pressure improve carotid baroreflex responses in conscious men. *AJP - Heart and Circulatory Physiology, 257,* H1389-H1395.

Piepoli, M., Clark, A. L., Volterrani, M., Adamopoulos, S., Sleight, P., & Coats, A. J. S. (1996). Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. *Circulation, 93,* 940-952.

Potts, J. T., Shi, X. R., & Raven, P. B. (1993). Carotid baroreflex responsiveness during dynamic exercise in humans. *AJP - Heart and Circulatory Physiology, 265,* H1928-H1938.

Potts, J. T. & Mitchell, J. H. (1998). Rapid resetting of carotid baroreceptor reflex by afferent input from skeletal muscle receptors. *AJP - Heart and Circulatory Physiology, 275,* H2000-H2008.

Potts, J. T. (2002). Neural circuits controlling cardiorespiratory responses: baroreceptor and somatic afferents in the nucleus tractus solitarius. *Clinical and Experimental Pharmacology and Physiology, 29,* 103-111.

Potts, J. T., Paton, J. F. R., Mitchell, J. H., Garry, M. G., Kline, G., Anguelov, P. T., & Lee, S. M. (2003). Contraction-sensitive skeletal muscle afferents inhibit arterial baroreceptor signalling in the nucleus of the solitary tract: role of intrinsic GABA interneurons. *Neuroscience, 119,* 201-214.

Potts, J. T. (2006). Inhibitory neurotransmission in the nucleus tractus solitarii: implications for baroreflex resetting during exercise. *Experimental Physiology, 91,* 59-72.

Querry, R. G., Smith, S. A., Stromstad, M., Ide, K., Raven, P. B., & Secher, N. H. (2001). Neural blockade during exercise augments central command's contribution to carotid baroreflex resetting. *AJP - Heart and Circulatory Physiology, 280,* H1635-H1644.

Raven, P. B., Fadel, P. J., & Ogoh, S. (2006). Arterial baroreflex resetting during exercise: a current perspective. *Experimental Physiology, 91,* 37-49.

Rotto, D. M. & Kaufman, M. P. (1988). Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *Journal of Applied Physiology, 64,* 2306-2313.

Rotto, D. M., Schultz, H. D., Longhurst, J. C., & Kaufman, M. P. (1990a). Sensitization of group III muscle afferents to static contraction by arachidonic acid. *Journal of Applied Physiology, 68,* 861-867.

Rotto, D. M., Hill, J. M., Schultz, H. D., & Kaufman, M. P. (1990b). Cyclooxygenase blockade attenuates responses of group IV muscle afferents to static contraction. *AJP - Heart and Circulatory Physiology, 259,* H745-H750.

Sala-Mercado, J. A., Ichinose, M., Hammond, R. L., Ichinose, T., Pallante, M., Stephenson, L. W., O'Leary, D. S., & Iellamo, F. (2007). Muscle metaboreflex attenuates spontaneous heart rate baroreflex sensitivity during dynamic exercise. *AJP - Heart and Circulatory Physiology, 292,* H2867-H2873.

Seals, D. R. & Victor, R. G. (1991). Regulation of muscle sympathetic nerve activity during exercise in humans. *Exercise and Sport Sciences Reviews, 19,* 313-349.

Sinoway, L. I. & Li, J. (2005). A perspective on the muscle reflex: implications for congestive heart failure. *Journal of Applied Physiology, 99,* 5-22.

Smith, S. A., Querry, R. G., Fadel, P. J., Gallagher, K. M., Stromstad, M., Ide, K., Raven, P. B., & Secher, N. H. (2003a). Partial blockade of skeletal muscle somatosensory afferents attenuates baroreflex resetting during exercise in humans. *The Journal of Physiology Online, 551,* 1013-1021.

Smith, S. A., Mammen, P. P. A., Mitchell, J. H., & Garry, M. G. (2003b). Role of the exercise pressor reflex in rats with dilated cardiomyopathy. *Circulation, 108,* 1126-1132.

Smith, S. A., Mitchell, J. H., Naseem, R. H., & Garry, M. G. (2005a). Mechanoreflex mediates the exaggerated exercise pressor reflex in heart failure. *Circulation, 112,* 2293-2300.

Smith, S. A., Williams, M. A., Mitchell, J. H., Mammen, P. P. A., & Garry, M. G. (2005b). The capsaicin-sensitive afferent neuron in skeletal muscle is abnormal in heart failure. *Circulation, 111,* 2056-2065.

Smith, S. A., Williams, M. A., Leal, A. K., Mitchell, J. H., & Garry, M. G. (2006). Exercise pressor reflex function is altered in spontaneously hypertensive rats. *The Journal of Physiology Online, 577,* 1009-1020.

Spyer, K. M. (1994). Annual review prize lecture. Central nervous mechanisms contributing to cardiovascular control. *The Journal of Physiology Online, 474,* 1-19.

Stebbins, C. L., Brown, B., Levin, D., & Longhurst, J. C. (1988). Reflex effect of skeletal muscle mechanoreceptor stimulation on the cardiovascular system. *Journal of Applied Physiology, 65,* 1539-1547.

Sterns, D. A., Ettinger, S. M., Gray, K. S., Whisler, S. K., Mosher, T. J., Smith, M. B., & Sinoway, L. I. (1991). Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation, 84,* 2034-2039.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation, 93,* 1043-1065.

Vorluni, L. & Volianitis, S. (2008). Baroreflex control of sinus node during dynamic exercise in humans: effect of muscle mechanoreflex. *Acta Physiologica, 192,* 351-357.

Walgenbach, S. C. & Donald, D. E. (1983). Inhibition by carotid baroreflex of exercise-induced increases in arterial pressure. *Circulation Research, 52,* 253-262.

Williamson, J. W., Mitchell, J. H., Olesen, H. L., Raven, P. B., & Secher, N. H. (1994). Reflex increase in blood pressure induced by leg compression in man. *The Journal of Physiology Online, 475,* 351-357.

Williamson, J. W., McColl, R., & Mathews, D. (2003). Evidence for central command activation of the human insular cortex during exercise. *Journal of Applied Physiology, 94,* 1726-1734.