NEURAL CARDIOVASCULAR CONTROL DURING EXERCISE: INFLUENCE OF SEX AND OVARIAN HORMONES

by

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ABSTRACT

Cardiovascular control during exercise results from three main mechanisms, namely central command (descending neural input), skeletal muscle afferent feedback (metabo - and mechanoreflex) and the arterial baroreflex. The studies outlined in this thesis sought to examine the potential sex- and ovarian hormone influences in the neural cardiovascular control during exercise. It was observed that the activation of metabolically sensitive skeletal muscle afferents (i.e. muscle metaboreflex) by partial restriction of blood flow to the exercising skeletal muscle contributes to the exercise tachycardia via a reduction in cardiac baroreflex sensitivity from rest during dynamic exercise. Importantly, the magnitude of this metaboreflex-mediated reduction in cardiac baroreflex responsiveness was not different between men and women during the early and late follicular phases of the ovarian cycle. Baroreflex perturbation during dynamic exercise, by means of hypotensive and hypertensive stimuli to the carotid baroreceptors, revealed that baroreflex control of blood pressure was similarly maintained during exercise in men and women. Finally it was demonstrated that the sympathetic vasoconstriction in the exercising limb is similarly blunted in men and women. Overall, the results of this thesis suggest that there are no differences between men and women in baroreflex function and sympathetic vascular responsiveness during dynamic exercise.

SYNOPSIS

Young women have been shown to exhibit attenuated pressor responses during both static and dynamic exercise compared to men of a similar age. However, the reasons for this sex-specific blood pressure (BP) response remain unclear. Three main neural control mechanisms are known to be essential for eliciting the appropriate cardiovascular response to exercise: central command (descending neural input), skeletal muscle afferent feedback (metabo- and mechanoreflex) and the arterial baroreflex. Input from these neural control mechanisms converges at central cardiovascular control centres in the brain and collectively modulates the autonomic nervous outflow to the heart and the vasculature.

The first experimental study of this thesis (Chapter 4), aimed to determine whether the activation of metabolically sensitive skeletal muscle afferents (muscle metaboreflex) is a potential mechanism for the decrease in spontaneous cardiac baroreflex sensitivity (cBRS) during dynamic exercise. Young men performed leg cycling (low and moderate workloads; protocol 1) and rhythmic handgrip exercise (protocol 2) under free-flow conditions and with partial flow-restriction (bilateral thigh cuff or graded arm cuff inflation) to evoke muscle metaboreflex activation during exercise, followed by post exercise ischemia (PEI; isolated muscle metaboreflex). Leg cycling-induced increases in heart rate (HR) and BP were augmented by partial flow-restriction, while HR and BP remained elevated during PEI. Leg cycling evoked an intensity dependent decrease in cBRS (16±2, 7±1, 2±0.2 ms·mmHg^{-1} at rest, low and moderate workloads; $P<0.05$), which was further reduced with partial flow-restriction (by -2.6±0.8 and -0.4±0.1 ms·mmHg^{-1} at low and moderate workloads). cBRS remained suppressed during PEI following leg cycling with partial flow-restriction (4±1 ms·mmHg^{-1}; $P<0.05$ vs. rest) and remained unchanged during handgrip with and without partial flow-restriction and PEI. These data

indicate that the activation of the muscle metaboreflex decreases cBRS during leg cycling exercise.

To examine whether there are sex- and/or ovarian hormone influences on the muscle metaboreflex-mediated decreases in cBRS the two protocols utilized in the first experimental study were performed in women during early follicular (EF; low estrogen) and late follicular (LF; high estrogen) phases of the ovarian cycle and compared to the men (Chapter 5&6). In contrast to men, free-flow leg cycling had no effect on BP in women at both ovarian phases, while HR increases and reductions in cBRS were comparable (EF: 21±5, 6±1, 1±0.1 ms·mmHg^{-1}, LF: 22±5, 6±1, 1±0.1 ms·mmHg^{-1} at rest, low and moderate workloads). Muscle metaboreflex activation during leg cycling evoked similar increases in HR and BP and reductions in cBRS in men and women, independent of the ovarian phase. Rhythmic handgrip under free-flow conditions evoked elevations in BP, which were greater in men than women EF and further attenuated in women LF (+12±2, +6±1 and +3±1 mmHg in men, women EF and women LF, respectively). However, Doppler ultrasound measures of FBF showed that similar reductions in flow elicit a comparable cardiovascular response in men and women, independent of the ovarian phase. Taken together, these data suggest that the muscle metaboreflex, engaged by partial flow-restriction during leg cycling and muscle metaboreflex sensitivity during rhythmic handgrip exercise are not different in sexes and these two ovarian phases.

Given that baroreflex control of the heart during exercise seems unaffected by sex and ovarian hormones, the forth experimental study (Chapter 7) investigated the influence of sex on carotid baroreflex (CBR) control of BP. Oscillatory neck pressure (NP) and neck suction (NS) was applied at 0.1 Hz at rest and during rhythmic handgrip exercise and the degree of CBR control over BP (Fast Fourier transformation) was determined. Oscillatory

NP and NS at rest and during exercise significantly increased spectral power of BP in both men and women without any significant sex-difference in the magnitude of the response either at rest or exercise, with either NP or NS. This suggests that baroreflex control of BP at rest and during rhythmic handgrip exercise is not affected by sex.

To investigate whether the attenuated exercise pressor response is attributable to an attenuated sympathetic responsiveness (transduction of sympathetic nerve activity to the peripheral vasculature) the final experimental study (Chapter 8) employed a cold pressor test (CPT) to evoke sympathetic vasoconstriction at rest and during rhythmic handgrip exercise (very low, low, moderate intensity) in men and women. Handgrip evoked increases in HR, FBF and forearm vascular conductance (FVC), while BP was unchanged. CPT-induced increases in BP and HR were comparable between rest and exercise and sexes. FVC was markedly reduced with the CPT at rest (−44±6% in men and −32±5% in women, effect of sex P=0.95), but this vasoconstrictor effect was progressively attenuated with increasing exercise intensity (−20±5% in men and −20±5% in women at moderate workloads). Collectively, these data indicate that sympathetic vasoconstriction during exercise is similarly blunted in the peripheral of men and women.

In summary, the results from this thesis suggest that the muscle metaboreflex contributes to the reductions in cBRS during leg cycling. However, there are no apparent sex-differences in the ability of the muscle metaboreflex to reduce cBRS during exercise. Thus, sex-differences in muscle metaboreflex function seem not to contribute to the reported sex-specific exercise pressor response. The similar neural vascular transduction of sympathetic nervous activity at rest and during exercise in men and women indicates that an attenuated sympathetic responsiveness does not explain the lower BP rise during exercise in women compared to men.

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Abstracts

(1) Hartwich D, Dear WE, Kirtley LA, Taylor SM, Waterfall JL, Fisher JP. Influence of ovarian cycle on muscle metaboreflex control of spontaneous cardiac baroreflex sensitivity in young women. J Physiol (2011) Proc Physiol Soc, 23 C43.

(2) Hartwich D, Dear WE, Grice TD, Kerr TJ, Waterfall JL, Fisher JP. Effect of muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity during exercise in humans. FASEB J March 17 (2011) 25:1056.6.

(3) Hartwich D, Kim A, Griffin HS, Balanos GM, Aldred S, Fadel PJ, Fisher JP. Influence of sex and menstrual phase on the middle cerebral artery blood flow velocity responses to dynamic exercise in humans. FASEB J March 17 (2011) 25:1024.11.

(4) Hartwich D, Fowler KL, Wynn LJ, Fisher JP. Differential responses to sympathetic stimulation in the cerebral and brachial circulations during rhythmic handgrip exercise in humans. J Physiol (2010) Proc Physiol Soc, 19 PC163.

ABBREVIATIONS

ANOVA	=	Analysis of variance
BMI	=	Body mass index
BP	=	Blood pressure
CBR	=	Carotid baroreflex
cBRS	=	Cardiac baroreflex sensitivity
CO	=	Cardiac output
CO_2	=	Carbon dioxide
CP	=	Chamber pressure
CPT	=	Cold pressor test
CVCi	=	Cerebrovascular conductance index
$CVCi_{CPT}$	=	Cerebrovascular conductance index during the cold pressor test
$CVCi_{SS}$	=	Cerebrovascular conductance index during steady state rhythmic handgrip exercise
ECG	=	Electrocardiogram
EF	=	Early follicular phase of the ovarian cycle
EX	=	Exercise
Ex120	=	Leg cycling at target heart rate of 120 beats·min^{-1}
Ex90	=	Leg cycling at target heart rate of 90 beats·min^{-1}
FBF	=	Forearm blood flow
FBV	=	Forearm blood velocity
FF	=	Free-flow
FVC	=	Forearm vascular conductance
FVC_{CPT}	=	Forearm vascular conductance during the cold pressor test
FVC_{SS}	=	Forearm vascular conductance during steady state rhythmic handgrip exercise
HR	=	Heart rate
HR-cBRS	=	Spontaneous cardiac baroreflex sensitivity calculated using heart rate

LF	=	Late follicular phase of the ovarian cycle
Ln	=	Natural logarithm
LoF	=	Low frequency power
LH	=	Luteinising hormone
MAP	=	Mean arterial pressure
MCA	=	Middle cerebral artery
MCA V_{mean}	=	Middle cerebral artery blood flow velocity
ML	=	Midluteal phase of the ovarian cycle
MSNA	=	Muscle sympathetic nerve activity
MVC	=	Maximum voluntary contraction
NIRS	=	Near-infrared spectroscopy
NP	=	Neck pressure
NS	=	Neck suction
PEI	=	Post exercise ischemia
$P_{ET}CO_2$	=	End-tidal pressure of carbon dioxide
PFR	=	Partial flow-restriction
RMSSD	=	square root of the mean of the sum of successive differences in R-R interval
RPE	=	Rating of perceived exertion
RRI	=	R-R interval
RRI-cBRS	=	Spontaneous cardiac baroreflex sensitivity calculated using R-R interval
SD	=	Standard deviation
SEM	=	Standard error mean
SV	=	Stroke volume

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CHAPTER 1: INTRODUCTION

This thesis is predominantly concerned with the examination of sex- and/or ovarian hormone influences on the neural cardiovascular control during dynamic exercise. The exercise-induced increase in blood pressure in postmenopausal women is reportedly exaggerated in comparison to their age matched male counterparts (Ogawa et al., 1992). Intriguingly, young premenopausal women exhibit attenuated elevations in BP during exercise when compared to young men (Fleg et al., 1995). This suggests that female reproductive hormones play a role in the cardiovascular responses to exercise. This may be important because epidemiological studies have reported that an exaggerated blood pressure response to exercise is associated with increased future cardiovascular risk. The reasons for these altered exercise pressor responses in women are unknown. The arterial baroreflex plays an important role in the moment-to-moment regulation of resting blood pressure and heart rate by altering sympathetic and parasympathetic nerve activity to the heart and the peripheral vasculature. During exercise the arterial baroreflex continues its contribution to the overall cardiovascular control by integration of central signals arising from the brain and peripheral signals from the skeletal muscle (see Figure 2.2).

The aim of this thesis is to investigate reasons and/or mechanisms which may explain the attenuated pressor response observed in young women. In that, the role of metabolically sensitive skeletal muscle afferents in the exercise pressor response, and control of the heart via modulation of the arterial baroreflex, will be studied in the context of sex- and ovarian hormone concentration. In addition, the arterial baroreflex control of blood pressure during exercise will be examined in men and women. Lastly, sex-differences in the transduction of sympathetic efferent activity to the vasculature will be investigated at rest and during exercise.

CHAPTER 2: LITERATURE REVIEW

2.1 Cardiovascular responses to exercise

2.1.1 Dynamic exercise

Dynamic exercise is characterised by force development in which the muscle shortens as it contracts. During dynamic exercise increases in heart rate (HR) and stroke volume (SV) are the driving factors of the increase in cardiac output (CO). CO, at rest is around 5-6 l/min and can increase 5-6 fold during heavy dynamic exercise to ensure adequate supply of oxygen and nutrients to the contracting skeletal muscle (Saltin et al., 1998). In addition, decreases in total peripheral resistance (TPR) are needed to adjust perfusion during dynamic exercise such that vasodilatation occurs in the contracting muscles, while vasoconstriction occurs in less active areas, such as the splanchnic and renal circulation (Rowell, 1993a). Together these cardiovascular adaptations allow a moderate increase in BP during dynamic exercise (Figure 2.1).

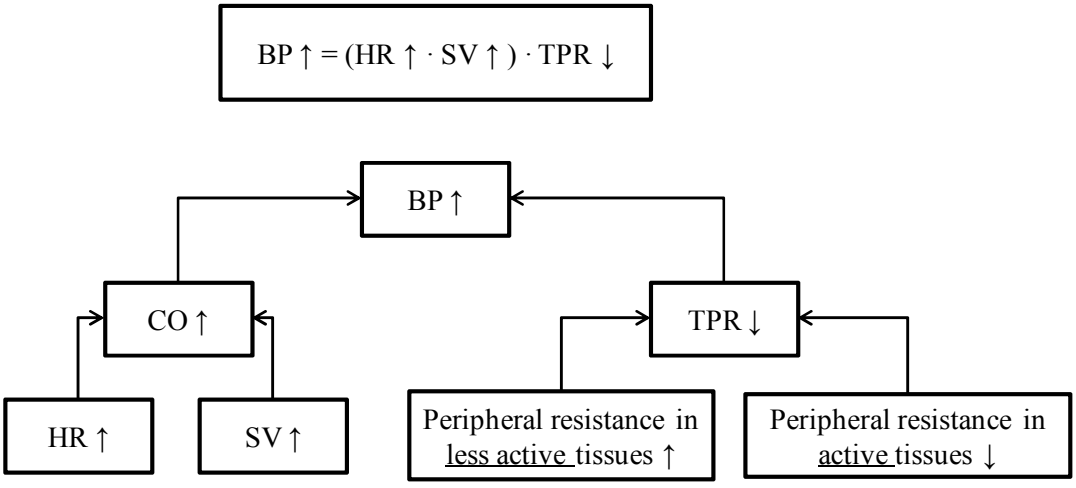

Figure 2.1. Cardiovascular responses during dynamic exercise. HR, heart rate; SV, stroke volume; CO, cardiac output; BP, blood pressure; TPR, total peripheral resistance.

2.1.2 Static exercise

Static exercise is characterised by force development without any change in muscle length or joint angle and is often referred to as an isometric contraction (i.e. iso = same, metric = length). During static exercise, these mechanical forces increase intramuscular pressure thus limiting the perfusion of the active skeletal muscles leading to an increase in TPR (Barcroft and Millen, 1939, Humphreys and Lind, 1963). Despite this, static muscle contractions evoke increases in CO and HR with either a decrease or no change in SV (Rowell, 1993a). As a consequence of the increased TPR and CO, the elevations in BP are much greater during static than dynamic exercise (Lind and McNicol, 1967).

The cardiovascular responses to static and dynamic exercise have been attributed to the actions and interactions of central neural signals arising from higher brain areas (i.e. central command; section 2.2), and skeletal muscle afferents (section 2.3). The latter is characterised by feedback from the exercising skeletal muscle in response to mechanical and metabolic stimuli (i.e. muscle mechanoreflex and muscle metaboreflex, respectively). The arterial baroreflex (section 2.4) plays an important role in the beat-to-beat regulation of arterial BP and HR, by modulating sympathetic and parasympathetic activity to the heart and the peripheral vasculature. During exercise the arterial baroreflex depends on input from central command and feedback from skeletal muscle afferents to ensure overall control of BP and HR. As a result exercise-induced increases in HR and BP are a complex interplay of parasympathetic withdrawal (i.e. reduced acetylcholine release) which causes an immediate rise in HR and increases in sympathetic nerve activity (i.e. release of noradrenaline) directed to the heart and the peripheral vasculature (Figure 2.2).

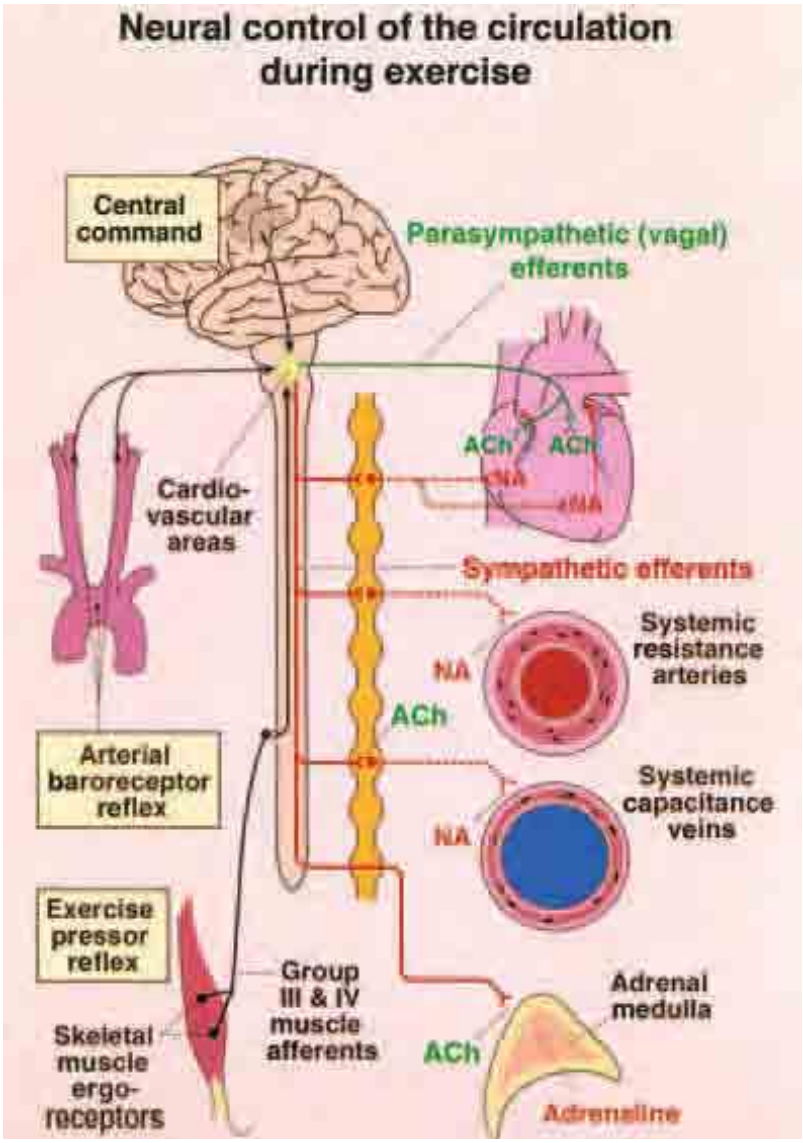

Figure 2.2. Neural cardiovascular control during exercise. ACh, acetylcholine; NA, noradrenaline. (Smith et al., 2006).

2.2 Central command

2.2.1 Evidence for central command

In the late 19th and early 20th century, Johansson (1893) and Krogh and Lindhard (1917, 1913) first demonstrated that cardiac and respiratory changes occur almost immediately at the onset of voluntary exercise. Importantly, these rapid cardio-respiratory adaptations did not occur during passive movement or when muscle contractions were evoked by electrical stimulation suggesting they are initiated centrally by "*irradiation of impulses from the motor cortex*" (Krogh and Lindhard, 1913). Since the 1970s, the concept that descending signals from higher brain centres are capable of influencing the cardiovascular and respiratory responses to exercise in parallel to somatomotor activation of the skeletal muscle has been commonly designated 'central command' (Goodwin et al., 1972). Central command is classically defined as a 'feed-forward system' implying the control of cardiovascular activity without continuous feedback.

2.2.2 Early studies of central command

Using a non-pharmacological experimental approach Goodwin and colleagues (1972) sought to modulate central command by mechanically opposing or assisting the active muscle contraction. High frequency muscle vibration, known to activate muscle spindles, was applied to the biceps brachii during either voluntary arm flexion (e.g. assisted the muscle contraction; decreased central command) or extension (e.g. opposed muscle contraction; increased central command). Cardiorespiratory responses were augmented during increased central command (arm extension with biceps brachii vibration) and attenuated during decreased central command (arm flexion with biceps brachii vibration), when compared to arm flexion or extension without tendon vibration. The authors

concluded that cardiorespiratory responses to exercise are clearly related to central command. However, a potential limitation of this study might derive from the different fibre type composition in the studied muscles. As such, activation of predominantly fast-twitch fibres has been shown to evoke augmented cardiovascular responses (Petrofsky et al., 1981). Therefore, it is arguable whether metabolic conditions and central command influences are comparable between biceps brachii and triceps brachii contractions. It cannot be excluded that tendon vibration co-activates smaller muscle afferent fibres (group III and IV), which may exert inhibitory influences on alpha-motorneurons and therefore alter central command input (Gandevia, 2001).

In an attempt to disassociate central command from feedback from the exercising skeletal muscles, partial neuromuscular blockade has been performed to weaken the exercising skeletal muscles (Asmussen et al., 1965, Freyschuss, 1970, Iwamoto and Kaufman, 1987, Leonard et al., 1985, Mitchell et al., 1989b, Secher, 1985). This allows the comparison of the cardiovascular responses from control and weakened muscle contractions at the same absolute and relative workloads. At the same relative force (e.g. 30% of maximum voluntary contraction (MVC) under control and 30% of MVC after partial neuromuscular blockade), central command is thought to be similar during weakened and control contractions and thus evoke similar increments in HR and BP. Conversely, at the same absolute force (e.g. 10% of the initial MVC before neuromusclular blockade), central command is expected to be higher during weakened contractions. Collectively, studies that employed this method suggest that augmented central command increases the contraction-induced increments in HR and BP (Mitchell et al., 1989b, Secher, 1985). In contradiction, during epidural anaesthesia that blocks muscle fibres and sensory feedback from the skeletal muscles, the increments in HR and BP were similar at the same absolute force (30% of MVC) between contractions under control and epidural anaesthesia

(Mitchell et al., 1989a, Strange et al., 1993). Therefore, it can be concluded that central command alone cannot explain the exercise-induced cardiovascular changes. Rather, it has been suggested that there is a redundancy between central command and afferent feedback from the exercising skeletal muscle, when one of them is blocked or inhibited (Mitchell et al., 1989a).

Care must be taken when interpreting the results of studies using partial neuromuscular blockade because different blocking agents (e.g. tubocuranine, decamethonium) preferentially block either slow- or fast-twitch fibres. Petrofsky et al. (1981) demonstrated that fast-twitch fibre recruitment evoked a greater pressor response. This means that the augmented increases in HR and BP in response to exercise at the same absolute workload could be instead caused by increased fast-twitch fibre availability and thus a greater quantity of metabolites produced after neuromuscular blockade (Saltin and Gollnick, 1983). In addition, increased stress, due to the loss of control over a limb and/or respiratory changes (e.g. Valsalva manoeuvre) might have confound the findings, as both have been shown to increase sympathetic nerve activity, HR and BP (Pryor et al., 1990). Finally, the majority of the studies using neuromuscular blockade failed to determine the degree of muscle activation or whether concomitant inadvertent muscle contractions were performed to overcome the weakness. Given the linear relationship of force production and integrated electromyogram activity (Bigland and Lippold, 1954, Gandevia et al., 1993), the use of an electromyogram could overcome this limitation.

It is now widely accepted that central command is dependent on the subjects' perception of effort during physical activity or even the attempt of exertion, independent of the actual force produced (Mitchell, 1990). An increase in a person's perceived exertion is coupled with an increased cardiovascular response. However, other factors, such as increased pain may enhance the perception of effort and provide additional afferent

feedback to the cardiovascular control centres in the brainstem, modulating the pressor response (Williamson, 2010). This challenges the "old" concept of central command as a pure feed-forward mechanism.

2.2.3 Function of central command

The immediate increase in HR at exercise-onset was attributed to central command as it was delayed (approximately 1 heart beat) during electrically stimulated contractions (Iwamoto et al., 1987), whereas partial curarisation had no effect on this time course (Secher, 1985). Given the latency of the effects of parasympathetic and sympathetic signalling on HR, it has been assumed that immediate HR increases during exercise via central command are mediated by vagal withdrawal. For example, vagal withdrawal can affect HR within 1 second, while sympathetic HR changes take up to 20 seconds (Warner and Cox, 1962). In support of this, tachycardia was attenuated when vagal activity was blocked by atropine during contractions with partial neuromuscular blockade (i.e. attenuated muscle afferents, augmented central command) compared to control contractions (Mitchell et al., 1989b, Victor et al., 1989a).

Mark et al. (1985) have investigated the effects of central command on muscle sympathetic nerve activity (MSNA) during voluntary and involuntary contractions (e.g. electrically-induced biceps brachii contraction). MSNA rose during involuntary contractions (i.e. no central command) and decreased during voluntary efforts. Furthermore, the magnitude of the rise in MSNA has been reported to be significantly smaller during contractions after partial neuromuscular blockade at the same absolute force (i.e. augmented central command and decreased muscle afferent activation) compared to control exercise (Victor et al., 1989a). Taken together, these studies indicate, that central command has a modest effect on sympathetic outflow to the skeletal muscle vasculature

during low to moderate intensities, although it may become more important during high exercise intensities (>75% of MVC) (Victor et al., 1995).

2.2.4 Neurocircuitry of central command

Early studies have demonstrated that electrical stimulation of diencephalic structures in the field of Forel, which is related to coordinated leg movements, initiated cardiovascular changes in parallel with somatomotor activation (Rushmer and Smith, 1959). Subsequently, electrical stimulation has been used to identify central command signals emanating from the hypothalamic, basal ganglia and mesencephalic locomotor regions in anaesthetised and unanaesthetised, decorticated animals (Eldridge et al., 1985, Eldridge et al., 1981). As cardiorespiratory changes associated with electrical stimulation persisted when animals are paralysed, the observed responses may be deemed to be independent of muscle afferent feedback. However, results from studies in anaesthetised animals have to be interpreted with care, as the direct translation into the conscious state may be questioned. To overcome this limitation, direct electrical stimulation of neural sites (e.g. deep brain stimulation), involved in the central command-induced cardiovascular and locomotor responses, has been performed in awake human patients during neurosurgery. Electrode recordings (e.g. electrophysiological activity) during volitional contractions have shown that subcortical structures such as the subthalamic nucleus, thalamus and the periaqueductal gray are highly involved in the combined facilitation of movement and increases in HR and BP (Green et al., 2007, Thornton et al., 2002). However, it must be noted that these data stem exclusively from patients (e.g. Parkinson disease) on drug therapy.

Positron-emission tomography and magnetic resonance imaging have been used to identify the central command neurociruitry. A linear relationship has been shown between

the increases in blood flow in brainstem nuclei and the insular cortex during incremental levels of exercise and the observed cardiovascular responses (Critchley et al., 2000, Nowak et al., 1999, Williamson et al., 1999). These areas have projections to the cardiovascular control centres or are itself heavily involved in cardiovascular regulation (Oppenheimer and Cechetto, 1990, Yasui et al., 1991). Employing hypnosis to manipulate perceived exertion during imagined exercise at different intensities (e.g. downhill or uphill cycling), allows investigation of low (e.g. imagined downhill cycling) and high (e.g. imagined uphill cycling) central command in the absence of feedback from the exercising skeletal muscles. Here, a greater central command activity with imagined uphill cycling resulted in a more pronounced increase in cerebral blood flow in the insular cortices, thalamic regions (bilaterally) and anterior cingulate cortex (Williamson et al., 2002, Williamson et al., 2001).

2.3 Skeletal muscle afferent feedback

2.3.1 Evidence for skeletal muscle afferent feedback

Alam and Smirk (1937) proposed that cardiovascular and respiratory responses to exercise cannot exclusively be explained by central command. They have found that exercise-induced increases in BP remained elevated above rest, for as long as the blood supply of the previously working limb was occluded (i.e. post exercise ischemia, PEI). In the absence of central command or mechanical feedback, the sustained BP is attributed to the production of metabolites during exercise, which remain trapped within the skeletal muscle during PEI (i.e. muscle metaboreflex isolation).

Coote and colleagues (1971) were the first to demonstrate the reflex nature of the exercise pressor response. They have used anaesthetised cats, in which muscular

contractions were produced by electrical stimulation of the distal end of the cut ventral roots (L6-S1). However, the muscle contraction-induced pressor response was abolished when the dorsal root (receiving sensory input from the skeletal muscle) was sectioned (Coote et al., 1971). Early investigations of sensory neurones had demonstrated that stimulation of fast conducting group I and II afferent fibres, which innervate proprioceptors (e.g. muscle spindles, Golgi tendon organs), elicit a slight cardiovascular response (Johansson, 1962, Koizumi et al., 1961). However, it cannot be assumed that solely the group I and II afferents were simulated in this study. Thus, McCloskey and Mitchell (1972) have argued that the small slow conducting group III and IV afferents play a more important role in mediating the exercise pressor response. Their evidence was based on two observations. First, the sustained cardiovascular and respiratory responses during anodal blockade preferentially blocks large myelinated fibres (e.g. group I and II). Second, the application of local anaesthetics to the dorsal root predominantly blocks small unmyelinated and thinly myelinated group III and IV afferent fibres and abolished the cardiovascular and respiratory responses (McCloskey and Mitchell, 1972). In support of these, the increases in HR, BP and ventilation elicited by succinylcholine infusion (i.e. neuromuscular blocking agent) were abolished when the input from group III and IV afferent fibres was blocked (Waldrop et al., 1984).

In humans, the effects of the muscle metaboreflex have also been examined by restricting blood flow to the exercising skeletal muscles (i.e. hypoperfusion). This increases muscle metabolite accumulation and augments the stimulation of metabolically sensitive muscle afferents (Rowell, 1993b). Using increasing levels of lower body positive pressure during cycling to obtain leg hypoperfusion, it has been found that BP, HR, lactate and catecholamine levels were increased, while venous pH and leg blood flow were decreased in proportion to box pressure and exercise intensity (Rowell et al., 1991,

Sundberg and Kaijser, 1992). Amann et al. (2011) have used lumbar intrathecal injections of fentanyl to impair muscle afferent feedback during leg-kicking exercise. They have shown attenuated increases in CO, HR, BP, blood flow, vascular conductance and oxygen consumption during exercise with fentanyl compared to control exercise. These results underscore the importance of muscle afferent feedback for an adequate cardiovascular, haemodynamic and respiratory response during dynamic exercise.

2.3.2 Skeletal muscle afferents – neurophysiological pathways

Group III and IV muscle afferents synapse first at the dorsal horn of the spinal cord (predominantly the lamina I and V of the according segments L4 to S3), which has been identified using horseradish peroxidase (Craig and Mense, 1983). From the spinal cord, neurones project to the brain stem. The immunocytochemical labelling of the protein c-FOS to visualise increases in central neural activity associated with exercise revealed that neuronal activity in the hypothalamic areas, the medial aspect of the nucleus tractus solitarius (NTS) and the rostral and caudal ventrolateral medulla (RVLM and CVLM) is augmented during exercise compared to rest (Iwamoto et al., 1996). As these results were obtained in conscious rats running on a treadmill, this reflects active central pathways during voluntary exercise unlike findings from anaesthetised animals (Waldrop and Stremel, 1989). Importantly, the NTS and the ventrolateral medulla also receive projections from the glossopharyngeal and the vagus nerve. This highlights the potential role of these central sites for the integration of cardiovascular adjustments during exercise.

2.3.3 Stimulus-dependent division of skeletal muscle afferents

Microscopic investigations have indicated that nerve endings of the group III afferents are commonly found close to collagen structures in the skeletal muscle, while group IV afferents are mostly located in close proximity to blood and lymph vessels (Andres et al., 1985). The anatomical location of these fibres matches closely the functional properties of these afferents. Using an anaesthetised cat model, afferent impulse responses of group III and IV fibres on the dorsal horn have been recorded during statically (Kaufman et al., 1983) and dynamically (Kaufman et al., 1984, Mense and Stahnke, 1983) simulated muscle contractions. Importantly, these two groups of afferents produced very distinct discharge properties. Group III fibres increased their discharge frequency more profoundly during mechanical distortion, whereas group IV afferents were predominantly activated by contraction related muscle metabolites and during contractions under blood flow restriction (46.7% vs. 12.5% group IV vs. group IV afferents). Therefore, reflex changes induced by chemical stimulation of afferents are referred to as the muscle 'metaboreflex', and reflex responses to mechanical stimulation are termed muscle 'mechanoreflex' (Mense and Stahnke, 1983, Paintal, 1960, Stebbins et al., 1988). Contraction-related metabolites accumulate during ischemic exercise, altering the chemical milieu of the interstitium, which in turn stimulates and/or sensitizes the nerve endings of group III, but predominantly of group IV afferent fibres, and evokes a pressor response. However, there are studies that have demonstrated that some group III afferents also respond to metabolic stimuli, while only a minority of group IV afferents respond to mechanical input (Adreani and Kaufman, 1998, Hayes et al., 2005).

2.3.4 Putative chemical substances stimulating skeletal muscle afferents

Despite extensive research attempting to identify the chemical stimulants of muscle afferents, the exact mechanisms are still not fully elucidated. For example, increased interstitial potassium concentrations have been associated with muscular contractions, suggesting a potential role of potassium in facilitating the exercise pressor response. Indeed, potassium ions reportedly stimulate both group III and IV afferents (Kaufman and Rybicki, 1987). Moreover, exercise-induced increases in venous potassium concentrations remained elevated during PEI (Fallentin et al., 1992). However, potassium ion concentration in venous blood during PEI has been shown to be reduced compared to extracellular concentrations in the gastrocnemius muscle (Hnik et al., 1976). This reduction was attributed to the ischemia-induced re-absorption of these ions. Therefore, venous potassium concentrations might not directly reflect interstitial concentrations. Rybicki et al. (1984) have measured the interstitial potassium concentration directly. They injected potassium into the gracilis muscle of anaesthetised dogs, which then evoked rises in HR and BP. However, the HR and BP increases were brief, while interstitial potassium concentrations remained elevated. Nevertheless, these findings imply that potassium contributes to the initiation of the reflex pressor response to exercise, but seems to be less significant as exercise continues.

Evidence also supports the important role of hydrogen ions in stimulating the exercise pressor response. Injection of lactic acid has been shown to increase the activity of muscle afferents (Rotto and Kaufman, 1988) and to elicit a reflex tachycardia (Thimm et al., 1984). Victor et al. (1988) have demonstrated a correlation between the exercise-induced increases in MSNA and decreases in intracellular pH, implying that both products of glycolytic muscle metabolism, lactic acid and hydrogen, contribute to the exercise-induced sympathetic reflex response. In support of this, patients with McArdle's disease, a

condition with a congenital defect of glycogen degradation (i.e. myophosphorylase deficiency), have been shown to elicit attenuated elevations in BP and MSNA during static exercise. These patients do not develop muscle acidosis, which might attenuate metaboreflex activation during exercise (Pryor et al., 1990). Sympathetic dysfunction in these patients can be excluded as increases in MSNA to Valsalva manoeuvres and cold pressor tests (CPT) were comparable to healthy subject responses. Conversely, Vissing et al. (2001) have demonstrated that patients with McArdle's disease indeed elicit increases in MSNA and BP during exercise, despite markedly lower interstitial lactate concentrations compared to healthy controls. Subsequent to this, a study was performed in a greater number of patients and control subjects and showed that less acidosis is associated with lower MSNA (Fadel et al., 2003b), in support of the findings of Pryor and colleagues. Considerable differences existed in the exercise modalities (e.g. duration and intensity) in these patient studies possibly causing differential reflex stimulation. Another candidate substance, the diprotonated form of phosphate has been suggested to activate the metaboreflex. This was evidenced by the close correlation between this substance and increases in both MSNA and BP during exercise and PEI (Boushel et al., 1998, Sinoway et al., 1994).

Alternatively, metabolites such as bradykinin (Stebbins and Longhurst, 1986) or capsaicin and arachidonic acid (Kaufman et al., 1982, Rotto et al., 1989) have been demonstrated to stimulate group III and IV muscle afferents and cause a pressor response. Also, the exercise-induced increase in interstitial adenosine triphosphate (ATP; (Costa et al., 2001)) has been shown to activate group III and IV via purinergic P2 receptors and to elicit cardiovascular responses (Hanna et al., 2002, Hanna and Kaufman, 2004). Both indicate a central role of ATP in the exercise pressor response. Importantly, substances such as phosphocreatine, adenosine diphosphate or sodium lactate have been found not to

contribute to exercise pressor response via stimulation of group III and IV afferents (Rotto and Kaufman, 1988, Victor et al., 1988).

In conclusion, despite evidence for substances contributing to the pressor response via activation of group III and IV afferents underlying mechanism(s) is still not fully understood. There appears to be a redundancy. As such, the pressor response could be elicited by another substance, when one substance is blocked. Alternatively, the exercise pressor response via metabolically stimulation of group III and IV afferents relies not only on the contribution of one substance but rather a combination of two or more synergistically acting metabolites (McCord and Kaufman, 2010).

2.3.5 BP and skeletal muscle afferent activation

One method to investigate the contribution of muscle afferents is the elimination of central command during exercise by using electrically evoked muscle contractions. Using this approach, similar increases in BP, MSNA and oxygen consumption have been found to occur during voluntary and electrically evoked muscle contractions (Bull et al., 1989, Davies and Starkie, 1985, Hultman and Sjoholm, 1982, Krogh and Lindhard, 1917). In addition, elevations in renal sympathetic nerve activity and BP during stimulated muscle contractions were diminished in decerebrate cats after dorsal root section (Victor et al., 1989b) and have been associated with increases in muscle metabolites (e.g. hydrogen) (Victor et al., 1988). These results indicate an important role of metabolically sensitive skeletal muscle afferents in mediating BP elevations, by increases in vascular tone via augmented MSNA. Increased CO is unlikely to contribute to the rise in BP, since HR remained unchanged during electrically evoked muscle contractions (Bull et al., 1989, Davies and Starkie, 1985) provided that SV does not change (Rowell, 1993b). However, it

is acknowledged that muscle metaboreflex activation during exercise (i.e. hypoperfusion) may indeed contribute to the rise in BP via elevations in CO (O'Leary et al., 2007).

Findings from studies using motor nerve stimulation or percutaneous muscle stimulation must be interpreted with care since the muscle fibre recruitment pattern during stimulation might deviate from those during voluntary contractions (Henneman et al., 1965). Indeed, a reversed recruitment order during electrically evoked muscle contractions has been demonstrated meaning that fast conducting muscle fibres are recruited before slow conducting fibres, unlike during voluntary exercise (Knaflitz et al., 1990). This is of interest because electrical stimulation of muscles with predominantly fast-twitch fibres evoked a greater pressor response than stimulation of muscles with mostly slow-twitch fibre contribution (Carrington et al., 1995). Therefore, the magnitude of the pressor response during evoked muscle contractions would be expected to exceed those observed during voluntary exercise.

2.3.6 HR and muscle afferent activation

As mentioned, the initial HR increase at the onset of exercise may be attributable to central command (Krogh and Lindhard, 1917). However, muscle mechanoreceptor activation may also contribute to the initial HR rise (Stebbins et al., 1988). Electrically-induced muscle contractions (i.e. absence of central command) have been demonstrated to elicit a delayed HR rise compared to voluntary contractions (Krogh and Lindhard, 1917). In contrast, Hollander and Bouman (1975) have found no temporal differences during electrically-evoked and voluntary contractions. However, their suggested existence of a 'muscle-heart reflex' triggering the tachycardia at exercise onset could not be subsequently replicated. Iwamoto et al. (1987) have confirmed the early findings from Krogh and Lindhard, by showing that partial neuromuscular blockade had no effect on the temporal

HR response. This indicates that central command mediates the initial cardiac response to exercise. These discrepancies might derive from the methodology employed to evoke muscle contractions, and/or the muscle group studied. For example, exercise-induced cardiovascular responses have been suggested to depend on muscle mass and/or fibre type distribution (Fallentin et al., 1985, Freund et al., 1978). Alternatively, muscle afferents might have been unintentionally co-activated during muscle stimulation in the study by Hollander and Bouman, thus possibly stimulating mechanically sensitive muscle afferents and reducing the time lag of tachycardia (Iwamoto and Kaufman, 1987).

Nobrega and Araujo (1993) have found that in the absence of central command (e.g. unloaded passive cycling), the independent activation of mechanoreflexes caused HR to increased at the onset of exercise. More recently, Gladwell and Coote (2002) have demonstrated a reflex tachycardia via activation of mechanosensitive muscle afferents with passive stretch of the triceps surae. They further observed a diminished cardiac response to passive stretch following administration of glycopyrrolate (Gladwell et al., 2005). These findings indicate that the muscle mechanoreflex contributes to the initial HR rise at exercise onset by cardiac vagal inhibition.

During PEI after electrically evoked and voluntary exercise (i.e. isolated muscle metaboreflex) HR has been shown to return to pre-exercise levels, while BP and MSNA remain elevated (Alam and Smirk, 1937, Bull et al., 1989, Victor et al., 1987b). O'Leary (1993) has shown in dogs that muscarinic blockade prevented the drop in HR during PEI. This indicates that despite sustained sympathetic activation of the heart, the reactivation of vagal activity (i.e. withdrawal of vagal inhibition), resulting from the cessation of central command, overcomes the sympathetic influences to the heart. These findings have been confirmed in humans during PEI after static handgrip (Fisher et al., 2010). Notably, during augmented muscle metaboreflex activation, via hypoperfusion of the hindlimbs of dogs

running on a treadmill, O'Leary (1993) has observed that HR increased despite pharmacological blockade of β-adrenoreceptors. This suggests that muscle metaboreflex activation might also be able to reduce cardiac parasympathetic activity and therefore increase HR during exercise. However, comparable studies in humans remain to be conducted.

2.3.7 Interaction between central command and skeletal muscle afferents

Amann et al. (2009) had subjects cycling at high intensity with and without intrathecal infusion of fentanyl, which selectively blocks afferent sensory activity (group III and IV), without affecting neuromuscular function (e.g. power output). They assumed that increased sensory feedback from the exercising muscles would be essential for the regulation of central motor drive to limit the development of peripheral fatigue. During sensory afferent blockade subjects showed a greater power output, greater muscle activity (e.g. greater central motor drive) and faster cycling performance in the first half of the time trial experiments followed by substantial muscle fatigue in the second half compared to the control trial. These results indicate that ascending sensory feedback from the exercising skeletal muscles can limit the magnitude of central motor drive in order to avoid peripheral fatigue development. This highlights the interaction of these to mechanism during high intensity exercise.

2.4 Arterial Baroreflex

2.4.1 Arterial baroreflex function

The arterial baroreflex is the primary mechanism involved in the short-term homeostatic control of BP. Arterial baroreceptors have been identified in the adventitia of

the aortic arch and carotid sinus. Increased action potentials induced by vessel stretch (e.g. due to rise in BP) are transferred via the carotid sinus nerve ascending in the glossopharyngeal nerve (ninth cranial nerve) through the bipolar petrous ganglion and terminate at the NTS. Activation of the NTS provides inhibitory signals to nearby brain stem nuclei, particularly to the nucleus ambiguous, RVLM and CVLM. This modulates the parasympathetic and sympathetic branches of the autonomic nervous system to maintain BP at its point of operation (Dampney et al., 2003). The arterial baroreflex buffers BP rises by evoking reflex bradycardia and peripheral vasodilation, while BP falls result in reflex tachycardia and peripheral vasoconstriction.

2.4.2 Arterial baroreflex function during exercise

The functional contribution of the arterial baroreflex during exercise has been a matter of extensive debate. The observation that HR and BP both increase with exercise led some to conclude that the baroreflex was switched off (Bristow et al., 1969). Furthermore, the baroreflex-mediated changes in HR in response to adrenaline infusions were attenuated from rest during exercise. Therefore it was suggested that arterial baroreflexes play only a minor role (if any) in the modulation of HR during exercise (Pickering et al., 1972). Ernsting and Parry (1957) have stimulated the carotid arterial stretch receptors by decreasing ambient pressure in a box enclosing the neck (i.e. neck suction; NS), which provides a hypotensive stimulus to the carotid baroreceptors. Conversely, neck pressure (NP) has been used to decrease carotid sinus transmural pressure, to provide a hypertensive stimulus to the carotid baroreceptors. Using this approach, Bevegard and Shepherd (1966) have shown similar baroreflex-induced decreases in BP and HR at rest and during leg cycling in response to NP. This indicates that arterial baroreflexes remain important for the establishment of BP during exercise. Coote and

Dodds (1976) have confirmed these observations in the decerebrate cat model. Furthermore, baro-denervation of either aortic or carotid sinus afferents in dogs has been shown to evoke exaggerated BP responses to treadmill running, compared to intact animals (Walgenbach and Donald, 1983). This indicates that intact arterial baroreflexes are necessary for an appropriate cardiovascular response to exercise. It implies an inhibitory effect of the arterial baroreflex on the exercise-induced increase in BP.

The reason for the arguably erroneous assumption that the baroreflex might be switched off during exercise (Bristow et al., 1969, Pickering et al., 1972), might be explained by the methodology of assessing baroreflex function, especially the inconsistent use of HR or RRI. Since, RRI changes in a linear manner during vagus nerve stimulation and HR alterations are described by a hyperbolic relationship, changes in RRI are now commonly used (Parker et al., 1984). Moreover, in early studies the full sigmoidal response range of the cardiac-baroreflex function curve has not been assessed (Bristow et al., 1969, Pickering et al., 1972).

Using the variable neck chamber technique, Potts and colleagues (1993) have found an upward movement of the full carotid baroreflex (CBR) cardiac response curve on the response arm and a rightward shift of the curve to higher operating pressures without a change in the maximal gain. Concomitantly, the operating point was relocated away from the point of maximal gain at the centre of the cardiac-baroreflex function curve (i.e. centring point) and towards the reflex threshold to a locus of lesser gain (Figure 2.3) (Gallagher et al., 2001b, Norton et al., 1999, Ogoh et al., 2003b, Ogoh et al., 2005b, Papelier et al., 1994, Potts et al., 1993). This implies that the baroreflex becomes more sensitive to hypertensive compared to hypotensive stimuli. During NP and NS, SV was unchanged both at rest and during exercise (Ogoh et al., 2003b) indicating that CBR-mediated changes in HR are the primary mechanism by which changes in CO are elicited.

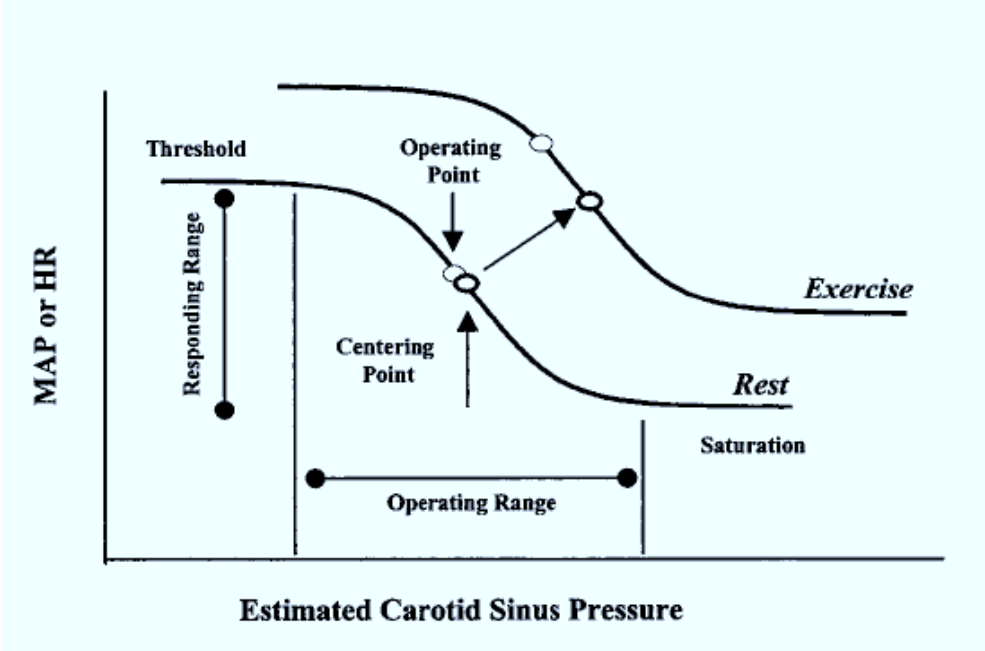

Figure 2.3. Schematic of the resetting of the baroreflex function curve during exercise. (Raven et al., 2002).

Although an upward and rightward resetting has also been observed for the baroreflex control of BP, a movement of the operating point towards the reflex threshold has been identified by some (Norton et al., 1999, Potts et al., 1993), but not all (Gallagher et al., 2001b, Ogoh et al., 2003b). The peak or nadir BP responses to NP and NS have been reported to occur later than the immediate HR responses and are associated with CBR-mediated changes in MSNA (Fadel et al., 2001, Ichinose et al., 2008, Keller et al., 2004, Ogoh et al., 2007). The pressor response to carotid sinus hypotension evoked by bilateral carotid occlusion in dogs running on a treadmill has been shown to be mediated solely by changes in TPR (Collins et al., 2001). In humans, Ogoh et al. (2002a, 2003b) have shown that resting CBR control of BP was predominantly (~80%) mediated by alterations of vascular tone and to a lesser extent (~20%) via changes in CO. However, during dynamic exercise baroreflex-mediated changes in BP were almost entirely elicited by alterations in vasomotor tone.

In recent times, numerous studies have elucidated the influence of central command and the skeletal muscle afferents on arterial baroreflex function. The main results of these studies will be discussed below.

2.4.3 Central command and arterial baroreflex function

Iellamo et al. (1997) have studied the influence of central command on spontaneous cardiac baroreflex sensitivity (cBRS) using the sequence technique (detailed description: Section 3.4.2). From the shallower slope of the regression line relating systolic BP and RRI (i.e. indicator of reduced cBRS) during stimulated dynamic quadriceps exercise (i.e. leg kicking) compared to voluntary exercise, central command was suggested to preserve cBRS. However, since spontaneous measures of cBRS only reflect the operating point gain of the cardiac-baroreflex function curve, it remained unclear whether central command only relocated the operating point upwards and/or resets the full baroreflex function curve.

Ogoh et al. (2002b) has demonstrated that attenuating central motor drive during exercise attenuates CBR resetting, without affecting the maximal gain. Adapting the experimental approach from Goodwin et al., they had subjects performing (i) knee extension with or without patellar tendon vibration to excite the primary afferents of the muscle spindles of the contracting quadriceps muscles at the same force (e.g. less central command with agonist vibration); and (ii) knee flexion with or without patellar tendon vibration to antagonise hamstring muscle contractions at the same force (e.g. more central command with antagonist vibration). In support of these findings, Gallagher et al. (2001c) have shown that increased central command during dynamic leg cycling with partial neuromuscular blockade also elicits a relocation of the operating point of the baroreflex function curve to a location of lesser gain closer to the reflex threshold. These findings indicate that central command contributes to the resetting of the baroreflex and to the

operating point movement during exercise. The latter is evidenced by a greater sensitivity to hypertensive than hypotensive stimuli during exercise.

2.4.4 Skeletal muscle afferents and arterial baroreflex function

Gallagher et al. (2001b) have activated skeletal muscle afferents by inflation of medical anti-shock trousers up to 100 mmHg. They have found that skeletal muscle afferents actively regulate the bidirectional carotid-vasomotor baroreflex resetting, while shifting the CBR-cardiac function curve only to the right to operate around higher pressures. These results are in accordance with the findings from Smith et al. (2003). They have used epidural anaesthesia to partially blocked muscle afferent feedback and demonstrated that skeletal muscle afferent feedback is requisite for a full baroreflex resetting during exercise. However, the application of medical anti-shock trousers or epidural anaesthesia both present models that do not distinguish between the contribution of mechanically and metabolically sensitive skeletal muscle afferents to the baroreflex resetting. Moreover, exercise with medical anti-shock trousers is likely to enhance the effort needed to perform the exercise task. This means that augmented activation of central command may contribute to the augmented CBR resetting.

The combined application of medical anti-shock trousers (to activate muscle afferents) and simultaneous neuromuscular blockade (to augment central command) has been demonstrated to elicit a greater resetting of the CBR than observed during isolated over-activation of either them alone (Gallagher et al., 2006). From this, it appears that the CBR resetting during exercise occurs by the combined actions and interaction of central command and the muscle afferent feedback.

2.4.5 Mechanoreflex and baroreflex

McWilliam and Yang (1991) have examined CBR function in the decerebrate cat model during selective electrical stimulation of group III and IV afferents. They found that a hypertensive stimulus evoked a prolongation of the RRI, which was attenuated during group III and IV afferent stimulation. Later, McMahon and McWilliam (1992) have confirmed these results and showed that the attenuated cardiac responses to baroreflex stimulation during electrically evoked muscle contractions could be blocked by atropine. This indicates that peripheral reflex activation inhibits the baroreflex-mediated cardiac vagal responses.

Iellamo et al. (1997) have determined cBRS in humans during electrically evoked exercise under free-flow conditions in an attempt to isolate mechanoreflex influences on cBRS (e.g. absence of central command and muscle metaboreflex). In agreement with (McWilliam and Yang, 1991) and (McMahon and McWilliam, 1992), they have found that spontaneous cBRS was reduced by the muscle mechanoreflex. During PEI with and without passive calf stretch, Drew et al. (2008b) have further demonstrated that mechanoreceptor activation evokes an upward and rightward resetting of CBR cardiac function curve without affecting CBR control of BP. This led them to suggest that stretch sensitive muscle afferents might be sensitised in the presence of muscle metabolites, but do not contribute to the resetting of the vasomotor baroreflex function curve during exercise. However, Hayes et al. (2005) have identified differential discharge properties of group III and IV muscle afferents in response to contraction and muscle stretch in animals. Moreover, far less group III muscle afferents were contraction sensitive. Whether the results from studies employing muscle stretch hold true for the contribution of the muscle mechanoreflex to baroreflex resetting during exercise, which to a large extent stimulates contraction sensitive skeletal muscle afferents, remains unclear. Thus, the effect of muscle

mechanoreflex activation on the reduction in cBRS during large muscle mass dynamic exercise needs investigation.

2.4.6 Metaboreflex and baroreflex

In an attempt to investigate the effect of the muscle metaboreflex on baroreflex function, Iellamo et al. (1997) have compared cBRS during electrically evoked exercise under free-flow or complete flow-occlusion. While free-flow electrically stimulated muscle contractions elicited a decrease in spontaneous cBRS, sensitivity was preserved with occluded circulation. Therefore, the authors suggested that the muscle metaboreflex may serve to maintain cBRS during exercise. Further, Spaak et al. (1998) have determined CBR function during PEI after static arm exercise and found no effect of the muscle metaboreflex on CBR cardiac sensitivity. This has since been replicated in subsequent studies (Cui et al., 2001, Fisher et al., 2010, Fisher et al., 2008, Ichinose et al., 2002, Iellamo et al., 1999b). In a recent study, Fisher et al. (2008) have examined whether the magnitude of metaboreflex activation would influence cBRS around the operating point of the CBR cardiac function curve. They reported that this was not the case during PEI after handgrip exercise. These results seem to indicate that the muscle metaboreflex does not contribute to the cardio-acceleration via reductions in cBRS during exercise in humans.

In contrast, recent work in dogs running on a treadmill has suggested that the muscle metaboreflex indeed reduces spontaneous cBRS (Sala-Mercado et al., 2007). The main difference between the above described studies in humans (Cui et al., 2001, Fisher et al., 2010, Fisher et al., 2008, Ichinose et al., 2002, Iellamo et al., 1999b) and the study by Sala-Mercado et al. (2007) concerns the methodology of metaboreflex assessment. In dogs, a pneumatic occluder is placed around the terminal aorta. This reduces blood flow to the working hindlimb muscles (i.e. hypoperfusion) and causes accumulation of metabolic by-

products in the exercising muscles, which stimulate metabolically sensitive afferents. In humans, the muscle metaboreflex is commonly investigated during PEI, by inflation of a pressure cuff to trap the metabolites accumulated during exercise (Alam and Smirk, 1937). At present, it is unclear whether hypoperfusion of dynamically exercising skeletal muscles similarly reduces cBRS in humans. If this was the case, it means that enhanced muscle metaboreflex activation contributes to the rise in HR during exercise via a decrease in cBRS. Criticism of this model has derived from the possibility that enhanced central command during partial flow-restriction might cause the decrease in cBRS, as the effort required to maintain running velocity is likely increased. However, no simple indication of central command such as a rating of perceived exertion is feasible in canines.

2.4.7 MSNA and baroreflex

In an attempt to determine whether the exercise-induced increases in MSNA during isometric handgrip exercise are modulated by the arterial baroreflex, Scherrer et al. (1990) have pharmacologically manipulated BP during exercise to control baroreflex input. They found MSNA responses to be augmented when the normal BP rises during exercise were suppressed by nitroprusside infusion. Conversely, noradrenaline infusion, to evoke exaggerated BP response during exercise, attenuated MSNA responses. Eckberg and Wallin (1987) have used short pulses of NP and NS (5 seconds) to elicit CBR perturbations. They found that during brief handgrip the increases in MSNA during NP and the augmented reductions of MSNA during NS were diminished. Baroreflex sensitivity in the control of MSNA is frequently expressed as the relationship between spontaneous fluctuations in diastolic BP and MSNA. While, MSNA baroreflex sensitivity has been demonstrated to be increased during isometric handgrip exercise (Kamiya et al., 2001), Fadel et al. (2001) have found it to be well preserved during dynamic arm cycling. In

addition, the CBR stimulus response curve for MSNA was reset similarly to the CBR curve of BP without a change in maximal gain. This suggests that CBR in control of MSNA is closely linked to muscle metaboreflex activation. This is evidenced by the gradual increase in sensitivity during isometric handgrip exercise (e.g. gradual increase in metabolite accumulation) and by the elevated sympathetic baroreflex sensitivity during PEI after isometric exercise (e.g. isolated muscle metaboreflex activation) (Cui et al., 2001, Ichinose et al., 2006).

In the context of the maintained baroreflex control of BP during exercise, the increased MSNA baroreflex sensitivity seems counterintuitive. In light of the dominant contribution of vascular responses to baroreflex-mediated changes in BP, Ogoh et al. (2009) suggested that the transduction of MSNA into peripheral vascular responses (e.g. peripheral arc) during muscle metaboreflex activation might be attenuated to counteract for the increase in MSNA baroreflex sensitivity. Indeed, they have found that during PEI when MSNA-baroreflex sensitivity was increased (i.e. neural arc) the sensitivity of the peripheral baroreflex arc was attenuated. This suggests that neural and peripheral arc of the baroreflex are differentially modulated to ensure the maintenance of the overall sensitivity of baroreflex control of BP.

2.5 Muscle blood flow control during exercise

2.5.1 Exercise hyperaemia

Increases in skeletal muscle blood flow during dynamic exercise, commonly referred to as ‘exercise hyperaemia’, have been recognised for over 100 years. The search for the underlying mechanism includes the investigation of neuronal mechanisms, such as sympathetic cholinergic vasodilation, sympathetic withdrawal or vasodilation via the

release of acetylcholine. Alternatively, mechanical factors such as the 'muscle pump' have been determined (Sheriff et al., 1993). Finally, attempts have been made to identify vasodilator substances released by the muscle or endothelium, or carried by the blood. Since neural factors seem to be unlikely to contribute to the exercise hyperaemia, this section will focus on mechanical and metabolic determinants.

The 'muscle pump' is characterised by mechanical deformation of veins during muscle contractions. This causes an increase the arterio-venous pressure gradient after release of a contraction and thus a rise in blood flow. Therefore activation of the muscle pump increases venous return and promotes elevations in SV and CO. To mimic the muscle pump-induced emptying of veins rhythmical inflation of a cuff around the forearm has been used. This manoeuvre increased blood flow, but only when a venous hydrostatic column existed (e.g. no blood flow increase when arm was above heart level) (Tschakovsky et al., 1996). In contrast, during passive knee extension with the legs placed above heart level, to minimise muscle pump contribution, the temporal properties of the blood flow increases were comparable with those during voluntary exercise (Wray et al., 2005). This suggests that although the muscle pump contributes to the blood flow rise and maintenance during exercise, it cannot account solely for this phenomenon.

Given the delay with which potential vasodilator metabolites can be produced and/or released, it is unlikely that these metabolites contribute to the immediate hyperaemia at exercise onset. Rather, they may be more important in the modulation of skeletal muscle blood flow during steady state exercise. Putative candidates include nitric oxide, prostaglandins, adenosine and/or ATP, which are released from myocytes, vascular endothelium or the red blood cells. Blockade of nitric oxide synthase, an enzyme catalysing nitric oxide production, has been demonstrated to attenuate the exercise-induced increases in forearm blood flow (FBF) (Gilligan et al., 1994). This indicates that nitric

oxide plays an important role in the hyperaemic response to exercise. However, these results are not universal and the magnitude of flow reductions varied from 0-30% (Dyke et al., 1995, Hillig et al., 2003, Schrage et al., 2004, Shoemaker et al., 1997). Possible reasons might derive from the exercise mode (e.g. arm or leg exercise), the infused blocking agent (e.g. L-NMMA or L-NAME) or the method applied to measure blood flow (e.g. strain gauge plethysmography, thermodilution or Doppler ultrasound). In particular, the use of strain gauge plethysmography to assess exercising blood flow is problematic, because blood flow can only be measured between contractions or after exercise. Therefore it is not a precise reflection of exercising skeletal muscle blood flow (Dinenno and Joyner, 2003).

Boushel et al. (2002) have found that administration of indomethacin to block prostaglandin synthesis, elicited a 10-20% reduction in blood flow during leg exercise. It was suggested that both nitric oxide and prostaglandins contributed at least in part to the exercise hyperaemia, but that there might be a redundancy or interaction between them. To investigate this, concomitant blockade of nitric oxide synthase and prostaglandins has been performed during forearm (Schrage et al., 2004) and leg exercise (Mortensen et al., 2007, Mortensen et al.). The reduction in exercising blood flow during combined blockade was greater than during single blockade of these substances, although vasodilation was not completely blocked. Moreover, adenosine was proposed as a putative vasodilator, since exercising blood flow was reduced by approximately 20% during adenosine receptor blockade (Radegran and Calbet, 2001). However, adding adenosine receptor blockade to the double block (i.e. nitric oxide synthase plus prostaglandins), did not further decrease exercising blood flow. This implies that an additive effect of these vaso-relaxants can be discounted.

Another putative vasodilator mechanism relates to erythrocytes, the ‘carrier’ of oxygen. When oxygen is removed from haemoglobin to the muscle nitric oxide and/or

ATP are released and vasodilation occurs through activation of the endothelium (Ellsworth et al., 1995). In line with this, a tight relationship has been demonstrated to exist between exercise-induced increases in blood flow and alterations in venous plasma ATP concentration (Gonzalez-Alonso et al., 2002). However, the blockade of nitric oxide only marginally affected exercising blood flow. Moreover, in patients with cystic fibrosis, a condition in which ATP release is attenuated, vasodilator responses were similar compared to age matched controls (Schrage et al., 2005).

Taken together, pharmacological blockade of one system (e.g. nitric oxide) or multiple systems (e.g. nitric oxide, prostaglandins and adenosine) produces only attenuated exercise hyperaemia, but does not abolish it completely. Thus it is suggested that multiple redundant pathways contribute to the hyperaemic response, so that when one is blocked or absent, blood flow is maintained to ensure sufficient blood supply (Joyner, 2011). Alternatively, exercise hyperaemia might relate to a substance which has not been found yet.

2.5.2 Muscle afferent feedback and blood flow

Muscle perfusion is a major determinant of the exercise pressor response. Especially the accumulation and washout of muscle metabolic by-products (e.g. muscle metaboreflex stimulation) depends on skeletal muscle blood flow. The large pressor response seen during isometric handgrip exercise has been attributed to reductions in blood supply induced by mechanical compression of the vessels enclosed by the isometrically contracting muscles (Barcroft and Millen, 1939). The pressor response to exercise under ischemic conditions was attenuated compared to free-flow conditions (Alam and Smirk, 1937). This indicates perhaps that a mismatch between blood supply and demand causes a metabolic error signal, which is ‘sensed’ by metabolically sensitive afferents, which

initiate a pressure rise in order to restore blood flow (Rowell, 1993b). Wyss et al. (1983) had dogs running on a treadmill, equipped with an occluder around the terminal aorta to gradually reduce hindlimb blood flow during exercise. During mild exercise initial occlusions led to stepwise reductions in blood flow without affecting BP and HR. Larger flow restrictions caused a marked pressor response. However, during higher intensity running smaller reductions in flow were necessary to evoke a pressor response. It was concluded that there is a threshold associated with muscle perfusion, which when passed activates the muscle metaboreflex. This interaction between blood flow reductions and pressor response led to the assumption that initially central command mediates the pressor response. Once the metabolic error is detected, the muscle metaboreflex restores blood flow (although not completely) by an increase in BP (e.g. flow-raising effect of the muscle metaboreflex). Conversely, Joyner (1991) asked subjects to perform rhythmic handgrip exercise with the arm placed in a positive pressure box to restrict blood flow. This evoked increases in BP and concomitant decreases in venous oxygen saturation, while blood flow remained partially restricted (e.g. no flow-correction). In a recent study, Casey and Joyner (2009) have applied the dog model from Wyss and colleagues in humans. They reported that hypoperfusion (-37-41%) as induced by inflation of a balloon in the brachial artery in the rhythmically exercising forearm evoked a restoration of forearm blood flow (75%) in the absence of BP changes. This indicates that the restoration of blood flow during mild exercise relies primarily on local dilator responses and not reflex rises in BP. It is more likely that the muscle metaboreflex-induced increases in sympathetic activity during large muscle mass dynamic exercise serves to maintain BP by restricting blood flow in the active skeletal muscle. Thus excessive local vasodilation is avoided, which would lead to a drop in BP. The challenge of maintaining a balance between increased muscle perfusion during exercise and maintaining BP control will be discussed below.

2.5.3 Autonomic control of skeletal muscle blood flow

Previous studies only measured from the resting limb assuming a general efferent sympathetic outflow (Victor and Seals, 1989). However, to determine autonomic control of blood flow, MSNA to the exercising skeletal muscle would need to be measured. To overcome this limitation, Hansen et al. (1994) asked subjects to perform static toe extension followed by PEI and measured MSNA from the peroneal nerve of the contracting and resting leg. They found that MSNA increased within the second minute of exercise equally in the active and non-active leg. These bilateral increases in MSNA were maintained during PEI. This suggests that MSNA recordings from the quiescent limb are representative for efferent sympathetic outflow to the active muscles.

During exercise, increases in sympathetic tone and exercise hyperaemia seem to oppose each other (Figure 2.4). Controversy exists regarding the effects of increased sympathetic outflow on the perfusion of the active skeletal muscle. Joyner et al. (1992) asked subjects to perform graded levels of rhythmic handgrip before and after elimination of sympathetic outflow by local anaesthetic infusion (i.e. lidocaine). They reported that forearm blood flow was increased at rest and during all exercise intensities after sympathetic blockade compared to control conditions. Similarly, O'Leary et al. (1997) have demonstrated greater increases in exercising limb blood flow after elimination of vascular α-adrenergic receptors (i.e. sympathetic blockade) in dogs running on a treadmill. These findings suggest that skeletal muscle blood flow is under constant sympathetic restraint and that sympathetic constrictor action overrides the exercise-induced production of local vasodilator substances. However, as mentioned earlier, the use of venous occlusion plethysmography, as by Joyner et al., only allows flow measurements in a quiescent muscle. Thus it is questionable whether these flow values are representative for the active skeletal muscle.

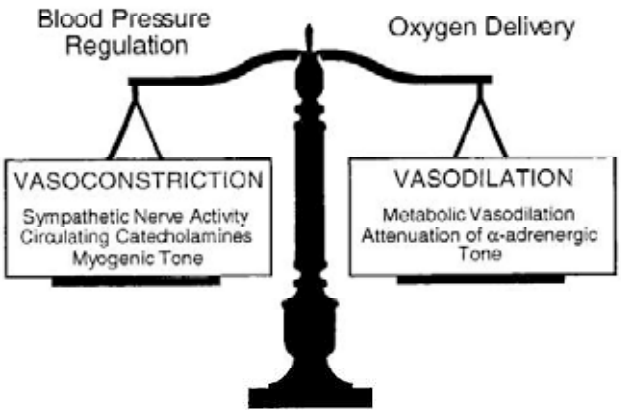

Figure 2.4. Illustration of the desired balance between vasoconstriction and vasodilation during exercise. (Buckwalter and Clifford, 2001).

Remensnyder et al. (1962) have proposed that vasodilator substances released from the contracting skeletal muscle are able to counteract sympathetic vasoconstrictor stimuli to enable sufficient muscle perfusion. They have observed diminished responsiveness to direct (i.e. sympathetic nerve stimulation) and reflex sympathetic stimulation (i.e. infusion of norepinephrine) in the vasculature of the contracting compared to the resting limb. This phenomenon is termed "functional sympatholysis" and has been repeatedly observed (Hansen et al., 2000, Rosenmeier et al., 2003, Wray et al., 2004b). Moreover, these blunted sympathetic vasoconstrictor responses have been shown to be positively related to exercise intensity (Watanabe et al., 2007). Sympathetically-mediated increases in vascular have been demonstrated to occur in large resistance arterioles via α_1-adrenergic receptors and in small arterioles by α_2-adrenergic receptor activation (Thomas et al., 1994). VanTeeffelen and Segal (2003) have reported that sympathetic vasomotor responsiveness was very heterogeneous from proximal to distal branches of the resistance network of skeletal muscles. They observed that perivascular sympathetic nerve activation resulted in restricted blood flow to exercising muscle by constriction of feed arteries and 1st order

arterioles, while in 2nd and 3rd order arterioles dilation prevailed. This selective vasomotor sensitivity to sympathetic activation presents a very attractive explanation. Thus, downstream dilation in the exercising muscle helps to meet the muscles metabolic demands (e.g. oxygen extraction), while upstream constriction serves to maintain BP during exercise.

In order to identify putative mechanisms of functional sympatholysis several local vasodilator substances have been investigated, such as nitric oxide or prostaglandins, and cellular signal transduction, specifically via metabolically regulated ATP sensitive potassium channels (Hansen et al., 2000). Given the potential important role of ATP sensitive potassium channels, it could be questioned, whether the magnitude of functional sympatholysis is dependent on the muscle fibre profile. Further investigations are warranted to explore this fact.

Degtyarenko and Kaufman (2000) have found that central command blunts group III and IV muscle afferent input to the dorsal horn in decerebrate cats. They suggested that central motor activation might contribute to the attenuated vasoconstrictor responses in the exercising skeletal muscle (i.e. functional sympatholysis). However, this has not been explored in humans. Modulation of central command input could be accomplished during leg flexion and extension with or without patellar tendon vibration (Ogoh et al., 2002b). Simultaneous femoral artery blood flow measurements and MSNA recordings with and without sympathetic stimuli (e.g. noradrenaline infusion or CPT) would complete this experimental approach. The sympathetically mediated changes in leg vascular conductance (e.g. functional sympatholysis) observed during assisted leg extension with patellar tendon vibration (e.g. less central command) would be expected to be smaller compared with conductance changes during leg flexion and patellar tendon vibration (e.g. greater central command). In this regard it is worthwhile to address the question whether muscle mass

modulates the strength of functional sympatholysis. It has been suggested that central command is dependent on muscle mass contribution (Mitchell et al., 1980, Mitchell et al., 1981). However, at present no relevant human data is available.

In an attempt to investigate the baroreflex control of blood flow, Keller et al. (2003) have determined the effects NP and NS on leg vascular conductance at rest and during one legged knee-extension. The decreases in perfusion during NP were attenuated in the exercising compared to the non-exercising leg and to rest (Keller et al., 2004, Keller et al., 2003, Wray et al., 2004b). Since CBR control of BP was well maintained from rest to exercise, the attenuated vasoconstriction was attributed to sympathetic restraint (e.g. functional sympatholysis) within the exercising leg muscles (Keller et al., 2004). Wray et al. (2004b) have confirmed and extended these results. They showed a diminished reduction in the microvasculature perfusion in the exercising leg during NP. At present, there is no study available that determines whether microcirculatory perfusion changes in response to hypertensive stimuli are similarly increased at rest and during exercise. In conclusion the arterial baroreflex appears to be a vital controller of muscle blood flow by mediating tissue specific vascular effects to maintain overall systemic BP during exercise.

2.6 Sex-differences in cardiovascular control at rest and during exercise

Epidemiological studies have demonstrated a lower prevalence of heart disease and a lower incidence and severity of hypertension in premenopausal women compared to men, which are diminished following the menopause (Lerner and Kannel, 1986, Schenck-Gustafsson, 1996). These findings suggest significant cardio-protective effects of female reproductive hormones. Recognition of the differences and similarities in men and women with regards to the neural, cardiovascular, and haemodynamic functions mean that sex-differences in the physiological responses to exercise are arguably inevitable. However,

our understanding of the cardiovascular responses to exercise is often based on investigations undertaken in men, but exercise performance is notably greater in men compared to women and men are stronger and faster than women. In physiological terms, this is expressed by the greater peak exercise performance and higher maximal oxygen consumption in men (Higginbotham et al., 1984, Sullivan et al., 1991). Anthropometric characteristics and body composition are markedly different between men and women, such as women have a lower body weight, less muscle and lean body mass compared to men, whereas their percentage of body fat is higher (Charkoudian and Joyner, 2004) (Table 2.1).

Table 2.1. Selective anatomical and physiological differences between men and women.

Anthropometric differences:	
Body weight	Men > Women
Muscle mass	Men > Women
Lean body mass	Men > Women
% body fat	Men < Women
Muscle strength	Men > Women
Cardiac differences:	
SV (rest and exercise)	Men > Women
Left ventricular mass	Men > Women
CO (maximal exercise)	Men > Women
Blood differences:	
Haemoglobin concentration	Men > Women
Haematocrit	Men > Women
Total blood volume	Men > Women
Lung capacity	Men > Women
Oxygen consumption & peak exercise performance	Men > Women

From a cardiac perspective, heart volume, left ventricular mass and resting SV as well as maximal exercise SV and CO are smaller in women than men (Kucher et al., 2001, Olivetti et al., 1995, Roberts and Roberts, 1980). Even expressing maximal CO or oxygen consumption relative to body weight or per kg lean body mass only partly diminishes the

attenuated performance and cardiac function in women (Higginbotham et al., 1984). This implies "*that men are, nevertheless, better working machines than women*" (Astrand, 1956). Moreover, the lung capacity is reportedly smaller in women than men (Harms and Rosenkranz, 2008). In combination with the smaller blood volume, the lower haematocrit and less haemoglobin (de Simone et al., 1991) this explains the hindered oxygen transport in women compared to men (e.g. lower maximal oxygen consumption in women).

2.6.1 Control of HR

Comparisons of the resting HR in men and women have shown that HR tends to be higher in men (Ogawa et al., 1992, Stoney et al., 1987). Sex differences in the autonomic control of the heart (e.g. balance between sympathetic and parasympathetic tone) have been extensively investigated using HR variability measurements. Time domain indices of HR variability, predominantly a measurement of parasympathetic tone (Task, 1996), have been shown to be greater in women compared to men (Sinnreich et al., 1998). This indicates that vagal control of beat-to-beat changes in HR is greater in women. Estimations of the HR variability in the frequency domain concentrate mostly on low frequency (0.04 – 0.15 Hz) and high frequency (0.15 – 0.40 Hz) spectral power. The first is considered to provide an estimation of both cardiac-sympathetic and parasympathetic control, while the latter is indicative of cardiac vagal control. The ratio between low and high frequency spectral power may provide some insight in the balance between sympathetic and parasympathetic cardiac control (Malliani et al., 1991). Commonly, low frequency spectral power is higher and high frequency power lower in resting men compared to women, while the ratio of both seems higher in men (Huikuri et al., 1996, Kuo et al., 1999, Pikkujamsa et al., 2001, Sevre et al., 2001, Sinnreich et al., 1998). However, this is not a universal finding (Laitinen et al., 1998, Ramaekers et al., 1998). These findings confirm the dominance of

cardiac vagal control in women and suggest a greater sympathetic influence on the heart in men. The latter is supported by 2 to 6-fold higher circulating adrenaline levels in men (Evans et al., 2001). A possible explanation for the inconsistency of the results might stem from the very heterogeneous study populations (e.g. exclusion criteria for study participation differed widely), the particularly wide age range (from 18 to 79 years), the duration of the RRI recordings (short- or long-term measurements), and often a lack of control for hormonal status in women (e.g. no distinction between pre- and postmenopausal women). The impact of female reproductive hormones (e.g. estrogen) on the autonomic cardiac control has been demonstrated by Du et al. (1994). They have found a more pronounced reduction in HR in female than male rats in response to vagus nerve stimulation, which was abolished after ovariectomy. In addition, a similar HR variability has been found in postmenopausal women and an age matched group of men (Liu et al., 2003). Intriguingly, postmenopausal women replacing estrogen orally have been demonstrated to exhibit greater high frequency spectral power and reduced low-to-high frequency ratios compared to men and women without hormonal replacement (Liu et al., 2003). These results suggest that estrogen plays a crucial role in the autonomic control of the heart, presumably mediated by a higher level of acetylcholine release following nerve stimulation (Du et al., 1994).

Results from power spectral analyses of HR variability need to be interpreted with care. The parasympathetic contribution to high frequency fluctuations in HR has been demonstrated in terms of abolished respiratory sinus arrhythmia after vagus nerve blockade in dogs (Katona and Jih, 1975) and pharmacological blockade of parasympathetic outflow to the heart with atropine (Pomeranz et al., 1985). However, the interpretation of changes in low frequency spectral power is less clear, as noradrenaline spillover from the heart, a measure of cardiac sympathetic activation, does not correlate with low frequency

fluctuations in HR and/or RRI (Kingwell et al., 1994, Saul et al., 1990). Thus, it is questionable whether the greater low frequency spectral power (e.g. sympathetic chronotrophic cardiac control) observed in men, represents indeed a cardiac sympathetic preponderance.

2.6.2 Sex and hormonal influences on baroreflex control of HR

Examination of sex-differences in resting cardiac baroreflex function has predominantly focused on measurements of cBRS using pharmacological approaches (e.g. modified Oxford technique) to evoke BP decreases and increases by infusion of vasodilator (sodium nitroprusside) and vasopressor (noradrenaline hydrochloride) agents. Using this method, Chen and DiCarlo (1996) have demonstrated that cBRS was 40% greater in female than male rats. However, the results in humans are rather controversial, reporting greater, similar or lower cBRS in women relative to men (Abdel-Rahman et al., 1994, Beske et al., 2001, Laitinen et al., 1998, Tanaka et al., 2003, Tank et al., 2005). One potential reason for these contrasting results may be related to the method of drug administration (e.g. bolus injection vs. sustained infusion) (Abdel-Rahman et al., 1994). Furthermore, some authors have only presented the baroreflex-mediated cardiac responses to BP elevation (Laitinen et al., 1998), thereby neglecting important information about the directional sensitivity of the cardiac response (Young et al., 2008). This information is also lost by the combination of the resultant HR responses to hypo- and hypertensive stimuli, commonly performed to obtain a measure of cBRS. Further limitation derives from the potential side effects of these drugs in that the baroreflex itself buffers the evoked changes in BP resulting in inaccurate measures of cBRS (Diaz and Taylor, 2006). An alternative approach to investigate cardiac baroreflex function in men and women could be the

separate application of hyper- and hypotensive stimuli to the carotid sinus, using the neck chamber technique.

Animal studies have shown that intravenous estrogen administration resulted in increased cBRS and parasympathetic tone while decreasing sympathetic nervous activity (el-Mas and Abdel-Rahman, 1998, Saleh and Connell, 2000, Saleh et al., 2000c). While these animal data indicate that estrogen improves cardiac baroreflex function via increases in vagal tone and sympatholysis, the results in humans are equivocal. Indeed, it has been found that high levels of estrogen and progesterone during the luteal phase have no effect on cBRS compared to the early follicular (EF) phase of the ovarian cycle (Minson et al., 2000a). Similarly, cBRS was unchanged in postmenopausal women after estrogen replacement therapy (Hunt et al., 2001). In line with the findings in animals, Tanaka et al. (2003) have demonstrated that cBRS was significantly elevated during the late follicular (LF) compared to both EF and luteal phases of the ovarian cycle. In summary, these studies suggest that estrogen can increase cBRS in both animals and human, but that progesterone may inhibit this rise in cBRS when both hormones are elevated at the same time.

2.6.3 Control of BP

The autonomic nervous system is an important factor for the tonic control of BP, with the sympathetic nervous system as the key determinant (Christou et al., 2005). BP, particularly systolic BP, has been reported to be lower in young women compared to men at the same age (Christou et al., 2005, Ogawa et al., 1992, Stoney et al., 1987). Moreover, there is a tendency of elevated noradrenaline concentrations (Evans et al., 2001) and higher MSNA (Ng et al., 1993) in men indicating lower sympathetic tone in females. Barnett et al. (1999) have found a smaller increase in low frequency BP fluctuations in women

compared to men, which are suggested to represent sympathetically-mediated BP control. Thus, they presumed that women have a lower autonomic support of BP. Direct experimental evidence stems from Christou et al. (2005), who have used trimethaphan infusion (i.e. ganglionic blockade) to block parasympathetic and sympathetic nervous outflow in both sexes. They showed that the resultant reductions in BP were lower in women than men.

Sympathetic nerve activity is also important for the control of TPR, which has been often reported to be higher in women than men (Christou et al., 2005, Lambert et al., 2007). But this is not an exclusive finding (Convertino, 1998). Although, in men, TPR is positively related to MSNA, and MSNA shows an inverse relationship with CO (Charkoudian et al., 2005), these relationships are not evident in women (Hart et al., 2009, Hogarth et al., 2007). This indicates that there are profound sex-differences in the resting autonomic control of BP. In this regard, arterial baroreflexes may play an important role in the overall beat-to-beat regulation of BP via the autonomic nervous system.

2.6.4 Sex and hormonal influences on baroreflex control of BP

To date, the majority of studies examining arterial baroreflex function have used pharmacological approaches that do not allow an assessment of the BP responses to baroreflex perturbation. Therefore, it remains unclear whether there are sex-differences in the baroreflex-mediated control of BP. As mentioned, alterations in TPR are the predominant means by which the baroreflex elicits changes in BP (Collins et al., 2001, Ogoh et al., 2002a, Ogoh et al., 2003b). However, these studies were not designed to investigate potential sex-differences. Nevertheless, there is plenty of evidence to suggest that sex-differences might exist in the baroreflex-mediated changes in vascular tone.

The lower sympathetic tone in women compared to men (Jones et al., 1996, Ng et al., 1993) may result from alterations in baroreflex buffering of changes in sympathetic nerve activity and/or sympathetic baroreflex sensitivity. Tank et al. (2005) have observed similar increases and decreases in systolic BP to stepwise infusion of sodium nitroprusside and adrenaline in men and women. Although they have not observed sex-differences in the baroreflex buffering of changes in MSNA, the reflex function curve was found to operate at a lower prevailing BP in women. However, Hogarth et al. (2007) have used the close relationship between MSNA and short-term changes in diastolic BP to estimate sympathetic baroreflex sensitivity during Valsalva manoeuvres, which provide a pressure rising stimulus. They found that sympathetic baroreflex sensitivity was greater in women than men. These contrasting findings are likely to be related to the methodology of baroreflex assessment. Tank et al. included hypo-and hypertensive stimuli in their calculation after pharmacological BP perturbation, while Hogarth et al. examined sympathetic baroreflex sensitivity only during pressure rises.

A greater adrenergic receptor responsiveness to sympathetic stimulation in men has been demonstrated by means of larger decreases in FBF in response to noradrenaline infusions in men compared to women (Kneale et al., 2000, Kneale et al., 1997, Luzier et al., 1998). Despite similar increases in MSNA to sympatho-excitatory manoeuvres such as the CPT, women were found to exhibit an attenuated increase in vascular resistance (Hogarth et al., 2007). These findings demonstrate that sympathetic vasoconstriction is impaired in women compared to men. In combination with the lower autonomic support of BP in women, it might be presumed that baroreflex control of BP is altered in women compared to men.

Estrogen influences on the sympathetic arc of the arterial baroreflex, have been investigated in ovariectomized rats. Administration of 17-β estradiol has been reported to

inhibit renal and splanchnic sympathetic nervous outflow and increase sympathetic baroreflex sensitivity (He et al., 1998). But attenuated baroreflex-mediated sympatho-excitation (e.g. sympathoinhibition) to a hypotensive stimulus has been found in pregnant animals, when progesterone concentrations are largely elevated (Heesch and Rogers, 1995). In humans, sympathetic baroreflex sensitivity is greater in women during the luteal (high estrogen and progesterone) compared to the EF phases (low estrogen and progesterone) of the ovarian cycle. This means that for a given change in BP the changes in MSNA were greater when estrogen and progesterone were both elevated (Minson et al., 2000a). Importantly, the static handgrip-induced increase in MSNA evoked comparable increases in vascular resistance during these phases. Therefore, they concluded that the increased sympathetic baroreflex sensitivity during the luteal phase cannot simply be explained by enhanced neural vascular transduction. Further investigations by Hunt et al. (2001) have reported increased sympathetic baroreflex sensitivity in postmenopausal women supplementing with estrogen compared to untreated postmenopausal women. These results suggest that progesterone might increase MSNA and therefore off-set the sympatho-inhibitory effects of estrogen when both hormones are elevated. Alternatively, the vasodilatory effects of estrogen, which will be discussed in detail in a later section, might have counteracted the increases in MSNA observed during the luteal phase and lead to the maintenance in vascular resistance observed in the experiments by Minson and colleagues (2001a).

While afferent discharge from baroreceptors was not influenced by hormonal changes in pregnant rats, it was suggested that female hormones mediate their effects via central mechanisms (Heesch and Foley, 2001). Injection of estrogen into brain stem nuclei associated with central cardiovascular control (e.g. NTS and RVLM) decreased both baseline and adrenaline-induced increases in sympathetic outflow and BP (Saleh et al.,

2000a). Although animal studies provide evidence of the potential mechanisms by which ovarian hormones might modulate sympathetic baroreflex function there is clearly a need for further research in humans.

2.6.5 Sex and hormonal influences on the cardiovascular response to exercise

Increases in SV and ejection fraction (fraction of blood ejected with each heart beat) with exercise have been reported to be attenuated in women (Hossack and Bruce, 1982, Martin et al., 1991). This seems to be true exclusively for maximal dynamic exercise as ejection fraction was similar in men and women at submaximal exercise intensities (Higginbotham et al., 1984). For a given absolute workload the exercise-induced increases in HR were greater in women compared to men (Astrand, 1956). However, if HR has been compared at a workload relative to the individual maximum during static and dynamic exercise there were no sex-differences existent (Ettinger et al., 1996, Fleg et al., 1995, Higginbotham et al., 1984). Therefore, lower increases in SV and similar HR increases elicit a lower CO response to exercise in women. Fluctuations of female reproductive hormones during the ovarian cycle have been shown not to affect the cardiac response to static (Ettinger et al., 1996) and dynamic exercise (Kuwahara et al., 2005). This has been confirmed by studies in postmenopausal women receiving supplemental estrogen (Pines et al., 1998).

Young healthy women show attenuated BP responses to both static (Ettinger et al., 1996, Jarvis et al., 2011, Petrofsky and Lind, 1975) and dynamic (Fleg et al., 1995, Green et al., 2002, Ogawa et al., 1992, Sullivan et al., 1991) exercise compared to young healthy men. However, the reasons for this are unclear. Possible explanations include an attenuated vasoconstriction in inactive tissues during exercise (e.g. kidney, splanchnic), a reduced ability to adjust skeletal muscle blood flow (e.g. altered endothelium-dependent

vasodilation), attenuated sympathetic outflow and/or attenuated adrenergic receptor sensitivity on the vascular smooth muscle. Fleg et al. (1995) have found no differences in systemic vascular resistance during cycling at similar relative workloads in men and women. However, when concentrating on skeletal muscle blood flow a greater increase in vascular conductance in the exercising limb has been observed in females compared to males (Parker et al., 2007, Rogers and Sheriff, 2004).

Despite a lower resting MSNA in women, the question remains whether this difference is sustained during exercise. MSNA responses to static handgrip have been shown to be attenuated in women compared to men (Ettinger et al., 1996, Jarvis et al., 2011, Jones et al., 1996). However, whether these results can be translated to dynamic exercise with a large muscle mass remains to be eluded. MSNA measures are very sensitive to movement which limits the maintenance of good quality recordings during large muscle dynamic exercise. Ichinose et al. (2008) have measured MSNA from the median nerve of the arm during an incremental bout of leg cycling and observed an approximate 8-fold increase in MSNA from very mild to exhaustive exercise. Application of this experimental protocol in the context of sex-differences in the BP control during exercise would provide valuable information, including the potential sex-differences in the baroreflex control of MSNA during exercise.

Female sex hormones may influence the cardiovascular response to exercise (Astrand, 1956). However, increases in CO, HR and SV during submaximal dynamic exercise have been reported not to differ in women during LF and luteal phases. However, women maintained exercise at high workloads for longer when concentrations of estrogen and progesterone were high, which coincided with lower lactate concentrations (Jurkowski et al., 1981). These results suggest that sex hormones do not modulate central hemodynamic responses to exercise. But Jurkowski et al. (1981) proposed that estrogen

may affect carbohydrate metabolism such that in the presence of high estrogen concentrations glycogen is metabolised more sparsely.

The increases in BP during submaximal and maximal dynamic exercise were found to be more pronounced in older than younger women (Fleg et al., 1995, Martin et al., 1991, Ogawa et al., 1992, Petrofsky and Lind, 1975, Younis et al., 1990). Green and colleagues (2002) have observed a 14 mmHg reduction in peak exercise systolic BP after hormonal replacement in postmenopausal women compared to age matched postmenopausal women not taking hormones. This suggests that female reproductive hormones may contribute to the attenuated BP response observed in young premenopausal women. To identify which female hormone causes the altered BP response, Lewandowski et al. (1998) have tested young ovariectomized women with and without estrogen replacement during running. They have found that BP increases were attenuated in women with estrogen supplementation. This suggests that estrogen is the main hormone influencing the exercise pressor response. No ovarian cycle effect (LF versus luteal phase) on exercise-induced BP rises was reported (Lewandowski et al., 1998). This indicates that progesterone may oppose the estrogenic effect (e.g. inhibition of BP rise) on exercise pressor response, because progesterone is elevated during the luteal but low during the LF phase, while estrogen concentration is similarly high in both phases.

In contrast, during static exercise, BP responses have been reported to be unaffected by the ovarian cycle (Petrofsky et al., 1976). However, exercise-induced increases in MSNA have been found to be attenuated when estrogen levels were high during the LF relative to the EF phases (Ettinger et al., 1998). Similar observations were reported by Fadel et al. (2004a) in postmenopausal women before and after short-term estrogen replacement during dynamic handgrip exercise. These results indicate that the effect of female reproductive hormones is dependent on the exercise modality (e.g.

isometric vs. dynamic), potentially due to the apparent differences in skeletal muscle perfusion. A nonspecific generalized influence of the ovarian cycle on sympathetic activation appears unlikely as comparable increases in MSNA and BP during a CPT have been reported (Ettinger et al., 1998, Fadel et al., 2004a). Given the exercise-modality specific blood flow responses and the fact that estrogen has been described to exert vasodilation via actions on the endothelium (Hashimoto et al., 1995), it may be suggested that the attenuated BP response to dynamic exercise is caused by an estrogen-mediated reduction in TPR. In addition, whether estrogen affects the increases in MSNA during dynamic exercise involving large muscle masses remains subject to further investigation. Performance of MSNA recording from the median nerve during leg cycling (Ichinose et al., 2008) in women across the ovarian cycle could be an adequate experimental model to address this question.

2.6.6 Sex and hormonal influences on central command

The scientific literature examining the influence of central command on the sex-differences in the pressor response to exercise is very sparse. Although, the influence of muscle fibre composition on central command is a field of controversy, the relatively higher percentage of slow-twitch fibres in female skeletal muscle may reduce the magnitude of central command input needed to evoke a similar force compared to men. According to the theoretical model proposed by Hobbs and Gandevia (1985), increased central command is necessary to activate more motor units, causing higher pressor responses. As higher firing frequencies are needed to activate fast-twitch fibres compared to slow-twitch fibres (e.g. low threshold for activation), more central command may be needed to produce the same force in a muscle with a higher proportion of fast-twitch fibres

as reported in men compared to women (Komi and Karlsson, 1978). In this way, a greater central command input may contribute to the greater exercise pressor response in men.

Hayes and colleagues (2002) attempted to identify the effect of estrogen on cardiovascular responses to electrical stimulation of the mesencephalic locomotor region in the decerebrate, paralysed cat model. Although, estrogen receptors have been identified in several brain areas involved in the central cardiovascular control, their expression in the midbrain, thalamus and insular cortex has been shown to be apparently rather sparse (Pfaff and Keiner, 1973). Intriguingly, intravenously injected 17β-estradiol attenuated the increases in HR and BP when central command was evoked in isolation. However, the mechanism(s) of the estrogen-mediated attenuation of the exercise pressor response remain unclear. Moreover, no human data is available on this phenomenon.

2.6.7 Sex and hormonal influences on the muscle afferent feedback

A lower muscle metabolite accumulation during exercise may lead to an attenuated pressor response via less stimulation of metabolically sensitive muscle afferents. Indeed, previous studies have shown that substrate metabolism differs between men and women, by virtue of a greater ability to oxidize fat and a rather sparingly operating glycogen metabolism in women (Gorski et al., 1976, Kendrick and Ellis, 1991). In line with this, muscle function analyses have revealed slower contractile characteristics and muscle biopsies from the vastus lateralis have indicated a smaller cross-sectional area of slow-twitch and fast-twitch fibres in women compared to men (Komi and Karlsson, 1978). These results suggest that when men and women perform the same relative work, women would rely more on aerobic metabolism. Thus their muscle metabolite production would be lower, which leads to less muscle metaboreceptor stimulation and a smaller pressor response. Indeed, during static handgrip exercise at the same relative workload followed by

PEI the increases in MSNA and BP were found to be smaller in women compared to men (Ettinger et al., 1996). This suggests that the muscle metaboreflex is attenuated in women. Ettinger et al. (1996) have also assessed muscle metabolic status and have identified that the attenuated exercise pressor response in women coincided with lower pH and less accumulation of dihydrogen phosphate ions during PEI. This indicates that the female exercising muscles exhibit less acidosis and less metaboreflex activation. However, they have used a very heterogeneous female study sample, which included both pre- and postmenopausal women, with some on hormonal replacement therapy and some not, and without controlling for the ovarian cycle phase. This is important in light of potential influences of female reproductive hormones on skeletal muscle afferents, which will be discussed below. Despite the advantages of PEI (Section 2.3.1), autonomic control of HR during PEI differs markedly from exercise conditions. During PEI after handgrip exercise HR returns to resting levels via vagal reactivation, while HR is elevated during exercise via vagal withdrawal and increased cardiac sympathetic activation (Robinson et al., 1966). Therefore, it remains unclear whether sex-differences in the muscle metaboreflex function during PEI are also present when the muscle metaboreflex is engaged during exercise. Moreover, data from human studies are derived predominantly during small muscle mass static exercise (e.g. handgrip). Therefore, the applicability to large muscle mass dynamic exercise remains to be determined.

Laprad et al. (1999) have overcome some of these limitations by having male and female dogs running on a treadmill while gradually enhancing muscle metaboreflex activation by stepwise inflation of a pneumatic occluder around the terminal aorta supplying the exercising hindlimb. They observed similar increases in HR, BP, and CO and decreases in renal vascular conductance for a given reduction in terminal aortic blood flow. There was no effect of sex on metaboreflex threshold and sensitivity indicating that

sex does not influence the muscle metaboreflex in dynamically exercising dogs. However, it remains unclear whether these results in canines are translatable into humans.

The influence of estrogen on the skeletal muscle afferents has been elegantly investigated in the decerebrate cat model. Ventral root stimulation has been performed with and without direct application of 17β-estradiol on the dorsal root of the spinal cord (L6-S1) (Schmitt and Kaufman, 2003a, Schmitt and Kaufman, 2003b). The exercise pressor response was observed to be similarly attenuated by approximately 50% in male and female estrogen-treated animals compared to the pre-treatment response. However, to obtain a similar effect in male cats a higher dose of estradiol was necessary. This indicates that estrogen sensitivity in the spinal cord of female cats is augmented. Moreover, these results point towards the possibility that estrogen is able to interact with the afferent signalling pathway of thin group III and IV skeletal muscle afferent fibres. However, central effects of estrogen might also modulate the pressor response. In animals, estrogen receptor populations (ERα and/or β) have been identified in many brain areas associated with the neural integration of peripheral reflexes (i.e. metabo- and mechanoreflex) including the NTS, the RVLM and CVLM (Asarian and Geary, 2007, Vanderhorst et al., 2005). Furthermore, bilateral injection of estrogen into these nuclei has been shown to decrease HR, BP and sympathetic outflow an increase parasympathetic nerve activity (Saleh et al., 2000a). Despite this, it remains unclear whether these central effects of estrogen at rest are transferable to humans and/or to exercise conditions.

2.6.8 Sex and hormonal influences on arterial baroreflex function during exercise

A potential explanation for the attenuated BP response to exercise in women might be an attenuated resetting of the arterial baroreflex function curve or increased baroreflex

sensitivity in control of BP. However, the influences of sex-and/or ovarian hormone concentrations on the baroreflex control of BP during exercise have not been investigated.

Central command and skeletal muscle afferents contribute to the integrated baroreflex resetting and control of BP and HR during exercise in men. The decreases in cBRS during dynamic exercise can be attributed to the actions of the muscle metaboreflex (Sala-Mercado et al., 2007). The muscle metaboreflex has been suggested to be attenuated in women compared to men during static exercise, potentially depending on the actions of female reproductive hormones (Ettinger et al., 1996, Ettinger et al., 1998) However, it remains to be determined whether differences in the muscle metaboreflex activation between men and women also affect the reduction in cBRS during dynamic exercise, including examination of the influence of female reproductive hormones.

2.6.9 Sex differences and hormonal influences on exercising blood flow

The enhanced skeletal muscle vasodilation during exercise in females (Parker et al., 2007, Rogers and Sheriff, 2004) may be explained by differences in vascular reactivity, vascular receptor responsiveness and/or differences in endothelium-dependent and independent dilatory mechanisms. Rogers et al. (2004) have found a reduced basal tone and greater flow-induced dilation in isolated arterioles from female compared to male rats. While abolition of the prostaglandin-induced vasodilatation completely abolished perfusion in these male rats, blood flow was reduced by only 75% in female rats. Moreover, estrogen treatment in ovariectomized rats running on a treadmill increased exercising hindlimb blood flow and attenuated the BP increase during exercise (Huang et al., 1998). The estrogenic influences on BP were abolished after inhibition of prostaglandins. However, the estrogen-mediated increases in blood flow were abolished after blockade of nitric oxide synthase. Taken together, these animal data indicate that the

sex-specific vasodilator response depends on estrogen-mediated increase in nitric oxide. In humans, Proctor et al. (2004) have observed up to 38% reduction in peak exercise femoral blood flow and vascular conductance in older women compared to young women. Such a decline was not present in old compared to young men. These findings support the notion that increased exercise blood flow can at least in part be attributed to the vasodilator actions of estrogen possibly via enhanced nitric oxide release from the endothelium (Sudhir et al., 1996).

The aforementioned sex-differences in sympathetic activity and sympathetic control at rest and during exercise provide support for a sex-specific sympathetic control of blood flow during exercise. In animal and human investigations, the sympathetic vasoconstrictor responses to infusion of noradrenaline or the CPT have been shown to be attenuated in women compared to men (Cheng and Gruetter, 1992, Hogarth et al., 2007, Kneale et al., 2000, Kneale et al., 1997). This suggests that adrenergic receptor responsiveness is blunted in women. Despite sympathetic activation, blood flow increases during exercise. This has been attributed to the sympatholytic effect of some muscle metabolites (i.e. functional sympatholysis). However, previous studies investigating functional sympatholysis have been performed predominantly in men and were not designed to determine the influence of sex- and/or ovarian hormone concentrations. Hence, it remains unclear whether and how the attenuated adrenergic responsiveness at rest and the greater exercise hyperaemia observed in women affect the blunted sympathetic vasoconstrictor response during exercise.

Sex-differences in the blood flow responses during dynamic exercise might also influence the exercise pressor response, because blood flow is the major determinant of the muscle metaboreflex. Greater blood flow during dynamic exercise potentially reduces the accumulation of contraction-related metabolites (e.g. increased metabolite washout). This

may lead to less stimulation of metabolically sensitive afferents that causes an attenuated pressor response. An increased washout of muscle metabolites may also reduce the inhibitory influences of the muscle metaboreflex on the baroreflex control of HR and BP during exercise. Sex-differences in the pressor response to static exercise and PEI have been reported to be abolished when exercise was performed during complete circulatory occlusion (Ettinger et al., 1996). However, it remains unclear whether the influences of sex and/or female reproductive hormones on exercise hyperaemia alter the integrated cardiovascular response during dynamic exercise.

2.7 Summary of literature review

The arterial baroreflex plays an important role in the beat-to-beat regulation of BP and HR, by modulating sympathetic and parasympathetic activity to the heart and the peripheral vasculature. During exercise, the arterial baroreflex is reset rightwards and upwards to operate around a higher BP without a change in maximal gain. Central command and skeletal muscle afferents are essential contributors to the baroreflex resetting and the upwards movement of the operating point of the baroreflex function curve towards the reflex threshold, to a locus of lesser gain. Exercise-induced increases in sympathetic nerve activity cause vasoconstriction and together with increases in cardiac output total peripheral resistance falls, precipitating an increase in BP. As outlined in this review, the numerous anatomical, neural, cardiovascular and haemodynamic differences between men and women at rest have been extensively investigated. However, the reason(s) for the attenuated pressor responses during static and dynamic exercise in young healthy women compared to men remain to be determined. Given, the numerous autonomic and vascular effects of estrogen and the fact that the sex-differences in the exercise pressor response are abolished after menopause, a role of female reproductive hormones needs to be considered.

2.8 Purpose of the present study

The aims of this thesis are to:

- Develop a non-invasive experimental model to examine the effects of augmented muscle metaboreflex activation on baroreflex function during dynamic exercise in humans
- Examine the influences of sex and/or ovarian hormone concentration on the muscle metaboreflex and on the muscle metaboreflex-mediated effects on baroreflex function during exercise
- Investigate potential sex-differences in carotid baroreflex function during exercise
- Examine whether there are sex-differences in the neural-vascular transduction of sympathetic outflow from rest to exercise (e.g. magnitude of sympatholysis)

CHAPTER 3: GENERAL METHODS

3.1 Ethical approval and consent process

All experimental procedures were performed in accordance with the Declaration of Helsinki and received approval from the College of Life & Environmental Sciences ethical review committee at the University of Birmingham. All participants provided written informed consent for participation after receiving a detailed verbal and written explanation of the experimental procedures and measurements. All subjects were recreationally active (e.g. elite athletes were excluded), engaging in not more than two to three days of low to moderate exercise. Subjects were free of any cardiovascular, respiratory and metabolic disease. None of the participants took any prescribed or over the counter medication. This was determined by a medical health questionnaire. Before each experimental session subjects refrained from caffeinated beverages, alcohol and strenuous physical activity at least 12 hr. Experiments were conducted in a laboratory with an ambient temperature of 22 to 24 °C with external stimuli minimized.

3.2 Recorded variables

3.2.1 Heart rate & blood pressure

In all studies, RRI was continuously monitored using a three lead electrocardiogram (Diascope DS 512, S&W Medioteknik AS, Albertslund, Denmark) in the lead II position displayed on an oscilloscope, from which HR was derived. Beat-to-beat arterial BP was obtained non-invasively from the finger using photoplethysmography (PortaPres Model-2, TNO Biomedical Instrumentation, Amsterdam, The Netherlands) (Imholz, 1996). In addition, brachial artery BP was measured using an automated sphygmomanometer (SunTech Tango^{+}, SunTech Medical, Morrisville, USA) (Cameron et al., 2004). Prior to study commencement, readings of the diastolic BP from the Portapres

were matched with the measurements obtained from the brachial artery by the SunTech device on the left upper arm. Mean BP was calculated as:

$$\text{Mean BP} = \text{diastolic BP} + 1/3\ (\text{systolic BP} - \text{diastolic BP}).$$

During studies including handgrip exercise (Chapters 4 & 6-8) brachial BP was obtained at rest and used for the correction of the Portapres values. During leg cycling exercise studies (Studies 1&2; Chapters 4&5) automated sphygmomanometer BP measurements were performed continuously throughout the protocol and used for the correction of the Portapres-derived BP values for each protocol stage.

3.2.2 Peripheral blood flow measurements

In chapters 4&6, forearm blood flow velocity (FBV) from the brachial artery of the right arm was obtanied by Doppler ultrasound (Philips Envisor, Andover, MA, USA). A linear array Doppler ultrasound probe with a operating frequency of 2 MHz was placed on the medial aspect of the upper arm approximately five to eight cm proximal to the antecubital fossa over the brachial artery and in an insonation angle of 60 degree maintained relative to the skin (Dinenno and Joyner, 2003). FBV was measured in Duplex mode from the velocity waveform. Online tracing of the waveform allowed for beat-to-beat recordings of the time averaged velocity mean, which were stored on S-VHS videotape for offline analysis. The diameter of the brachial artery was determined in two dimensional B-Mode at the end of each protocol step. It was recorded in loops over three cardiac cycles and stored on the ultrasound device for offline analysis. Then the average of three measurements of arterial diameter made during diastole was taken as the diameter for that time point (Schrage et al., 2005). During exercise, diastolic cross-sectional measurements were obtained between contractions. FBF (ml/min) was then calculated using the formula:

$$FBF = FBV \times \pi \times (diameter/2)^2 \times 60.$$

Forearm vascular conductance (FVC; ml/min/kg/mmHg) was calculated using the following equation: FVC = FBF / mean BP.

In chapters 7&8, FBV was obtained from the brachial artery of the arm using a Super Dopplex II (Huntleigh Healthcare Ltd, Cardiff, UK) device with an operating frequency of 8 MHz. Doppler probe placement and insonation angle were the same as above. The diameter measurements in Chapter 8 were performed using the linear array Doppler probe mentioned above.

3.2.3 Lean forearm arm mass

FBF was normalized to the lean forearm mass. Forearm volume was assessed by measurements of forearm length and circumferences (Figure 3.1) using the following formula (Jones and Pearson, 1969):

$$\text{Forearm mass} = 1/3 \times \text{length ab} \times (\text{area a} + \text{area ab} + \text{area b}).$$

Forearm mass was then calculated from the anthropometric forearm volume multiplied by it the muscle density (1.06 $g \cdot cm^{-3}$) (Donato et al., 2006). It was corrected for skinfold thickness, which was measured using skin fold callipers at four different sites; proximal and distal, dorsal and ventral forearm.

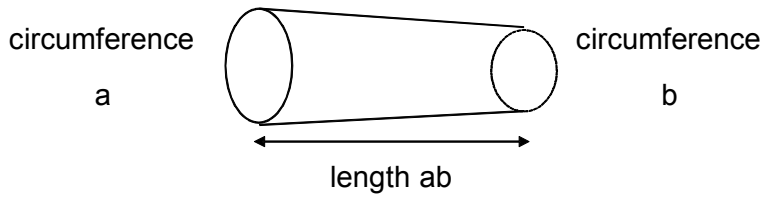

Figure 3.1. Schematic of a truncated cone (e.g. forearm) to calculate forearm muscle mass.

3.2.4 Cerebral blood flow

In the fifth study (Chapter 8), cerebral perfusion was assessed by measurement of blood flow velocity (V_{mean}) in the left middle cerebral artery (MCA). The MCA was insonated through the temporal 'window' in front of the ear and above the zygomatic arch using a 2 MHz pulsed wave transcranial Doppler ultrasound probe (Multidop X, DWL, Sipplingen, Germany) with online spectrum analysis. After the optimal signal was achieved, the probe was attached to an adjustable headband and fixed in position using adhesive ultrasonic gel (Tensive, Parker Laboratories, Orange, NJ, USA). MCA V_{mean} was expressed in cm/s, and the cerebral vascular conductance index (CVCi) was calculated as MCA V_{mean} / mean BP.

3.2.5 Expired carbon dioxide

In the fifth study (Chapter 8), end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) was obtained from a capnogram acquired by means of a nasal cannula connected to a rapid response infrared CO_2 analyser (Servomex 1440, Crowborough, East Sussex, UK). Before each experimental session, the CO_2 analyser was calibrated using gases with known concentrations of CO_2.

3.2.6 Near infrared spectroscopy

In the forth study (Chapter 7), tissue oxygenation measurements were performed using near-infrared spectroscopy (NIRS; OxiplexTS, ISS Inc., Champaign, USA). NIRS utilises near-infrared light in order to detect oxygen absorption-dependent changes of haemoglobin and myoglobin. Due to the nearly identical absorption spectra of haemoglobin and myoglobin it is not possible to distinguish between them (Hansen et al., 1996). In addition, tissue oxygenation measurements using NIRS are limited to the skeletal

muscle microcirculation. Due to the absorption properties of large arteries and veins, NIRS provides a beat-to beat estimate of oxygen delivery relative to the consumption (Wray et al., 2004a). A sensor with two optic fibre bundles with an optode separation of three cm was placed on the skin over the region of the flexor digitorum profundus, the muscle mainly involved in forearm exercise, approximately three cm below the antecubital fossa. The probe was secured to the forearm using elastic bandages and covered with a towel to minimize light penetration of the optodes. NIRS signals at two wavelengths (750 and 830 nm) were sampled at a rate of 100 Hz and translated into optical densities using known algorithms (Hansen et al., 1996) and digitally stored for later analysis.

3.3 Interventions

3.3.1 Rhythmic handgrip exercise

In the first and third to fifth study (Chapter 4 & 6-8), participants were seated in a semi-recumbent position on a medical examination table with a custom made handgrip dynamometer held in the right hand, while the arm was supported on an adjustable bedside table. MVC was determined as the highest force produced during three-to-five efforts, each separated by one minute. The force exerted by the subject during the experimental protocol, expressed as a percentage of maximum, was continuously recorded and displayed on a computer screen positioned in front of the subject at eye level. Rhythmic handgrip exercise was performed at a duty cycle of 1 second contraction – 2 seconds relaxation (20 contractions per minute) in all studies apart from study 4 (Chapter 7), where a contraction cycle (1 ½ seconds contraction 1 ½ seconds relaxation) was used.

3.3.2 Dynamic leg cycling

In the first and second study (Chapter 4&5), participants performed leg cycling exercise in a semi-recumbent position at a constant rate of 60 revolutions per minute using a customised electrically braked cycle ergometer (Angio, Lode, Groeningen, The Netherlands).

3.3.3 Partial flow-restriction

In the first to third study (Chapters 4-6), blood flow to the exercising skeletal muscle was partially restricted by (i) bilateral thigh cuff inflation to 100 mmHg during leg cycling or (ii) upper arm cuff inflation to 80, 100 and 120 mmHg during rhythmic handgrip exercise. A rapid inflation device was used to obtain and sustain the target pressure during exercise (E20, Hokanson, Bellevue, WA, USA). Cuff inflation evokes a reduction in blood flow to the exercising skeletal muscle, which causes accumulation of muscle metabolites, thus activating metabolically sensitive skeletal muscle afferent nerve fibres (i.e. muscle metaboreflex).

3.3.4 Oscillatory neck pressure & neck suction

In the forth study (Chapter 7), the neck chamber technique was used with five second pulses of NP at +40 and NS at -60 Torr to selectively unload (i.e. NP; simulated carotid hypotension) and load (i.e. NS; simulated carotid hypertension) the carotid baroreceptors. These intensities of stimulation were chosen to produce near maximal physiological amounts of carotid hypotension and hypertension. For the application of NP and NS a malleable lead neck collar was fitted around the anterior two-third of the neck. Pressure changes in the neck collar were generated from a custom made variable pressure

source. A pressure transducer (model BTEM5P35OD, Sensortechnics, Puchheim, Germany) connected to a port on the neck collar accurately amplified the applied stimulus.

NP and NS were applied in an oscillatory manner at 0.1 Hz (i.e. 5 seconds on, 5 seconds off) for 5 minutes to determine the degree of CBR control over RRI and BP (Wray et al., 2004a). The oscillations in carotid sinus transmural pressure were applied at 0.1 Hz to separate the CBR-entrainment of cardiovascular and haemodynamic variables from breathing-induced cardiac oscillations (e.g. respiratory sinus arrhythmia), which occur predominantly at frequencies between 0.15 to 0.5 Hz (Pagani et al., 1997, Saul et al., 1991).

To familiarize the subjects with the neck chamber 5 second pulses of NP were initially applied at a very low level followed by pulses of gradually increasing pressure intensity until the target of 40 Torr was reached. In a next step NP was applied in an oscillatory manner (5 seconds on; 5 seconds off) for 30 seconds to ensure the subjects maintained the breathing rate observed without CBR stimulation. The familiarisation with NS to a target pressure of -60 Torr was conducted in a similarly fashion.

3.3.5 Cold pressor test

In the fifth study (Chapter 8), the CPT was employed to stimulate the sympathetic nervous system (Hines and Brown, 1933, Victor et al., 1987a). The subject's bare foot was passively placed in ice-cold water at 4 °C for 2 minutes. Prior to each CPT the water temperature was measured using a thermometer and by adding or removing ice from the bucket to ensure the CPT was always performed in the right temperature.

3.4 Data analysis

3.4.1 Spike

For all five studies, all signals were sampled by an analogue-to-digital converter (Cambridge Electronic Design 1401plus, Cambridge, UK) at 1 kHz. Data was recorded and displayed using Spike 2 software (CED, Cambridge, UK). Customized Spike 2 script files were used offline to determine beat-to-beat values for systolic BP, diastolic BP, mean BP, HR and RRI (all studies) and FBV (only study 5 in Chapter 8) and the text output was imported into Microsoft Excel.

3.4.2 Spontaneous cardiac baroreflex sensitivity

In the first to third study (Chapter 4-6), the sequence technique was used to estimate spontaneous cBRS (Fisher et al., 2009, Iellamo et al., 1997). A customized Spike 2 script file was used off-line to detect sequences of three or more consecutive beats, during which systolic BP and RRI changed in the same direction, or systolic BP and HR changed in an opposite direction (i.e. arterial baroreflex sequences). A linear regression was applied to each individual sequence and only those sequences in which r^2 was greater than 0.85 were accepted. The slope values derived from the regression analysis provide an index of cardiac vagal tone. This is evidenced by the almost completely abolished slope of the regression line after injection of atropine, a potent anticholinergic drug, blocking vagal activity to the heart (Parati et al., 2000, Parlow et al., 1995).

3.4.3 Heart rate variability

In the first study (Chapter 4), time domain HR variability was estimated using the square root of the mean of the successive differences between adjacent RRI's (RMSSD)

(Task, 1996). RMSSD has been suggested to reflect short-term high frequency variability of HR and therefore provides a measure of cardiac parasympathetic activity (i.e. vagal tone) (Chess et al., 1975).

3.4.4 Stroke volume & cardiac output

In the fifth study (Chapter 8), SV was calculated off-line from the BP waveform (re-sampled at 100Hz) using a Modelflow software program incorporating the BeatScope version 1.0 software (TNOTPD, Biomedical Instrumentation, Amsterdam, The Netherlands) (Jansen et al., 2001). CO was calculated using the formula:

$$CO = SV \cdot HR$$

The Modelflow method has been shown to reliably estimate rapid changes in CO during a variety of experimental protocols, including exercise (Bogert and van Lieshout, 2005, Kim et al., 2011).

3.4.5 Post hoc analysis & significance level

Statistical test performed in each study are separately described in the method section of each experimental chapter. To investigate significant main effects and interactions, post hoc analysis was generally employed using Students t-tests with Bonferoni correction. The level of statistical significance was set at $P<0.05$. SPSS for Windows (IBM Corporation, Somers, NY, USA) was used for all statistical analyses. Data are presented as mean ± standard error of measurements (SEM).

CHAPTER 4: EFFECT OF MUSCLE METABOREFLEX ACTIVATION ON SPONTANEOUS CARDIAC BAROREFLEX SENSITIVITY DURING DYNAMIC EXERCISE IN HUMANS

4.1 Introduction

The arterial baroreflex plays a key role in maintaining short-term arterial BP homeostasis by adjusting efferent autonomic outflow to the heart and the peripheral vasculature. During dynamic exercise, the arterial baroreflex is reset and remains operative around the prevailing BP and HR (Bevegard and Shepherd, 1966, Coote and Dodds, 1976, Papelier et al., 1994, Potts et al., 1993). This resetting is attributable to the actions and interactions of neural signals arising from higher brain centres (central command) (Gallagher et al., 2001c, Iellamo et al., 1997, McIlveen et al., 2001, Ogoh et al., 2002b) and feedback from group III and IV sensory afferents in response to metabolic and mechanical stimuli within exercising skeletal muscles (muscle metaboreflex and mechanoreflex) (Gallagher et al., 2001b, Iellamo et al., 1997, McIlveen et al., 2001, Smith et al., 2003).

Concomitant with the resetting of the arterial baroreflex during dynamic exercise, cardiac baroreflex sensitivity (cBRS) has been shown to be reduced when estimated from the relationship between spontaneous fluctuations in BP and HR (Iellamo et al., 1999b, Ogoh et al., 2005b, Sala-Mercado et al., 2010, Sala-Mercado et al., 2007). Such measures of cBRS have been reported to be associated with the operating point (i.e. point at which HR is regulated) of the full baroreflex stimulus-response curve and during dynamic exercise the gain or sensitivity at the operating point is reduced (Fisher et al., 2007, Fisher et al., 2009, Ogoh et al., 2005b). This is because, during dynamic exercise, the operating point is relocated away from the point of maximal baroreflex sensitivity at the centre of the baroreflex function curve (i.e. centring point) and towards the reflex threshold to a locus of lesser gain (Ogoh et al., 2005b). This phenomenon has been attributed to an exercise-induced reduction in cardiac parasympathetic tone (Ogoh et al., 2005b). However, the precise mechanism(s) underlying the reduction in spontaneous cBRS reported during

dynamic exercise is unclear. Notably, the effect of the muscle metaboreflex on spontaneous cBRS remains particularly controversial and incompletely understood.

Humans studies have reported that cBRS is unchanged during isolated activation of the muscle metaboreflex during a period of post-exercise ischemia (PEI) following static handgrip (Cui et al., 2001, Fisher et al., 2010, Fisher et al., 2008, Ichinose et al., 2002, Iellamo et al., 1999b, Spaak et al., 1998), calf plantar flexion (Drew et al., 2008a) or single leg extensor exercise (Iellamo et al., 1999a). This technique involves the inflation of a suprasystolic pressure cuff proximal to the exercising muscles to arrest the circulation just prior exercise cessation, thus trapping exercise metabolites within the muscle and sustaining the activation of metabolically sensitive muscle afferents (Alam and Smirk, 1937). In canines, muscle metaboreflex activation has been evoked by hypoperfusion of the exercising skeletal muscle, via inflation of a pneumatic occluder placed around the terminal aorta (Sala-Mercado et al., 2010, Sala-Mercado et al., 2007). In contrast to studies in humans, activation of the muscle metaboreflex by hypoperfusion of the active skeletal muscles of dogs during treadmill running has been shown to evoke a reduction in spontaneous cBRS (Sala-Mercado et al., 2007). Although species-related differences in cardiac autonomic control may contribute to such discrepant findings, an alternative explanation for these contrasting observations relates to methodological differences employed to activate the muscle metaboreflex and/or the exercise modality utilized.

Cardiac autonomic activity may be profoundly different when the muscle metaboreflex is isolated during PEI compared to when it is activated by flow restriction during exercise. Augmented muscle metaboreflex activation during dynamic exercise, at a time when central command and muscle mechanoreflex are also active, causes an elevation in HR due to an increase in cardiac sympathetic activity and/or reduction in cardiac parasympathetic activity (Bonde-Petersen et al., 1978, O'Leary, 1993, Sala-Mercado et al.,

2010, Sala-Mercado et al., 2007, Sun et al., 1993, Sundberg and Kaijser, 1992, Wyss et al., 1983). In contrast, HR has been shown to remain at baseline levels during isolated muscle metaboreflex activation with PEI following handgrip, single calf plantar flexion or single leg extensor exercise (no central command or muscle mechanoreflex) (Cui et al., 2001, Drew et al., 2008a, Fisher et al., 2010, Fisher et al., 2008, Ichinose et al., 2002, Iellamo et al., 1999a, Iellamo et al., 1999b, Spaak et al., 1998). A potential explanation for this is that the robust reactivation of cardiac parasympathetic tone during PEI masks the potential tachycardic effect of an elevation in cardiac sympathetic activity (Fisher et al., 2010, O'Leary, 1993). Such differences in cardiac autonomic activity may mean that the mode of muscle metaboreflex activation (i.e. post vs. during exercise) differentially affects spontaneous cBRS, and the elevated cardiac parasympathetic tone during PEI may obscure the inhibitory actions of muscle metaboreflex activation on cBRS. Furthermore, the seminal work of Alam and Smirk (1938) has identified that when PEI was used following dynamic calf plantar flexion of both legs, HR remained elevated, unlike following handgrip exercise where no change in HR from baseline was noted. Such apparent specificity in the metaboreflex control of HR, indicates that the question of whether muscle metaboreflex activation reduces cBRS during dynamic exercise of a large muscle mass (e.g. leg cycling) cannot be accurately addressed by studies using small muscle mass exercise (e.g. handgrip).

It is presently unknown whether activation of the muscle metaboreflex by restricting skeletal muscle perfusion during exercise evokes a decrease in spontaneous cBRS in humans, as previously reported in canines. To address this, the sequence technique was used to calculate spontaneous cBRS during low and moderate leg cycling under conditions of both free-flow and partial flow-restriction, and during PEI. cBRS was also examined during rhythmic handgrip with free-flow and partial flow-restriction, and

followed by PEI. It was hypothesised that spontaneous cBRS would be decreased by metaboreflex activation during leg cycling with partially restricted flow and during PEI, but such muscle metaboreflex-mediated changes in cBRS would not be evident during handgrip.

4.2 Methods

4.2.1 Subjects

Fifteen male subjects volunteered to participate in the present study. Their mean age, weight and height (mean±standard deviation) was 22±4 years, 78±9 kg and 181±5 cm, respectively.

4.2.2 Experimental measurements

In both protocols HR and BP were measured continuously and mean BP was calculated (see Section 3.2.1. for details). During protocol 2 (rhythmic handgrip exercise) FBV and diameter from the brachial artery of the right arm were obtained by Doppler ultrasound (Philips Envisor, Andover, MA, USA) with velocity measurements taken as an average over 10 cardiac cycles, and 3 measures made at each experimental phase. FVC calculated and corrected for lean forearm mass (see Sections 3.2.2 & 3.2.3 for details).

4.2.3 Experimental procedures

Protocol 1: Leg cycling exercise. Subjects performed leg cycling exercise in a semi-recumbent position at a constant rate of 60 revolutions per minute using a customised electrically braked cycle ergometer (Angio, Lode, Groeningen, The Netherlands). After a 3 minute resting baseline period, subjects performed 10½ minutes of leg cycling at a low

intensity workload (Ex90; target HR of 90 beats·min^{-1}; 26±4 Watts), followed by 10½ minutes of leg cycling at a moderate intensity workload (Ex120; target HR of 120 beats·min^{-1}; 105±7 Watts) (Figure 4.1). The first 3½ minutes of each workload were used to adjust the resistance in order to reach the target HR, following which the workload was kept constant for 7 minutes. Two trials were conducted in a counterbalanced order and separated by at least 30 minutes to ensure the baseline physiological status was re-established. In one trial, bilateral thigh cuffs were inflated to 100 mmHg (E20, Hokanson, Bellevue, WA, USA) during the last 3 ½ minutes of each exercise workload (at low and moderate intensity) in order to partially restrict the blood flow to the exercising muscles and engage the muscle metaboreflex (see Section 3.3.3 for details). The other trial served as a time control, as the thigh-cuffs were not inflated and exercise was performed under free-flow conditions. Ratings of perceived exertion (RPE) were obtained during leg cycling using the standard 6 – 20 Borg scale (Borg, 1998). Ten seconds before the end of exercise in both trials, thigh cuff pressure was increased to 230 mmHg and a 3 ½ minute period of PEI was undertaken to isolate muscle metaboreflex activation.

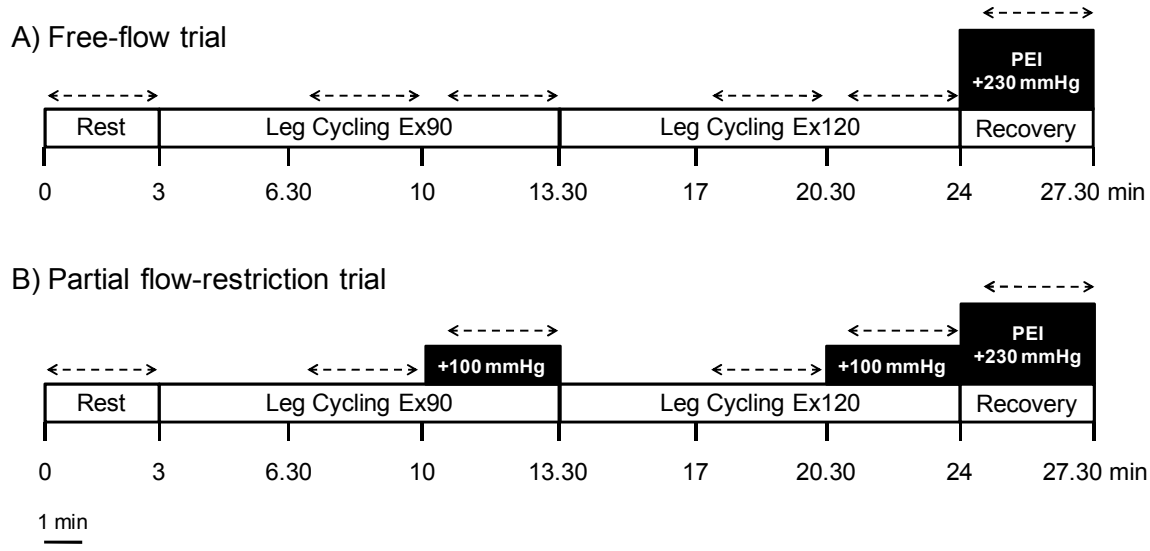

Figure 4.1. Schematic representation of experimental protocol 1, comprising of leg cycling exercise under free-flow conditions (Trial A) and with partial flow-restriction (Trial B). Dashed lines in indicate time of data acquisition.

In a subset of eight subjects, two identical trials of moderate intensity leg cycling exercise were performed in a counterbalanced order (3 ½ minutes warm-up, followed by 3 ½ minutes at 139±14 Watts). In one trial a period of PEI followed exercise, while in the other trial recovery was conducted under free-flow conditions.

Protocol 2: Rhythmic handgrip exercise. Subjects performed rhythmic handgrip exercise in a semi-recumbent position using a custom built handgrip dynamometer held in the right hand, while the arm was supported on an adjustable bedside table. After MVC was determined (see Section 3.3.1) subjects rested for 15 minutes. Rhythmic handgrip exercise was performed at a duty cycle of 1 second contraction – 2 seconds relaxation (20 contractions per minute) to allow blood velocity and diameter measurements during exercise (Dinenno and Joyner, 2003, Hartwich et al., 2010). After a 3 minute resting baseline period, subjects performed 17 ½ minutes of rhythmic handgrip exercise at 35% of MVC (Figure 4.2). The first 3 ½ minutes were used to attain steady state exercise conditions. Two trials were conducted in a counterbalanced order and separated by at least 20 minutes, to ensure the baseline physiological status was re-established. In one trial, after 7 minutes of handgrip exercise a pressure-cuff around the exercising upper arm was rapidly inflated to 80 mmHg (E20, Hokanson, Bellevue, WA, USA). After 3 ½ minutes arm cuff pressure was increased to 100 mmHg, and after a further 3 ½ minutes arm cuff pressure was increased to 120 mmHg. This manoeuvre was performed in order to restrict the blood flow to the exercising muscles and engage the muscle metaboreflex. The other trial served as a time control, as the pressure cuff was not inflated during handgrip and exercise was performed under free-flow conditions. Ten seconds before the end of exercise, the arm cuff pressure was increased to 230 mmHg and a 3 ½ minute period of PEI undertaken. An RPE was obtained during handgrip exercise using the standard 6 – 20 Borg scale (Borg, 1998).

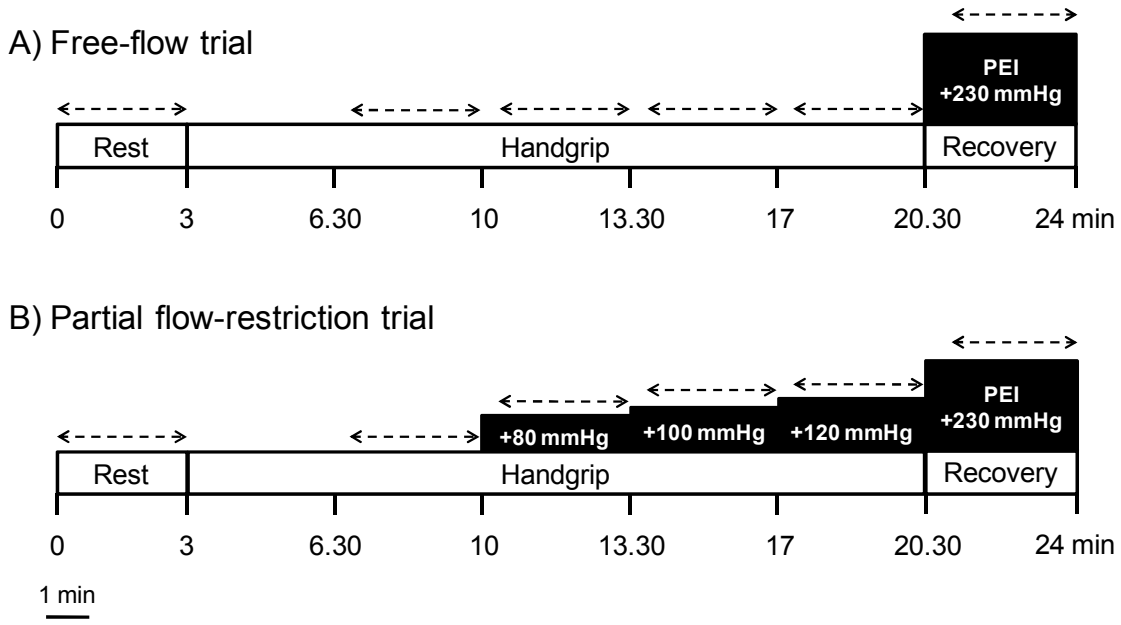

Figure 4.2. Schematic representation of experimental protocol 2, comprising of rhythmic handgrip exercise under free-flow conditions (Trial A) and handgrip exercise with partial flow-restriction (Trial B). Dashed lines in indicate time of data acquisition.

4.2.4 Data analysis

After determination of beat-to-beat values for each variable (systolic, diastolic, mean BP, HR, RRI), spontaneous cBRS was calculated using the sequence technique (Section 3.4.2) and time domain HR variability was calculated (Section 3.4.3). For each trial, cBRS and cardiovascular data were averaged over a 3 minute period for each of the experimental phases (indicated with dashed lines in Figure 4.1 and 4.2). Physiological data were statistically analysed using two-way repeated measures ANOVA, in which the main factors were *trial* (free-flow or partial flow-restriction) and *time* (Protocol 1 - Rest, Ex90, Ex90 free-flow or partially restricted-flow, Ex120 and Ex120 free-flow or partially restricted-flow, PEI; Protocol 2 - Rest, Ex, Ex free-flow or partially restricted-flow +80, Ex free-flow or partially restricted-flow +100, and Ex free-flow or partially restricted-flow +120).

4.3 Results

4.3.1 Protocol 1: Leg cycling exercise.

Resting HR, RRI, BP, RMSSD and cBRS were similar in both the free-flow and restricted-flow trials (Figures 4.3 and 4.4, Table 4.1). Dynamic leg cycling at a low intensity workload elicited a significant increase in HR (+22±2 b·min^{-1}) and systolic BP, while diastolic BP and mean BP remained unchanged from rest (Figure 4.3, Table 4.1). During moderate intensity leg cycling, HR (+55±3 b·min^{-1}), systolic BP and mean BP (+12±2 mmHg) were all significantly elevated above resting levels, while diastolic BP tended to fall. RRI was significantly decreased during exercise in an intensity dependent manner (Table 4.1). cBRS was decreased from rest during low intensity exercise, and further decreased from rest during moderate intensity exercise (RRI-cBRS, 16±2, 7±1, 2±0.2 ms·mmHg^{-1} at rest, low and moderate leg cycling, respectively; $P<0.05$; Figure 4.4). RMSSD was similarly reduced in an exercise intensity dependent manner (Table 4.1). RPE was significantly increased from low (8±0.4 au) to moderate (13±0.5 au) intensity leg cycling.

Muscle metaboreflex activation, with partial flow-restriction to the exercising skeletal muscles, evoked a significant decrease in RRI and increase in HR during both low and moderate intensity exercise (+6±2 b·min^{-1} and +13±2 b·min^{-1}, $P<0.05$ vs. free-flow trial; Figure 4.4). Muscle metaboreflex activation also increased mean BP by +17±1 mmHg and +17±2 mmHg at low and moderate exercise intensities ($P<0.05$ vs. free-flow trial; Figure 4.4). cBRS was significantly attenuated by muscle metaboreflex activation during low and moderate intensity leg cycling (RRI-cBRS, -2.6±0.8 and -0.4±0.1 ms·mmHg^{-1}; $P<0.05$ vs. free-flow trial; Figures 4.5&4.6). Notably, the muscle metaboreflex-mediated reduction in cBRS was more pronounced during low intensity leg

cycling than during moderate intensity leg cycling (Figure 4.5). Muscle metaboreflex activation also reduced RMSSD during low but not moderate intensity leg cycling (Table 4.1). The relationship between RRI and RRI-cBRS, and HR and HR-cBRS were non-linear (Figure 4.6). The effect of dynamic exercise with or without partial flow-restriction on cBRS were similar irrespective of whether cBRS was analysed using either HR or RRI. Partial flow-restriction during exercise evoked a marked increase in RPE at both low (11±0.5 au) and moderate (16±0.4 au) exercise intensities ($P<0.05$ vs. free-flow trial).

Isolated muscle metaboreflex activation during PEI following leg cycling with partial flow restriction maintained a pronounced elevation in mean BP (+32±2 mmHg) and HR (+44±3 $b \cdot min^{-1}$) from rest ($P<0.05$), while cBRS remained suppressed ($P<0.05$ vs. rest) irrespective of whether cBRS was analysed using either HR or RRI. RMSSD also remained suppressed during PEI following leg cycling with partial flow restriction (Table 4.1). In the subset of subjects who performed separate trials of leg cycling under free-flow conditions followed either PEI or recovery under free-flow conditions, cBRS and RMSSD were significantly reduced during PEI (RRI-cBRS, 6±2 and 15±4 $ms \cdot mmHg^{-1}$, RMSSD, 26±12 vs. 54±15 ms; $P<0.05$ PEI vs. free-flow recovery). In contrast, the increase from rest in mean BP and HR was significantly greater during PEI compared to recovery under free-flow conditions (+32±2 vs. +3±2 mmHg, +29±6 vs. +15±3 $b \cdot min^{-1}$; $P<0.05$ PEI vs. free-flow recovery).

Table 4.1. Selected physiological variables at rest and low and moderate leg cycling during the free-flow and partial flow-restriction experimental trials

		Rest	Ex 90	Ex 90 FF/PFR	Ex 120	Ex 120 FF/PFR	PEI
Systolic BP (mmHg)							
	FF	121 ± 3	132 ± 3 *	134 ± 3 *	163 ± 5 *†	164 ± 5 *†	161 ± 5 *†
	PFR	119 ± 2	132 ± 3 *	148 ± 3 *†#	163 ± 5 *†	185 ± 4 *†‡#	170 ± 4 *†#
Diastolic BP (mmHg)							
	FF	60 ± 1	60 ± 2	59 ± 1	57 ± 2	57 ± 2	82 ± 2 *†‡
	PFR	64 ± 2 #	62 ± 3	77 ± 2 *†#	58 ± 2	74 ± 2 ‡#	86 ± 3 *†‡
RRI (ms)							
	FF	961 ± 37	701 ± 10 *	693 ± 10 *	504 ± 5 *†	492 ± 5 *†‡	673 ± 20 *‡
	PFR	965 ± 39	710 ± 12 *	651 ± 15 *†#	508 ± 7 *†	444 ± 7 *†‡#	569 ± 20 *†‡#
RMSSD (ms)							
	FF	54 ± 5	25 ± 4 *	24 ± 3 *	6 ± 1 *†	5 ± 1 *†	26 ± 4 *‡
	PFR	59 ± 6	26 ± 3 *	17 ± 3 *†#	6 ± 1 *†	5 ± 2 *†	16 ± 3 *‡#

Values are mean±SEM. FF, free-flow; PFR, partial flow-restriction; BP, blood pressure; RRI, R-R interval; RMSSD, square root of the mean of the sum of successive differences in R-R interval; Ex90, leg cycling at target HR of 90 $b \cdot min^{-1}$; Ex120, leg cycling at target HR of 120 $b \cdot min^{-1}$; PEI, post exercise ischemia. * $P<0.05$ vs. Rest of corresponding trial; † $P<0.05$ vs. Ex90 of corresponding trial; ‡ $P<0.05$ vs. Ex120 of corresponding trial; # $P<0.05$ vs. FF trial at corresponding time point.

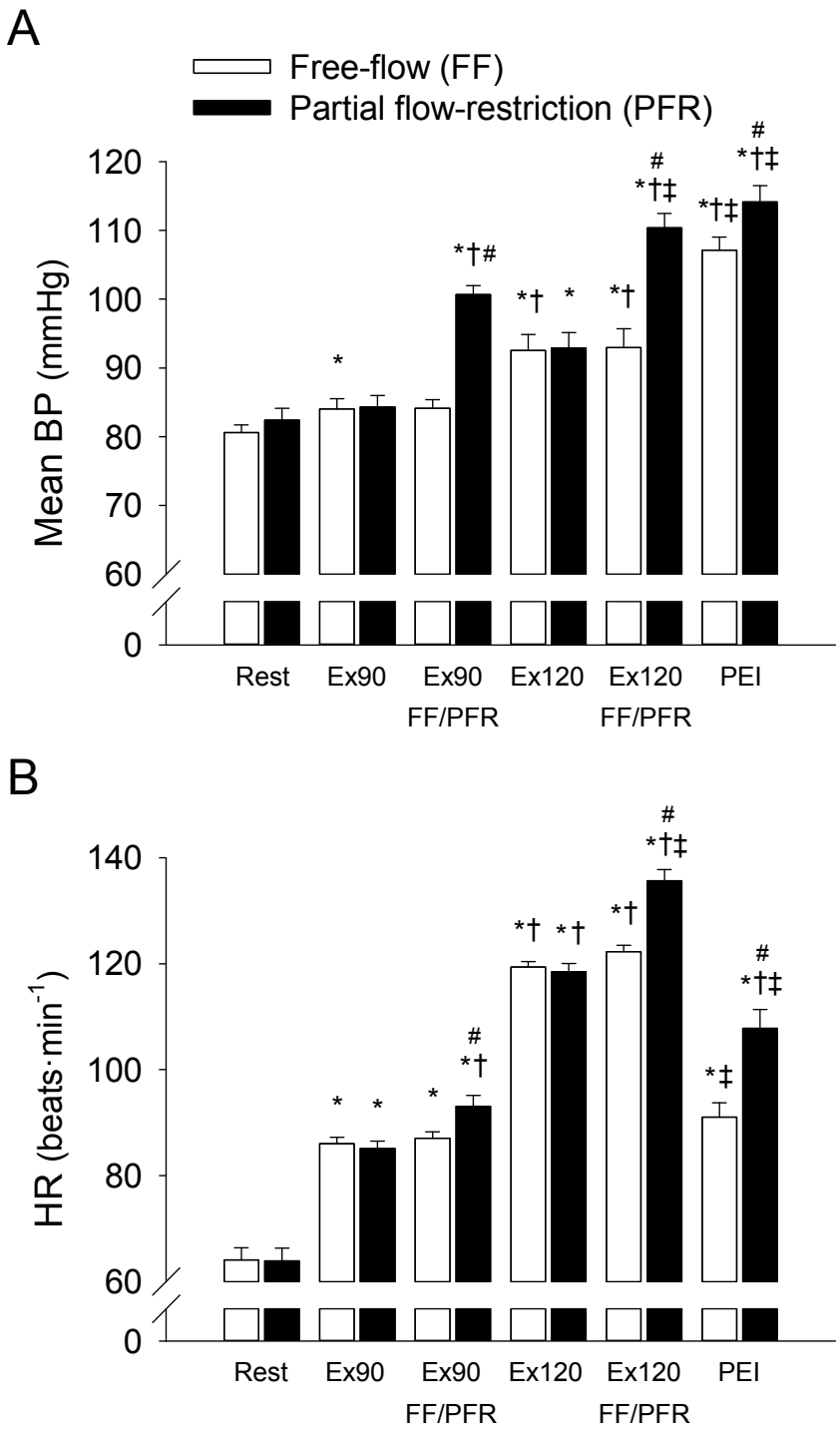

Figure 4.3. Mean arterial blood pressure (BP; panel A) and heart rate (HR; panel B) at rest and during dynamic leg cycling under free-flow conditions (white bars) and with partial flow-restriction (black bars). FF, free-flow; PFR, partial flow-restriction; BP, blood pressure; RRI, R-R interval; Ex90, leg cycling at target HR of 90 b·min^{-1}; Ex120, leg cycling at target HR of 120 b·min^{-1}; PEI, post exercise ischemia. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex90 of corresponding trial; ‡ P<0.05 vs. Ex120 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

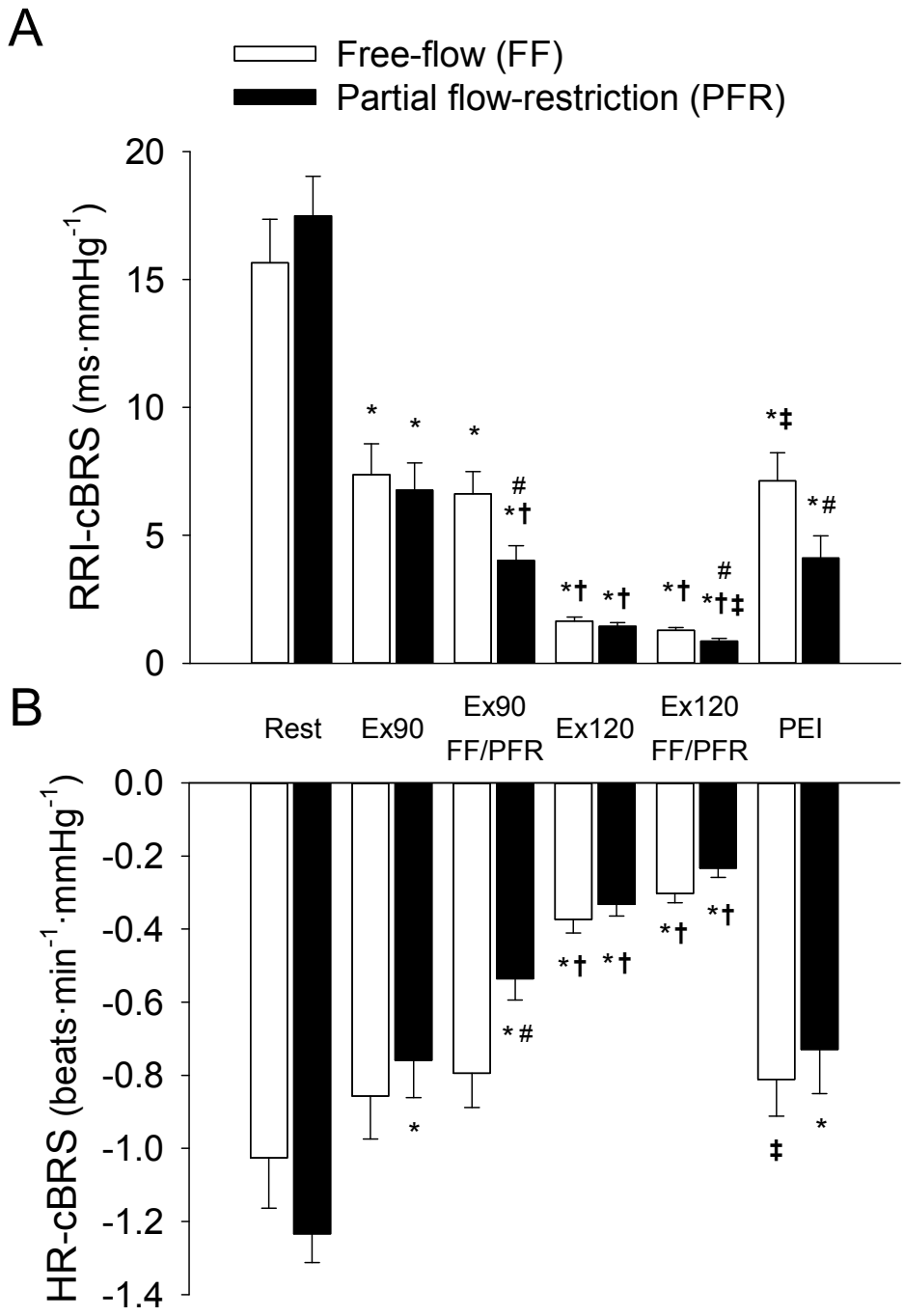

Figure 4.4. Spontaneous cardiac baroreflex sensitivity (cBRS) at rest and during dynamic leg cycling under free-flow conditions (white bars) and with partial flow-restriction (black bars). Spontaneous cBRS calculated using either R-R interval (RRI-cBRS, panel A) or heart rate (HR-cBRS, panel B). FF, free-flow; PFR, partial flow-restriction; BP, blood pressure; RRI, R-R interval; Ex90, leg cycling at target HR of 90 b·min^{-1}; Ex120, leg cycling at target HR of 120 b·min^{-1}; PEI, post exercise ischemia. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex90 of corresponding trial; ‡ P<0.05 vs. Ex120 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

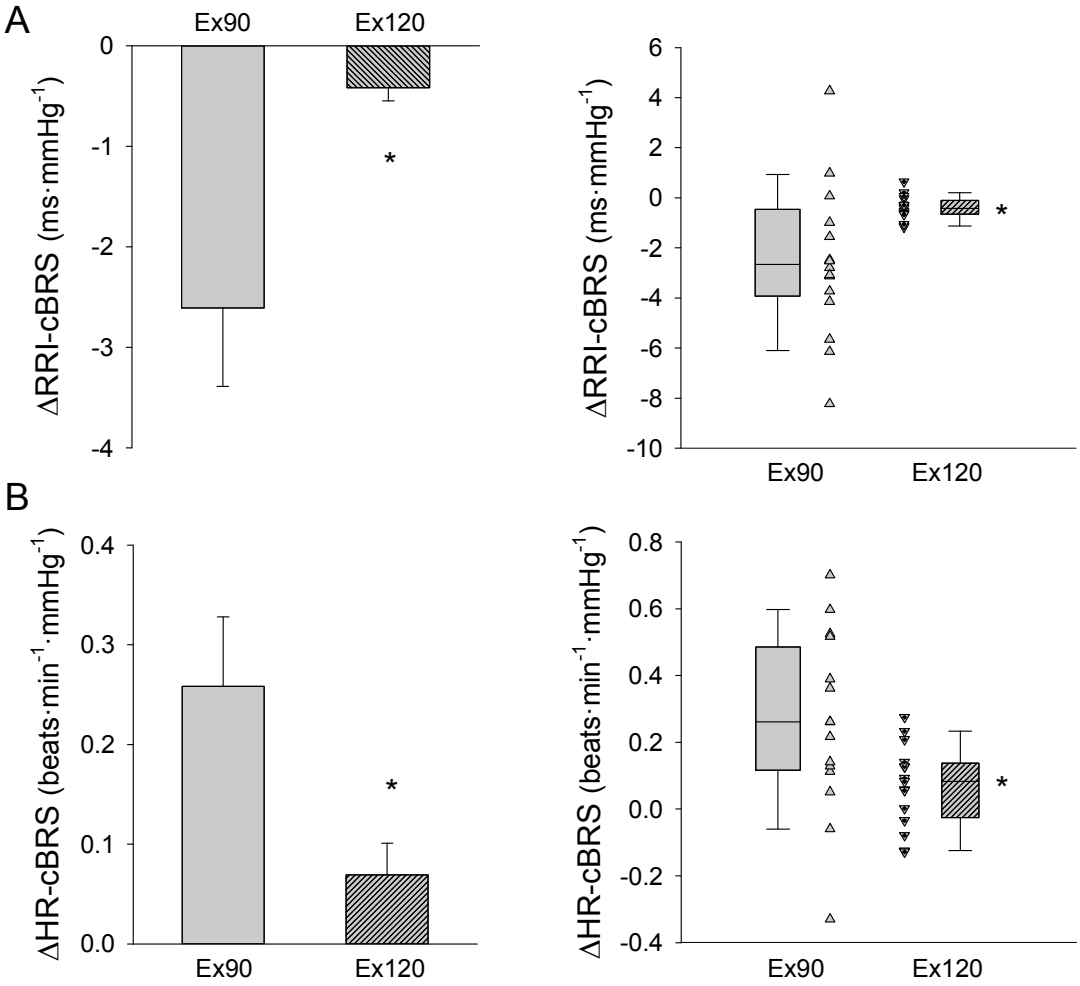

Figure 4.5. Change in spontaneous cardiac baroreflex sensitivity (cBRS) induced by partial flow-restriction versus free-flow trial at low (grey bars) and moderate (grey hatched bars) exercise intensities. Boxes on right indicate the average reduction in cBRS at low (grey box) and moderate (grey hatched box) exercise intensities. Whiskers indicate the 5th and 95th percentile. Individual data are presented in triangles during low (upwards triangles) and moderate (downwards triangles) intensity leg cycling. Spontaneous cBRS calculated using either R-R interval (RRI-cBRS, panel A) or heart rate (HR-cBRS, panel B). * $P<0.05$, significantly different from Ex90.

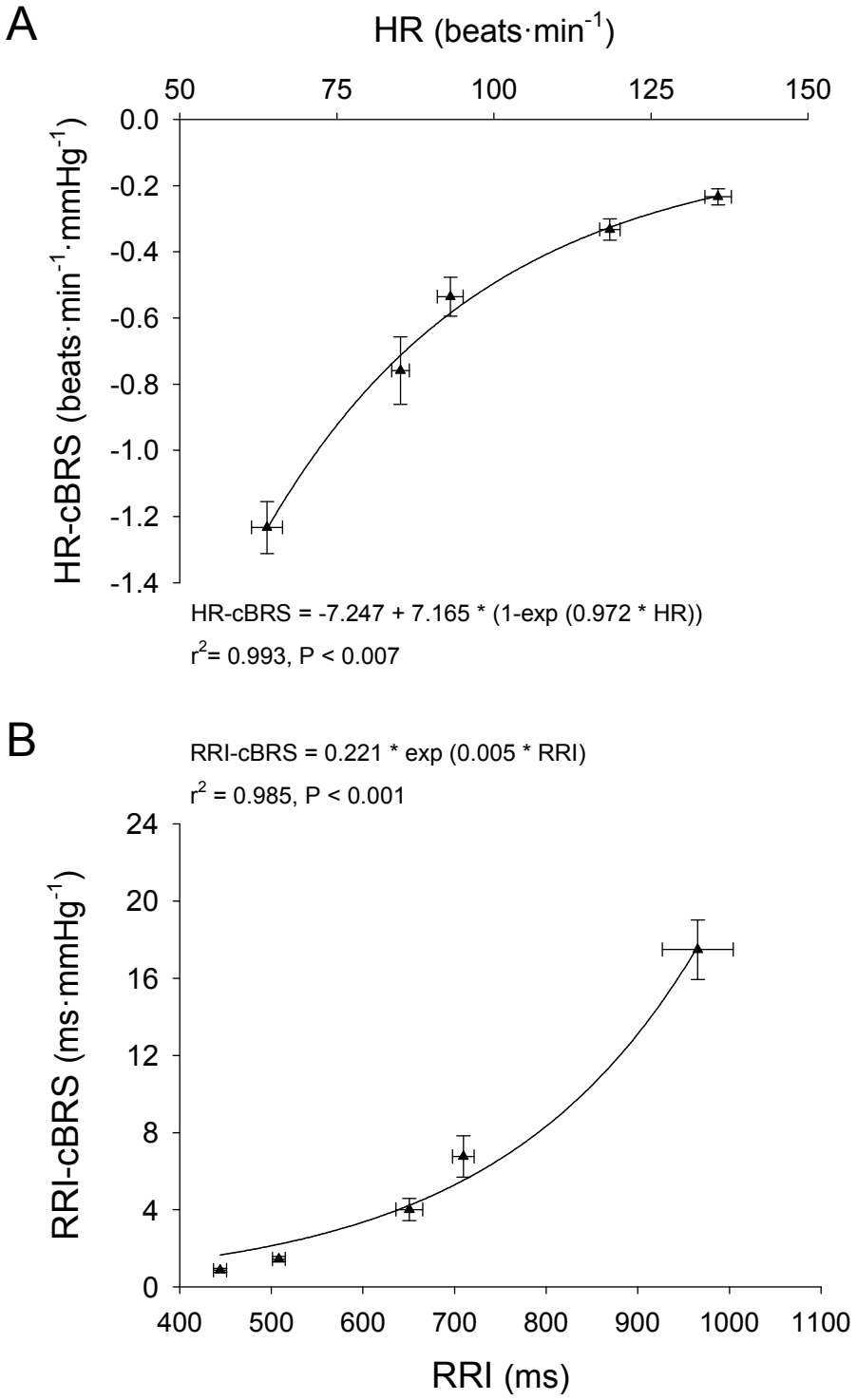

Figure 4.6. Relationship between heart rate (HR) and spontaneous cardiac baroreflex sensitivity (HR-cBRS, panel A) and R-R interval and cBRS (RRI-cBRS, panel B). Triangles represent group average data during the partial flow-restriction trial.

4.3.2 Protocol 2: Rhythmic handgrip exercise

Resting BP, HR, FBF, RRI, RMSSD and cBRS were similar in both the free-flow and restricted-flow trials (Figures 4.7&4.8, Table 4.2). Rhythmic handgrip exercise elicited a significant increase in BP, HR and FBF from rest, under free flow conditions, while RRI was reduced and cBRS remained unchanged (Figures 4.7&4.8, Table 4.2). Arm cuff inflation during rhythmic handgrip exercise evoked a graded reduction in FBF compared to the free-flow trial, such that FBF during rhythmic handgrip was reduced by -126±24, -187±21 and -211±19 $ml \cdot min^{-1} \cdot kg^{-1}$ during the Ex+80, Ex+100 and Ex+120 conditions ($P<0.05$; equivalent to a reduction of -24±4, -35±2, and -38±2 %, from the respective free-flow value, during the Ex+80, Ex+100 and Ex+120 conditions; Figure 4.7). Muscle metaboreflex activation, during incremental partial flow-restriction to the exercising skeletal muscles, evoked an increase in mean BP and HR ($P<0.05$ vs. free-flow trial; Figure 4.7), whereas cBRS was unchanged ($P>0.05$ vs. free-flow trial; Figure 4.8). RPE was 10±1 au during the early phase of rhythmic handgrip under free-flow conditions and increased progressively to 12±1, 13±1, and 15±0.5 au, during the Ex+80, Ex+100 and Ex+120 conditions. Isolated muscle metaboreflex activation, during PEI following rhythmic handgrip exercise, partially maintained the exercise-induced increase in mean BP, while HR returned to baseline (Figure 4.7). cBRS and RMSSD were unchanged from rest during PEI following rhythmic handgrip exercise (Table 4.2, Figure 4.8), indicated by no main effects of phase ($P=0.486$ and $P=0.246$ for RRI-cBRS and HR-cBRS, respectively) and trial ($P=0.113$ and $P=0.159$ for RRI-cBRS and HR-cBRS, respectively) and no significant interactions ($P=0.183$ and $P=0.664$ for RRI-cBRS and HR-cBRS, respectively).

Table 4.2. Selected physiological variables at rest, handgrip exercise and post exercise ischemia during the free-flow and graded partial flow-restriction experimental trials

		Rest	Ex	Ex FF/+80	Ex FF/+100	Ex FF/+120	PEI
Systolic BP (mmHg)							
	FF	127 ± 4	145 ± 4 *	146 ± 4 *	148 ± 5 *	152 ± 5 *$	140 ± 4 *$§
	PFR	126 ± 4	145 ± 5 *	149 ± 5 *	153 ± 5 *†‡	162 ± 6 *†‡$#	152 ± 5 *#
Diastolic BP (mmHg)							
	FF	63 ± 2	72 ± 2 *	72 ± 2 *	73 ± 2 *	75 ± 2 *	68 ± 2 *†‡$§
	PFR	60 ± 2 #	69 ± 2 *#	71 ± 2 *†	74 ± 2 *†‡	79 ± 3 *†‡$#	72 ± 3 *#
RRI (ms)							
	FF	1024 ± 44	927 ± 48 *	920 ± 47 *	916 ± 48 *	903 ± 48 *	1034 ± 49 †‡$§
	PFR	1016 ± 40	906 ± 39 #	888 ± 36 *†	858 ± 36 *†	831 ± 37 *†‡$	1009 ± 54 *†‡$#
RMSSD (ms)							
	FF	59 ± 6	60 ± 8	62 ± 9	67 ± 9	67 ± 9	61 ± 7
	PFR	56 ± 6	58 ± 8	58 ± 9	56 ± 8	57 ± 9	63 ± 8

Values are mean±SEM. FF, free-flow; PFR, partial flow-restriction; BP, blood pressure; RRI, R-R interval; RMSSD, square root of the mean of the sum of successive differences in R-R interval; Ex, rhythmic handgrip exercise at 35% of maximum voluntary contraction; Ex+/-80, Ex+/-100 and Ex+/-120, handgrip exercise with or without upper arm cuff inflation to 80, 100 and 120 mmHg, respectively; PEI, post exercise ischemia. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex of corresponding trial; ‡ P<0.05 vs. Ex FF/+80 of corresponding trial; $ P<0.05 vs. Ex FF/+100 of corresponding trial; § P<0.05 vs. Ex FF/+120 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

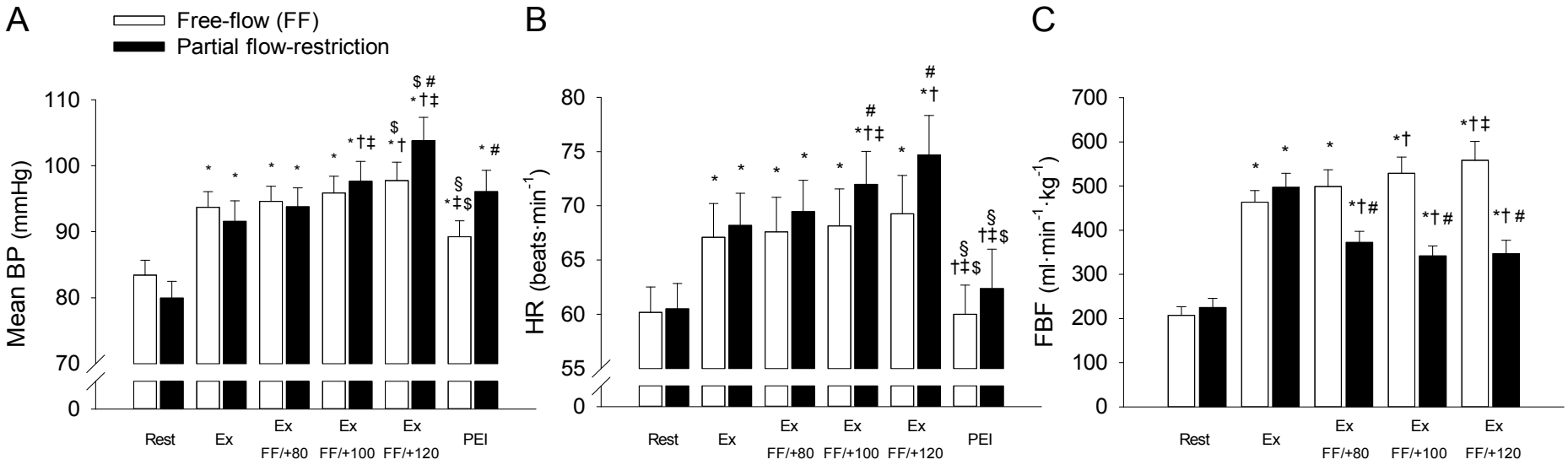

Figure 4.7. Mean arterial blood pressure (BP; panel A), heart rate (HR; panel B) and forearm blood flow (FBF; panel C) at rest, during rhythmic handgrip exercise under free-flow conditions (white bars) and with partial flow-restriction (black bars). FF, free-flow; PFR, partial flow-restriction; BP, blood pressure; RRI, R-R interval; Ex, rhythmic handgrip exercise at 35% of maximum voluntary contraction; Ex FF/+80, Ex FF/+100 and Ex FF/+120, handgrip exercise without or with upper arm cuff inflation to 80, 100 and 120 mmHg, respectively; PEI, post exercise ischemia. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex of corresponding trial; ‡ P<0.05 vs. Ex FF/+80 of corresponding trial; $ P<0.05 vs. Ex FF/+100 of corresponding trial; § P<0.05 vs. Ex FF/+120 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

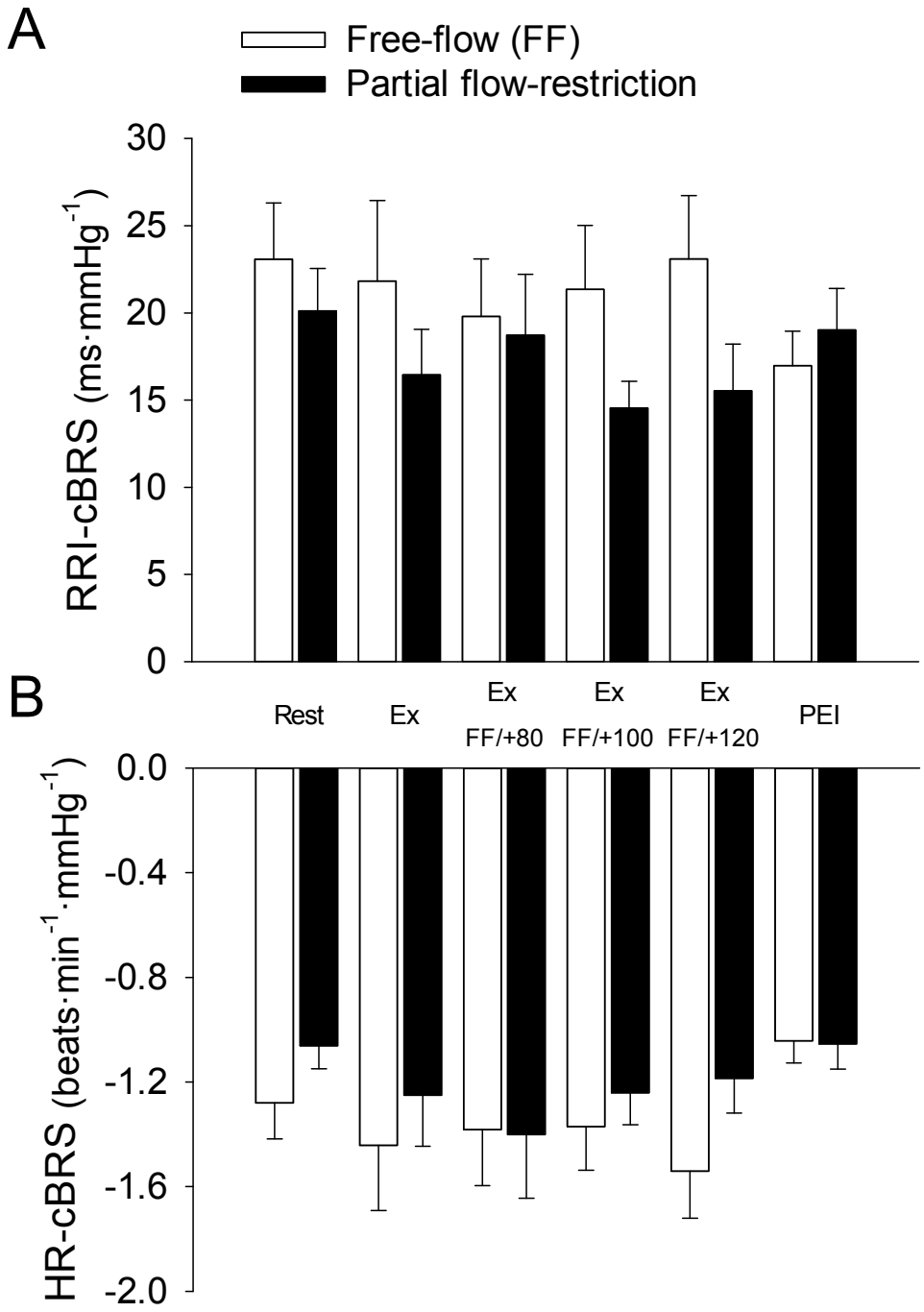

Figure 4.8. Spontaneous cardiac baroreflex sensitivity (cBRS) at rest, during handgrip exercise under free-flow conditions (white bars) and with graded partial flow-restriction (black bars). Spontaneous cBRS calculated using either R-R interval (RRI-cBRS, panel A) or heart rate (HR-cBRS, panel B). FF, free-flow; PFR, partial flow-restriction; RRI, R-R interval; Ex, rhythmic handgrip exercise at 35% of maximum voluntary contraction; Ex FF/+80, Ex FF/+100 and Ex FF/+120, handgrip exercise without or with upper arm cuff inflation to 80, 100 and 120 mmHg, respectively; PEI, post exercise ischemia.

4.4 Discussion

The aim of the present study was to determine whether activation of the muscle metaboreflex by restricting skeletal muscle perfusion during exercise evokes a decrease in spontaneous cBRS in humans. The major novel findings of the present investigation are two-fold: (i) Activation of the muscle metaboreflex during leg cycling exercise by partial restriction of blood flow to the active skeletal muscles evoked a decrease in cardiac baroreflex responsiveness (cBRS). (ii) While pronounced reductions in cBRS were observed during isolated activation of the muscle metaboreflex with PEI following leg cycling exercise, cBRS was unchanged during PEI following rhythmic handgrip exercise. Collectively, these data indicate that activation of metabolically sensitive muscle afferents contributes to the exercise-induced decrease in cBRS during leg cycling exercise in humans. They also reveal that the effect of muscle metaboreflex activation on cBRS is specific to the exercise modality studied.

An exercise-intensity dependent decrease in cBRS was observed during dynamic leg cycling in support of previous work in dogs (Sala-Mercado et al., 2010, Sala-Mercado et al., 2007) and humans (Iellamo et al., 1998, Ogoh et al., 2005b). Such a reduction in cBRS is potentially attributable to the activation of central command, the muscle mechanoreflex and the muscle metaboreflex, which are powerful modulators of cardiac autonomic activity and baroreflex function, both independently and interactively. The aim of the present study was to investigate whether the activation of the muscle metaboreflex is a potential mechanism for the exercise-mediated reduction in spontaneous cBRS during dynamic exercise of a large muscle mass (i.e. leg cycling). Previous work in humans from our group (Fisher et al., 2010, Fisher et al., 2008) and others (Cui et al., 2001, Ichinose et al., 2002, Iellamo et al., 1999b, Spaak et al., 1998) have demonstrated that cBRS is unchanged from baseline during isolated muscle

metaboreflex during a period of PEI following handgrip exercise. This is seemingly in contrast to the findings of the present study and those undertaken in exercising canines (Sala-Mercado et al., 2010, Sala-Mercado et al., 2007), where augmented activation of the muscle metaboreflex elicited by partial restriction of blood flow to the active skeletal muscles has been shown to reduce cBRS. Thus, it appears that methodological differences in the mode of muscle metaboreflex activation (post vs. during exercise) or indeed exercise modality can differentially affect spontaneous cBRS.

It is well established that activation of metabolically sensitive afferents elicits an increase in sympathetic nerve activity to the heart and peripheral vasculature (Fisher et al., 2010, Mark et al., 1985, O'Leary, 1993). These autonomic adjustments cause pronounced increases in HR and BP when the muscle metaboreflex is engaged by hypoperfusion of the dynamically exercising muscles using partial terminal aortic occlusion in dogs (O'Leary, 1993, Sala-Mercado et al., 2010, Sala-Mercado et al., 2007, Wyss et al., 1983), or using lower body positive pressure in cycling humans (Bonde-Petersen et al., 1978, Sun et al., 1993, Sundberg and Kaijser, 1992). However, during isolated muscle metaboreflex activation with PEI following handgrip exercise, BP remains elevated while HR returns to resting levels (Fisher et al., 2010, Fisher et al., 2008, Mark et al., 1985). There is evidence to suggest that the restoration of HR from end-exercise levels under such circumstances is related to the loss of the inhibitory actions of central command and/or the muscle mechanoreflex on cardiac parasympathetic activity at the end of exercise (Fisher et al., 2010, O'Leary, 1993). In addition, the elevation of BP during PEI may facilitate the robust reactivation of cardiac parasympathetic tone via the arterial baroreflex, thus masking the potential tachycardic effects of an elevation in cardiac sympathetic activity (Coote, 2010). Therefore, it is possible that during PEI the elevated cardiac parasympathetic tone obscures the

inhibitory actions of muscle metaboreflex activation on cBRS. In the present study, previous observations that cBRS was unchanged during PEI following handgrip exercise (Cui et al., 2001, Fisher et al., 2010, Fisher et al., 2008, Ichinose et al., 2002, Iellamo et al., 1999b, Spaak et al., 1998) were replicated. The current results further document that cBRS is not altered by augmented muscle metaboreflex activation during handgrip exercise (partial flow-restriction). However, in contrast cBRS was attenuated by muscle metaboreflex activation during both leg cycling (partial flow-restriction) and PEI following leg cycling in the present study. These observations are congruent with (i) the ground breaking work of Alam and Smirk (1938) who have demonstrated that HR remained elevated during PEI following dynamic calf plantar flexion of both legs, but not during PEI following handgrip, and (ii) Blonde-Petersen et al. (1978) who have shown that HR also remained elevated during PEI following leg cycling exercise. Taken together, these findings indicate that when examining the interaction between the muscle metaboreflex and cBRS, the exercise modality employed may be more important than the means by which muscle metaboreflex activation is achieved. As such, one cannot accurately elucidate the effect of muscle metaboreflex activation on cBRS during dynamic exercise of a large muscle mass (e.g. leg cycling) using studies employiong small muscle mass exercise (e.g. handgrip). Of note, Iellamo et al. (2006) have reported a maintained cBRS during PEI after leg cycling. However, these observations should be interpreted with care since the study sample comprised a unique cohort of four astronauts. As the authors acknowledge, possibly due to the timing of the experiments (e.g. close to launch) and the age of the participants (45 years compared with 22 years in the present study), resting cBRS was remarkably low (~4 ms/mmHg compared with ~16 ms/mmHg in the present study). This may have reduced the chances

of detecting a reduction in cBRS with metaboreflex activation. This means that direct comparisons with the present study are difficult.

The sequence technique was used in the present study to provide an estimate of the integrated cBRS (i.e. carotid and aortic baroreceptors). This method utilizes the natural and spontaneous beat-to-beat fluctuations in BP and the corresponding short-term, baroreflex-mediated change in HR (identified according to established criteria). There is strong evidence to suggest that spontaneous measures of cBRS predominantly represent alterations in cardiac parasympathetic efferent activity (Fisher et al., 2010, Ogoh et al., 2005b, Parlow et al., 1995). Indeed, cBRS, estimated using the sequence technique, is virtually abolished by administration of an anti-cholinergic drug (e.g. atropine, glycopyrrolate) (Fisher et al., 2010, Parlow et al., 1995). Importantly, reductions in cBRS during dynamic exercise are also linked to cardiac parasympathetic withdrawal (Ogoh et al., 2005b). Thus it is tempting to speculate that activation of the muscle metaboreflex during leg cycling and PEI following leg cycling attenuates cBRS via a direct reduction in cardiac parasympathetic tone. O'Leary (1993) has reported that hypoperfusion of the hindlimbs of dogs running on a treadmill increased HR despite pharmacological blockade of β-adrenoreceptors. These findings support the notion that muscle metaboreflex activation during leg cycling reduces cardiac parasympathetic activity thus increasing HR and potentially decreasing cBRS (Sun et al., 1993). In the present study decreases in spontaneous cBRS and RMSSD with muscle metaboreflex activation were more marked at low intensity exercise (HR ~90 $b \cdot min^{-1}$) than during moderate intensity exercise (HR ~120 $b \cdot min^{-1}$). This possibly reflects the greater cardiac parasympathetic activity available to be inhibited at lower exercise intensities by the muscle metaboreflex (Robinson et al., 1966). Furthermore, both cBRS and RMSSD were also reduced during isolated activation of the muscle metaboreflex during PEI.

Skeletal muscle afferents have been reported to converge on barosensitive cells within the NTS and cardiac vagal motoneurons within the brain stem and can act to decrease cardiac baroreflex responsiveness (Iwamoto and Kaufman, 1987, McWilliam and Yang, 1991, Potts, 2006, Potts and Mitchell, 1998). However, the available evidence in humans indicates that those skeletal muscle afferents evoking alterations in cardiac parasympathetic activity and cBRS are mechanosensitive rather than metabolically sensitive (Gladwell and Coote, 2002, Gladwell et al., 2005). Thus, whether the muscle metaboreflex alters cBRS during leg cycling via a direct effect remains incompletely understood.

An alternative mechanism by which the bilateral thigh cuff inflation manoeuvre used to evoke a hypoperfusion of the exercising muscle during leg cycling may indirectly modulate cBRS is via an increase in intramuscular pressure and consequent stimulation of mechanically sensitive muscle afferents (Kaufman and Rybicki, 1987). Isolated activation of the muscle mechanoreflex by passive stretch of the calf muscles has been reported to cause a reduction in spontaneous cBRS (Drew et al., 2008a), and an increase HR (+5±3 $b \cdot min^{-1}$) due to a reduction in cardiac parasympathetic nerve activity (Gladwell and Coote, 2002, Gladwell et al., 2005). However, an increase in HR with passive stretch was not evident when cardiac parasympathetic tone was reduced with either glycopyrrolate administration, mild rhythmic handgrip exercise or carotid baroreceptor unloading (Gladwell et al., 2005). Therefore, it is unlikely that mechanoreflex activation could wholly account for the reduction in cBRS observed in the present study when blood flow was obstructed to the exercising muscles during low and moderate leg cycling (target HR of 90 and 120 $b \cdot min^{-1}$, respectively), as cardiac parasympathetic tone would be expected to be significantly withdrawn at these workloads (Robinson et al., 1966). Furthermore, it has been suggested that stretch

sensitive group III and IV muscle afferents are a distinct population from those activated by muscular contraction (Hayes et al., 2005). Muscle mechanoreflex activation has also been experimentally elicited by external calf muscle compression (Bell and White, 2005). However, the cuff inflation pressure previously used was much greater than in the present study (300 vs. 100 mmHg) and did not cause any HR increase. Muscle afferent responses to mechanical stimuli have also been suggested to be augmented by concomitant accumulation of metabolites in the interstitium (Kaufman and Rybicki, 1987). Thus, augmenting metabolite accumulation within the exercising muscle may increase the firing of mechanosensitive muscle afferents. In the present study, the increases in HR and BP with bilateral thigh cuff inflation during leg cycling were in excess of the modest cardiovascular responses previously observed in response to experimental sensitization of muscle mechanoreflex (Bell and White, 2005, Cui et al., 2008, Drew et al., 2008a, Fisher et al., 2005, Middlekauff and Chiu, 2004). Furthermore, in the present study, cBRS was attenuated during PEI following leg cycling, when the exercise-induced activation of mechanically sensitive muscle afferents was presumably absent. In light of these collective findings one might suggest that the activation of mechanically sensitive muscle afferents is unlikely to explain the decrease in cBRS with partial restriction of blood flow to the active skeletal muscles during leg cycling observed in the present study. Nevertheless their possible contribution cannot be definitively rule out.

An alternative explanation for the reduction in cardiac baroreflex responsiveness elicited by hypoperfusion of the active skeletal muscles during leg cycling is an increase in central command. It is possible that bilateral thigh cuff inflation during leg cycling may decrease mechanical efficiency, thus altering motor unit recruitment strategies and augmenting central command. Moreover, skeletal muscle afferent feedback may exert

an inhibitory influence to spinal and supraspinal areas of the central nervous system (Gandevia, 2001). Activation of metabolically sensitive muscle afferents during exercise may inhibit alpha motor neurons innervating the skeletal muscle, reducing their excitability (Amann et al., 2009). This means that additional central drive is required to maintain the requisite exercise intensity. In support of this, RPE, a measure of the participants' sense of effort, historically related to central command (Mitchell, 1990), was significantly increased by bilateral thigh cuff inflation during leg cycling in the present study. In humans, central command predominantly alters HR via withdrawal of parasympathetic tone (Mitchell et al., 1989b) and evokes a movement of the operating point towards the threshold of the carotid baroreflex function curve (i.e. a point of reduced sensitivity) (Gallagher et al., 2001c, Ogoh et al., 2002b). As such, a muscle afferent-induced increase in central command provides a plausible explanation for the further reduction in parasympathetic tone and spontaneous cBRS observed during partial flow-restriction during leg cycling in the present study. However, it should be noted that in exercising dogs, muscle afferent blockade has been shown to abolish the cardiovascular response to unilateral iliac arterial occlusion during exercise (Kozelka et al., 1987, Pomeroy et al., 1986). Whether such observations can be translated to humans remain unclear. Thus, on the basis of the available evidence it appears reasonable that in humans muscle metaboreflex activation may indirectly reduce spontaneous cBRS during leg cycling via an increase in central neural drive.

It is possible that muscle metaboreflex-mediated elevations in plasma noradrenaline may attenuate parasympathetic control of HR and consequently reduce cBRS (Miyamoto et al., 2003). Miyamoto et al. (2003) have demonstrated that spontaneous cBRS was attenuated by noradrenaline infusion during vagus nerve stimulation in anaesthetized rabbits. Although a directly comparable study has not been

performed in humans, Taylor et al. (2001) have reported that administration of the β-adrenergic receptor antagonist atenolol augmented respiratory sinus arrhythmia. This indicated that cardiac sympathetic nerve activity may oppose cardiac parasympathetic nerve activity in resting humans. However, Ogoh et al. (2005b) have demonstrated that β-adrenergic blockade had a minimal affect on spontaneous cBRS during dynamic exercise. As such, it appears unlikely that muscle metaboreflex mediated sympathoexcitation reduced cBRS in the present study.

Since the sequence technique involves analysis of spontaneous fluctuations in BP and HR (or RRI) the full arterial baroreflex stimulus-response curve could not be evaluated. For this reason, it cannot be concluded whether the maximal sensitivity of the baroreflex has been manipulated by the intervention, or whether the operating point of the reflex has shifted to a non-linear region of the baroreflex function curve. It has been suggested that spontaneous measures of cBRS provide the same data as the sensitivity of the baroreflex at the operating point (Ogoh et al., 2005b, Parati et al., 2000). For this reason, the present findings are important as they imply that the physiologically active region of the baroreflex (i.e. the operating point) operates with a reduced sensitivity during dynamic exercise, possibly due to activation of metabolically sensitive muscle afferents.

A reduction in blood flow to the exercising skeletal muscles has been effectively shown to evoke increases in HR and BP using either graded clamping of the terminal aorta blood flow in treadmill running dogs (Sala-Mercado et al., 2010, Sala-Mercado et al., 2007, Sheriff et al., 1993) or noninvasively using lower body positive pressure in cycling humans (Eiken et al., 1992, Gallagher et al., 2001a, Sun et al., 1993, Sundberg and Kaijser, 1992). In the present study, a comparable cardiovascular response was elicited by bilateral thigh cuff inflation to 100 mmHg during dynamic leg cycling

exercise. Previous reports indicate that this manoeuvre evokes a reduction in limb blood flow, a mismatch between oxygen delivery and demand, an accumulation of workload related muscle metabolites and the activation of metabolically sensitive skeletal muscle afferents (Eiken and Bjurstedt, 1987, Eiken et al., 1992). The present measurements of FBF during rhythmic handgrip confirmed that inflation of a cuff proximal to the exercising muscles significantly reduced their perfusion. As a suitable methodology (e.g. femoral venous thermodilution) was not accessible, leg blood flow during cycling exercise could not be measured. Thus a limitation of the present study is that the magnitude of the flow restriction during low and moderate cycling workloads could not be quantified or compared. In addition, it is acknowledged that restriction of venous outflow from the exercising muscle via thigh-cuff inflation to 100 mmHg is also likely to evoke venous congestion. This in turn may stimulate mechanosensitive afferents located in the walls of the vasculature within the skeletal muscle (Cui et al., 2009, Haouzi et al., 1999, McClain et al., 1993). Although stimulation of such sensory afferents has been shown to evoke a cardiovascular response (Cui et al., 2009, Haouzi et al., 1999, McClain et al., 1993), it is presently unclear if they modulate cBRS.

The present study examined the effect of muscle metaboreflex activation on the arterial baroreflex. However, it is recognised that this is a two-way interaction. Work by Waldrop and Mitchell (1985) and Sheriff et al. (1990) has indicated that the arterial baroreflex attenuates the pressor response evoked by muscle afferent activation. More recently, Kim et al. (2005) have demonstrated in dogs that following barodenervation the muscle metaboreflex-induced increase in CO was attenuated (due to a decrease in stroke volume). They have also shown that the pressor response was greater compared to the baro-intact condition. Further studies in humans are required to examine the

impact of the arterial baroreflex on the strength and mechanisms by which the muscle metaboreflex modulates BP during exercise (i.e. CO vs. TPR).

In chronic disease conditions that are characterised by a hypoperfusion of the active skeletal muscles (e.g. peripheral vascular disease, chronic heart failure), the muscle metaboreflex is not activated in isolation from central command and the muscle mechanoreflex. Thus, experimental reductions in muscle blood flow to augment the muscle metaboreflex during exercise may more realistically mimic such clinical conditions. As such, in light of the cardioprotective properties of parasympathetic tone (Billman, 2006), the muscle metaboreflex-mediated reduction in cBRS observed in the present study may have clinical significance for patient populations in whom increased muscle metaboreflex sensitivity (Piepoli et al., 1996, Scott et al., 2002) and decreased cBRS (Grassi et al., 1995) have been identified.

4.5 Conclusion

In summary, the findings of the present study indicate that the activation of the muscle metaboreflex during dynamic leg cycling exercise using partial restriction of the blood flow to the active skeletal muscles, or during PEI, elicits a decrease in cardiac baroreflex responsiveness. Overall, the present findings suggest that the activation of metabolically sensitive muscle afferents plays an important role in the decrease in cBRS during dynamic exercise involving a large muscle mass in humans.

CHAPTER 5: METABOREFLEX CONTROL OF SPONTANEOUS CARDIAC BAROREFLEX SENSITIVITY DURING DYNAMIC EXERCISE: INFLUENCE OF SEX AND OVARIAN HORMONES IN HUMANS

5.1 Introduction

As reported in the previous chapter in humans, and as recently demonstrated in dogs running on a treadmill (Sala-Mercado et al., 2010, Sala-Mercado et al., 2007), the activation of metabolically sensitive skeletal muscle afferents plays an important role in the exercise-induced reduction in spontaneous cBRS. However, the potential influences of sex and/or ovarian hormone concentration remain unclear, as these previous studies have been conducted predominantly in males.

Sex-differences appear to exist in the arterial baroreflex control of HR at rest. This is indicated by greater cBRS in female than male rats (Chen and DiCarlo, 1996). However, in humans results are rather inconsistent, with studies reporting either increased, decreased or unchanged cBRS in women compared to men (Abdel-Rahman et al., 1994, Beske et al., 2001, Convertino, 1998, Tank et al., 2005). Also, all these studies have been conducted at rest, and it is therefore unclear at whether sex-differences in the arterial baroreflex control of HR exist during exercise. Accumulation of metabolic by-products activates metabolically sensitive skeletal muscle afferents (i.e. muscle metaboreflex) (Mark et al., 1985, O'Leary, 1993). Since women have reportedly a greater proportion of slow-twitch fibres, produce less lactate and have a greater capacity to oxidize fat compared to men (Komi and Karlsson, 1978, Simoneau and Bouchard, 1989, Sullivan et al., 1991), it is reasonable to assume that these factors might attenuate the activation of metabolically sensitive skeletal muscle afferents during exercise in women. Thus, sex-differences in muscle metaboreflex activation may lead to differences in cBRS during exercise. However, it is presently unknown whether there are sex-differences in the muscle metaboreflex-induced reduction in cBRS during dynamic exercise.

The ovarian hormone estrogen is known to have a multitude of effects on cardiovascular regulatory mechanisms at rest and during exercise. For example, injection of estrogen into brainstem nuclei associated with the central autonomic cardiovascular control (e.g. NTS) has been demonstrated to increase vagal tone and to decrease sympathetic nerve traffic at rest and in response to hypertensive stimuli (Du et al., 1994, Saleh et al., 2000b, Saleh and Connell, 2000). In humans, elevated estrogen concentration during the late follicular phase (LF; when progesterone concentrations are low) has been shown to enhance resting cBRS when compared to the early follicular (EF; low estrogen and progesterone concentrations) and the midluteal phase (high estrogen and progesterone concentrations) of the ovarian cycle (Minson et al., 2000a, Tanaka et al., 2003). Similarly, ovariectomy in female rats has been shown to evoked a reduction in resting cBRS, which was prevented with subcutaneous estrogen administration (17-β estradiol) (el-Mas and Abdel-Rahman, 1998). This suggests that estrogen has beneficial effects on resting arterial baroreflex control of HR. In female cats, estrogen application on the spinal cord elicited an attenuated pressor response (+38 vs. +23 mmHg pre- and post-estrogen application, respectively) to electrically induced static muscle contractions (Schmitt and Kaufman, 2003a). Moreover, 17-β estradiol infusion attenuated the rise in HR and BP during electrical stimulation of the mesencephalic locomotor region (i.e. brain site associated with central command activation) in male cats (Hayes et al., 2002). Moreover, in humans elevated estrogen concentrations during the ovarian cycle have been associated with attenuated MSNA responses to static exercise (Ettinger et al., 1998). As the exercise-induced reduction in spontaneous cBRS has been attributed to a cardiac parasympathetic withdrawal mechanism (Ogoh et al., 2005b), estrogen might play an important role in the potential sex-specific changes in cBRS as well as the muscle metaboreflex-mediated reduction in

cBRS during dynamic exercise. However, it is presently unclear whether estrogen affects the reduction in cBRS observed during dynamic exercise and/ or during augmented muscle metaboreflex activation.

The aim of the present study was to determine the potential influence of sex and ovarian hormones on the muscle metaboreflex-mediated cardiovascular responses (i.e. HR and BP) and reductions in cBRS. To investigate this, the experimental model established in the previous chapter was utilised. Specifically, HR, BP and cBRS were assessed during dynamic leg cycling with and without partial flow-restriction in men and women (protocol 1) and in women during the EF and LF phases of the ovarian cycle (protocol 2). It was hypothesised that; (i) muscle metaboreflex activation during leg cycling would evoke an attenuated pressor response in women compared to men and (ii) reductions in cBRS during enhanced muscle metaboreflex activation would be attenuated in women, particularly when estrogen is elevated.

5.2 Methods

5.2.1 ***Subjects***

Twelve healthy female and fifteen healthy male subjects (data for men has been presented in Chapter 4) from the University of Birmingham volunteered to participate in the present study (Table 5.1).

5.2.2 Experimental measurements

To determine whether sex and/or ovarian hormone concentration influence muscle metaboreflex-induced effects on spontaneous cBRS and the pressor response

during dynamic exercise, beat-to-beat HR and BP were recorded and mean BP calculated (see Section 3.2.1 for details).

Table 5.1. Subject characteristics

	Men (n=15)	Women (n=12)
Age (years)	22 ± 1	20 ± 0.6 *
Weight (kg)	78 ± 2	61 ± 2 *
Height (cm)	181 ± 1	165 ± 2 *
BMI ($kg \cdot m^{-2}$)	24 ± 1	22 ± 1
Low Workload (W)	24 ± 4	21 ± 2
Moderate Workload (W)	105 ± 7	71 ± 3 *
EF day		3 ± 0.4
LF day		13 ± 0.5

Values are mean±SEM. BMI, body mass index; EF, early follicular phase; LF, late follicular phase. * $P<0.05$ different from men.

5.2.3 Experimental procedures

Protocol 1 (Men versus Women): Men and women during the EF phase of the ovarian cycle performed leg cycling exercise under free-flow and partial flow restriction (see Chapter 4, Section 4.3.3 for details).

Protocol 2 (Women EF versus LF): All twelve women performed leg cycling under free-flow and partial flow-restriction twice during their ovarian cycle. The order was counterbalanced: One session was conducted during the EF phase (range, day 1-5; estrogen and progesterone both low) and the other during the LF phase (range, day 10-15; estrogen high and progesterone low) of the ovarian cycle. The peak in estrogen concentration was indirectly measured using ovulation kits (Clearblue®, SPD Swiss Precision Diagnostics GmbH, Petit Lancy, Switzerland). Ovulations kits detect concentrations of luteinising hormone (LH) in the urine. This rise in LH occurs simultaneously with an increase in estrogen concentration during the LF phase of the

ovarian cycle. As the estrogen peak occurs before the LH surge, women were tested before the ovulation kit detected the LH peak. The resistance necessary to reach the target HR of 90 and 120 beats·min^{-1}, was not different between the two experimental visits.

5.2.4 Data analysis

Spontaneous cBRS was calculated using the sequence technique (see Section 3.4.2 for details). For each trial, cBRS and cardiovascular data (systolic, diastolic, and mean BP, HR and RRI) were averaged over a 3 minute period for each of the 6 experimental phases (Figure 4.2 in Chapter 4).

Protocol 1 (Men versus Women): Physiological data were statistically analysed using three-way repeated measures ANOVA. The main factors were *trial* (free-flow, partial flow-restriction), *time* (Rest, Ex90, Ex90 free-flow or partially restricted-flow, Ex120 and Ex120 free-flow or partially restricted-flow) and *sex* (men, women) as the between subject factor. Statistical analyses of differences within one sex-group used two-way repeated measures ANOVA. The main factors were *trial* (free-flow, partial flow-restriction) and *time* (e.g. Ex90). Similarly, changes in spontaneous cBRS induced by partial flow-restriction versus free-flow trial at low and moderate exercise intensities in men and women were analysed using two-way repeated measures ANOVA with *sex* and *time* as the main factors.

Protocol 2 (Women EF versus LF): Physiological data were statistically analysed using three-way repeated measures ANOVA. The main factors were *trial* (free-flow or partial flow-restriction), *time* (Rest, Ex90, Ex90 free-flow or partially restricted-flow, Ex120 and Ex120 free-flow or partially restricted-flow) and *phase* (EF or LF). Statistical analyses of differences within one cycle phase were performed using

two-way repeated measures ANOVA. The main factors were *trial* (free-flow, partial flow-restriction) and *time* (e.g. Ex90). Similarly, changes in spontaneous cBRS induced by partial flow-restriction versus free-flow trial at low and moderate exercise intensities during EF and LF phases of the ovarian cycle were analysed using two-way repeated measures ANOVA, with *phase* and *time* as the main factors.

5.3 Results

5.3.1 Protocol 1: Men versus Women under free-flow conditions

Resting HR, RRI, mean BP and cBRS were similar in men and women in both the free-flow and restricted-flow trials, while systolic BP was greater in men compared to women (Table 5.2; Figures 5.1&5.2). During low intensity dynamic leg cycling, systolic BP and HR increased significantly in men and women (+22±2 $b \cdot min^{-1}$), while diastolic BP was unchanged from rest (Table 5.2; Figure 5.2). Mean BP rose slightly in men (+3±1 mmHg), but not in women and systolic BP rose significantly more in men than women ($P<0.05$; Table 5.2). Dynamic leg cycling at moderate intensity elicited a further increase in systolic BP, mean BP and HR (+55±3 and +59±3 $b \cdot min^{-1}$ in men and women, respectively; Table 5.2; Figure 5.1), while diastolic BP tended to fall in both groups. The magnitude of the rise in HR was similar between the groups, while the increases in systolic and mean BP were greater in men than in women (+12±2 and +4±1 in men and women, respectively; Table 5.2). RRI decreased in an exercise dependent manner in both men and women (Table 5.2). cBRS was decreased from rest during low intensity exercise, and further decreased from rest during moderate intensity exercise ($P<0.05$). This reduction in cBRS was not different between men (RRI-cBRS, 16±2, 7±1 and 2±0.2 $ms \cdot mmHg^{-1}$ at rest, low and moderate leg cycling, respectively) and

women (RRI-cBRS, 21±5, 6±1 and 1±0.1 ms·mmHg^{-1} at rest, low and moderate leg cycling, respectively; Figure 5.3). The significant increase in RPE from low (8±0.4 au) to moderate (13±0.5 au) intensity leg cycling was similar in men and women.

At low intensity leg cycling men and women reached the target HR at an average of 24±4 and 21±3 Watts, respectively. However, the resistance to achieve the expected HR of 120 during moderate leg cycling was significantly greater in men than women (105±7 and 72±4 Watts in men and women, respectively; $P<0.05$).

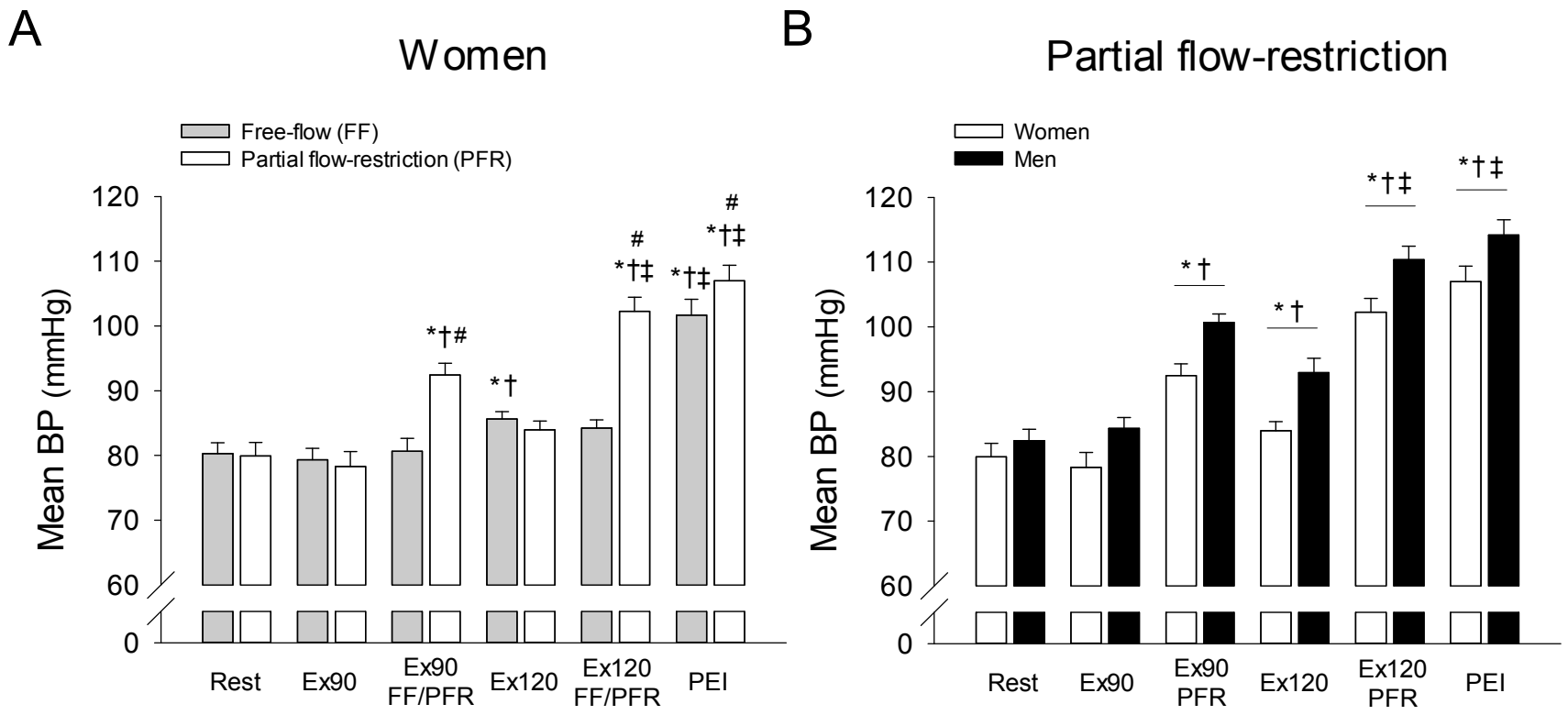

Figure 5.1. Panel A shows mean blood pressure (BP) responses in women at rest and during dynamic leg cycling under free-flow conditions (grey bars) and with partial flow-restriction (white bars). Panel B shows mean BP responses at rest and during dynamic leg cycling with partial flow-restriction in women (white bars) and men (black bars). FF, free-flow; PFR, partial flow-restriction; Ex90, leg cycling at target HR of 90 $b \cdot min^{-1}$; Ex120, leg cycling at target HR of 120 $b \cdot min^{-1}$. * $P<0.05$ vs. Rest of corresponding trial; † $P<0.05$ vs. Ex90 of corresponding trial; ‡ $P<0.05$ vs. Ex120 of corresponding trial; # $P<0.05$ vs. FF trial at corresponding time point.

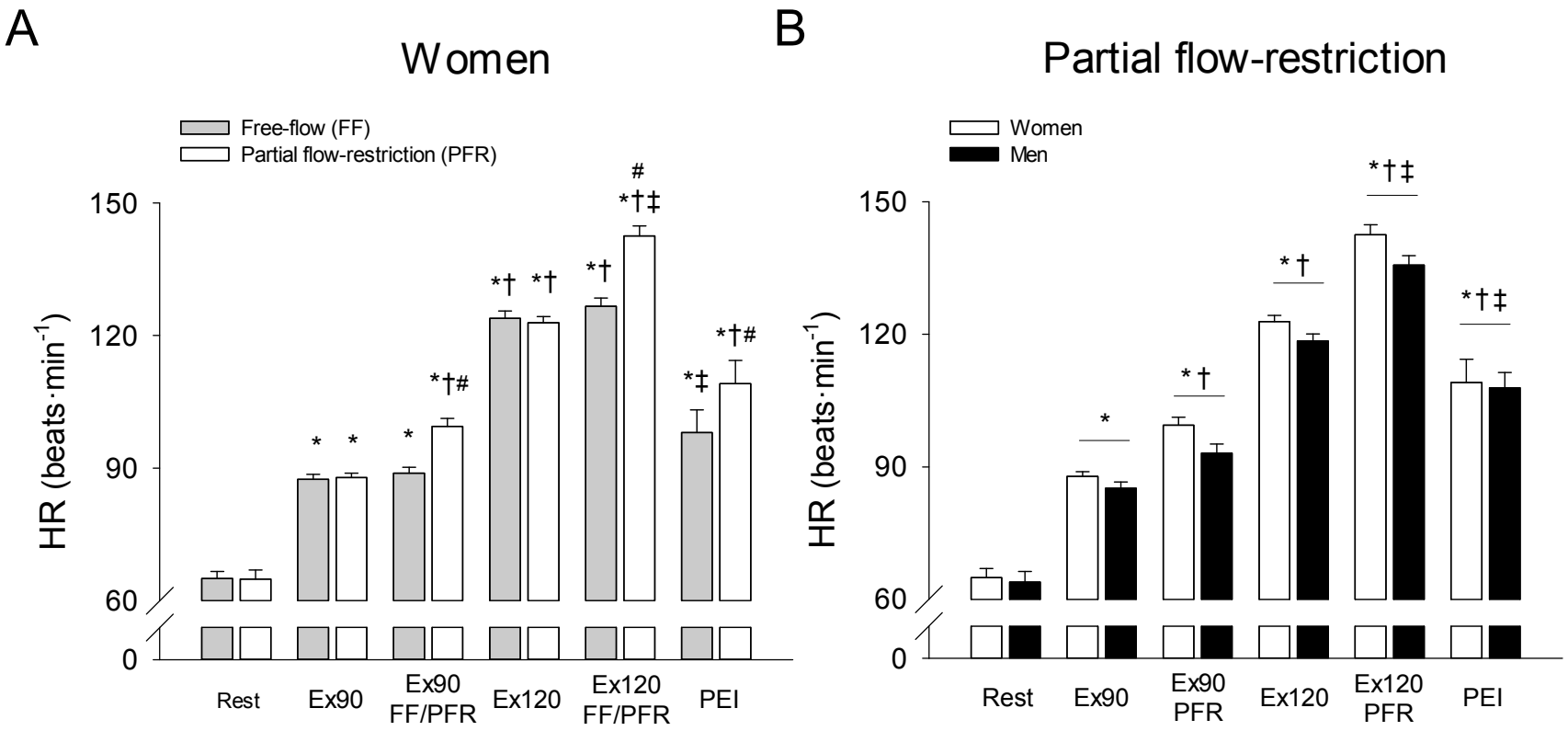

Figure 5.2. Panel A shows heart rate (HR) responses in women at rest and during dynamic leg cycling under free-flow conditions (grey bars) and with partial flow-restriction (white bars). Panel B shows HR responses at rest and during dynamic leg cycling with partial flow-restriction in women (white bars) and men (black bars). FF, free-flow; PFR, partial flow-restriction; Ex90, leg cycling at target HR of 90 b·min^{-1}; Ex120, leg cycling at target HR of 120 b·min^{-1}. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex90 of corresponding trial; ‡ P<0.05 vs. Ex120 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

5.3.2 Protocol 1: Men versus Women during muscle metaboreflex activation

Muscle metaboreflex activation by partially restricting exercising skeletal muscle blood flow evoked a significant decrease in RRI and increases in HR, systolic and mean BP in men and women ($P<0.05$). The muscle metaboreflex-induced increase in mean BP during the low and moderate exercise workload was comparable in men and women ($P>0.05$; Table 5.2; Figure 5.1). Similarly the magnitude of the intensity dependent changes in HR was not different between men and women (Table 5.2; Figure 5.2). cBRS was significantly attenuated by muscle metaboreflex activation during low intensity leg cycling in men and women (RRI-cBRS, -2.6±0.8 ms·mmHg^{-1} in men and -2.0±0.4 ms·mmHg^{-1} in women; $P<0.05$ vs. free-flow trial; Figures 5.3, 5.4&5.9). The significant muscle metaboreflex-mediated reduction in cBRS during moderate intensity leg cycling in men (-0.4±0.1 ms·mmHg^{-1}; $P<0.05$ vs. free-flow trial) was absent in women (Figure 5.3). The effect of dynamic exercise with or without partial flow-restriction on cBRS was similar irrespective of whether cBRS was analysed using either HR or RRI in both men and women. During PEI, BP and HR were significantly elevated above resting values, while RRI and cBRS were decreased from rest during PEI ($P<0.05$). PEI after the partial flow-restriction trial evoked a higher systolic and diastolic BP and HR, and a lower RRI and cBRS compared to PEI after the free-flow trial ($P<0.05$; Table 5.2; Figures 5.1-5.4). Dynamic leg cycling with partial flow-restriction evoked a significant rise in RPE at both low (13±0.5 and 12±1 au in men and women) and moderate (16±0.4 and 16±1 au in men and women) exercise intensities ($P<0.05$ vs. free-flow trial).

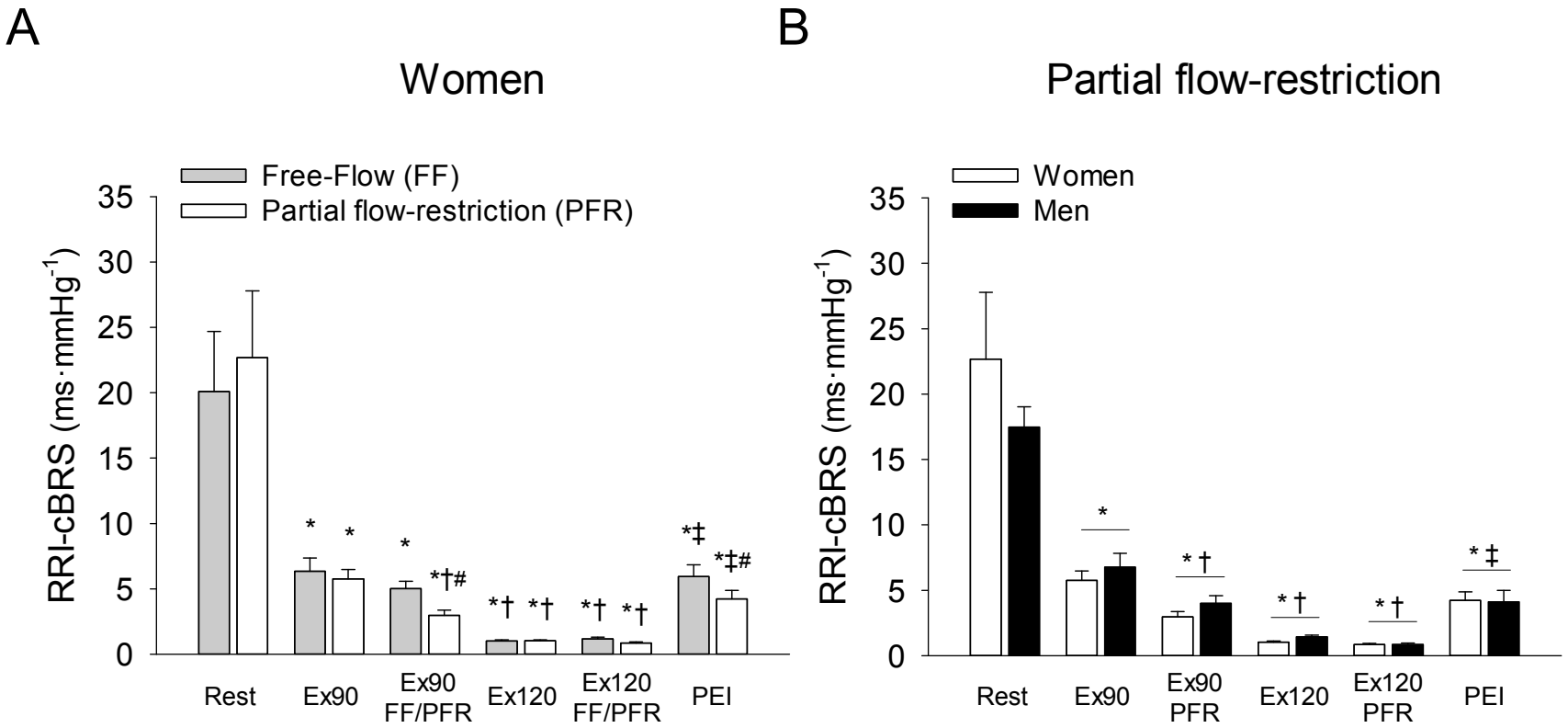

Figure 5.3. Panel A indicates spontaneous cardiac baroreflex sensitivity (cBRS) calculated using R-R interval (RRI-cBRS) in women at rest and during dynamic leg cycling under free-flow conditions (grey bars) and with partial flow-restriction (white bars). Panel B indicates spontaneous RRI-cBRS in women (white bars) and men (black bars) at rest and during dynamic leg cycling with partial flow-restriction. PFR, partial flow-restriction; Ex90, leg cycling at target HR of 90 $b \cdot min^{-1}$; Ex120, leg cycling at target HR of 120 $b \cdot min^{-1}$. * $P<0.05$ vs. Rest of corresponding trial; † $P<0.05$ vs. Ex90 of corresponding trial; # $P<0.05$ vs. FF trial at corresponding time point.

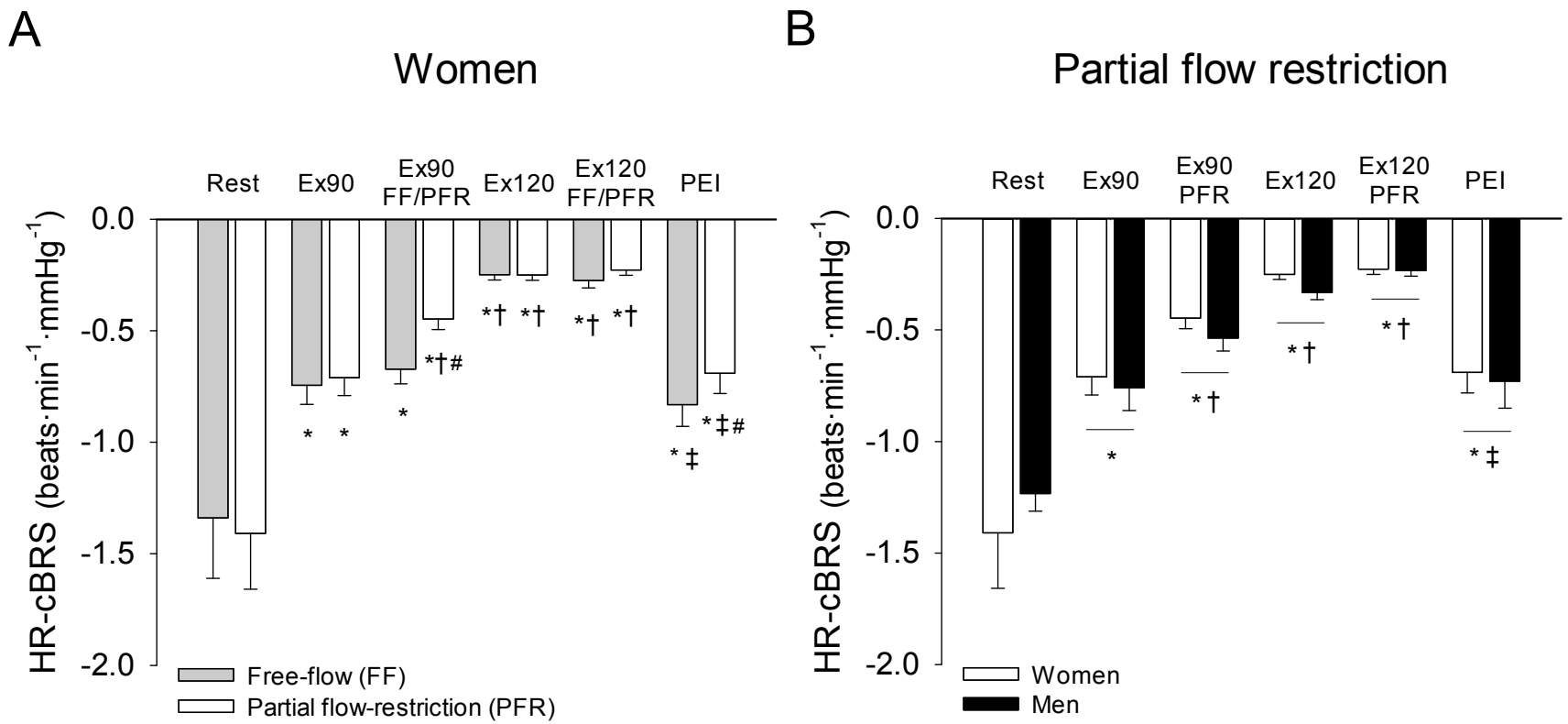

Figure 5.4. Panel A indicates spontaneous cardiac baroreflex sensitivity (cBRS) calculated using heart rate (HR-cBRS) in women at rest and during dynamic leg cycling under free-flow conditions (grey bars) and with partial flow-restriction (white bars). Panel B indicates spontaneous HR-cBRS in women (white bars) and men (black bars) at rest and during dynamic leg cycling with partial flow-restriction. PFR, partial flow-restriction; Ex90, leg cycling at target HR of 90 b·min^{-1}; Ex120, leg cycling at target HR of 120 b·min^{-1}. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex90 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

Table 5.2. Selected physiological variables at rest and low and moderate intensity leg cycling during the free-flow and partial flow-restriction experimental trials in men and women during early and late follicular phase of the ovarian cycle

	Rest		Ex 90		Ex 90 FF/PFR		Ex 120		Ex 120 FF/PFR		PEI	
	FF	PFR	FF	PFR	FF	PFR	FF	PFR	FF	PFR	FF	PFR
Systolic BP (mmHg)												
Men	121 ± 3	119 ± 2	132 ± 3	132 ± 2	134 ± 3	148 ± 3	163 ± 5	163 ± 5	164 ± 5	185 ± 4	161 ± 5	170 ± 4
Women EF *†$‡	111 ± 3	111 ± 3	117 ± 3	117 ± 3	118 ± 2	133 ± 3	142 ± 4	143 ± 4	143 ± 4	170 ± 6	143 ± 4	152 ± 4
Women LF *†$	111 ± 3	111 ± 3	117 ± 3	118 ± 3	117 ± 2	133 ± 3	141 ± 4	143 ± 4	143 ± 4	170 ± 6	143 ± 4	159 ± 5
Diastolic BP (mmHg)												
Men	60 ± 1	64 ± 2	60 ± 2	62 ± 3	59 ± 1	77 ± 2	57 ± 2	58 ± 2	58 ± 2	74 ± 2	82 ± 2	86 ± 3
Women EF *†$	65 ± 2	65 ± 2	60 ± 2	58 ± 3	59 ± 3	75 ± 2	58 ± 3	58 ± 3	58 ± 3	71 ± 3	81 ± 3	84 ± 3
Women LF *†$	65 ± 2	65 ± 2	60 ± 2	59 ± 3	60 ± 2	75 ± 2	58 ± 2	58 ± 3	59 ± 3	72 ± 3	79 ± 4	83 ± 3
RRI (ms)												
Men	961 ± 37	965 ± 39	701 ± 10	710 ± 12	693 ± 9	651 ± 15	504 ± 5	508 ± 7	492 ± 5	444 ± 7	673 ± 20	569 ± 20
Women EF *†$	936 ± 25	940 ± 29	689 ± 9	686 ± 8	679 ± 10	607 ± 12	486 ± 7	489 ± 5	475 ± 7	422 ± 7	631 ± 26	570 ± 26
Women LF *†$	944 ± 30	997 ± 32	680 ± 11	693 ± 14	672 ± 10	613 ± 15	492 ± 7	497 ± 8	478 ± 8	421 ± 8	632 ± 24	576 ± 18

Values are mean±SEM. EF, early follicular phase; LF late follicular phase; FF, free-flow; PFR, partial flow-restriction; BP, blood pressure; RRI, R-R interval; Ex90, leg cycling at target HR of 90 $b \cdot min^{-1}$; Ex120, leg cycling at target HR of 120 $b \cdot min^{-1}$. * $P<0.05$ effect of time; † $P<0.05$ effect of trial; $ $P<0.05$ time * trial effect; ‡ $P<0.05$ effect of sex (men vs. women EF). Two separate 3 way-repeated measures ANOVA were performed to compare men vs. women EF and women EF vs. women LF.

5.3.3 Protocol 2: Women EF versus LF under free-flow conditions

Resting HR, RRI, BP and cBRS were similar in women during EF and LF phases of the ovarian cycle and were not different between the free-flow and the partial-flow restriction trial at each visit (Table 5.2; Figures 5.1-5.8). Low intensity leg cycling evoked a significant increase in HR (+22±2 and +24±2 $b \cdot min^{-1}$ during EF and LF phases of the ovarian cycle, respectively; Figure 5.6) and systolic BP ($P<0.05$; Table 5.2), while diastolic and mean BP remained unchanged from rest (Table 5.2; Figures 5.1&5.5). Moderate intensity leg cycling significantly increased HR (+59±3 and +58±2 $b \cdot min^{-1}$ during EF and LF phase of the ovarian cycle, respectively), systolic and mean BP (+4±1 and +7±1 mmHg during EF and LF phases of the ovarian cycle, respectively), while diastolic BP tended to fall from rest similarly during EF and LF phases (Table 5.2; Figures 5.1, 5.2 & 5.5, 5.6). RRI decreased relative to the exercise intensity during both EF and LF phases of the ovarian cycle ($P<0.05$; Table 5.2). cBRS was decreased from rest during the low exercise intensity workload and further reduced during moderate intensity leg cycling, without any differences between the EF (RRI-cBRS, 20±5, 6±1 and 1±0.1 $ms \cdot mmHg^{-1}$ at rest, during low and moderate intensity leg cycling; Figures 5.3&5.4) and LF phases (RRI-cBRS, 22±5, 6±1, 1±0.1 $ms \cdot mmHg^{-1}$ at rest, during low and moderate intensity leg cycling; Figures 5.7&5.8). During both phases of the ovarian cycle RPE was similarly increased from low (8±0.4 and 8±0.3 au during EF and LF phases) to moderate intensity leg cycling (13±0.4 and 13±0.5 au during EF and LF phases).

5.3.4 Protocol 2: Women EF versus LF during muscle metaboreflex activation

Enhanced muscle metaboreflex activation, during partial flow-restriction to the exercising skeletal muscles at low intensity leg cycling, evoked significant decreases in RRI and increases in HR (+11±1 and +9±2 $b \cdot min^{-1}$ during EF and LF phases of the ovarian cycle, respectively) and mean BP (+12±1 and +15±1 mmHg during EF and LF phases of the ovarian cycle, respectively; Figure 5.5&5.6). During moderate intensity leg cycling RRI was further decreased and HR (+16±1 and +17±2 $b \cdot min^{-1}$ during EF and LF phases of the ovarian cycle, respectively; $P<0.05$; Table 5.2; Figures 5.6) and mean BP (+18±2 and +18±2 mmHg during EF and LF phases of the ovarian cycle, respectively; $P<0.05$; Table 5.2; Figures 5.1&5.5) further increased in comparison to the free-flow trial. The magnitude of the decrease in RRI and the increase in HR and mean BP were similar between the EF and LF phases of the ovarian cycle ($P>0.05$). cBRS was significantly attenuated by muscle metaboreflex activation during low intensity leg cycling ($P<0.05$), but the magnitude of this reduction was similar during EF (RRI-cBRS: -2.0±0.4 $ms \cdot mmHg^{-1}$ vs. free-flow trial; Figures 5.3&5.4) and LF (RRI-cBRS: -2.5±0.4 $ms \cdot mmHg^{-1}$ vs. free-flow trial; Figures 5.7-5.9) phases of the ovarian cycle. During moderate intensity leg cycling muscle metaboreflex activation elicited no changes in cBRS in women during EF or LF phases of the ovarian cycle. The relationship between RRI and RRI-cBRS, and HR and HR-cBRS were non-linear and not affected by ovarian cycle phase (Figure 5.10). The effect of dynamic exercise with or without partial flow-restriction on cBRS in women during the EF and LF phases of the ovarian cycle were similar irrespective of whether cBRS was analysed using either HR or RRI. During PEI systolic, diastolic and mean BP and HR were significantly elevated above rest, while RRI and cBRS were markedly lower compared to rest ($P<0.05$). The magnitude of the muscle metaboreflex-induced changes in BP, HR, RRI

and cBRS were greater during PEI after the partial flow-restriction trial than after the free-flow trial, without any differences between the EF and LF phases of the ovarian cycle (Table 5.2; Figures 5.5-5.8). Partial flow-restriction during exercise evoked a marked increase in RPE at both low (12±1 au during EF and LF phases of the ovarian cycle) and moderate (16±1 au during EF and LF phases of the ovarian cycle) exercise intensities ($P<0.05$ vs. free-flow trial).

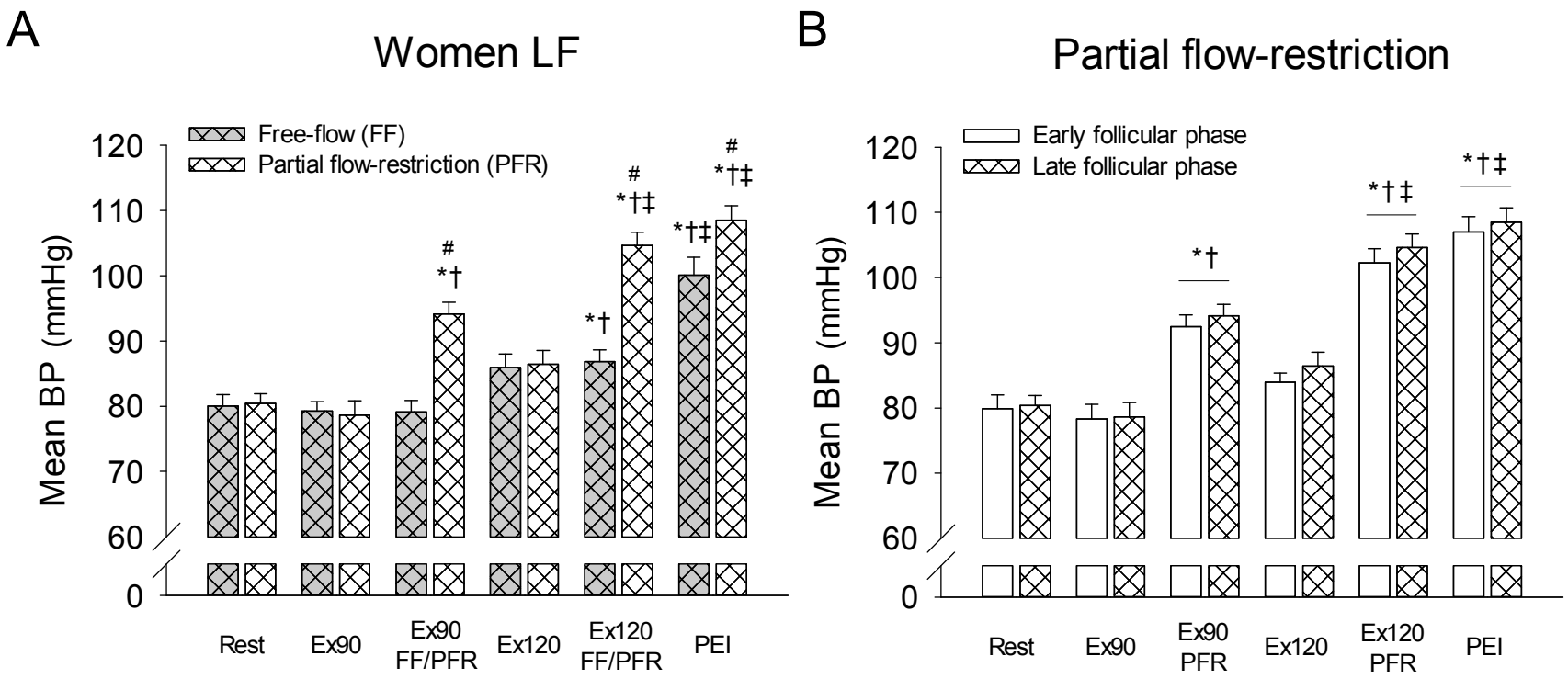

Figure 5.5. Panel A indicates mean blood pressure (BP) in women during the late follicular (LF) phase of the ovarian cycle at rest and during dynamic leg cycling under free-flow conditions (grey shaded bars) and with partial flow-restriction (white shaded bars). Panel B shows mean BP at rest and during dynamic leg cycling with partial flow-restriction in women during early (white bars) and late (white shaded bars) follicular phase of the ovarian cycle. FF, free-flow; PFR, partial flow-restriction; Ex90, leg cycling at target heart rate of 90 b·min^{-1}; Ex120, leg cycling at target heart rate of 120 b·min^{-1}. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex90 of corresponding trial; ‡ P<0.05 vs. Ex120 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

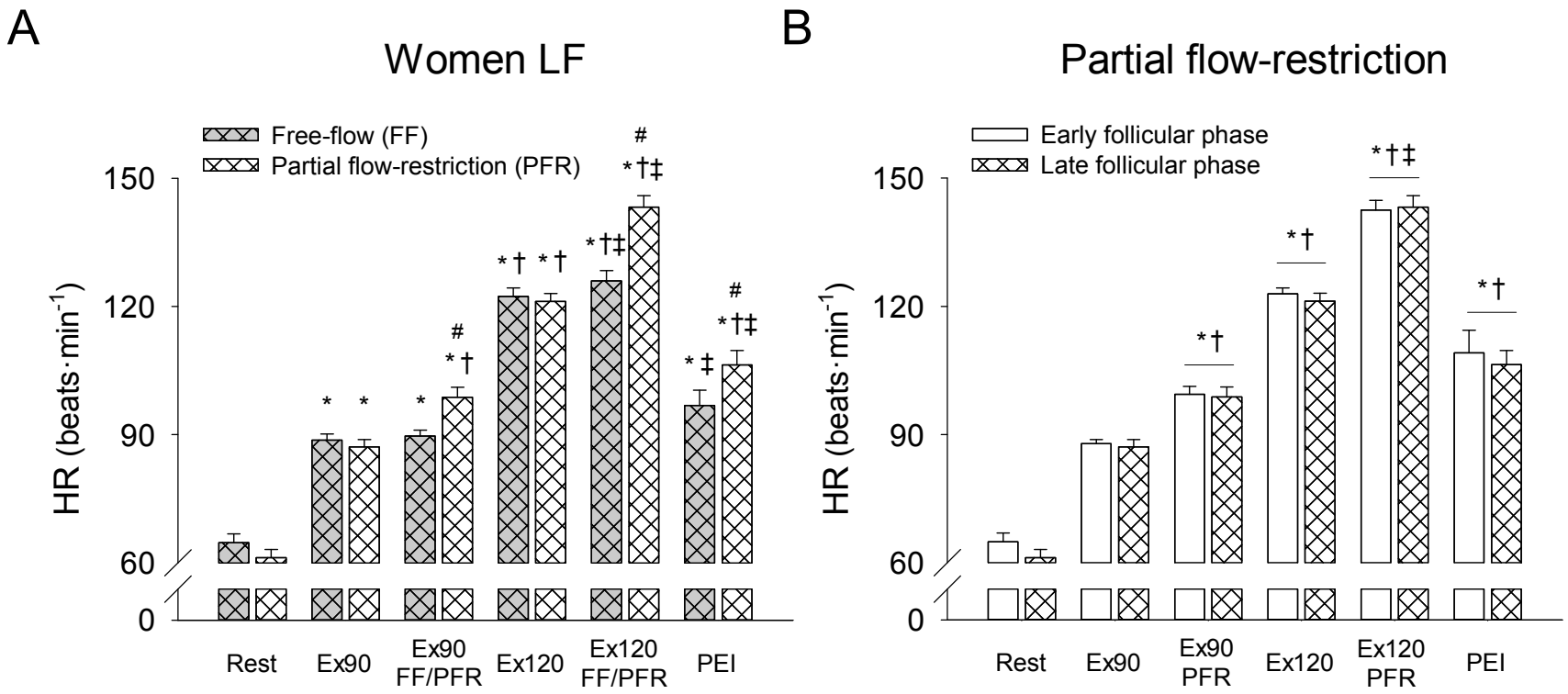

Figure 5.6. Panel A indicates heart rate (HR) in women during the late follicular phase (LF) of the ovarian cycle at rest and during dynamic leg cycling under free-flow conditions (grey shaded bars) and with partial flow-restriction (white shaded bars). Panel B shows HR at rest and during dynamic leg cycling with partial flow-restriction in women during early (white bars) and late (white shaded bars) follicular phase of the ovarian cycle. FF, free-flow; PFR, partial flow-restriction; Ex90, leg cycling at target HR of 90 $b \cdot min^{-1}$; Ex120, leg cycling at target HR of 120 $b \cdot min^{-1}$. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex90 of corresponding trial; ‡ P<0.05 vs. Ex120 of corresponding trial; # P<0.05 vs. FF trial at corresponding time point.

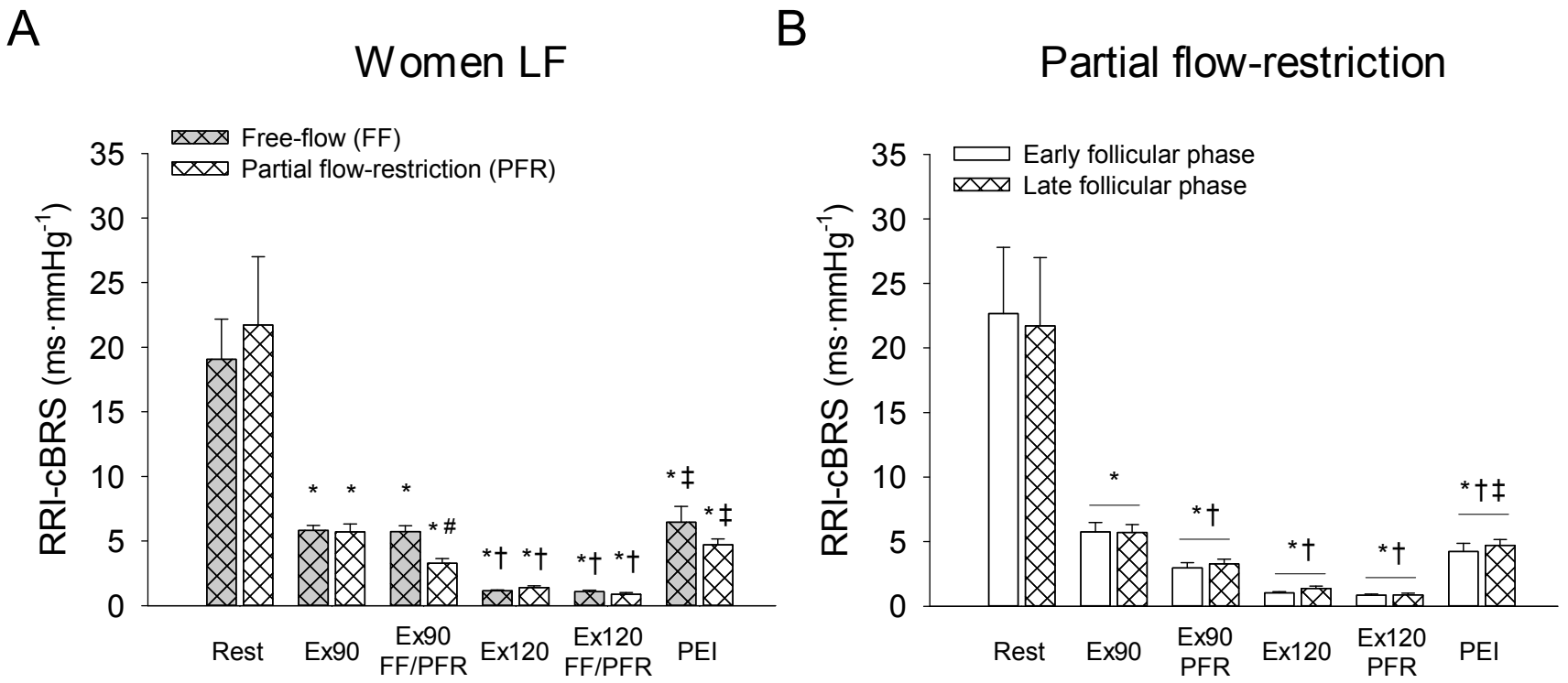

Figure 5.7. Panel A indicates spontaneous cardiac baroreflex sensitivity (cBRS) calculated using R-R interval (RRI-cBRS) in women during the late follicular (LF) phase of the ovarian cycle at rest and during dynamic leg cycling under free-flow conditions (grey shaded bars) and with partial flow-restriction (white shaded bars). Panel B shows spontaneous RRI-cBRS in women during the early (white bars) and late (white shaded bars) follicular phases at rest and during dynamic leg cycling with partial flow-restriction. FF, free-flow; PFR, partial flow-restriction; Ex90, leg cycling at target heart rate of 90 $b \cdot min^{-1}$; Ex120, leg cycling at target heart rate of 120 $b \cdot min^{-1}$. * $P<0.05$ vs. Rest of corresponding trial; † $P<0.05$ vs. Ex90 of corresponding trial; # $P<0.05$ vs. FF trial at corresponding time point.

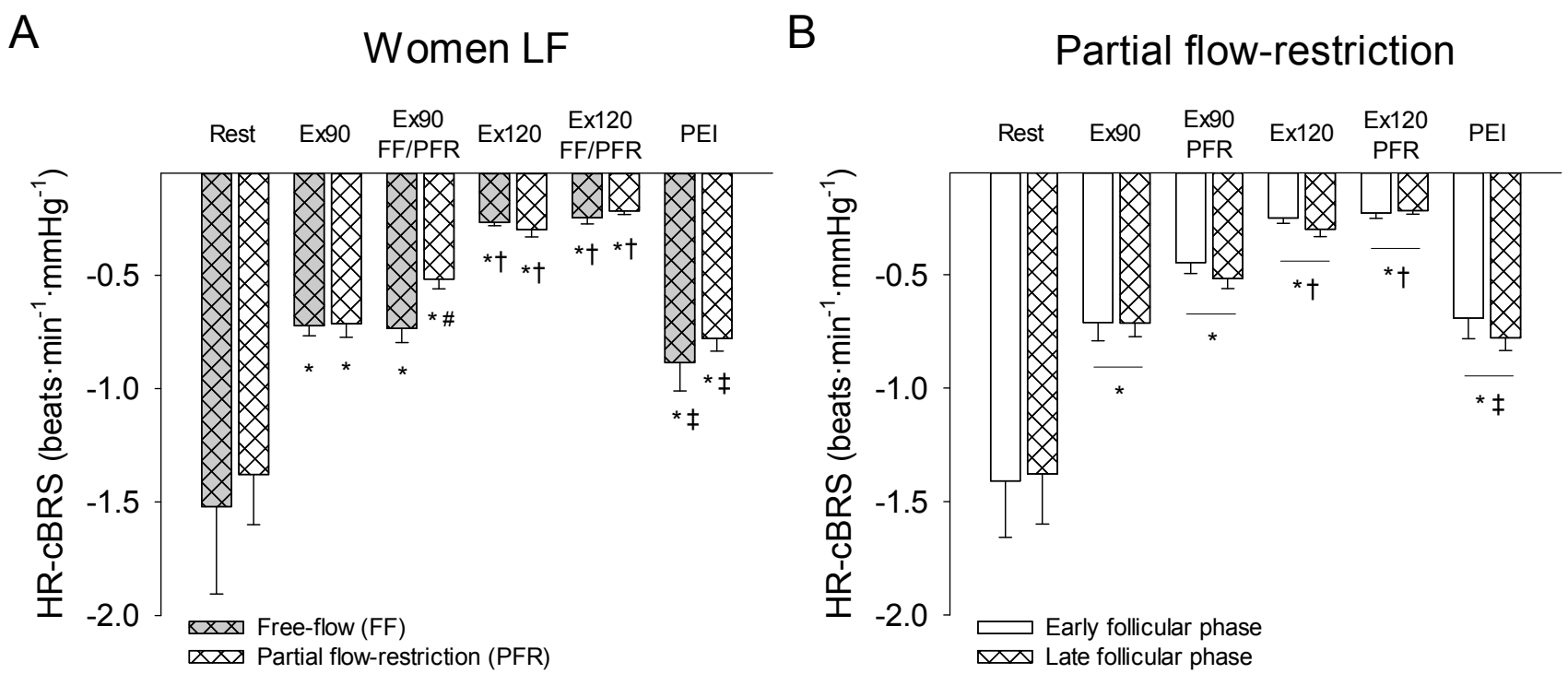

Figure 5.8. Panel A indicates spontaneous cardiac baroreflex sensitivity (cBRS) calculated using heart rate (HR-cBRS) in women during the late follicular (LF) phase of the ovarian cycle at rest and during dynamic leg cycling under free-flow conditions (grey shaded bars) and with partial flow-restriction (white shaded bars). Panel B shows spontaneous HR-cBRS in women during the early follicular (white bars) and LF (white shaded bars) phases at rest and during dynamic leg cycling with partial flow-restriction. FF, free-flow; PFR, partial flow-restriction; Ex90, leg cycling at target heart rate of 90 $b \cdot min^{-1}$; Ex120, leg cycling at target heart rate of 120 $b \cdot min^{-1}$. * $P<0.05$ vs. Rest of corresponding trial; † $P<0.05$ vs. Ex90 of corresponding trial; # $P<0.05$ vs. FF trial at corresponding time point.

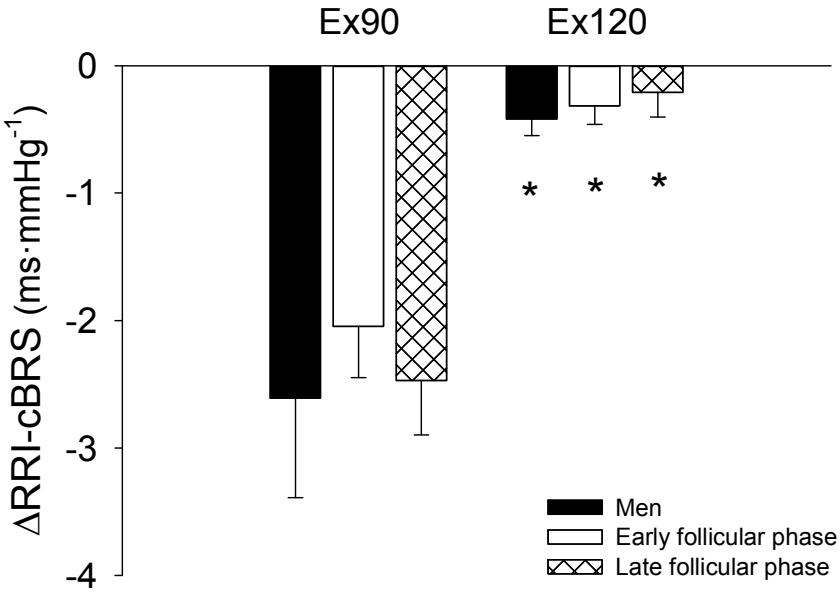

Figure 5.9. Change in spontaneous cardiac baroreflex sensitivity (cBRS) induced by partial flow-restriction versus free-flow trial at low and moderate exercise intensities in men (black bars), women during early follicular (white bars) and women during late follicular (shaded bars) phase of the ovarian cycle. Spontaneous cBRS calculated using R-R interval (RRI-cBRS). * $P<0.05$ vs. Ex90 of corresponding group.

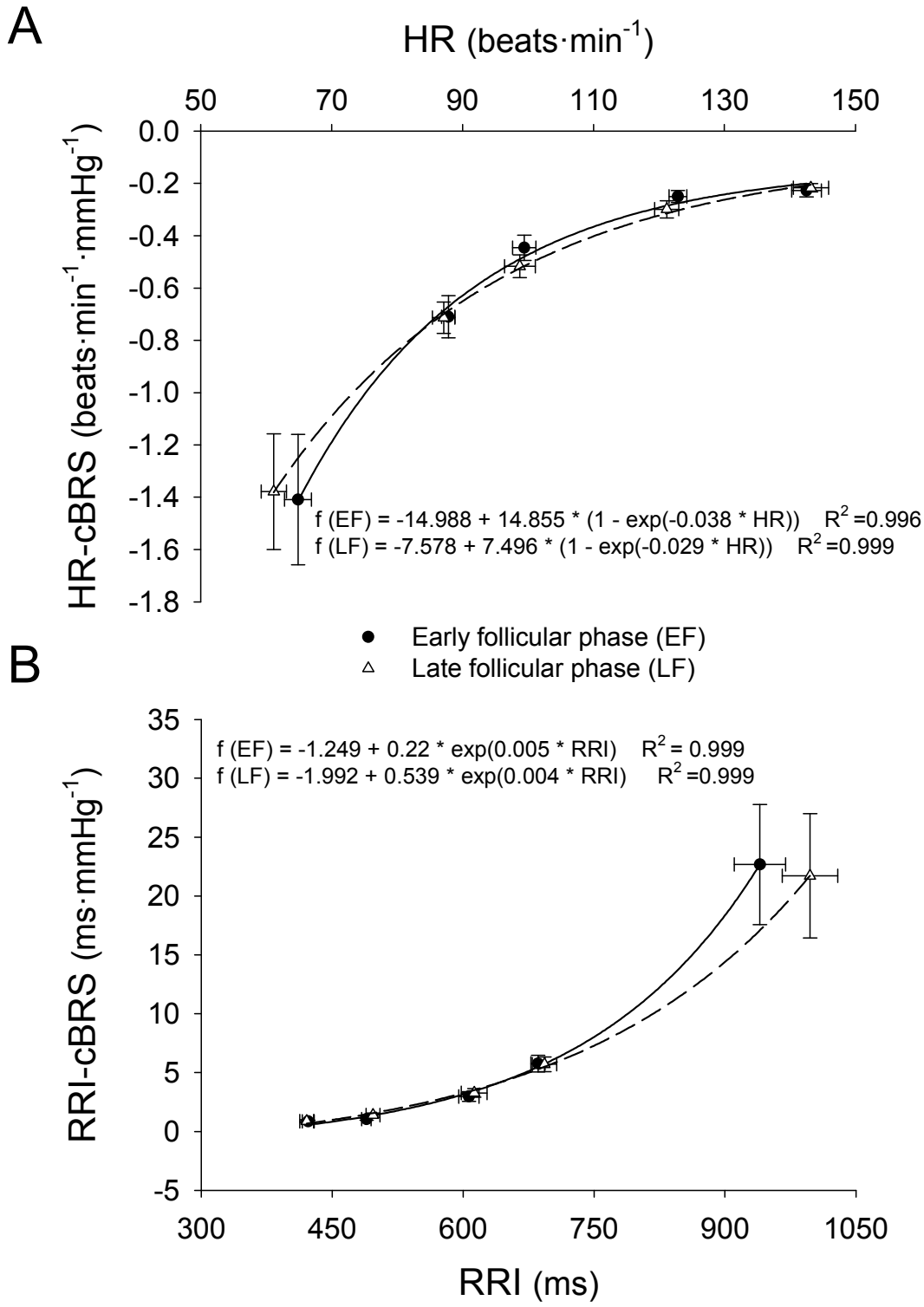

Figure 5.10. Relationship between heart rate (HR) or R-R interval (RRI) and spontaneous cardiac baroreflex sensitivity (HR-cBRS, panel A; RRI-cBRS, panel B). Black circles represent group average data of the partial flow-restriction trial during the early follicular (EF) and open triangles during the late follicular (LF) phase of the ovarian cycle.

5.4 Discussion

The purpose of the present study was to determine whether sex and/or ovarian hormone concentration influence the cardiovascular responses to muscle metaboreflex activation caused by partially restricting skeletal muscle blood flow during dynamic leg cycling. In agreement with the findings from the previous chapter, dynamic leg cycling evoked an intensity dependent reduction in spontaneous cBRS in men and women. cBRS was further decreased during muscle metaboreflex activation at the low exercise intensity in both sexes. Similarly, muscle metaboreflex activation also evoked marked increases in HR and BP in men and women. In contrast to the initial hypothesis, the reduction in cBRS and the increases in HR and BP during exercise with augmented muscle metaboreflex activation were not different between young men and women, or between the EF and LF phases of the ovarian cycle. As expected, exercise-induced increases in BP were greater in men than in women at both exercise intensities, but independent of the tested ovarian cycle phase. Increases in HR during exercise with and without augmented muscle metaboreflex activation were similar in men and women and in women at both phases of the ovarian cycle. Collectively, these findings indicate that there is no effect of sex on the muscle metaboreflex-induced pressor response or the reduction in spontaneous cBRS during dynamic leg exercise.

5.4.1 Effects of sex on the pressor response

It has been frequently reported that women show attenuated BP responses during static (Ettinger et al., 1996, Petrofsky and Lind, 1975) and dynamic (Fleg et al., 1995, Green et al., 2002, Ogawa et al., 1992, Sullivan et al., 1991) exercise compared to men. In agreement with previous reports, in the current study systolic and mean BP were greater in men than women at both low and moderate intensity leg cycling. The reason

for this attenuated pressor response in women is still unclear. One possible contributing mechanism might derive from sex-differences in the activation of the muscle metaboreflex. Indeed, muscle metaboreflex-mediated increases in BP and MSNA during static handgrip exercise and a subsequent period of PEI have been shown to be lower in women compared to men (Ettinger et al., 1996, Jarvis et al., 2011). Using nuclear magnetic resonance spectroscopy, Ettinger et al. (1996) have demonstrated that exercise-induced reductions in pH were less marked in women than men (e.g. lower acidity) after two minutes of static handgrip at the same relative workload. Since metabolite accumulation (e.g. increased acidity) is thought to activate the muscle metaboreflex, Ettinger et al. presumed that attenuated metaboreflex activation was the main factor driving the attenuated BP and MSNA responses observed in women (Ettinger et al., 1996, Jarvis et al., 2011). Indeed, women have been shown to produce less lactate when performing the same relative work, to express a greater relative percentage of slow oxidative muscle fibres and are characterised by a greater capacity to oxidize fat (Komi and Karlsson, 1978, Simoneau and Bouchard, 1989, Sullivan et al., 1991). However, in the present study, muscle metaboreflex activation during low and moderate intensity leg cycling exercise evoked a similar rise in mean BP and HR in women and men. The reasons for this discrepancy are unclear. Potential explanations might derive from the differences in the exercise modality (e.g. static vs. dynamic) and/or the modality of muscle metaboreflex activation. For example, Ettinger et al. (1996) have performed PEI after static handgrip exercise. Conversely, in the present study, activation of metabolically sensitive skeletal muscle afferents was augmented by partial flow-restriction during and complete circulatory occlusion after dynamic leg cycling. Static forearm contractions have been shown to evoke distinct increases in MSNA even at low exercise intensities (Mark et al., 1985, Victor et al., 1989a) leading

to a marked pressor response (Barcroft and Millen, 1939), compared to the rather modest rises in MSNA and BP during low intensity dynamic exercise (Victor et al., 1989a). However, whether the sympathetic responses to leg cycling with and without augmented muscle metaboreflex activation were attenuated in women cannot be answered on the basis of the present findings. As highlighted in the previous chapter, it appears that the effects of the muscle metaboreflex during PEI following exercise involving either large or small muscle mass are not comparable.

Previous studies have shown that muscle metaboreflex activation by graded reductions in terminal aortic blood flow in treadmill running dogs (Sala-Mercado et al., 2010, Sala-Mercado et al., 2007, Sheriff et al., 1993) or by application of lower body positive pressure (Eiken et al., 1992, Gallagher et al., 2001b) effectively elicits significant increases in HR and BP. In the present study, bilateral thigh cuff inflation to 100 mmHg also evoked marked increases in HR and BP at both exercise intensities in men and women. This suggests that this manoeuvre evokes a reduction in limb blood flow, a mismatch between oxygen delivery and demand and an accumulation muscle metabolites leading to the activation of metabolically sensitive skeletal muscle afferents (Eiken and Bjurstedt, 1987, Eiken et al., 1992). However, since no direct measurement of the reduction in leg blood flow induced by bilateral thigh cuff inflation was available in the present study, it cannot be confidently assumed that blood flow to the exercising legs was similarly decreased during external leg compression in men and women. Hence, some uncertainty remains of whether the magnitude of muscle metaboreflex activation was comparable between the groups. Further studies are warranted to fully answer this question. Leg kicking may present an alternative experimental approach. It allows the simultaneous assessment of leg perfusion during thigh cuff inflation using either Doppler ultrasound or near-infrared spectroscopy. In addition, the measurement

of efferent sympathetic nerve activity could be performed using the leg kicking model. This would provide further insight into potential sex-differences in the autonomic cardiovascular control via the muscle metaboreflex.

Central command is one important neural mechanism that contributes to the cardiovascular adjustments during exercise. Previously central command has been shown to evoke increases in MSNA (Victor et al., 1995). Hence, a reduced activation of central command might explain the attenuated pressor response in women during exercise. During PEI after leg cycling, in the absence of central command, HR and BP were similarly elevated in men and women. However, the RPE, which quantifies the participants sense of effort and are historically related to central command (Mitchell, 1990), were not different between men and women during exercise. This suggests that a differential contribution of central command is unlikely to explain the lower BP responses in women during low intensity leg cycling. However, since these assumptions are based only on subjective ratings, a fully differential activation of central command in men and women cannot be excluded.

It is acknowledged that bilateral thigh cuff inflation is likely to have restricted venous outflow in the exercising muscles. As the cuff pressure exceeded diastolic BP at any time point during the study, venous distension very likely occurred, thus activating mechanically sensitive group III afferents (i.e. muscle mechanoreflex) located in the walls of venules (Haouzi et al., 1999). Differences in the venous responsiveness to blood pooling may affect the magnitude of mechanoreceptor activation during thigh cuff inflation while cycling. Indeed, greater venous compliance has been reported in men (Monahan and Ray, 2004). Therefore, enhanced mechanoreceptor activation and thus a greater pressor response in men than women might be expected. However, BP responses were similar in men and women during bilateral thigh cuff inflation during

low and moderate intensity leg cycling. Importantly, the above mentioned sex-differences in venous compliance (Monahan and Ray, 2004) are based on observations at rest. Therefore, the present results appear to indicate that a differential venous compliance does not affect the pressor response during exercise, potentially due to the muscle pump largely affecting venous blood flow.

5.4.2 Effects of sex on cBRS

This is the first study to investigate potential sex-differences in the cardiac baroreflex function and the effect of the muscle metaboreflex control of the heart during dynamic exercise. Previous studies at rest have yielded inconsistent results, reporting increased, decreased or unchanged cBRS in women compared to men (Abdel-Rahman et al., 1994, Beske et al., 2001, Chen and DiCarlo, 1996, Convertino, 1998, Tank et al., 2005). As such, there is no clear indication of whether there are differences in the baroreflex control of HR between men and women. In the present study, no sex-differences in cBRS were observed at rest. In addition, the exercise-induced decreases in cBRS during dynamic exercise reported from studies predominantly in men (Iellamo et al., 1998, Ogoh et al., 2005b, Sala-Mercado et al., 2010, Sala-Mercado et al., 2007) occurred to a similar extent in women.

The muscle metaboreflex has been proposed to contribute to the reduction in spontaneous cBRS during exercise (Sala-Mercado et al., 2010, Sala-Mercado et al., 2007). Based on the assumption that the muscle metaboreflex might be attenuated in women (Ettinger et al., 1996, Jarvis et al., 2011), it was anticipated that the reductions in spontaneous cBRS during bilateral thigh cuff inflation would be attenuated in women compared to men. However, the magnitude of the muscle metaboreflex-induced decrease in cBRS was consistent in women compared to men. As described in the

previous chapter, the reduction in cBRS evoked by muscle metaboreflex activation via bilateral cuff inflation may result indirectly from an increase in central motor drive (Amann et al., 2009, Gandevia, 2001). Importantly, the increases in RPE during bilateral thigh cuff inflation at low and moderate intensity leg cycling were not different in men and women. Therefore, a differential reduction in spontaneous cBRS in men and women based on varying input from central command seems unlikely. Taken together, the absence of any sex-differences in spontaneous cBRS during leg cycling with partial flow-restriction indicates that the muscle metaboreflex does not differentially affect baroreflex control of HR during leg cycling in men and women.

5.4.3 Effects of ovarian cycle phase on pressor response and cBRS

In protocol 2 the potential influence of the female reproductive hormone estrogen on the cardiovascular responses to exercise with and without augmented muscle metaboreflex activation, and the subsequent modulation of spontaneous cBRS, were investigated. Previous studies have suggested that estrogen is a powerful modulator of cardiovascular and autonomic control mechanisms at rest and during exercise (el-Mas and Abdel-Rahman, 1998, Hayes et al., 2002, Saleh and Connell, 2000, Schmitt and Kaufman, 2003a). For example, estrogen has been shown to enhance cBRS at rest in animals (el-Mas and Abdel-Rahman, 1998) and humans (Tanaka et al., 2003). Moreover, spinal application of 17-β estradiol evoked an attenuation of the pressor response during electrically evoked muscle contractions (Schmitt and Kaufman, 2003a, Schmitt and Kaufman, 2003b) and central command-induced increases in HR and BP were smaller in cats after infusion of 17-β estradiol (Hayes et al., 2002). Given this, it was hypothesised that the pressor response to dynamic leg cycling with and without augmented muscle metaboreflex activation would be lower in women during

the LF (when estrogen is high) compared to the EF phases of the ovarian cycle (when estrogen concentration is low). Secondly, the muscle metaboreflex-induced reductions in spontaneous cBRS were expected to be attenuated during the high estrogen phase of the ovarian cycle compared to the low hormone phase (e.g. EF phase). However, in contrast to the initial hypotheses the present findings do not support an effect of ovarian hormone concentrations (during the EF and LF phases) on the cardiovascular responses or the reduction in cBRS caused by muscle metaboreflex engagement during leg cycling.

The present study is the first to determine potential female hormone influences on the baroreflex control of the heart during isolated muscle metaboreflex activation during a period of PEI following dynamic leg exercise in humans. Previous animal experiments have suggested that estrogen has the ability to attenuate the cardiovascular responses to a hypertensive stimulus, such as the administration of noradrenaline (Saleh et al., 2000a, Saleh et al., 2000b). The noradrenaline-induced reduction in sympathetic nerve activity and increase in vagal tone were greater in the estrogen treated animals compared to the control animals. This augmented vagal responsiveness has been suggested to be at least in part a result of estrogen eliciting a greater acetylcholine release (Du et al., 1994). Moreover, estrogen has been repeatedly shown to enhance cBRS (el-Mas and Abdel-Rahman, 1998). In the previous chapter it has been reported that during PEI after leg cycling, in the absence of central command- and the muscle mechanoreflex, the muscle metaboreflex is able to reduce spontaneous cBRS, thus decreasing cardiac vagal tone. Given the greater estrogen-induced vagal reactivity, one might suggest that reductions in cBRS during PEI would be attenuated in women during the LF compared to the EF phases of the ovarian cycle. Indeed, the reductions in cBRS during PEI in the present study were related to the magnitude of muscle metaboreflex

activation (e.g. more metabolites accumulated during PEI after the partial flow-restriction trial evoked greater reduction in spontaneous cBRS). However, no effect of ovarian hormone concentration on the baroreflex control of the heart during isolated muscle metaboreflex activation in women was found. These findings suggest that estrogen fluctuations during these two ovarian phases do not affect the autonomic control of the heart via baroreflex mechanism.

Alternative explanations for these seemingly unexpected findings might derive from the fact that most of the work examining the effects of estrogen on the neural cardiovascular control mechanisms during exercise stems from animal investigations (Hayes et al., 2002, Schmitt and Kaufman, 2003a, Schmitt and Kaufman, 2003b). Thus, there might be species differences. In addition, in the above mentioned studies, estrogen has been administered exogenously, via either venous infusion (Hayes et al., 2002) or applied directly on the spinal cord (Schmitt and Kaufman, 2003a, Schmitt and Kaufman, 2003b). Therefore, the comparison of past and present findings may be limited by the fact that the muscle metaboreflex was investigated during physiologically fluctuating hormone concentrations during the EF and LF phases in the present investigation. Taken together, it is tempting to speculate, that variations in endogenous estrogen concentration during the ovarian cycle are not sufficient to unmask the various reported modulatory effects of estrogen on autonomic, cardiovascular control and baroreflex function during exercise in humans (Du et al., 1994, Saleh et al., 2000a, Saleh et al., 2000b)

The strength of the muscle metaboreflex has been shown to rely on the amount of stimulating metabolites and the degree of washout of these metabolic by-products from the exercising skeletal muscle (Sheriff et al., 1987). Although muscle metabolic state was not determined in the present study, the observation of unchanged exercise

workloads at low and moderate intensity leg cycling during the EF and LF phase of the ovarian cycle, may indirectly imply that the muscle metaboreflex was similarly activated. In addition, muscle metabolism analyses during exercise (e.g. ^{13}P-NMR Spectroscopy) have shown no effect of the ovarian cycle phase on exercise-induced changes in metabolic state (Ettinger et al., 1998). Consequently, it may be speculated that differential muscle metaboreflex activation during the EF and LF phases of the ovarian cycle is unlikely.

Another possible explanation for the similar pressor response and cBRS during the EF and LF phases of the ovarian cycle may be that estrogen affected cardiovascular and/or arterial baroreflex mechanisms during leg cycling, but that in order to provide an adequate pressor response to maintain exercise, alternative pathways/mechanisms were activated in substitution (i.e. concept of redundancy). One might speculate that central command and/or the muscle mechanoreflex might have compensated for any potential estrogen-mediated attenuation in muscle metaboreflex afferent neural input to the central cardiovascular control areas (e.g. NTS). Indeed, animal studies reporting inhibitory influences of estrogen on cardiovascular responses during exercise via central command and/or the skeletal muscle afferents have been performed in isolation of each of these mechanisms (Hayes et al., 2002, Schmitt and Kaufman, 2003a, Schmitt and Kaufman, 2003b). For example, animal preparations have used either direct ventral root stimulation or direct electrical stimulation of the mesencephalic locomotor region in decerebrate cats, to either isolate central command or sensory afferent feedback from the contracting muscle. Hence, it is likely that in the presence of both central motor drive and neural feedback from the exercising skeletal muscle, the effects of estrogen might be overridden leading to comparable cardiovascular responses during the EF and LF phases of the ovarian cycle in women.

5.4.4 Limitations

One limitation of the present study is that the quantification and comparison of the blood flow during free-flow and partial flow-restriction were not possible because the appropriate technique (e.g. femoral venous thermodilution) was not accessible. Therefore, it remains unknown whether metaboreflex activation was equivalent in men and women and in women during the EF and LF phases of the ovarian cycle.

Plasma levels of estrogen and progesterone was not determined in the present study. Hence, no direct evidence is available that estrogen concentrations were elevated above EF values during the LF phase. Nevertheless, ovulation kits were used to indirectly detect the peak of estrogen (Minson et al., 2000a), which occurs shortly before the surge in LH (Nielsen et al., 2001). Therefore, it can be confidentially assumed that women partaking in the present investigation were studied in the LF phases, when estrogen is elevated above concentrations during the EF phases of the ovarian cycle.

5.4.5 Alternative approaches

Although in the present study, physiological fluctuations in estrogen during the EF and LF phases did not modify the pressor response during muscle metaboreflex activation as indicated by animal experiments (Schmitt et al., 2010, Schmitt and Kaufman, 2003a), it cannot be fully excluded that estrogen does not exert these effects in humans. Future studies conducted in post-menopausal women with or without hormonal replacement might provide a closer insight on the impact of estrogen on the cardiovascular and cardiac baroreflex responses to muscle metaboreflex activation during dynamic exercise. A new experimental paradigm of administering an antagonist of the gonadotrophin releasing hormone with subsequent controlled administration of

either estrogen or progesterone (or a combination of both) might further our understanding of the effect of ovarian hormones on the exercise-induced cardiovascular responses (Stachenfeld, 2008, Stachenfeld et al., 2003). The advantage of this new approach lies in the ability to selectively study the effect of estrogen in the absence of any, potentially minimal background influence of progesterone as apparent during the LF phase of the ovarian cycle (Stachenfeld, 2008, Stachenfeld et al., 2003).

5.5 Conclusion

In summary, the results of the present study show that young men and women exhibit not only similar muscle metaboreflex-induced increases in HR and BP, but also comparable reductions in spontaneous cBRS when the muscle metaboreflex is activated during exercise and following exercise during PEI. In addition, no differences were observed in the cardiovascular responses and cBRS during the EF and LF phases of the ovarian cycle. Collectively, these findings suggest that there is no effect of sex on the muscle metaboreflex-induced pressor response or reductions in spontaneous cBRS during dynamic leg exercise in humans.

CHAPTER 6: METABOREFLEX CONTROL OF SPONTANEOUS CARDIAC BAROREFLEX SENSITIVITY DURING RHYTHMIC HANDGRIP: INFLUENCE OF SEX AND OVARIAN CYCLE PHASE

6.1 Introduction

The data from the previous study (Chapter 5) suggested that there is no effect of sex on the muscle metaboreflex-induced pressor response or the reductions in spontaneous cBRS during dynamic leg exercise in humans. The major limitation of the previous study was the lack of direct blood flow measurements during leg cycling with and without partial flow-restriction. Thus, without the quantification blood flow during external cuff inflation, the direct comparison of the hypoperfusion-induced pressor responses and reductions in cBRS during dynamic exercise were limited. Previous studies have indicated that once the muscle metaboreflex threshold is passed there was a linear increase in systemic cardiovascular responses including BP, HR, CO and TPR, while cBRS gradually decreased, with progressive further decreases in skeletal muscle blood flow (Ichinose et al., 2011, Sala-Mercado et al., 2010, Wyss et al., 1983). This relationship can be regarded as representative of the muscle metaboreflex sensitivity in control of these variables.

The aim of the present study was to determine the potential sex-differences in the relationship between the magnitude of blood flow restriction (i.e. metaboreflex activation) and the systemic cardiovascular responses or spontaneous cBRS during exercise. To investigate this, young men and women during the EF and LF phases of the ovarian cycle performed rhythmic handgrip exercise under free-flow and partial flow-restriction elicited by gradual inflation of a pressure cuff placed around the exercising upper arm. It was expected that BP responses to rhythmic handgrip exercise would be attenuated in women compared to men. Moreover, equivalent reductions in forearm blood flow (FBF) in men and women were hypothesised to evoke an attenuated pressure

rise in women (i.e. attenuated muscle metaboreflex sensitivity) coinciding with elevated estrogen concentrations during the ovarian cycle (i.e. LF phase).

6.2 Methods

6.2.1 Subjects

Eleven female and fourteen male healthy subjects (data for men has been presented in Chapter 4) from the University of Birmingham volunteered participate in the present study (Table 6.1).

6.2.2 Experimental measurements

HR, BP, FBV and brachial artery diameter were measured and FBF and mean BP calculated (see Sections 3.2.1&3.2.2 for details). FBV measurements were taken when velocity was stable and FBF calculated and corrected for lean forearm muscle mass, to account for any sex-differences (see Section 3.2.3 for details).

Table 6.1. Subject characteristics

	Men (n = 14)	Women (n = 11)	
Age (years)	22 ± 1	19 ± 0.3 *	
Weight (kg)	78 ± 2	60 ± 2 *	
Height (cm)	180 ± 1	165 ± 2 *	
BMI ($kg \cdot m^{-2}$)	24 ± 1	22 ± 1 *	
Forearm mass (g)	949 ± 40	480 ± 28 *	
MVC (kg)	57 ± 2	EF: 34 ± 1 *	LF: 34 ± 2 *
Ovarian cycle day		EF: 3 ± 0.4	LF: 12 ± 0.5

Values are mean±SEM. BMI, body mass index; MVC, maximum voluntary contraction; EF, early follicular phase; LF, late follicular phase. * $P<0.05$ different from men.

6.2.3 Experimental procedures

Protocol 1: Young men and women in the EF phase of the ovarian cycle (day 1-5) were seated in a semi-recumbent position on a medical examination table with a custom made handgrip dynamometer held in the right hand, while the arm was supported on an adjustable bedside table. Following instrumentation and assessment of MVC (57±2 kg and 34±1 kg in men and women, respectively; $P<0.05$) (see Section 3.3.1 for details) subjects rested for 15 minutes followed by rhythmic handgrip exercise at 35% MVC under free-flow and partial flow-restriction (see Section 4.2.3 and Figure 4.2 for details).

Protocol 2: Eleven women performed the rhythmic handgrip exercise under free-flow and graded partial flow-restriction twice during their ovarian cycle. The order of trials was counterbalanced. Women were studied in the EF (days 1-5; estrogen and progesterone both low) and the LF (days 10-15; estrogen high and progesterone low) phases of the ovarian cycle. The peak estrogen concentration was detected indirectly using ovulation kits (see Section 5.2.3 for details).

6.2.4 Data analysis

For both protocols beat-to-beat HR, BP and FBV were recorded and mean BP, FBF and spontaneous cBRS calculated (see Sections 3.2.1&3.4.2 for details). For each trial, cBRS and cardiovascular data (apart from FBF, see Section 3.2.2) were averaged over a 3 minute period for each of the five experimental phases (see Figure 4.2 in Chapter 4 for details). FBV was averaged over 3x10 cardiac cycles during each experimental phase.

Physiological data were statistically analysed using three-way repeated measures ANOVA. The main factors were *condition* (free-flow, graded partial flow-restriction),

time (Rest, Ex, Ex+/-80 free-flow or partially restricted-flow, Ex+/-100 free-flow or partially restricted-flow and Ex+/-120 free-flow or partially restricted-flow) and *sex* (men, women) for protocol 1 or cycle *phase* (women EF, women LF) for protocol 2. Statistical analysis of differences within one *sex*-group or within one ovarian cycle *phase* were performed using two-way repeated measures ANOVA, with *condition* (free-flow, partial flow-restriction) and *time* (e.g. Ex+80) as the main factors. The relationship between the changes in FBF (free-flow vs. partial flow restriction at 80, 100 and 120 mmHg) and mean BP (or HR) in men and women and women during EF and LF phases were compared by application of least square linear regression lines for each individual response. Unpaired t-tests compared the average slope of the regression lines for men and women EF (protocol 1) and for women EF and women LF (protocol 2) after regressions $r^2<0.5$ were removed.

6.3 Results

6.3.1 Protocol 1: Sex-differences during handgrip under free-flow conditions

The body weight, height, BMI and lean forearm mass were lower in women compared to men. As expected, women exerted a lower maximal force than men. At rest mean BP, HR, RRI and cBRS were not different between men and women (Table 6.2). During free-flow rhythmic handgrip exercise, mean BP, HR and FBF increased, while RRI decreased significantly from rest in both men and women ($P<0.05$ from Rest). The exercise-induced rise in mean BP was greater in men compared to women (+12±2 vs. +6±1 mmHg in men and women, respectively; $P<0.05$ from Rest; Table 6.2). In men, mean BP and HR were elevated above steady state exercise levels during the last 3½ minutes of rhythmic handgrip exercise under free-flow conditions, while FBF rose

continuously as a function of exercise duration (P<0.05; Figures 6.1-6.3). In women, mean BP and FBF remained unchanged at steady state exercise levels, while HR was slightly higher during the last 3½ minutes of free-flow exercise (P<0.05). The brachial artery diameter was significantly greater in men than women and increased slightly in both sexes during free-flow handgrip exercise. cBRS was unchanged from rest to free-flow rhythmic handgrip exercise and remained unchanged throughout in both men and women (Table 6.3). RPE increased slightly, but significantly over time in men (9±0.4 to 12±1 au) and women (10±1 to 11±1 au).

6.3.2 Protocol 1: Sex-differences during graded muscle metaboreflex activation

Graded muscle metaboreflex activation by means of arm cuff inflation of +80, +100 and +120 mmHg evoked successive increases in mean BP and HR in proportion to the level of external pressure in both men and women (P<0.05 from Ex during partial flow-restriction trial). The magnitude of the change in mean BP compared to the free-flow trial was not different between men (-1±1, +1±1 and +6±2 mmHg during rhythmic handgrip exercise at +80, +100 and +120 mmHg upper arm cuff pressure, respectively) and women (-1±1, +5±2 and +10±2 mmHg during rhythmic handgrip exercise at +80, +100 and +120 mmHg upper arm cuff pressure, respectively; Figures 6.1&6.4, Table 6.2). The magnitude of the increase in HR during graded muscle metaboreflex activation compared to the free-flow conditions were similar in men and women (+5±2 and +6±1 beats·min^{-1} during Ex +/- 120 in men and women, respectively; Figures 6.2&6.4; Table 6.2). The changes in RRI mimicked the response in HR in a reciprocal manner (Table 6.2).

Application of graded upper arm cuff pressure significantly reduced FBF in both men and women (P<0.05; Figures 6.3&6.4). In women, FBF decreased successively

with every 20 mmHg increase in cuff pressure by -138±23, -215±27 and -307±39 $ml \cdot min^{-1} \cdot kg^{-1}$ from Ex of partial flow-restriction trial during Ex+/-80, Ex+/-100 and Ex+/-120 mmHg, respectively; $P<0.05$), while FBF decreased with no statistically significant difference between time points in men (-126±24, -187±21 and -211±19 $ml \cdot min^{-1} \cdot kg^{-1}$ from Ex of partial flow-restriction trial during Ex +/-80, Ex+/-100 and Ex+/-120 mmHg, respectively). Relative to the progressive rise in FBF during the free-flow trial in men, upper arm cuff inflation evoked a progressive reduction in FBF during the first two pressure levels, with no further effect of 120 mmHg external cuff pressure (equivalent to a reduction of -24±4, -35±2, and -38±2 %, from the respective free-flow value, during the Ex+/-80, Ex+/-100 and Ex+/-120 condition), while women elicited a stepwise decrease in FBF ($P<0.05$; equivalent to a reduction of -24±5, -36±5, and -51±5 %, from the respective free-flow value, during the Ex+/-80, Ex+/-100 and Ex+/-120 conditions; Figures 6.3&6.4). RPE increased in proportion to the increase in upper arm cuff pressure without any differences between the men and women (during Ex+120, to 15.0±0.5 in men and to 16.1±0.8 in women).

Spontaneous cBRS was unaffected by the reduction in FBF in both men and women (Table 6.2). This was independent of whether HR (HR-cBRS) or RRI (RRI-cBRS) were used for the estimation of cBRS. Figures 6.5 and 6.6 present muscle metaboreflex sensitivity, as characterised by the relationship between the changes in FBF and the changes in mean BP (or HR) evoked by gradual partial flow-restriction in comparison to the free-flow trial. In men and women, there were no differences in the averaged slope (-0.04±0.02 and -0.05±0.02 $mmHg \cdot ml^{-1} \cdot min^{-1} \cdot kg^{-1}$ in men and women, respectively; $P>0.05$) of the regression lines for the FBF/mean BP relationship. Similarly, no differences were obtained in the sensitivity of the muscle metaboreflex in

control of HR in men and women (-0.05±0.02 and -0.02±0.01 beats·min^{-1}·ml^{-1}·min^{-1}·kg^{-1} in men and women respectively; P>0.05).

6.3.3 Protocol 2: EF versus LF phase during free-flow conditions

Between the EF and LF phases there was no difference in the MVC (34±1 and 34±2 kg during EF and LF phase, respectively). Similar to findings for women EF, mean BP, HR and FBF and brachial artery diameter increased, while RRI decreased from rest during free-flow rhythmic handgrip exercise in women during the LF phase (P<0.05). These variables remained unchanged as free-flow exercise continued in women LF (Table 6.3; Figures 6.1 – 6.3). The exercise-induced increases in HR and BP during free-flow conditions were smaller in women during the LF (+3±1 mmHg) than EF phases (+6±1 mmHg; Table 6.3), while FBF was not affected by the ovarian cycle phases. Spontaneous cBRS remained at resting levels during rhythmic handgrip exercise in both phases of the ovarian cycle independent of whether HR (HR-cBRS) or RRI (RRI-cBRS) were used for the estimation of cBRS (Table 6.3). Similar to women in the EF phase, RPE increased slightly over time during the free-flow condition in LF women.

6.3.4 Protocol 2: EF versus LF phase during graded muscle metaboreflex activation

Graded muscle metaboreflex activation by upper arm cuff inflation during rhythmic handgrip exercise in women during the LF phase evoked increases in mean BP and HR proportional to the successive increase in cuff pressure (P<0.05; Table 6.3: Figures 6.1&6.2). Similarly to women during the EF phase, the HR and BP responses during the highest pressure application were significantly greater than during the free-flow trial at the corresponding time point (P<0.05; Figures 6.1&6.2). FBF decreased

during graded metaboreflex activation in proportion to the applied cuff pressure in LF women ($P<0.05$; equivalent to a reduction of -28±6, -36±6, and -54±4 %, from the respective free-flow value during the Ex+/-80, Ex+/-100 and Ex+/-120 condition; Figure 6.3&6.4). There was no effect of ovarian cycle phase on the magnitude of the reduction in FBF during the graded partial flow-restriction trial or in the rise in HR and BP or the decrease in FBF when the free-flow trial and the graded partial flow restriction were compared (Figure 6.4).

Spontaneous cBRS was not affected when the muscle metaboreflex was activated by successive increases of external upper arm cuff pressure in women during the EF or LF phase of the ovarian cycle. The gain of the muscle metaboreflex expressed as the ratio between the reduction in FBF and the rise in BP (or HR) during graded muscle metaboreflex activation was not different in women during the EF (-0.05±0.02 and $mmHg \cdot ml^{-1} \cdot min^{-1} \cdot kg^{-1}$ for BP and -0.02±0.01 $beats \cdot min^{-1} \cdot ml^{-1} \cdot min^{-1} \cdot kg^{-1}$ for HR) and LF phases (-0.03±0.03 $mmHg \cdot ml^{-1} \cdot min^{-1} \cdot kg^{-1}$ for BP and -0.04±0.01 $beats \cdot min^{-1} \cdot ml^{-1} \cdot min^{-1} \cdot kg^{-1}$ for HR) (Figures 6.5&6.6). RPE successively increased in proportion to the applied upper arm cuff pressure during the graded partial flow-restriction trial, without differences between the EF and LF phases.

Table 6.2. Selected physiological responses to rhythmic handgrip exercise in men and women

	Rest		Ex		Ex +/- 80		Ex +/- 100		Ex +/- 120	
	FF	PFR	FF	PFR	FF	PFR	FF	PFR	FF	PFR
Mean BP (mmHg) *‡$#										
Men	82 ± 2	80 ± 3	94 ± 2	92 ± 3	95 ± 2	94 ± 3	96 ± 3	98 ± 3	98 ± 3	104 ± 4
Women EF	81 ± 1	79 ± 1	86 ± 2	84 ± 2	88 ± 2	87 ± 2	87 ± 2	92 ± 2	88 ± 2	97 ± 2
HR (beats·min^{-1}) *†$										
Men	60 ± 2	60 ± 2	67 ± 3	68 ± 3	68 ± 3	69 ± 3	68 ± 3	72 ± 3	69 ± 4	75 ± 4
Women EF	66 ± 3	67 ± 3	70 ± 3	73 ± 3	71 ± 2	74 ± 3	72 ± 2	77 ± 2	73 ± 2	79 ± 3
RRI (ms) *†$										
Men	1024 ± 44	1016 ± 40	927 ± 48	906 ± 39	920 ± 47	888 ± 36	916 ± 48	858 ± 36	903 ± 48	831 ± 37
Women EF	937 ± 43	915 ± 42	869 ± 31	835 ± 32	855 ± 29	823 ± 33	851 ± 29	794 ± 29	836 ± 27	774 ± 27
HR-cBRS (beats·min^{-1}·mmHg^{-1})										
Men	-1.3 ± 0.1	-1.1 ± 0.1	-1.4 ± 0.2	-1.3 ± 0.2	-1.4 ± 0.2	-1.4 ± 0.2	-1.4 ± 0.2	-1.2 ± 0.1	-1.5 ± 0.2	-1.2 ± 0.1
Women EF	-1.5 ± 0.2	-1.7 ± 0.2	-1.6 ± 0.2	-1.2 ± 0.2	-1.4 ± 0.2	-1.2 ± 0.1	-1.5 ± 0.2	-1.6 ± 0.2	-1.5 ± 0.3	-1.7 ± 0.2
RRI-cBRS (ms·mmHg^{-1}) *†$										
Men	23 ± 3	20 ± 2	22 ± 5	16 ± 3	20 ± 3	19 ± 3	21 ± 4	15 ± 2	23 ± 4	16 ± 3
Women EF	24 ± 6	25 ± 4	21 ± 4	14 ± 2	18 ± 3	14 ± 2	18 ± 3	18 ± 3	19 ± 6	17 ± 3
FBF (ml·min^{-1}·kg^{-1}) *†$#										
Men	195 ± 19	225 ± 21	438 ± 31	497 ± 32	474 ± 44	372 ± 25	503 ± 43	342 ± 23	531 ± 50	347 ± 30
Women EF	158 ± 23	159 ± 24	552 ± 46	563 ± 48	546 ± 38	425 ± 50	537 ± 39	348 ± 40	531 ± 37	256 ± 23
Diameter (cm) *†‡$#										
Men	0.46 ± 0.01	0.46 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.48 ± 0.01
Women EF	0.37 ± 0.01	0.36 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.37 ± 0.01

Values are mean±SEM. Ex, steady state rhythmic handgrip; Ex+/-80, +/-100, +/-120, handgrip exercise with or without cuff inflation to 80, 100 or 120 mmHg; FF, free-flow; PFR, partial flow-restriction; EF; early follicular phase; BP, blood pressure; HR, heart rate; RRI, R-R interval; cBRS, cardiac baroreflex sensitivity calculated using either HR (HR-cBRS) or RRI (RRI-cBRS); FBF, forearm blood flow. * $P<0.05$ effect of time; † $P<0.05$ effect of condition (FF vs. PFR); ‡ $P<0.05$ effect of sex (Men vs. Women EF); $ $P<0.05$ interaction time vs. condition; # $P<0.05$ interaction time vs. sex.

Table 6.3. Physiological responses to rhythmic handgrip exercise in women during the early and late follicular phase of the ovarian cycle

	Rest		Ex		Ex +/- 80		Ex +/- 100		Ex +/- 120	
	FF	PFR	FF	PFR	FF	PFR	FF	PFR	FF	PFR
Mean BP (mmHg) *†$#										
Women EF	81 ± 1	79 ± 1	86 ± 2	84 ± 2	88 ± 2	87 ± 2	87 ± 2	92 ± 2	88 ± 2	97 ± 2
Women LF	78 ± 1	80 ± 2	80 ± 2	84 ± 2	82 ± 2	85 ± 2	82 ± 2	91 ± 2	84 ± 2	97 ± 2
HR (beats·min^{-1}) *$#										
Women EF	66 ± 3	67 ± 3	70 ± 3	73 ± 3	71 ± 2	74 ± 3	72 ± 2	77 ± 2	73 ± 2	79 ± 3
Women LF	67 ± 3	65 ± 3	71 ± 3	70 ± 3	73 ± 3	72 ± 2	72 ± 3	75 ± 3	73 ± 3	79 ± 3
RRI (ms) *$										
Women EF	937 ± 43	915 ± 42	869 ± 31	835 ± 32	855 ± 29	823 ± 33	851 ± 29	794 ± 29	836 ± 27	774 ± 27
Women LF	918 ± 43	953 ± 39	860 ± 37	874 ± 31	846 ± 38	851 ± 26	849 ± 34	815 ± 26	839 ± 32	775 ± 26
HR-cBRS (beats·min^{-1}·mmHg^{-1})										
Women EF	-1.5 ± 0.2	-1.7 ± 0.2	-1.6 ± 0.2	-1.2 ± 0.2	-1.4 ± 0.2	-1.2 ± 0.1	-1.5 ± 0.2	-1.6 ± 0.2	-1.5 ± 0.3	-1.7 ± 0.2
Women LF	-1.2 ± 0.1	-2.0 ± 0.5	-1.4 ± 0.2	-1.4 ± 0.2	-1.4 ± 0.2	-1.5 ± 0.1	-1.6 ± 0.1	-1.7 ± 0.2	-1.3 ± 0.2	-1.7 ± 0.1
RRI-cBRS (ms·mmHg^{-1})										
Women EF	24 ± 6	25 ± 4	21 ± 4	14 ± 2	18 ± 3	14 ± 2	18 ± 3	18 ± 3	19 ± 6	17 ± 3
Women LF	17 ± 2	32 ± 9	18 ± 3	19 ± 3	18 ± 3	18 ± 2	19 ± 2	20 ± 3	18 ± 3	17 ± 2
FBF (ml·min^{-1}·kg^{-1}) *†$										
Women EF	158 ± 23	159 ± 24	552 ± 46	563 ± 48	546 ± 38	425 ± 50	537 ± 39	348 ± 40	531 ± 37	256 ± 23
Women LF	191 ± 22	184 ± 24	600 ± 57	608 ± 43	612 ± 64	417 ± 32	595 ± 60	347 ± 26	614 ± 63	268 ± 21
Diameter (cm) *†$#										
Women EF	0.37 ± 0.01	0.36 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.37 ± 0.01
Women LF	0.36 ± 0.02	0.36 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.01

Values are mean±SEM. Ex, steady state rhythmic handgrip exercise; Ex+/-80, +/-100, +/-120, handgrip exercise with or without cuff inflation to 80, 100 or 120 mmHg; FF, free-flow; PFR, partial flow-restriction; EF; early follicular phase; LF Late follicular phase; BP, blood pressure; HR, heart rate; RRI, R-R interval; cBRS, cardiac baroreflex sensitivity calculated using either HR (HR-cBRS) or RRI (RRI-cBRS); FBF, forearm blood flow. * P<0.05 effect of time; † P<0.05 effect of condition (FF vs. PFR); $ P<0.05 interaction time vs. condition; # P<0.05 interaction condition vs. cycle phase.

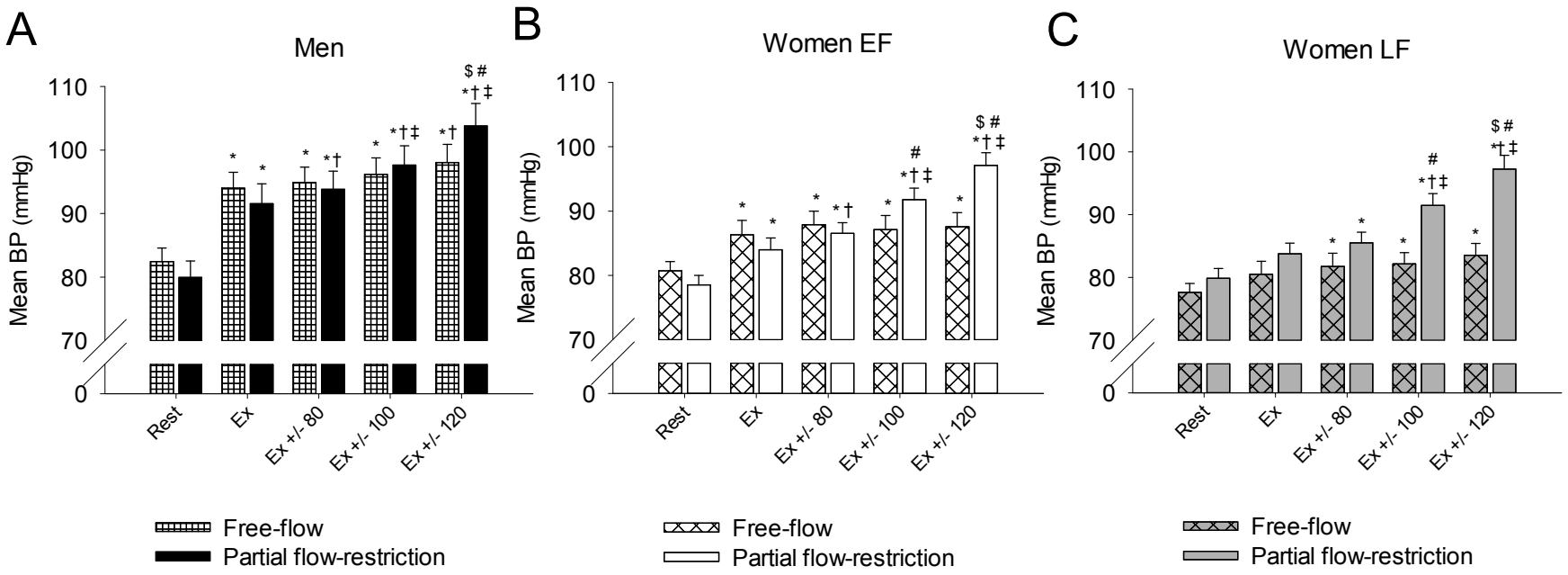

Figure 6.1. Mean arterial blood pressure (BP) responses to rhythmic handgrip exercise in men (panel A) and women during the early (EF; panel B) and late (LF; panel C) follicular phase of the ovarian cycle. Ex+/-80, +/-100, +/-120, handgrip exercise with or without cuff inflation to 80, 100 or 120 mmHg. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex of corresponding trial; ‡ P<0.05 vs. Ex 80 of corresponding trial; $ P<0.05 vs. Ex 100 of corresponding trial; # P<0.05 vs. free-flow trial at corresponding time point.

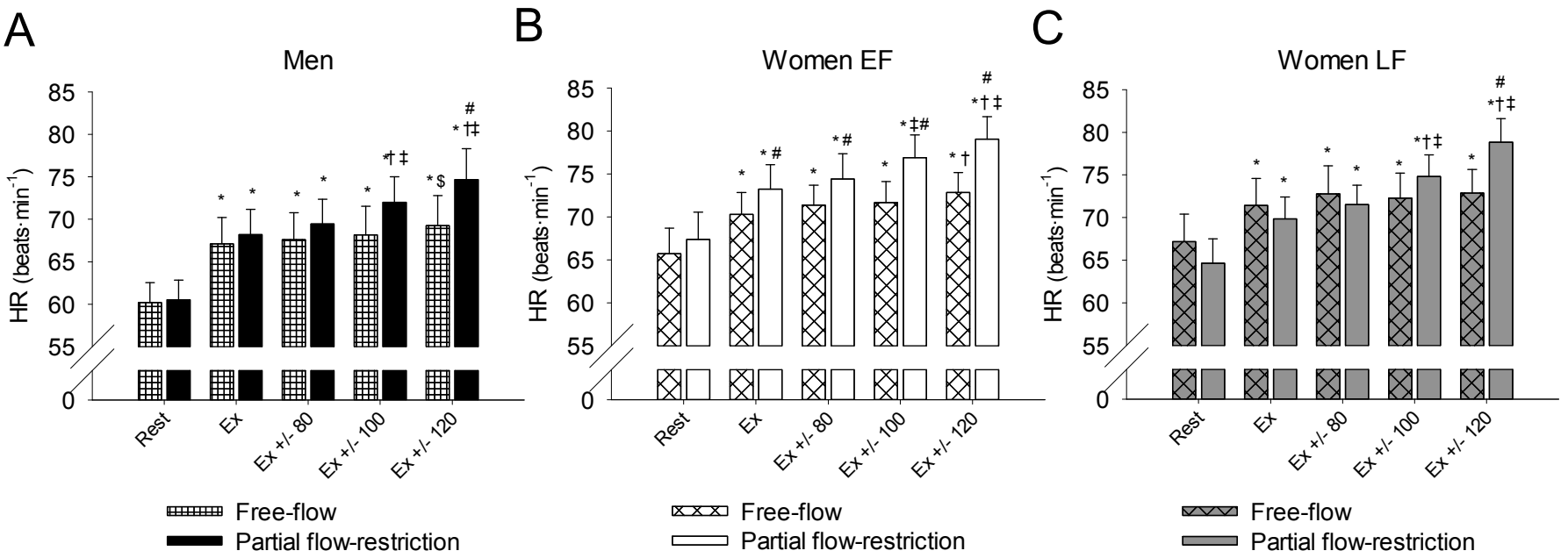

Figure 6.2. Heart rate (HR) responses to rhythmic handgrip exercise in men (panel A) and women during the early (EF; panel B) and late (LF; panel C) follicular phase of the ovarian cycle. Ex+/-80, +/-100, +/-120, handgrip exercise with or without cuff inflation to 80, 100 or 120 mmHg. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex of corresponding trial; ‡ P<0.05 vs. Ex 80 of corresponding trial; $ P<0.05 vs. Ex 100 of corresponding trial; # P<0.05 vs. free-flow trial at corresponding time point.

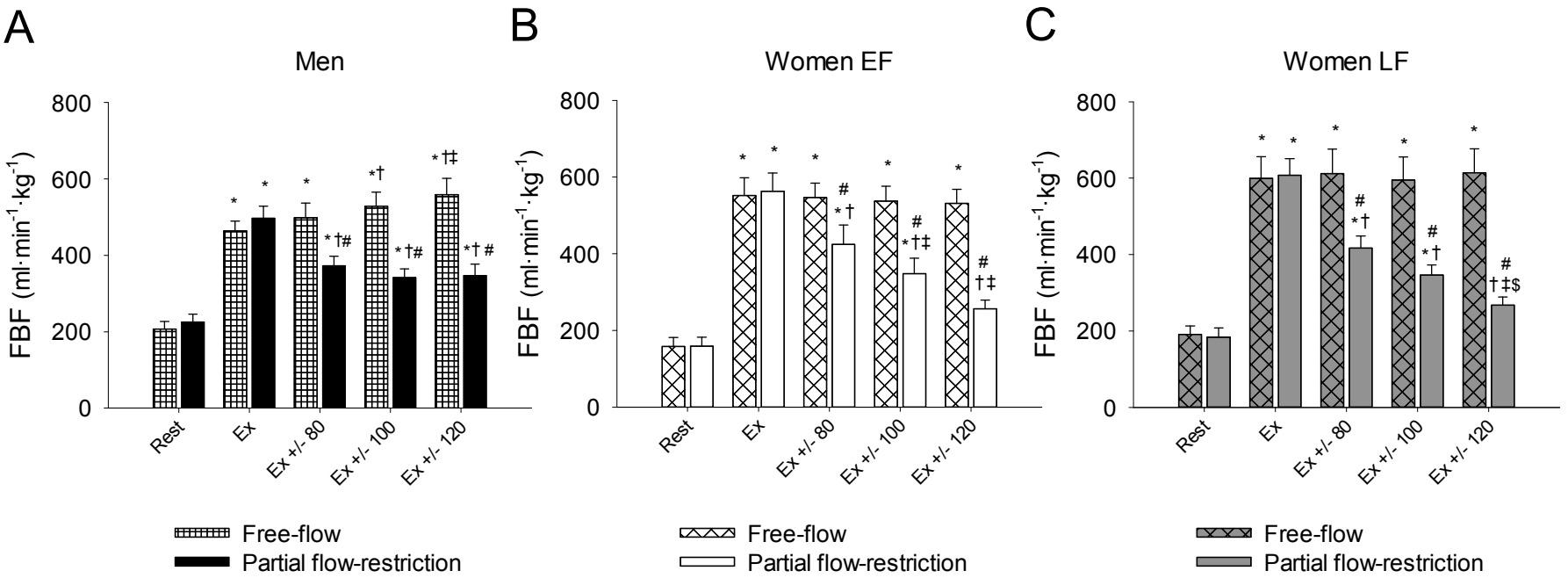

Figure 6.3. Forearm blood flow (FBF) responses to rhythmic handgrip exercise in men (panel A) and women during the early (EF; panel B) and late (LF; panel C) follicular phase of the ovarian cycle. Ex+/-80, +/-100, +/-120, handgrip exercise with or without cuff inflation to 80, 100 or 120 mmHg. * P<0.05 vs. Rest of corresponding trial; † P<0.05 vs. Ex of corresponding trial; ‡ P<0.05 vs. Ex 80 of corresponding trial; $ P<0.05 vs. Ex 100 of corresponding trial; # P<0.05 vs. free-flow trial at corresponding time point.

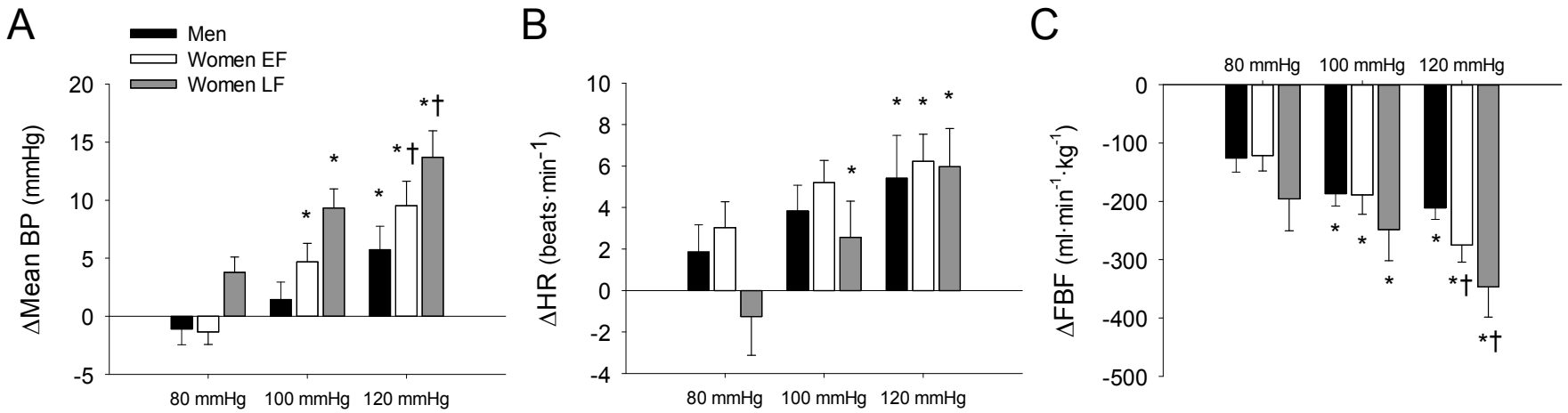

Figure 6.4. Changes in mean blood pressure (BP; panel A), heart rate (HR; panel B) and forearm blood flow (FBF; panel C) induced by graded partial flow-restriction (upper arm cuff inflation to 80, 100 and 120 mmHg) versus free-flow trial in men (black bars), women during the early (EF; white bars) and late follicular phase (LF; grey bars) of the ovarian cycle. * P<0.05 vs. 80 mmHg time point of corresponding group; † P<0.05 vs. 100 mmHg time point of corresponding group.

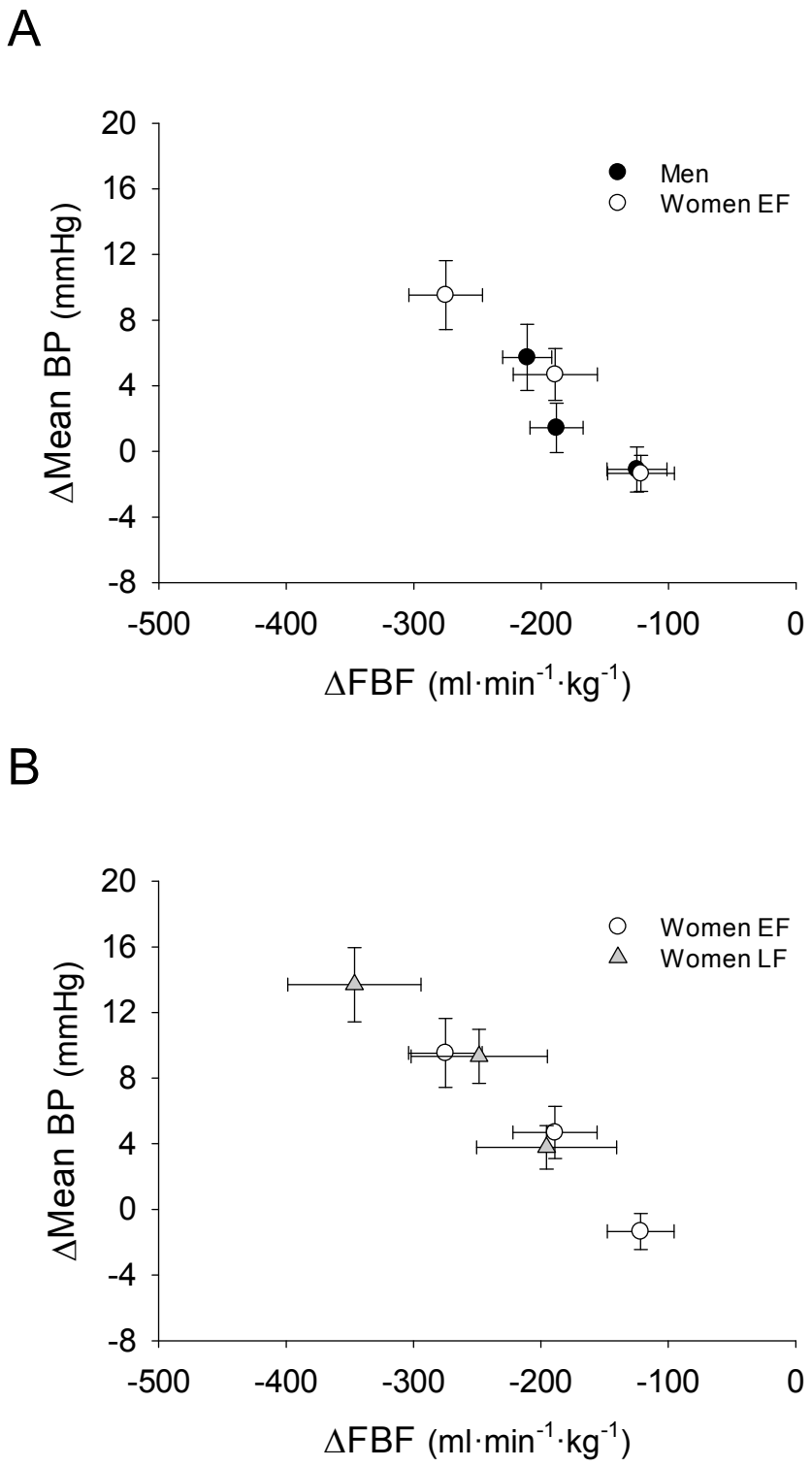

Figure 6.5. Relationship between the changes in forearm blood flow (FBF) and mean blood pressure (BP) induced by graded partial flow-restriction (upper arm cuff inflation to 80, 100 and 120 mmHg) vs. free-flow during rhythmic handgrip exercise. Values for men (black circles) and women during the early follicular phase (EF; white circles) are presented in panel A. Panel B shows the response relationship in women during the early and late follicular phase (LF; grey triangles) of the ovarian cycle.

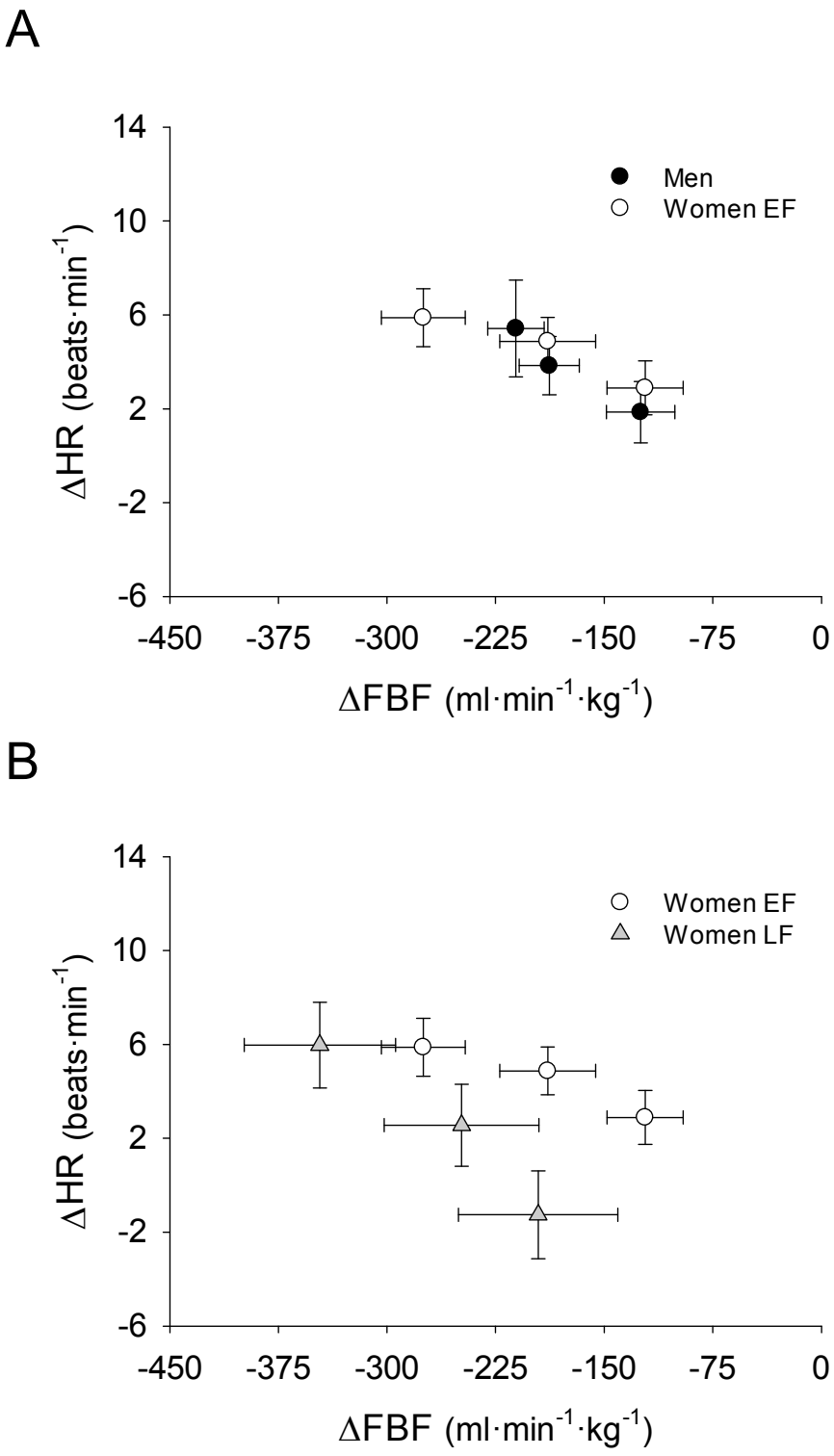

Figure 6.6. Relationship between the changes in forearm blood flow (FBF) and heart rate (HR) induced by graded partial flow-restriction (upper arm cuff inflation to 80, 100 and 120 mmHg) vs. free-flow during rhythmic handgrip exercise. Values for men (black circles) and women during the early follicular phase (EF; white circles) are presented in panel A. Panel B shows the response relationship in women during the early and late follicular phase (LF; grey triangles) of the ovarian cycle.

6.4 Discussion

The purpose of the present study was to investigate the potential influence of sex and/or female reproductive hormones on the sensitivity of the muscle metaboreflex when activated by graded reductions in skeletal muscle blood flow during handgrip exercise. The main novel findings of the present study are the following: (i) Increases in HR and BP during exercise with graded muscle metaboreflex engagement were similar in men and women and not different between the EF and LF phases of the ovarian cycle; (ii) In contrast to the initial hypothesis, the slopes of the relationship between the reductions in FBF and the changes in either HR or BP (i.e. muscle metaboreflex sensitivity) were not different in men and women and comparable in the EF and LF phases of the ovarian cycle; (iii) Similar to the observation in men (see Chapter 4 for details), cBRS remained unchanged from rest during rhythmic handgrip exercise with and without partial flow-restriction in women during both phases of the ovarian cycle. Taken together, muscle metaboreflex activation when engaged by similar reductions in skeletal muscle perfusion does not evoke differential cardiovascular responses (i.e. similar metaboreflex sensitivity) in men and women during low and high estrogen phases of the ovarian cycle.

6.4.1 Influence of sex on cardiovascular responses to free-flow and partial flow-restriction

Despite the fact that previous studies in humans have suggested that metaboreflex activation during static forearm exercise is attenuated in women (Ettinger et al., 1996, Jarvis et al., 2011), muscle metaboreflex sensitivity for the control of BP and HR were not different in men and women in the present study. However, these

findings are in agreement with a study in canines that also reported no sex-differences in the strength and mechanisms of the muscle metaboreflex (Laprad et al., 1999). Conversely to the experiments in humans, the study by Laprad et al. (1999) and the present data provide direct measurements of blood flow. Although, FBF was not statistically different in men and women during the free-flow trial, FBF increased slightly over time during exercise in men, but not in women. Thus, the muscle metaboreflex-induced effects were expressed as the difference between the free-flow and the partial flow-restriction trial. This allows an accurate comparison, as the level of the stimulus (i.e. flow restriction) was standardised.

In treadmill running dogs instrumented with a pneumatic occluder around the terminal aorta, the muscle metaboreflex has been characterised as a reflex mechanism, which is not tonically active (Wyss et al., 1983). According to this model, after passing the reflex threshold the magnitude of the metaboreflex-induced increases in HR, BP, CO and TPR is proportional to the extent of decrease in skeletal muscle blood flow (i.e. linear relationship). The slope of this relationship can provide an estimate of muscle metaboreflex responsiveness in control of these parameters. A recent study in humans has used graded increases in external cuff pressure during rhythmic handgrip (Ichinose et al., 2011). They identified an exercise intensity dependent shift in the muscle metaboreflex threshold, necessary to engage the muscle metaboreflex, from a 60% reduction in FBF at very low rhythmic handgrip exercise (5% MVC) to a 40% reduction in FBF at low exercise intensities (15% MVC). In the present study, during 35% MVC an approximately 25% reduction in FBF during the first cuff pressure (+80 mmHg) occurred, which increased progressively in proportion to the cuff pressure. Although it was beyond the scope of the present study to identify the muscle metaboreflex threshold, the clear pressor response during partial flow-restriction may suggest that this

threshold was passed. Therefore, the estimation of linear regression lines to plot the relationship between the reduction in FBF and the concurrent changes in mean BP and HR presents a reasonable approach to obtain an estimate of muscle metaboreflex responsiveness (i.e. slope of the regression line) and the subsequent comparison in men and women during EF and LF phases of the ovarian cycle.

Compared to men, women have been reported to have a greater oxidative capacity suggesting that during a similar exercise workload women accumulate a smaller amount of metabolites associated with anaerobic muscle metabolism than men (Komi and Karlsson, 1978, Simoneau and Bouchard, 1989). An attenuated metabolite accumulation would activate the muscle metaboreflex to a lower degree resulting in an attenuated pressor response in women compared to men (Ettinger et al., 1996). However, in the present study, muscle metaboreflex engagement evoked similar increases in HR and mean BP in men and women. One possible explanation might derive from the fact that during rhythmic handgrip exercise the engaged muscle mass is very low. It is therefore possible that differences in metabolite accumulation were not sufficient to affect the metaboreflex-mediated control of BP and HR. However, no estimate of muscle fibre-type distribution and/or the changes in metabolic profile of the exercising forearm muscles was available in the present study. Thus, by employing a relative workload (e.g. 35% MVC) and the observation that FBF was similarly reduced during pressure cuff inflation in men and women, it may be concluded that it is unlikely that differences in the magnitude of metabolite accumulation were present. However, to fully exclude this possibility the assessment of the muscle metabolic profile during exercise would be necessary using for example ^{31}P-nuclear magnetic resonance spectroscopy (Ettinger et al., 1996).

6.4.2 Influence of ovarian cycle phase on cardiovascular responses to free-flow and partial flow-restriction

The autonomic effects of estrogen include enhanced parasympathetic nerve activity, attenuated sympathetic nerve activity and enhanced cBRS resulting in a dampened pressor response during pharmacologically induced elevations in BP (He et al., 1998, Saleh et al., 2000a, Saleh and Connell, 2000). In humans, resting vagal tone has been reported to be elevated in postmenopausal women supplementing with estrogen compared to placebo-treated women (Virtanen et al., 2000). Moreover, sympathetic nerve activity is reportedly greater in the midluteal than the EF phase of the ovarian cycle (Minson et al., 2000a). In agreement, BP responses were only attenuated in LF women during free-flow exercise. However, HR and BP were similar in women between these two ovarian phases at rest and during graded muscle metaboreflex activation. One potential explanation for these partly discrepant findings could be that the utilized exercise intensity (i.e. 35% MVC) was insufficient to increase efferent sympathetic activity. In support of this, it has been demonstrated that exercise intensities below 40% MVC are not accompanied by elevations in MSNA (Seals, 1989b, Victor and Seals, 1989, Victor et al., 1987b). Moreover, the choice of rhythmic handgrip exercise very likely allowed the washout of metabolites, which could have limited sympathetic activation via the muscle metaboreflex. In addition, the magnitude of vagal withdrawal during both free-flow and partial flow-restriction might have been too small to detect any modulatory effects of estrogen. In support of that, spontaneous cBRS which is thought to represent a measure of cardiac vagal activity (Parati et al., 2000, Parlow et al., 1995, Task, 1996) was unaffected by the ovarian cycle phase at rest, during exercise under free-flow and partial flow-restriction.

6.4.3 Influence of sex on cBRS during free-flow and partial flow-restriction

It has been repeatedly shown that during PEI following forearm exercise, BP remains elevated, while HR returns back to resting levels (Alam and Smirk, 1937, Cui et al., 2001, Fisher et al., 2008, Papelier et al., 1997, Victor et al., 1987b). The majority of recent studies have also demonstrated no effect of muscle metaboreflex activation on cBRS using this technique after arm exercise (Cui et al., 2001, Fisher et al., 2010, Fisher et al., 2008, Iellamo et al., 1999b). Although PEI is a well-established experimental model to examine the role of the muscle metaboreflex in isolation from central command and muscle mechanoreflex, it arguably represents an artificial situation. Thus, in the present study, metabolically sensitive afferents were activated by gradually reducing skeletal muscle blood flow to the exercising forearm muscles during rhythmic handgrip exercise. In the current study, HR and BP rose in proportion to the degree of FBF restriction. These results are in agreement with previous studies, in which blood flow was reduced using a positive pressure box around the exercising arm (Daley et al., 2003, Joyner, 1991). Together, these results support the notion that the magnitude of muscle metaboreflex activation was indeed successively graded with increasing cuff pressure. However, cBRS was unchanged from rest during stepwise engagement of metabolically sensitive afferents. Similar observations including the unchanged operating point gain of the carotid-cardiac baroreflex stimulus-response curve have been reported during graded metabolite accumulation (e.g. magnitude of metaboreflex activation) during PEI after arm exercise (Fisher et al., 2010, Fisher et al., 2008). A close correlation has been demonstrated between the spontaneous cBRS sensitivity assessed by the sequence technique and the operating point gain of the full cardiac baroreflex stimulus response curve (Ogoh et al., 2005b). Thus, the results from the present study suggest that cardiac baroreflex responsiveness around the operating point

was well preserved when the muscle metaboreflex was engaged during rhythmic handgrip exercise.

6.4.4 Influence of ovarian cycle phase on cBRS during free-flow and partial flow-restriction

Although, estrogen receptors have been identified in several brain areas involved in the central cardiovascular control their expression in the brain areas associated with central command, such as the midbrain, thalamus and insular cortex is rather sparse (Pfaff and Keiner, 1973, Spary et al., 2009). However, Hayes and colleagues (2002) have observed that estrogen attenuated the central command-induced increases in BP, HR and sympathetic nerve activity during intravenous injection of 17β-estradiol on cardiovascular responses to electrical stimulation of the mesencephalic locomotor region in decerebrate, paralysed male cats. In contrast, in the present study RPE were similar, while BP was attenuated during free-flow exercise in LF women (e.g. high estrogen). Of note, estrogenic effects on the central command-induced cardiovascular responses were not determined in isolation, as performed in animals. Therefore, potential estrogen-mediated differences in the central command-induced pressor response might have been masked or overridden by augmented sensory afferent feedback from the exercising skeletal muscle (e.g. muscle mechanoreflex) that was activated in parallel with central command during voluntary exercise. Alternatively, profound species-differences and/or the use of fluctuations in endogenous (present study) versus exogenous (Hayes et al.) estrogen concentrations may explain the seemingly divergent results.

The lack of estrogenic influences on spontaneous cBRS during graded muscle metaboreflex engagement may alternatively be caused by the fact that fluctuations in

estrogen concentration were too small to evoke detectable effects on cardiovascular and haemodynamic variables during rhythmic handgrip exercise. Therefore, another model in which the magnitude of female hormone concentrations can be artificially altered, such as studying postmenopausal women with or without estrogen replacement therapy, might prove more successful.

6.4.5 Limitations

It cannot be ruled out completely that the muscle mechanoreflex, potentially activated by venous distension or muscle compression, has contributed to the tachycardia during rhythmic handgrip exercise with partial flow-restriction (Bell and White, 2005, Drew et al., 2008a, Kaufman and Rybicki, 1987) (see Sections 4.4 and 5.4.1 for details).

Another potential limitation of the present study is that plasma estrogen and progesterone concentrations were not measured. Hence, it can only be assumed that estrogen concentrations were significantly elevated during the LF phase of the ovarian cycle. However, the use of ovulation kits allows a very accurate, although indirect, estimate of the time of peak estrogen concentrations. Also, it is a well established and widely used methodology (Minson et al., 2000a). Moreover, only the effects of estrogen were considered to influence muscle metaboreflex control during exercise. However, progesterone has been shown to exert sympatholytic effects (Heesch and Rogers, 1995) by modulating the neurotransmitter release in medullary regions (e.g. RVLM) involved in the baroreflex mediated sympathetic inhibition (Potts, 2006). Whether progesterone affects the cardiovascular responses and/or arterial baroreflex control of HR and BP via modulation of the muscle metaboreflex warrants investigation. For example further

studies should include the midluteal phase of the ovarian cycle, when both estrogen and progesterone concentrations are elevated.

6.4.6 Alternative approaches

It is acknowledged that the experimental model employed in the present study is not ideal in that rhythmic handgrip exercise at 35% MVC was insufficient to reduce cBRS. This was presumably due to the small muscle mass activated during handgrip exercise. Therefore, one-legged kicking that involves a larger muscle mass performed under free-flow conditions and with partial flow-restriction might be a next step. In addition, femoral blood flow measurements are feasible during leg kicking (Andersen et al., 1985). These would provide a good model to investigate sex-differences during dynamic exercise with graded muscle metaboreflex activation. Although not measured in the present study, the inclusion of efferent sympathetic nervous activity might have added important information. For example, the muscle metaboreflex has been shown to raise BP via increases in MSNA during handgrip exercise (Mark et al., 1985, Victor and Seals, 1989) and MSNA responses to exercise have repeatedly been described to be attenuated in women compared to men during forearm exercise (Ettinger et al., 1996, Jarvis et al., 2011). Moreover, the inclusion of lower cuff pressures (0-50 mmHg) during rhythmic handgrip exercise (Daley et al., 2003, Ichinose et al., 2011) might provide additional insight regarding the threshold and/or breakpoint for muscle metaboreflex activation. Therefore, additional MSNA recordings, a greater range of cuff pressures and potentially a measure of the muscle metabolic profile (e.g. venous blood samples) during leg-kicking should be applied to address the presently unanswered questions.

6.5 Conclusion

In summary, for the first time in humans, this study determined the potential influence of sex and/or ovarian hormone concentration on the muscle metaboreflex-induced pressor response and effects on cBRS. The relationships between the changes in HR and BP and the reductions in FBF during rhythmic handgrip exercise with partial flow-restriction were similar in men and women and during the EF and LF phases of the ovarian cycle, while cBRS remained unchanged compared to rest in all groups. These data indicate that there are no sex-differences in muscle metaboreflex sensitivity and further suggest that previously observed sex-differences in the muscle metaboreflex-induced cardiovascular responses might potentially be related to differences in muscle perfusion as similar blood flow reductions evoked similar pressor responses in men and women.

CHAPTER 7: SEX DIFFERENCES IN THE DYNAMIC CAROTID BAROREFLEX FUNCTION AT REST AND DURING DYNAMIC EXERCISE

7.1 Introduction

7.1.1 Baroreflex control of BP and HR

The arterial baroreflex plays an important role in the beat-to-beat regulation of arterial BP and HR, by modulating sympathetic and parasympathetic activity to the heart and the peripheral vasculature. The data from the previous studies (see Chapters 5&6) indicated that there are no sex-differences in cardiac baroreflex responsiveness at rest and during exercise. However, there is some controversy in the literature, reporting either augmented, attenuated or unchanged cardiac baroreflex function in men compared to women at rest (Beske et al., 2001, Chen and DiCarlo, 1996, Convertino, 1998, Tanaka et al., 2003, Tank et al., 2005). Only a few studies have investigated the differences in the arterial baroreflex control of BP. These have found that women have a reduced effectiveness to buffer changes in BP via a baroreflex mechanism and lower autonomic support of BP (Christou et al., 2005, Schmitt et al., 2010). However, the influence of sex on the arterial baroreflex control of BP remains incompletely understood.

In both animals (Bevegard and Shepherd, 1966, Coote and Dodds, 1976) and humans (Papelier et al., 1994, Potts et al., 1993) the arterial baroreflex has been shown to be reset to operate around the higher exercising BP thus continuing to regulate BP during exercise. The resetting of the arterial baroreflex during exercise has been shown to dependent on the exercise intensity. It is also very closely related to the actions and interactions of neural signals arising from higher brain areas (i.e. central command) (Iellamo et al., 1997, McIlveen et al., 2001, Ogoh et al., 2002b, Querry et al., 2001) and the reflex feedback from skeletal muscle afferents (Gallagher et al., 2001b, Iellamo et al., 1997, Potts and Mitchell, 1998, Smith et al., 2003), which is characterised by the

stimulation of metabolically and mechanically sensitive skeletal muscle afferents (Kaufman and Rybicki, 1987).

Barodenervation has been shown to lead to exaggerated contraction-induced BP responses in cats and rats (Waki et al., 2003, Waldrop and Mitchell, 1985). This indicates that a fully operating arterial baroreflex is necessary to elicit adequate baroreflex resetting and appropriate cardiovascular responses during exercise. Indeed, men exhibit a greater pressor response compared to women during both static and dynamic exercise (Ettinger et al., 1996, Fleg et al., 1995). However, the reasons for these differences remain unclear. One potential explanation might be a reduced arterial baroreflex function in women during exercise. While the last studies (Chapter 5&6) indicated that cardiac baroreflex function at rest and during exercise is not different in men and women, there are no data available on sex-differences in the arterial baroreflex control BP during exercise. Although the arterial baroreflex continues to control BP during exercise, it might be erroneous to propose that the sex-differences observed at rest are simply translatable to the exercise condition. Varying activation of the muscle afferents and/or central command between men and women, and their subsequent modulation of the arterial baroreflex might contribute to an altered arterial baroreflex control of BP during exercise (Ettinger et al., 1996, Jarvis et al., 2011).

7.1.2 Baroreflex control of peripheral haemodynamics

Vascular conductance has been shown to play an important role in the baroreflex control of BP at rest and during exercise (Keller et al., 2004, Keller et al., 2003, Ogoh et al., 2002a). Moreover, the arterial baroreflex is known to mediate peripheral haemodynamic changes, by altering muscle sympathetic nerve activity and blood flow (Ernsting and Parry, 1957, Fadel et al., 2001, Ogoh et al., 2002a, Wray et al., 2004a).

Application of a hypotensive stimulus to the carotid baroreceptors (e.g. neck pressure, NP) provides a sympatho-excitatory stimulus, which has been found to evoke a blunted vasoconstrictor response in the exercising skeletal muscle compared to rest (Keller et al., 2004, Keller et al., 2003). This phenomenon has been termed 'functional sympatholysis' (Remensnyder et al., 1962). Local accumulation of contraction related metabolites such as adenosine triphosphate, nitric oxide or diprotonated phosphate have been suggested to partially off-set sympathetic activation in the vasculature of the exercising skeletal muscles (Boushel et al., 1998, Dinenno and Joyner, 2003, Thomas et al., 1997). However, since studies have been predominantly performed in men, it remains unclear whether there are sex-differences in the sympathetic vasoconstrictor responses during exercise. Indeed, sympathetically mediated vasoconstriction at rest was found to be blunted in women compared to men (Hogarth et al., 2007, Kneale et al., 1997). Also, distinct differences seem to exist in the relationship between efferent sympathetic activity and vascular tone between the sexes (Hart et al., 2009, Hogarth et al., 2007). However, to date, it is unknown whether these differences persist during exercise.

7.1.3 Assessment of carotid baroreflex function

The arterial baroreflex is known to mediate changes in HR, BP, sympathetic nerve activity and the peripheral circulation in a dynamic manner (Eckberg et al., 1988, Wray et al., 2004a). However, most studies evaluating CBR function in humans, especially during exercise, have utilized static perturbations to simulate carotid sinus hypo- and hypertension (Fadel et al., 2001, Fisher et al., 2006, Keller et al., 2004, Norton et al., 1999, Ogoh et al., 2002a, Potts et al., 1993, Smith et al., 2003). In that, brief pulses (e.g. 5 seconds) of external positive (NP) and negative pressure (NS)

directly alter carotid transmural pressure, leading to decreased or increased carotid sinus nerve firing, respectively (Ludbrook et al., 1977). However, it has been shown that during static pulses of CBR stimulation using the neck chamber technique a counteraction from the extra-carotid baroreceptors (e.g. aortic and cardiopulmonary baroreceptors) occurs within 20 seconds, which overrides the CBR-mediated cardiovascular, neural and haemodynamic responses (Fadel et al., 2003a, Ogoh et al., 2003a). To overcome this potential limitation and to assess the dynamic nature of the carotid baroreflex, the present study used an intermittent, more prolonged stimulus of the carotid baroreceptors, namely oscillatory NP and NS. Bath et al. (1981) have demonstrated that simulated carotid hypertension (NS) in a sinusoidal manner (e.g. a series of 7.5 seconds on followed by 7.5 seconds off and so on) elicited a profound reduction in muscle sympathetic nerve activity which was not achieved by application of a static pulse. Since then, oscillatory perturbations of the carotid baroreceptors have been successfully shown to evoke carotid baroreflex-mediated entrainment of BP, HR and skin blood flow (Bath et al., 1981, Bernardi et al., 1997, Spadacini et al., 2006). In a recent study by Wray et al. (2004a), measurements of muscle sympathetic nerve activity (MSNA), HR, BP, leg blood flow and tissue oxygenation have been simultaneous performed during oscillatory NP at rest and during exercise. They have found that CBR control of MSNA and BP is well maintained from rest to exercise, while CBR-entrainment of the peripheral vasculature was decreased during exercise compared to resting conditions. However, the study of Wray et al. (2004) was not designed to determine potential sex-differences in the CBR control of cardiovascular and haemodynamic responses.

The aim of the present study was to determine potential sex-differences in the dynamic CBR control over cardiovascular and haemodynamic parameters at rest and

during exercise. To investigate this, the neck chamber technique was employed to selectively unload (NP) and load (NS) the carotid baroreceptors in an oscillatory manner (i.e. 5 seconds on, 5 seconds off) at rest and during low-level rhythmic handgrip exercise. It was anticipated that during exercise the CBR control of the peripheral circulation would be decreased compared to rest, while CBR control of BP would be maintained from rest to exercise. It was further hypothesised that women would elicit an attenuated CBR entrainment of BP and that sympatho-excitatory NP would be less effective in reducing skeletal muscle perfusion in women compared to men.

7.2 Methods

7.2.1 Subjects

Ten healthy young men and ten healthy young women were recruited from the University of Birmingham student community (Table 1). Six out of the ten women used hormonal contraception. In order to minimize any effects of ovarian hormone concentrations women were studied during the EF phase of the ovarian cycle (4±1 day).

Table 7.1. Subject characteristics

	Men (n=10)	Women (n=10)
Age (years)	23 ± 2	23 ± 4
Weight (kg)	81 ± 9*	67 ± 16
Height (cm)	184 ± 5*	169 ± 6
BMI ($kg \cdot m^{-2}$)	24 ±2	23 ± 5
MVC (kg)	53 ±6*	32 ± 7

Values are mean±SEM. BMI, body mass index; MVC, maximum voluntary contraction.
* $P<0.05$ different from men.

7.2.2 Experimental measurements

Figure 7.1 shows the experimental set-up used in the present study. HR and BP were measured and mean BP was calculated (see Section 3.2.1. for details). A strain-gauge pneumobelt placed around the abdomen was additionally used to visualise and monitor the respiratory movements throughout the experiment (Pneumotrace II, model 1132, UFI, Morro Bay, USA). To determine whether sex influences CBR control of BP and HR, beat-to-beat changes in BP and HR were assessed during the application of five minutes of oscillatory NP and NS at rest and during rhythmic handgrip exercise in young men and women (Figure 7.2).

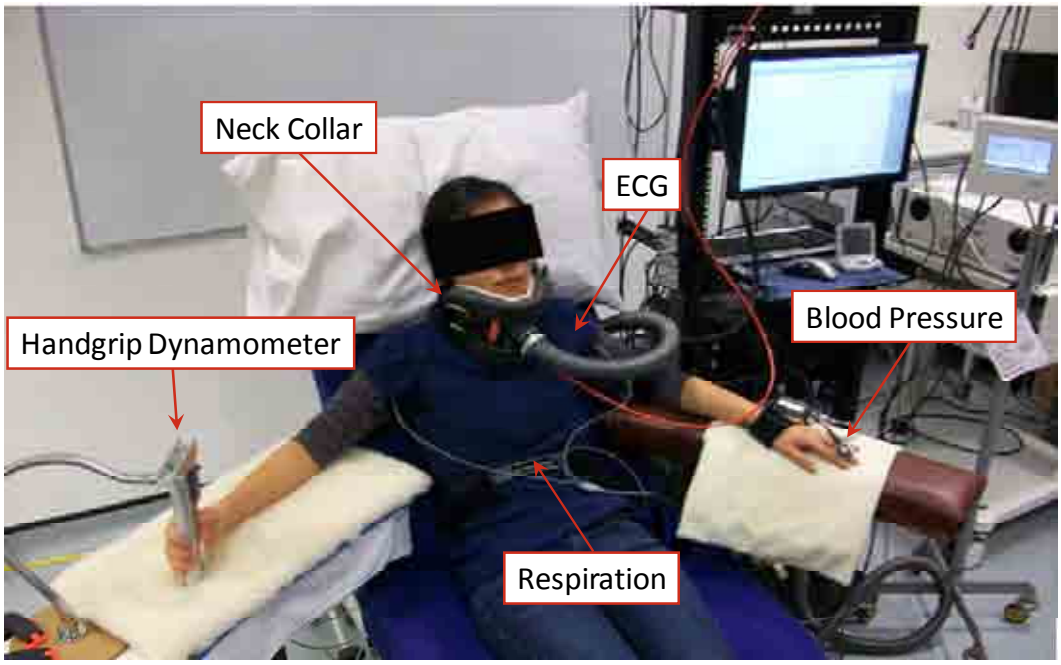

Figure 7.1. Laboratory set-up. ECG, electrocardiogram; neck collar allowed application of neck pressure (NP; simulated carotid hypotension) and neck suction (NS; simulated carotid hypertension).

Dynamic CBR function was assessed after familiarisation using the oscillatory NP and NS at rest and during exercise (see Section 3.3.4 for details). In a subset of three subjects FBV from the brachial artery of the right arm was obtained by Doppler

ultrasound (see Section 3.2.2 for details) and tissue oxygenation measurements were performed using NIRS (see Section 3.2.6 for details).

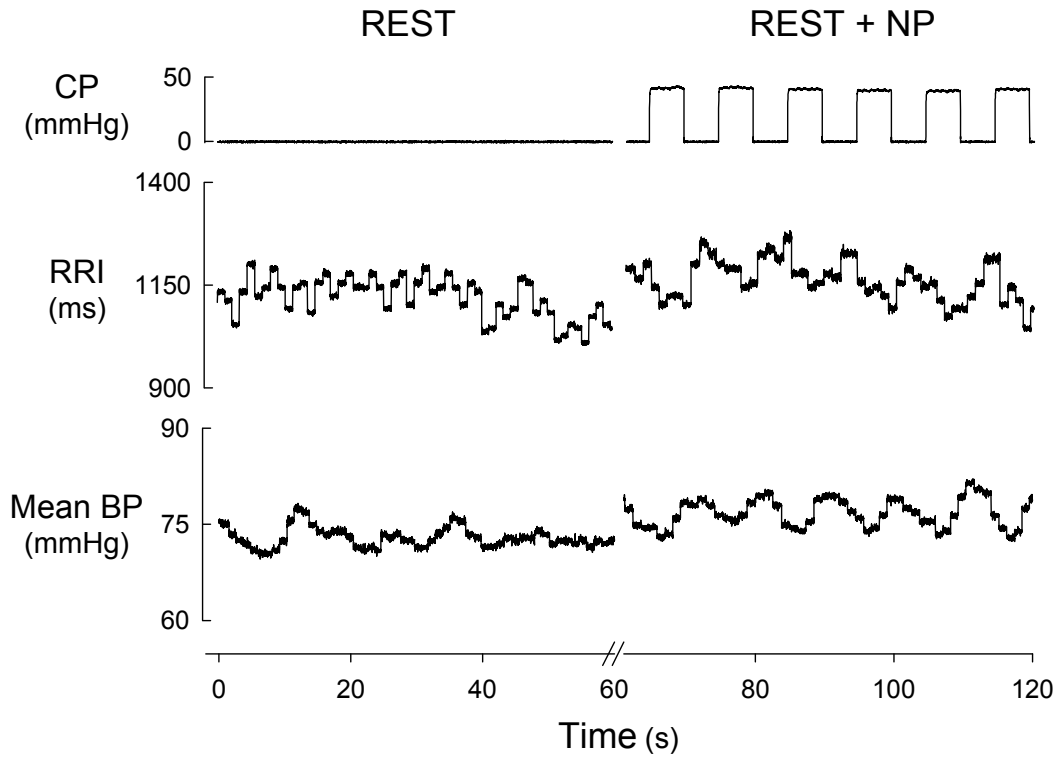

Figure 7.2. Original record of one representative subject showing chamber pressure (CP; top panel), R-R interval (RRI; middle panel) and mean blood pressure (mean BP; bottom panel). Oscillatory NP evoked carotid baroreflex entrainment of RRI and mean BP as indicated by the close coupling of fluctuations of these RRI and mean BP with oscillations in chamber pressure.

7.2.3 Experimental procedures

Subjects were seated in a semi-recumbent position on a medical examination table with a custom made handgrip dynamometer held in the right hand, while the arm was supported on an adjustable bedside table. Following instrumentation and assessment of MVC (see Section 3.3.1 for details) subjects rested for 15 minutes.

During the resting trial a 5 minute resting period was followed by 5 minutes of either NP or NS (Figure 7.3; panel A). 13 minutes of rhythmic handgrip exercise were

performed at a duty cycle of 1.5 seconds contraction – 1.5 seconds relaxation (20 contractions per minute) to allow FBV measurements during handgrip (Dinenno and Joyner, 2003). The exercise workload corresponded to 20% of MVC in order to minimise the potential for exercise-induced sympatho-excitation (Victor and Seals, 1989). Subjects started handgrip exercise after a 2 minute resting period. Following 3 minutes of exercise to reach cardiovascular and haemodynamic steady-state, 5 minutes of oscillatory NP or NS were applied (Figure 7.3; panel B). After the dynamic carotid baroreceptor perturbation was finished, subjects continued exercising for another 5 minutes. The order of oscillatory NP and NS application was randomized and counterbalanced. Exercise trials were separated by 15 minutes to avoid fatigue and ensure reestablishment of baseline HR and BP before commencing the next trial. RPE were obtained for each exercise trial.

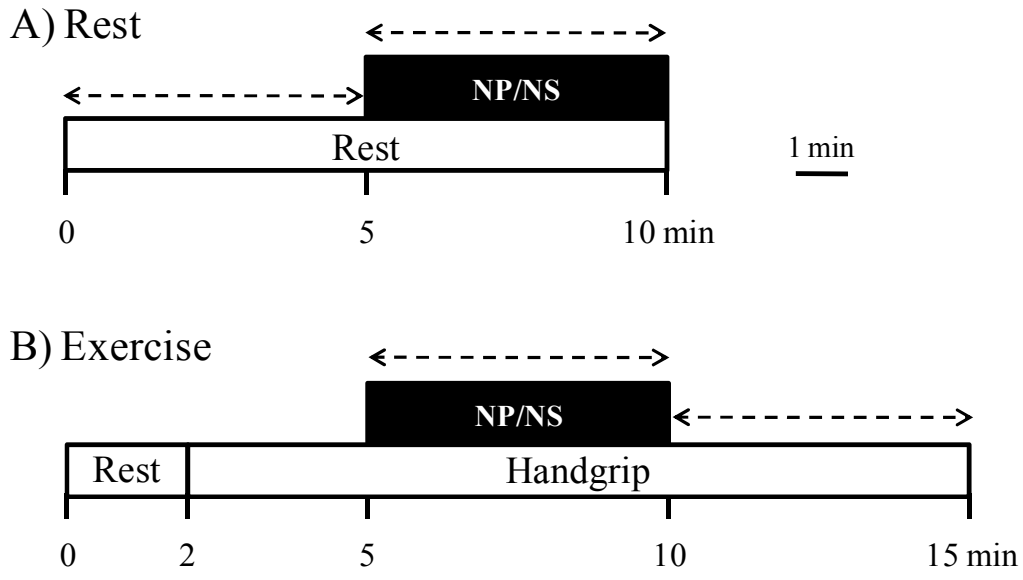

Figure 7.3. Schematic representation of the experimental protocol, comprising oscillatory neck pressure (NP) and neck suction (NS) at rest (panel A) and during rhythmic handgrip exercise (panel B). Dashed lines indicate time of data acquisition.

7.2.4 Data reduction & Spectral Power Analysis

Five minute averages were calculated for each section (Rest NoNP or NP, Ex NoNP or NP, Rest NoNS or NS and Ex NoNS or NS).

A Fast Fourier transformation was performed to calculate the spectral power of RRI and BP (Brown et al., 2003, Wray et al., 2004a) (Figure 7.4) using customized script files (Spike 2 Version 5, Cambridge Electronic Design [CED], Cambridge, UK). In detail, new waveforms were created from each RRI and the corresponding mean BP value. The resulting waveforms were linearly interpolated and re-sampled at 5.12 Hz. The voltage offset was removed to eliminate 0 Hz contamination of the spectrum. The Fast Fourier transform consisted of 512 bins, resulting in spectra with bin widths of 0.01 Hz. A Hann window was used to eliminate high frequency noise created by discontinuities in the waveform. The three bins spanning the low frequency range (LoF; 0.085-0.115 Hz) were selected and the area under the LoF peak used as a measure of power spectral density (Cooke et al., 1999, Wray et al., 2004a). Before statistical analyses were performed, the power spectra were transformed using natural logarithm (Ln), because some of the measured signals showed a skewed distribution (Bernardi et al., 1997, Wray et al., 2004a). In order to verify that the observed cardiovascular and haemodynamic entrainment was indeed evoked by the oscillatory input of NP and NS, an estimation of coherence was performed using DADiSP (DATAQ Instruments Inc., Akron, OH, USA). Coherence greater than 0.5 was assumed to provide a stable relationship between input (NP/NS) and output (cardiovascular and haemodynamic variables) (Spadacini et al., 2006).

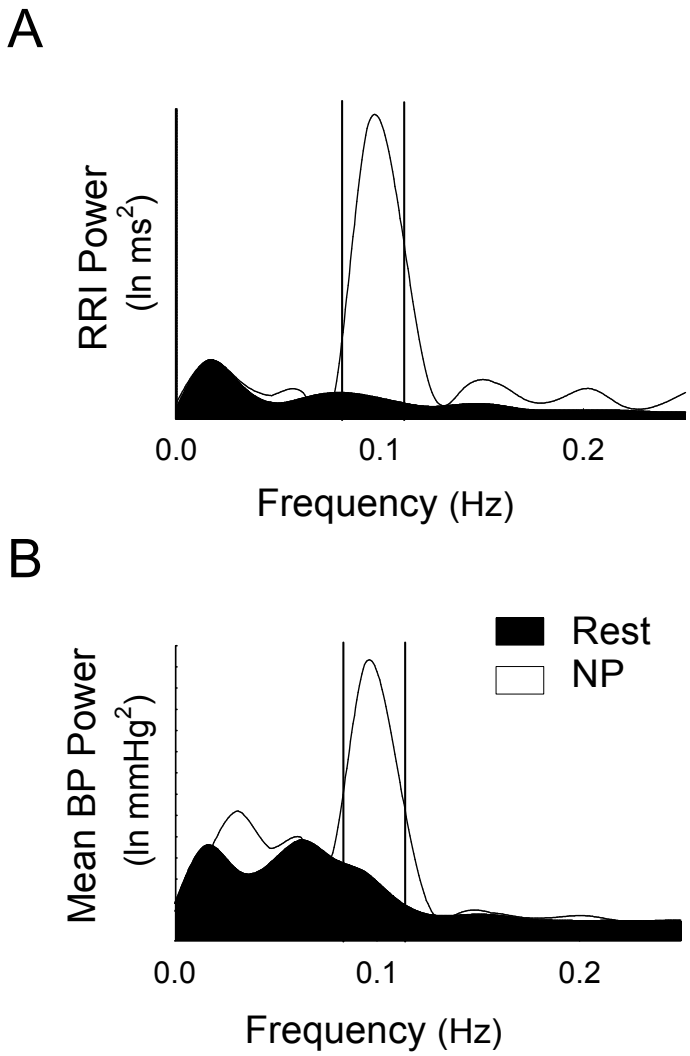

Figure 7.4. Power spectral density of RRI (panel A) and mean BP (panel B) at rest without (black area) and with (white area) neck pressure (NP). Vertical lines indicate the low frequency range (LoF; 0.085-0.115 Hz) of oscillatory carotid baroreflex stimulation. Five minutes of oscillatory NP elicited carotid baroreflex entrainment of RRI and mean BP indicated by the discrete peak in the power spectra at the stimulus frequency.

7.2.5 Statistical analysis

Group comparisons of the cardiovascular responses to exercise with and without oscillatory NP or NS were determined using three way-repeated measures ANOVA. The main factors were *sex* (men, women), *condition* (rest, exercise) and *pressure* (No NP or No NS and NP or NS). Absolute power spectral densities and changes in LoF power of each variable were compared using two-way repeated-measures ANOVA with the main factors *sex* and *condition* or *sex* and *pressure*.

7.3 Results

7.3.1 Systemic cardiovascular responses at rest and during exercise

Weight, height and MVC were higher in young men compared to women (Table 7.1). Low-level rhythmic handgrip exercise evoked significant increases in systolic, diastolic and mean BP and HR, while RRI was reduced (Tables 7.2&7.3). Of note, systolic BP was constantly lower in women than men at rest and during exercise, while diastolic BP (P= 0.080) and HR (P= 0.093) tended to be higher in women compared to men. RPE during low-level rhythmic handgrip exercise at 20% MVC was 12±1 au in men and 11±1 au in women.

7.3.2 Systemic cardiovascular responses to NP and NS

Oscillatory NP (i.e. simulated carotid hypotension) evoked increases in systolic, diastolic and mean BP ($P<0.05$). The magnitude of these increases was not different at rest and during exercise, or between the two groups (Table 7.2). HR and RRI were not affected by oscillatory NP. No interaction was found between condition (i.e. rest vs. exercise) and pressure (NoNP vs. NP; $P>0.05$).

Oscillatory NS (i.e. simulated carotid hypertension) decreased diastolic and mean BP and HR at rest and during exercise, while RRI was increased in both conditions ($P<0.05$; Table 7.3). The magnitude of these changes was greater during exercise compared to rest ($P<0.05$ condition effect). Systolic BP tended to increase with oscillatory NS at rest, whereas it decreased significantly during exercise. The RPE during handgrip was not affected by NP or NS.

Table 7.2. Cardiovascular responses at rest and during handgrip exercise without and with neck pressure in men and women

		Systolic BP *†‡ (mmHg)		**Diastolic BP** *† (mmHg)		**Mean BP** *† (mmHg)		**HR** * (beats·min^{-1})		**RRI** * (ms)	
		Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Rest											
	No NP	120 ± 2	112 ± 3	61 ± 2	67 ± 2	81 ± 2	82 ±2	58 ± 2	66 ± 4	1045 ± 26	941 ± 56
	NP	131 ± 4	118 ± 4	65 ± 3	68 ± 3	86 ± 6	84 ± 3	58 ± 1	66 ± 4	1050 ± 18	947 ± 58
Exercise											
	No NP	126 ± 3	118 ± 2	67 ± 3	74 ± 2	84 ± 2	87 ± 2	64 ± 2	70 ± 4	947 ± 29	882 ± 46
	NP	132 ± 4	124 ± 2	67 ± 3	76 ± 2	85 ± 3	90 ± 2	63 ± 2	69 ± 4	961 ± 25	896 ± 48

Values are mean±SEM. NP, neck pressure; BP, blood pressure; HR, heart rate; RRI, R-R interval; * P<0.05 effect of exercise; † P<0.05 effect of neck collar; ‡ P<0.05 effect of sex.

Table 7.3. Cardiovascular responses at rest and during handgrip exercise without and with neck suction in men and women

		Systolic BP *‡# (mmHg)		**Diastolic BP** *†# (mmHg)		**Mean BP** *†# (mmHg)		**HR** *†# (beats·min^{-1})		**RRI** *†# (ms)	
		Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Rest											
	No NS	119 ± 2	112 ± 2	62 ± 2	69 ± 2	81 ± 2	83 ± 2	58 ± 2	66 ± 4	953 ± 57	1046 ± 30
	NS	121 ± 3	116 ± 4	60 ± 2	67 ± 3	79 ± 2	82 ± 3	57 ± 2	64 ± 4	980 ± 56	1080 ± 33
Exercise											
	No NS	132 ± 5	119 ± 3	70 ± 2	75 ± 3	88 ± 3	88 ± 2	65 ± 2	72 ± 6	872 ± 56	929 ± 27
	NS	129 ± 4	117 ± 3	66 ± 2	71 ± 3	83 ± 2	84 ± 3	60 ± 2	68 ± 5	929 ± 61	1010 ± 31

Values are mean±SEM. NP, neck pressure; NS, neck suction; BP, blood pressure; HR, heart rate; RRI, R-R interval; * P<0.05 effect of exercise; † P<0.05 effect of neck collar; ‡ P<0.05 effect of sex; # P<0.05 interaction effect of condition *x* neck collar.

7.3.3 Power spectral analysis cardiovascular CBR entrainment

CBR entrainment of RRI and mean BP evoked by oscillatory NP at rest (Figure 7.2) was indicated by the distinct coupling of the fluctuations in RRI and mean BP with the positive pressure pulses. To estimate the magnitude of CBR entrainment, a Fast Fourier transformation was performed for each of the measured variables. Figure 7.4 shows the power spectra obtained at rest with and without NP for one representative subject. Oscillatory NP and NS was applied at a frequency of 0.1 Hz, which evoked an increase in LoF power of RRI and mean BP, as indicated by the larger area at the stimulus frequency in the spectra compared to rest without NP. As shown in Figures 7.5&7.6, oscillatory NP and NS produced a significant increase in LoF power of RRI and mean BP at rest and during exercise ($P<0.05$ vs. NoNP or NoNS). This increase occurred similarly in men and women.

To compare the magnitude of the increase in LoF power for each variable in men and women, changes in power spectral density were calculated from Rest NoNP to Rest+NP and from Ex NoNP to Ex+NP, and respectively for NS (Figures 7.7&7.8). Oscillatory NP applied at rest evoked a similar increase in LoF power of RRI in men (+3.2±0.5 Ln ms^2) and women (+2.3±0.5 Ln ms^2). In addition, during exercise with NP, the changes in LoF power of RRI were not different between the groups (+3.0±0.6 and +3.4±0.4 Ln ms^2 in men and women, respectively) or from the changes at rest ($P>0.05$). Similar results were obtained for the changes in LoF power of mean BP. When carotid hypertension was simulated using oscillatory NS, the LoF spectral power of RRI increased similarly at rest (+4.4±0.7 and +4.7±0.4 Ln ms^2 in men and women, respectively) and during exercise (+4.1±0.8 and +5.0±0.5 Ln ms^2 in men and women, respectively) without any difference between groups ($P>0.05$). LoF spectral power of

mean BP was also similarly increased at rest (+0.8±0.3 and +0.9±0.2 Ln $mmHg^2$ in men and women, respectively) and during exercise (+0.6±0.3 and +1.0±0.2 Ln $mmHg^2$ in men and women, respectively).

To verify that the observed fluctuations in RRI and mean BP were indeed closely correlated to the oscillatory changes in carotid sinus transmural pressure, coherence analysis was performed between chamber pressure and RRI or mean BP. The coherence in the LoF range (i.e. at stimulus frequency) at rest and during exercise for RRI and mean BP was above 0.5 in all subjects. This indicates that the observed cardiovascular entrainment was indeed evoked by the oscillatory input of NP and NS.

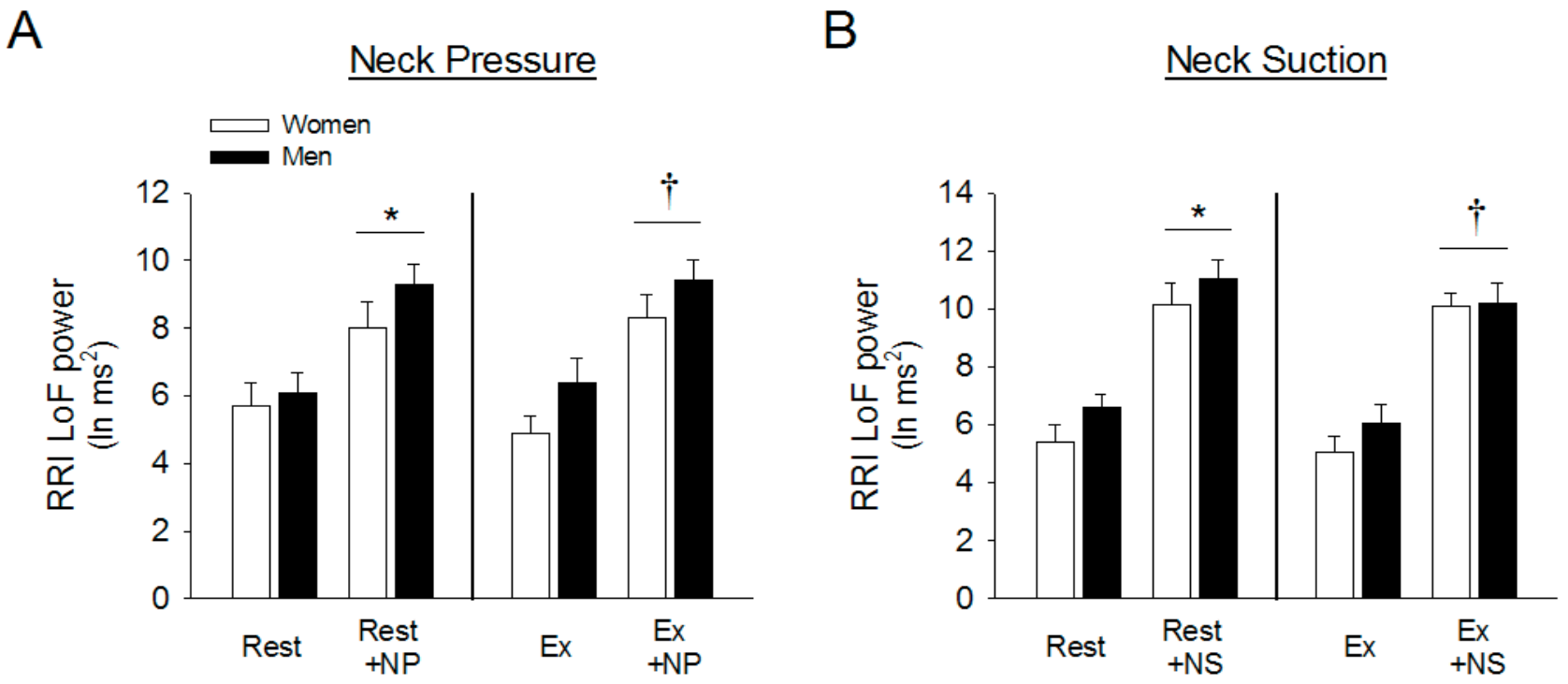

Figure 7.5. Absolute values of low frequency (LoF) power of R-R interval (RRI) without and with oscillatory neck pressure (NP; panel A) and neck suction (NS; panel B) at rest and during exercise (Ex) in women (white bars) and men (black bars). * P<0.05 from Rest; † P<0.05 from Ex.

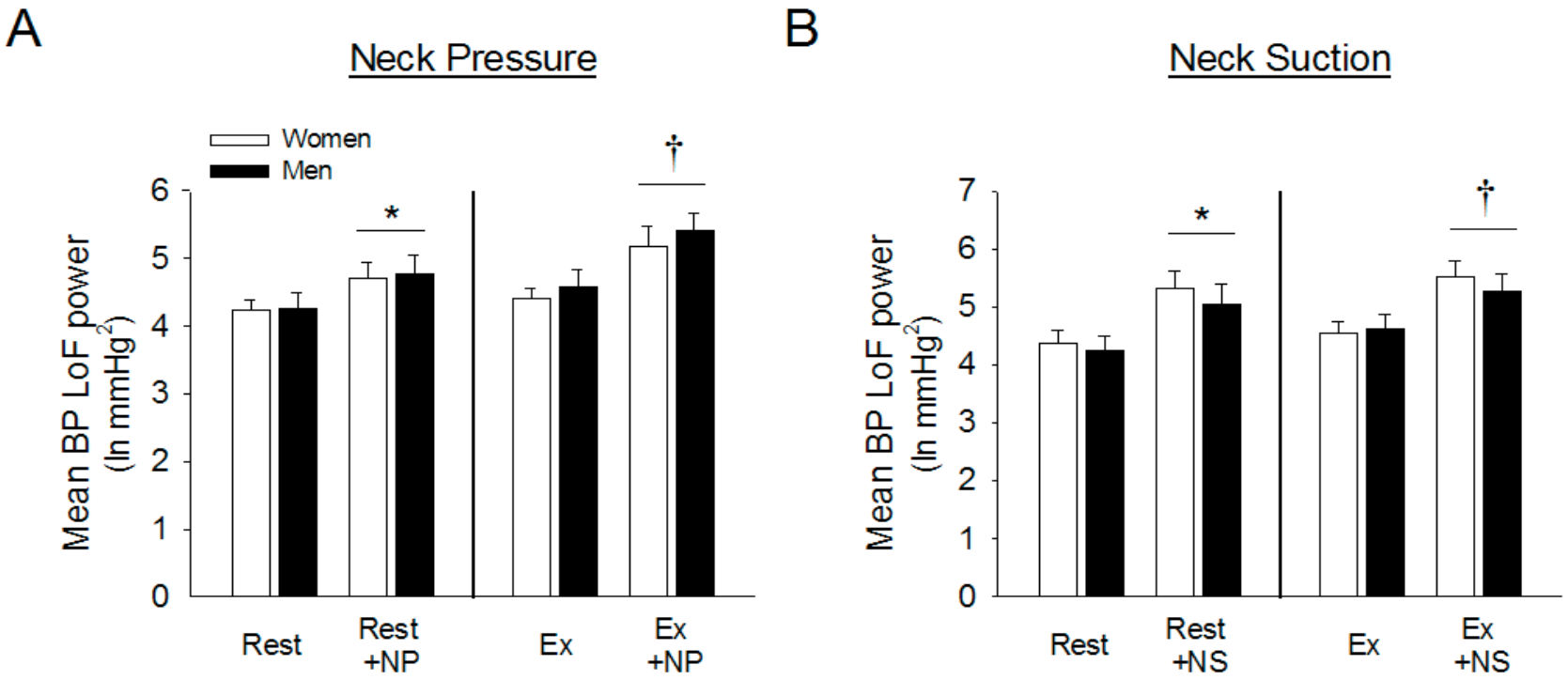

Figure 7.6. Absolute values of low frequency (LoF) power of mean blood pressure (BP) without and with oscillatory neck pressure (NP; panel A) and neck suction (NS; panel B) at rest and during exercise (Ex) in women (white bars) and men (black bars). * P<0.05 from Rest; † P<0.05 from Ex.

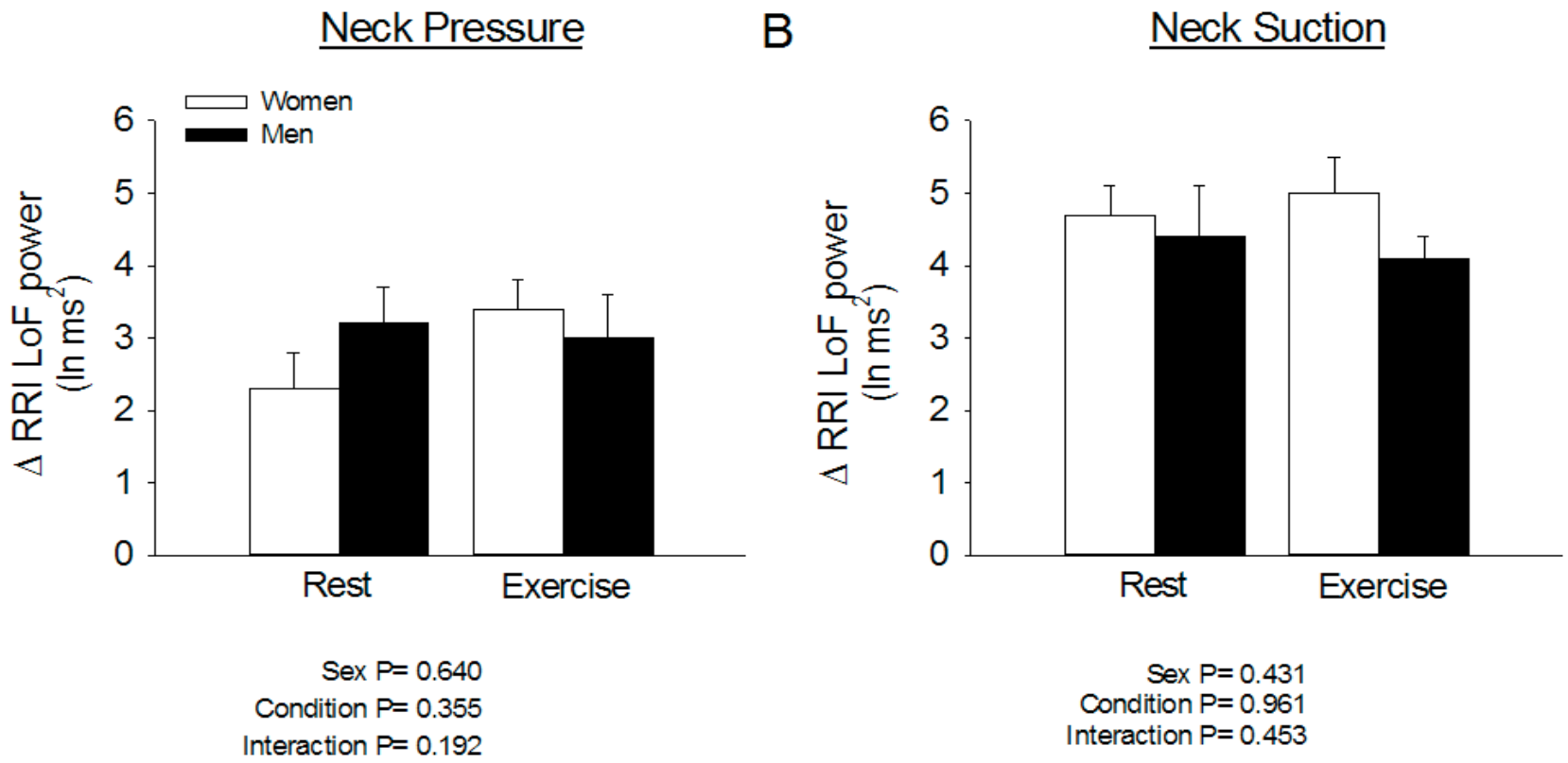

Figure 7.7. Changes in low frequency (LoF) power of R-R interval (RRI) at rest and during handgrip exercise with oscillatory neck pressure (Panel A) and neck suction (Panel B) at 0.1Hz. No significant differences were observed in ΔRRI LoF power between men and women, or rest and exercise.

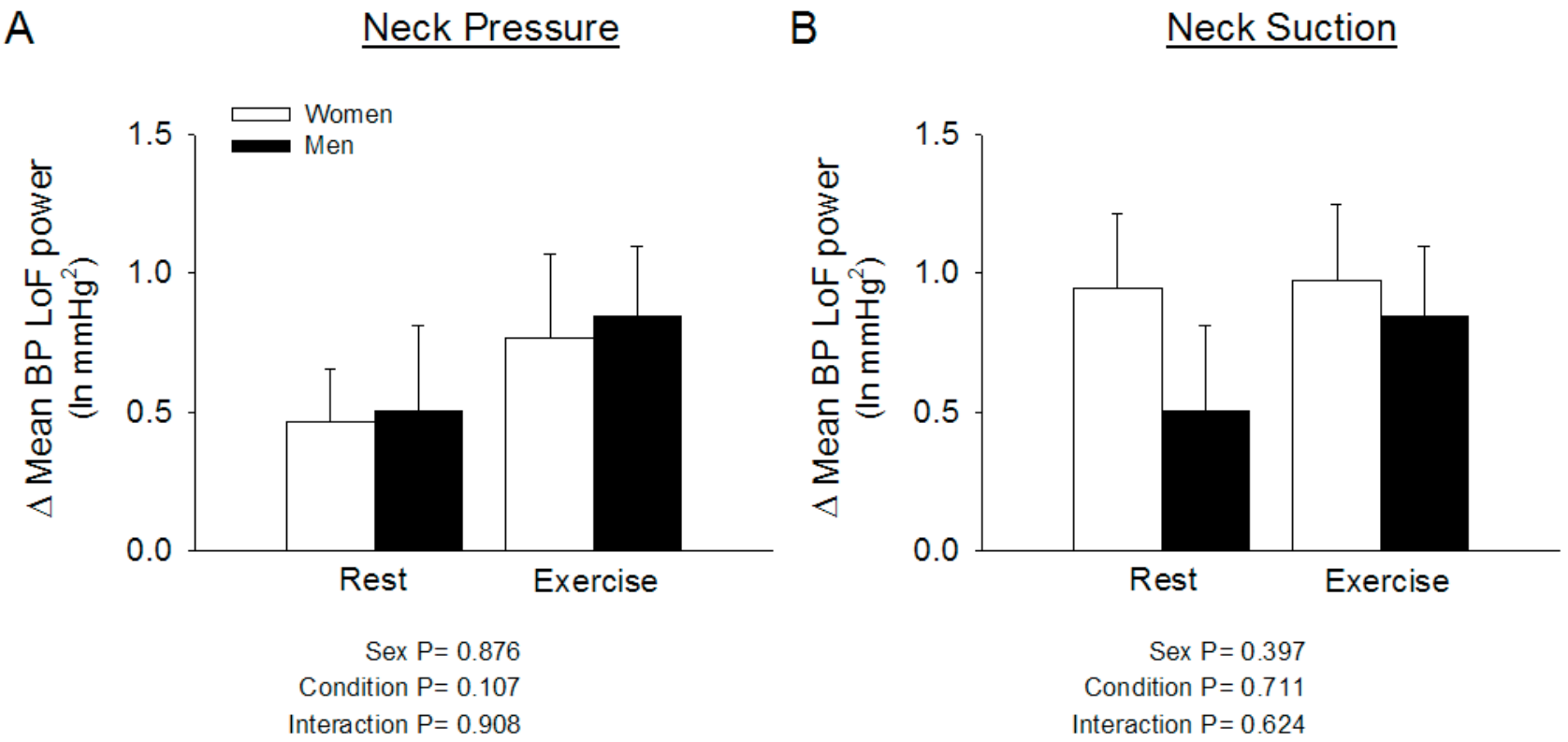

Figure 7.8. Changes in low frequency (LoF) power of mean blood pressure (BP) at rest and during handgrip exercise with oscillatory neck pressure (Panel A) and neck suction (Panel B) at 0.1Hz. No significant differences were observed in Δ Mean BP LoF power between men and women, or rest and exercise.

7.3.4 Power spectral analysis of haemodynamic CBR entrainment

In a subset of three subjects, an assessment of CBR entrainment of tissue oxygenation during oscillatory NP and NS was attempted at rest and during exercise. However, due to technical limitations (e.g. lack of sensitivity of the NIRS system), CBR control of tissue oxygenation could not be assessed (Figure 7.9). Measures of brachial artery blood flow velocity (FBV) were also attempted during oscillatory NP or NS at rest and during exercise in this subset. However, due to technical issues with the Dopplex Doppler ultrasound system, these measurements could not be performed. A detailed analysis of the underlying reasons will be addressed in the discussion section of this thesis chapter.

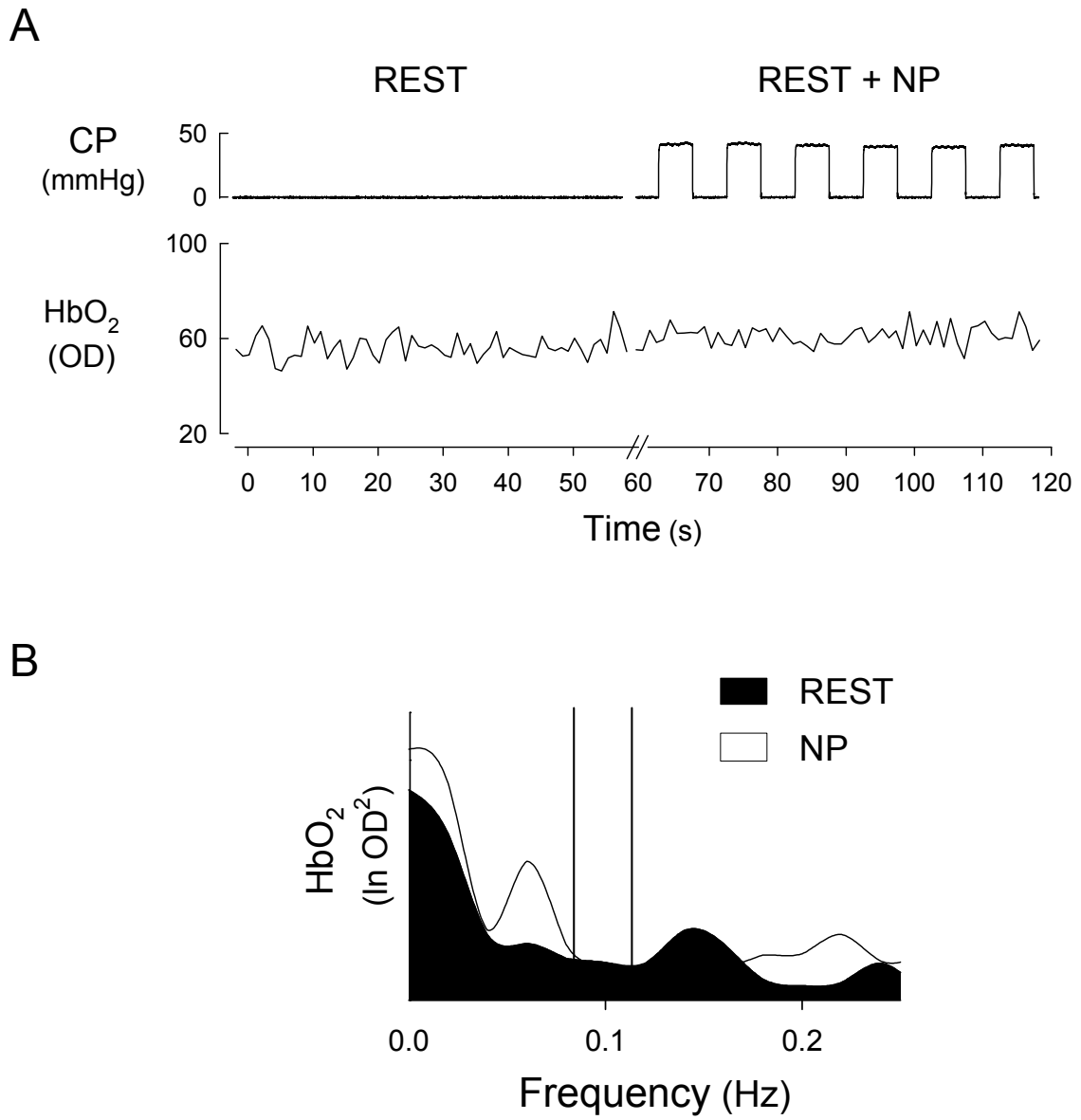

Figure 7.9. An original record of one representative subject with chamber pressure (CP; top) and tissue oxygenation (HbO_2; bottom) is presented in panel A. Panel B shows the power spectral density of tissue oxygenation (HbO_2) at rest without (black area) and with (white area) neck pressure (NP) (panel B). Vertical lines indicate the low frequency range (LoF; 0.085-0.115 Hz) of oscillatory CBR stimulation. Five minutes of oscillatory NP did not evoke carotid baroreflex entrainment of tissue oxygenation as indicated by the unchanged fluctuations of this variable during CP oscillations and the unchanged power spectra at the stimulus frequency.

7.4 Discussion

The purpose of the present study was to determine whether there are sex-differences in the CBR-mediated cardiovascular and haemodynamic control at rest and during exercise. It was observed that oscillatory unloading and loading of the carotid baroreceptors evoked a distinct increase in the spectral power of RRI and BP, both at rest and during low-level rhythmic handgrip exercise. The major novel finding of the present study is that the magnitude of the CBR-mediated changes were not different in men and women using either NP or NS, both at rest and during exercise. However, in contrast to the initial hypothesis CBR entrainment of BP was not attenuated in women compared to men. Collectively, the results of the present study suggest that there are no differences in the dynamic CBR control of RRI and BP between men and women at rest and during low-level rhythmic handgrip exercise.

7.4.1 Sex differences in resting CBR function

In the present study, women and men showed a similar CBR entrainment of RRI in response to both oscillatory NP and NS. Intriguingly, previous reports have suggested that women have greater vagal tone at rest and that cardiac function is reportedly dominated by sympathetic control mechanism in men (Huikuri et al., 1996, Kuo et al., 1999, Ryan et al., 1994). However, this is not a universal finding (Ramaekers et al., 1998, Sinnreich et al., 1998). With regards to potential sex-differences in cBRS, there is no consensus in the literature regarding whether women have a lower, greater or unchanged baroreflex control of the heart (Abdel-Rahman et al., 1994, Beske et al., 2001, Chen and DiCarlo, 1996, Convertino, 1998, Tanaka et al., 2003). The lack of sex-differences in LoF spectral power of RRI in response to oscillatory NP and NS in the present study and the comparable resting spontaneous cBRS in men and women

reported in chapters 5&6 suggest that the sensitivity of the CBR to control for changes in HR is similar in men and women. Importantly, the majority of previous studies (Abdel-Rahman et al., 1994, Beske et al., 2001, Chen and DiCarlo, 1996, Tanaka et al., 2003) used a pharmacological approach (i.e. Modified Oxford technique) to investigate cardiac baroreflex function. Using the Oxford technique, a depression and elevation of BP is elicited by infusion of sodium nitroprusside and noradrenaline, respectively. Cardiac baroreflex responsiveness is then characterised by the slope of the resultant HR changes. An important limitation using this approach derives from the potential side effects of these drugs in that the baroreflex itself buffers the evoked changes in BP resulting in inaccurate measures of cBRS (Diaz and Taylor, 2006). In addition, the cardiac responses to sodium nitroprusside and noradrenaline are commonly combined thus providing an overall estimate of cardiac baroreflex responsiveness. However, since the cardiac responses to a hypotensive stimulus can be impaired while responses to hypertensive stimuli might be maintained, one would miss out on important information by not separately investigating hypo- and hypertensive cardiac alterations (Young et al., 2008). To overcome these limitations, in the present study CBR control of the heart was assessed by separately investigating the cardiac responses to selective carotid hypo- and hypertension (e.g. NP and NS). However, the current results indicate that the dynamic CBR control of the heart seems unaffected by sex in response to both NP and NS.

Previous studies have demonstrated that autonomic control of resting BP is differently controlled in men and women as indicated by a lower resting sympathetic nerve activity (Jones et al., 1999, Ng et al., 1993, Taylor et al., 1998), a lower α-adrenergic support of BP (Convertino, 1998, Schmitt et al., 2010) and a lower baroreflex sensitivity in the control of sympathetic efferent outflow (Shoemaker et al., 2001) in women compared to men. Taken together, this strongly sugegsts a lower

sympathetic control of BP in women compared to men (Christou et al., 2005). However, these previous studies cannot ascertain the overall BP control elicited by the arterial baroreflex. Therefore, the present investigation is the first to directly examine the potential influence of sex on CBR control of BP. Overall, the current results refute differences in the dynamic CBR control of mean BP in men and women at rest, as indicated by the similar increase in LoF spectral power of mean BP in response to simulated oscillatory carotid hypo- and hypertension in men and women.

7.4.2 CBR function during exercise

In the current study, spectral power of mean BP increased significantly during oscillatory NP and NS at rest and during handgrip exercise, supporting the notion of the active regulation of BP via the CBR. Of note, the maintained LoF power of RRI during rhythmic handgrip exercise is seemingly in contrast to the exercise-induced reductions in cBRS observed in dogs (Sala-Mercado et al., 2007) and humans (Iellamo et al., 1998, Ogoh et al., 2005b). However, these observations seem to hold true only for dynamic exercise involving a large muscle mass (e.g. leg cycling, treadmill running).

Previously, Wray and colleagues (2004a) have found oscillatory NP-induced CBR entrainment of BP to be attenuated during leg-kicking exercise. Since these results are in contrast to the preserved CBR control of BP during exercise (Bevegard and Shepherd, 1966), the authors have argued that the operating point of the CBR might have moved towards the threshold of the reflex during exercise (Potts et al., 1993, Raven et al., 2006) thus limiting the possible magnitude of CBR entrainment of BP during exercise. However, a movement of the operating point of the CBR reflex function curve of BP is not a universal finding (Gallagher et al., 2001b, Ogoh et al., 2003b). Interestingly, in the present study, CBR control of mean BP was preserved

during exercise. One possible explanation might be founded on the exercise modality used. Wray and colleagues (2004a) have examined CBR function during very low intensity one-legged kicking (7 Watts), that evoked an increase in arterial BP of approximately +10 mmHg. However, in the present study, BP was raised only by +3±2 mmHg during rhythmic handgrip exercise at 20% MVC (Table 7.2&7.3). While both exercise modalities are of very low intensity, the greater BP response as shown by Wray et al. is possibly due to the greater muscle mass involved in leg kicking versus handgrip (Seals, 1989a). The smaller increase in mean BP may further indicate that in the present study the CBR was if at all reset only to a slightly higher exercising BP. This would mean that CBR entrainment of BP during oscillatory NP was not limited by a potential movement of the operating point towards the threshold.

Application of a hypotensive stimulus to the carotid sinus (NP) has been repeatedly reported to evoke a reflex tachycardia via increases in sympathetic efferent activity while application of hypertensive stimuli (NS) has been shown to elicit reflex bradycardia via reductions in sympathetic nerve activity (Ernsting and Parry, 1957, Fadel et al., 2001, Ichinose et al., 2002, Keller et al., 2004). However, in the present study, HR and RRI remained unchanged during oscillatory NP (Table 7.2). A possible explanation may derive from the methodology employed to simulate carotid sinus hypotension. The majority of previous studies have used single 5 second pulses of NP and have recorded the resultant peak HR or nadir RRI responses (Fadel et al., 2003a, Ogoh et al., 2003a). In contrast, in the present study, 5 second pulses of NP were applied in an oscillatory manner to produce an entrainment of cardiac responses over 5 minutes. The cardiac responses for dynamically applied NP represent an average over these 5 minutes; hence they do not only include the HR peaks, but also the periodically occurring decrease to pre-stimulus levels before the next NP pulse (e.g. 5 seconds on –

5 seconds off). Along those lines, CBR control of the heart has been described to be dominated by cardiac parasympathetic reflex responses (Ernsting and Parry, 1957, Pickering et al., 1972). This was supported by the short latency of the occurrence of peak HR in response to carotid sinus stimulation (Pickering et al., 1972). Taken together, in the present study, the relatively short time it takes for cardiac responses to subside after NP and the fact that absolute HR values represent an average over 5 minutes may explain the lack of tachycardia during oscillatory NP.

Alternatively, the cardiac CBR entrainment might have been influenced by the respiratory frequency and/or the respiratory cycle phase at which each pulse was applied (Eckberg et al., 1980). Indeed, a close relationship has been identified between the CBR and the respiratory control of the heart (e.g. respiratory sinus arrhythmia), as demonstrated by the blunted response on the sinus node when the CBR was stimulated during inspiration, whereas cardiac responses to baroreflex perturbation were augmented during expiration (Eckberg et al., 1980, Eckberg et al., 1985). Importantly, when CBR was stimulated in an oscillatory manner with either NP or NS the breathing frequency (on average 12 breaths per minute; ~0.2 Hz) remained unchanged at rest and during exercise. Therefore, it may be assumed that any effect of the respiratory cycle on the CBR entrainment of RRI was the same during control rest or exercise condition.

7.4.3 CBR function during exercise: influence of sex

Although extensive research has been performed examining sex-differences in the baroreflex control of HR and BP at rest, it is unclear whether sex affects CBR function during exercise. In agreement with previous observations (Gleim et al., 1991), in the current study, systolic BP responses to exercise were markedly lower in women than in men (Table 7.2&7.3). However, since resting systolic BP was also lower in

women, the magnitude of the exercise-induced systolic BP response was similar between groups. In addition, no differences were observed in the dynamic CBR control of BP during exercise between men and women (e.g. similar CBR sensitivity in the control of BP). According to the present data, a sex-specific alteration in CBR function during exercise *per se* is unlikely to explain the attenuated exercise-induced pressor response in women (Ettinger et al., 1996, Fleg et al., 1995, Gleim et al., 1991, Jarvis et al., 2011).

It is well established that the CBR control of BP and HR during exercise largely depends on the actions and interactions of neural signals arising from higher brain areas, known as central command, and input from metabolically and mechanically sensitive afferents in the exercising skeletal muscle. Of note, the results from the previous two studies of this thesis (Chapter 5&6) indicate firstly that the exercise-induced decreases in cBRS are similar in men and women. Secondly, the muscle metaboreflex-mediated reductions in cardiac baroreflex responsiveness during dynamic exercise are not different in men and women. In line with this, Laprad et al. (1999) also found no sex-differences in the strength and mechanisms of the muscle metaboreflex during exercise in dogs running on a treadmill. In contrast, activation of metabolically sensitive afferent during PEI after static handgrip exercise has been shown to evoke attenuated BP responses in women compared to men (Ettinger et al., 1996, Jarvis et al., 2011). This suggests that muscle metaboreflex activation is lower in women than men. The discrepancies in these studies are possibly due to the mode of muscle metaboreflex activation (during vs. post exercise) and/or muscle mass involvement (large vs. small) (see Sections 4.4 and 6.4.1 for more details). However, it remains unclear, how potential sex-differences in the activation of central command might influence CBR control of HR and BP during exercise.

7.4.4 Methodological considerations: haemodynamic measures

The lack of CBR entrainment measured by the spectral power of tissue oxygenation during oscillatory NP and NS can be explained by the large signal to noise ratio of the OxiTS near-infrared spectroscopy system. Reduction of the sampling frequency from 100 to 10 or 1 Hz did not improve signal quality. In addition, the properties of the sensors (i.e. very rigid, heavy) added inaccuracy and resulted often in temporary signal loss, especially during exercise. Taken together, although CBR entrainment of tissue oxygenation was not observed in the current study, this is suggested to be caused by equipment limitations and technical difficulties during oscillatory CBR stimulation and exercise-induced movement artefacts.

In order to gain a true spectral power estimate of FBV during the application of oscillatory NP and NS, a continuous data segment of minimum 5 minutes was needed. The Dopplex Doppler ultrasound system allows continuous blood flow velocity measurements. However, one limitation of this portable system is that it operates with a pen-probe, which does not provide the investigator with any visual feedback about the probe position relative to the vessel. Particularly during the application of oscillatory NP and NS, the signal quality suffered, due to the small but disruptive upper body movement, which accompanied the use of the neck collar, even though a probe holder was utilized. The described difficulties to obtain sustained blood velocity measurements are likely to have caused the lack of CBR entrainment of FBV at rest and during handgrip exercise as described previously (Wray et al., 2004a).

7.4.5 Limitations

The application of oscillatory NP and NS and the subsequent calculation of the spectral power of the end-organ responses (e.g. RRI and mean BP) using Fast Fourier

transformation only allowed an estimate of the CBR-sensitivity. Conversely, with the use of static pulses of NP and NS at different stimulus intensities the full CBR stimulus-response curve could be modelled (Kent et al., 1972). This would provide information about CBR response range, reflex threshold and saturation point and whether any of these parameters might be different in men and women. Therefore, further research is warranted to fully elucidate sex-differences in the CBR control of HR and BP. In addition, only one level of NP (+40 Torr) and NS (-60 Torr) was used in the present study. Therefore, the possibility of CBR stimulus intensity-dependent entrainment of RRI and mean BP cannot be excluded and warrants further investigation.

In the current study, women were scheduled in the EF phase of the ovarian cycle (day; 4±1) in order to minimize the potential effects of fluctuating female reproductive hormones (e.g. estrogen and progesterone). However, no control of contraceptive use was performed. Thus, women subjects presented a very inhomogeneous sample, because 6 out of 10 women were using various forms of hormonal contraception (e.g. contraceptive pill, implant or hormonal patch), while only four women were not using any form of contraception. This might be of importance since Minson et al. (2000a, 2000b) have reported that resting cardiac as well as sympathetic baroreflex sensitivity were differentially affected during the ovarian cycle (e.g. EF vs. luteal phases) in women with and without oral contraception. However, the authors have provided no direct comparison of baroreflex function in women without or with oral contraception. In addition, it remains unclear whether these influences of exogenous hormone application affect the CBR control of HR and BP during exercise.

7.4.6 Alternative approaches

It has been previously suggested that female reproductive hormones (e.g. estrogen, progesterone) may play an important role in the autonomic cardiovascular control at rest and during exercise (Charkoudian, 2001, Hayes et al., 2002, Hunt et al., 2001, Schmitt and Kaufman, 2003a, Schmitt and Kaufman, 2003b, Tanaka et al., 2003) and that sex-differences may be related to the prevailing concentrations of female hormones (Dart et al., 2002). The potential of estrogen to enhance resting cBRS has been reported in animals (el-Mas and Abdel-Rahman, 1998) and humans (Tanaka et al., 2003), although this is not a universal finding (Minson et al., 2000a). However, it remains unclear how estrogen affects CBR responsiveness in control of HR and BP. In that, preliminary results from a recent study have indicated a maintained CBR control of HR throughout the ovarian cycle (Kim et al., 2010). In addition, Kim et al. (2010) have reported that concomitant elevations in both estrogen and progesterone maintained CBR-mediated BP responses to hypertension (NP), but evoked enhanced BP decreases during simulated carotid hypotension (NS). This highlights a contribution of female sex hormones to the cardiovascular control via the CBR.

Women have been previously shown to exhibit attenuated exercise-induced increases in MSNA during the LF phase of the ovarian cycle, when estrogen concentrations are elevated, compared to the EF phase, when estrogen concentrations are low (Ettinger et al., 1998). Since the exercise-induced increases in BP seem to be unaffected by the ovarian cycle phase an attenuated CBR control of MSNA during the high estrogen phase might explain these differential responses. Moreover, in postmenopausal women progesterone treatment has been reported to lower HR variability, which is an indicator of cardiac parasympathetic activity (Christ et al., 2002). This suggests that progesterone has vagal inhibitory effects. However, the role of

estrogen and progesterone on the CBR control of BP during exercise remains unclear. Further investigations are needed to define the possible actions and interactions of female reproductive hormones and cardiovascular control mechanisms during exercise in humans.

7.5 Conclusion

In summary, the use of oscillatory NP and NS has been shown to successfully evoke CBR entrainment of RRI and mean BP by virtue of an increase in LoF spectral power of RRI and mean BP both at rest and during rhythmic handgrip exercise. The evaluation of the magnitude of CBR entrainment of RRI and mean BP in men and women at rest and during exercise has revealed no differences between these groups. The results of the present study suggest that a differential CBR control of HR and BP (e.g. CBR sensitivity) during exercise *per se* is unlikely to be the underlying factor of the attenuated pressor response during exercise in women.

CHAPTER 8: DIFFERENTIAL RESPONSES OF THE BRACHIAL AND CEREBRAL CIRCULATION TO SYMAPTHETIC ACTIVATION DURING EXERCISE IN MEN AND WOMEN

8.1 Introduction

8.1.1 Peripheral blood flow during exercise

Previous studies have reported sex-differences in the cardiovascular response to exercise indicating that healthy premenopausal women exhibit attenuated BP responses during physical activity compared to age matched men (Ettinger et al., 1996, Fleg et al., 1995). One possible mechanism, which could account for the lower pressor responses to exercise in women, might derive from differences in the translation of efferent neural activity (e.g. increased sympathetic nerve activity) into subsequent changes in vascular tone (e.g. sympathetic vasoconstriction). Sex-differences in the neurovascular coupling between sympathetic nerve discharge, noradrenaline release and/or the responsiveness of the vascular smooth muscle to the consequent α-adrenergic stimulation to evoke sympathetic vasoconstrictor responses might explain the attenuated BP response to exercise in women and/or the lower autonomic control of BP observed in women (Christou et al., 2005, Schmitt et al., 2010).

Previous studies have observed blunted α-adrenergic vasoconstriction in response to either exogenous elevation of catecholamines (e.g. infusion of the α_1-adrenergic agonist norepinephrine) or sympathoexcitation elicited by increases in endogenous catecholamines using the CPT in young women compared to men (Hogarth et al., 2007, Kneale et al., 1997). In addition, women have been shown to have lower autonomic nervous system control of BP at rest (Christou et al., 2005). The clearly positive linear relationship between MSNA and TPR observed in men was lacking in women (Hart et al., 2009, Hogarth et al., 2007). In summary, these data demonstrate that sympathetically-mediated vasoconstriction at rest is attenuated in women relative to

men. But it remains unclear whether these observations at rest can be translated to exercise conditions.

The sympathetic nervous system plays an important role mediating the exercise-induced cardiovascular adjustments. Sympathetic activation facilitates the rise in CO and evokes vasoconstriction in regions, such as renal and splanchnic vascular beds. This occurs in order to redistribute blood flow to the active skeletal muscles to meet their metabolic demand (Rowell, 1993a). Importantly, this sympathetically-mediated increase in vascular tone is blunted in the exercising skeletal muscle. This attenuated vasoconstrictor response has been attributed at least partly to the presence of locally produced metabolites acting to attenuate α-adrenergic receptor activation, a phenomenon which has been firstly described by Remensnyder and colleagues (1962) and is termed 'functional sympatholysis' (Hansen et al., 1996, Keller et al., 2004, Remensnyder et al., 1962, Schmitt et al., 2010, Tschakovsky et al., 2002, Watanabe et al., 2007). Blunting the sympathetic vasoconstriction in the exercising skeletal muscle serves to optimize nutritional supply by allowing skeletal muscle blood flow to rise despite increased sympathetic nerve activity. However, these previous studies have been conducted predominantly in males and were not designed to examine sex-differences. Therefore, the influence of sex on the sympathetically-mediated vasoconstrictor responses during exercise remains unclear.

The CPT is a well-established laboratory method to increase sympathetic nervous activity (Hines and Brown, 1933). It has been shown to evoke marked elevations in BP, HR, MSNA, catecholamine release and increases in peripheral vascular resistance (Cui et al., 2002, Dishman et al., 2003, Jacob et al., 2000, Thijssen et al., 2006, Victor et al., 1987a). Whether men and women respond differently to sympathetic activation via the CPT has been investigated reporting mainly similar

cardiovascular and hemodynamic changes in both sexes (Dishman et al., 2003, Ettinger et al., 1996, Jarvis et al., 2011, Jones et al., 1996). In contrast, a recent study found a greater CPT-mediated vasoconstrictor response in women (+88%) compared to men (+46%), while MSNA increases were comparable (Hogarth et al., 2007). However, in the study by Hogarth et al. smokers were included and one third of the female study population was postmenopausal. This is of importance as it has been suggested that the CPT response might be influenced by female hormone concentrations (Tersman et al., 1991).

8.1.2 Cerebral blood flow during exercise

As in the active skeletal muscles, increases in cerebral neuronal activity appear to be coupled with increases in cerebral perfusion during exercise (Secher et al., 2008). However, the role of the sympathetic nervous system in the regulation of cerebral perfusion both at rest and during exercise in humans remains controversial and incompletely understood (Ainslie and Tzeng, 2010, Ogoh and Ainslie, 2009). The cerebral vasculature is supplied by α-adrenergic nerves (Edvinsson et al., 1976, Lowe and Gilboe, 1971) which mainly originate in the superior cervical ganglia (Nielsen and Owman, 1967). They provide the anatomical apparatus for the sympathetic modulation of cerebral perfusion. Indeed, animal studies have suggested that direct stimulation of sympathetic nerves leads to vasoconstriction of cerebral blood vessels (Auer et al., 1983, Wagerle et al., 1983). There is selected evidence from human studies that systemic administration of α-adrenergic agonists (e.g. noradrenaline) (Brassard et al., 2010, Lucas et al., 2010, Olesen, 1972) and sympatho-excitatory manoeuvres such as the CPT (Micieli et al., 1994, Roatta et al., 1998), whole-body heating (Low et al., 2009) and cardiopulmonary baroreceptor unloading with lower body negative pressure

(Wilson et al., 2005) can evoke increases in cerebrovascular resistance (vasoconstriction) and/or reductions in cerebral perfusion.

Given the exercise-induced increases in cerebral neuronal activity it is possible that the sympathetic modulation of resting cerebrovascular tone may become blunted during muscular contractions, as it has been previously described for the skeletal muscle vasculature. In this regard, Gross et al. (1980) demonstrated in dogs running on a treadmill that the constrictor responses to arterial hypocapnia were blunted in the areas of cerebral vasculature associated with sensory-motor control. However, care should be taken when translating such observations to humans as substantial species differences have been shown in the cerebral vasomotor response to adrenergic nervous activation (Levine and Zhang, 2008). More recently, Brassard et al. (2010) have observed that during leg cycling, frontal lobe oxygenation and cerebral blood flow velocity responses to bolus injections of the α_1-adrenergic receptor agonist noradrenaline were attenuated. While suggestive of a metabolic modulation of adrenergic sensitivity, it is unclear whether these results are indicative of a direct effect of noradrenaline on the cerebral vasculature, or a secondary effect of an alteration in the systemic hemodynamic response (e.g. transient hypertension, reduced sympathetic nerve activity and cardiac output) (Ainslie and Tzeng, 2010). This is particularly relevant as noradrenaline does not appear to cross the blood brain barrier (Hardebo and Owman, 1980, Olesen, 1972). As such it is unknown whether exercise modulates the cerebrovascular responses to sympatho-excitatory manoeuvres which may elicit α-adrenergic receptor activation due to local release of endogenous catecholamines.

It has been previously suggested that cerebral perfusion might be influenced by sex-differences (Misteli et al., 2011). The majority of past studies have reported that women have a greater resting cerebral blood flow than men (Bakker et al., 2004, Gur et

al., 1982, Rodriguez et al., 1988). However, it is unclear whether these differences at rest are persistent during exercise. Although sympathetic vasoconstriction has been shown to be blunted in the peripheral circulation, it remains unknown whether these can be equally translated to the cerebral vasculature and cerebral reactivity to sympathetic activation during exercise in men and women.

Given the above, the purpose of the present study was to examine (i) whether the sympathetic vasoconstrictor responses in the peripheral circulation are different in men and women at rest and during exercise; and (ii) whether exercise modulates the cerebrovascular responses to sympatho-excitatory manoeuvres similarly in men and women. To investigate this, the cardiovascular and haemodynamic responses to an 'endogenous' sympathetic stimulus (i.e. CPT) were assessed at rest and during three levels of rhythmic handgrip exercise in young healthy men and women. Given the blunted α-adrenergic responsiveness in the peripheral circulation and the greater resting cerebral perfusion in women in relation to men, women were expected to exhibit an attenuated sympathetic vasoconstrictor response in the peripheral and cerebral vasculature during exercise (i.e. greater functional sympatholysis).

8.2 Methods

8.2.1 Subjects

Nine healthy female and ten healthy male subjects from the University of Birmingham volunteered to participate in the study (Table 8.1). In order to reduce the potential confounding effects of fluctuations in female reproductive hormones, women were tested during the EF phase of the ovarian cycle. Six out of the nine women were taking oral contraceptives (e.g. Microgynon or Yasmin).

Table 8.1. Subject characteristics

	Men (n=10)	Women (n=9)
Age (years)	23 ± 2	20 ± 0.4
Weight (kg)	76 ± 2	61 ± 3 *
Height (cm)	181 ± 2	163 ± 2 *
BMI ($kg \cdot m^{-2}$)	23 ± 1	23 ± 1
MVC (kg)	51 ± 3	29 ± 2 *
10% MVC (kg)	5 ± 0.3	3 ± 0.2 *
25% MVC (kg)	13 ± 0.7	7 ± 0.4 *
40% MVC (kg)	20 ± 1.1	12 ± 0.7 *
Day of OC		3.3 ±0.3
Oral contraceptives		6/9

Values are mean±SEM. BMI, body mass index; MVC, maximum voluntary contraction; OC, ovarian cycle. * $P<0.05$ different from men.

8.2.2 Experimental measurements

Figure 8.1 shows the laboratory set-up used in the present study. Beat-to-beat HR, BP and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) were measured (see Sections 3.2.1&3.2.5 for details). Mean BP, SV and CO were calculated (see Section 3.4.4 for details).

FBV from the brachial artery of the right arm was obtained by Doppler ultrasound and FBF calculated and corrected for lean forearm mass (see Section 3.2.2 for details). Regional cerebral perfusion was assessed by measurements of left middle cerebral artery (MCA) mean blood flow velocity (V_{mean}) and the cerebrovascular conductance index (CVCi) calculated (see Section 3.2.4 for details). The MCA was found at an average depth of 46-63 mm. Cerebrovascular parameters were obtained in 8 male and 7 female subjects from the study sample.

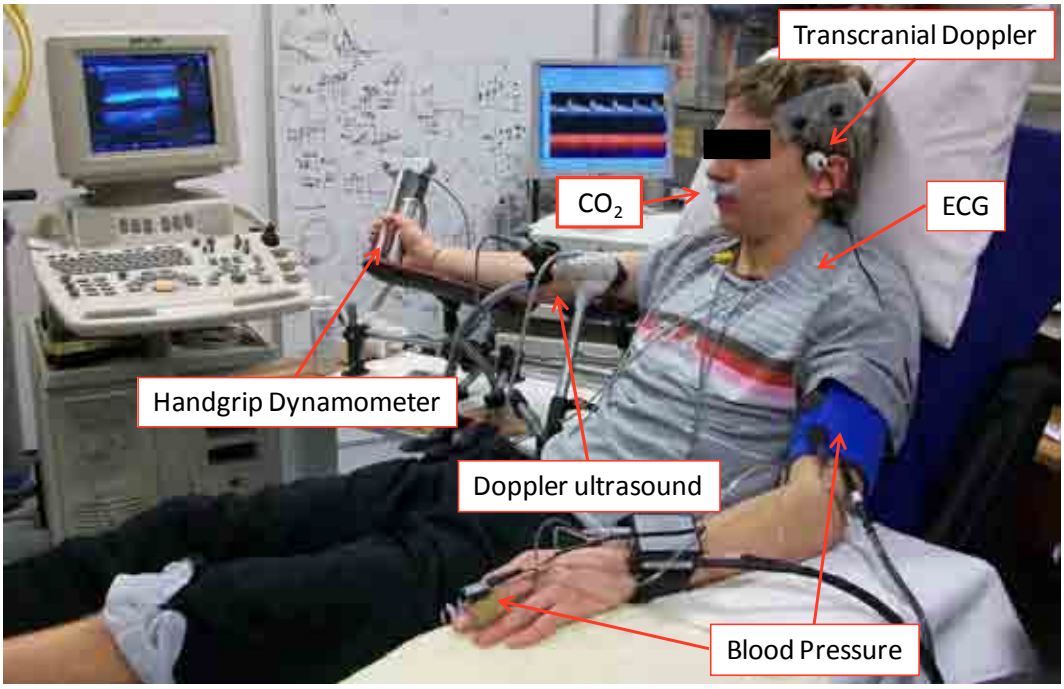

Figure 8.1. Laboratory set-up. ECG, electrocardiogram; CO_2, carbon dioxide.

8.2.3 Experimental procedures

Rhythmic Handgrip Exercise: Handgrip was performed at a duty cycle of 1 second contraction – 2 seconds relaxation (20 contractions per minute) to allow FBV and diameter measurements during exercise (Dinenno and Joyner, 2003). The exercise workloads corresponded to 10% (mild), 25% (low) and 40% (moderate) of MVC in order to minimise the potential for exercise-induced sympatho-excitation (Victor and Seals, 1989). Each exercise bout lasted for 7 minutes. The trials were separated by 15 minutes to avoid fatigue and to ensure reestablishment of baseline HR and BP before commencing the next trial. The exercise trials were conducted in a counterbalanced order. RPE were obtained for each exercise trial using the standard 6–20 Borg scale (Borg, 1998).

Cold Pressor Test: The CPT was employed to stimulate the sympathetic nervous system (Hines and Brown, 1933, Victor et al., 1987a) (see Section 3.3.5 for details). It was performed twice at rest. The first CPT tended to evoke exaggerated increases in BP

compared to subsequent tests and was therefore excluded from the statistical analysis (Schrage et al., 2005). As such, the cardiovascular and hemodynamic changes elicited by the second CPT were utilized for comparison with the subsequent CPT during exercise. A rating of perceived pain was determined for each CPT (Borg, 1998).

8.2.4 Experimental protocol

Subjects were seated in a semi-recumbent position on a medical examination table with a custom made handgrip dynamometer held in the right hand. The arm was supported on an adjustable bedside table. Following instrumentation and assessment of MVC (see Section 3.3.1 for details) subjects rested for 15 minutes. Then two CPT were applied at rest, each after 5 minutes of baseline determination of cardiovascular and haemodynamic variables (Figure 8.2). After a 15 minutes rest period the first handgrip exercise trial was performed. A CPT was applied during the last 2 minutes of each handgrip exercise bout (5^{th}-7^{th} minute). Brachial artery diameter measurements were obtained at the 4^{th} minute of both rest and handgrip exercise, and at the 1^{st} minute of CPT. FBV was averaged over the 30 s spanning the diameter measurements.

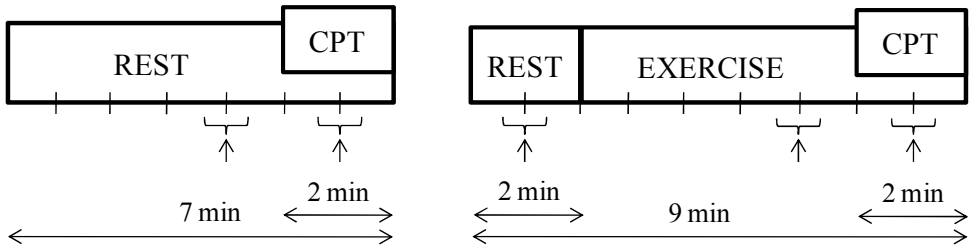

Figure 8.2. Schematic representation of experimental protocols used at rest and during three levels of rhythmic handgrip exercise (10, 25 and 40% of maximum voluntary contraction). Arrows indicate the time of brachial artery diameter measurements and curly brackets indicate the 30s used for cardiovascular and cerebrovascular measurements. CPT, cold pressor test.

8.2.5 Data analysis

To assess the magnitude of CPT mediated vasoconstriction the percentage change in FVC was calculated (Schrage et al., 2005):

$$\%\Delta FVC = (FVC_{CPT} - FVC_{SS}) / FVC_{SS} \times 100,$$

Here, FVC_{CPT} represents FVC during CPT and FVC_{SS} represents steady-state prior to CPT (i.e. either at rest or exercise). Similarly, the percentage change in CVCi was calculated:

$$\%\Delta CVCi = (CVCi_{CPT} - CVCi_{SS}) / CVCi_{SS} \times 100.$$

Cardiovascular data were statistically analysed using three-way repeated measures ANOVA. The main within-subjects factors were *trial* (rest, 10%, 25% and 40% MVC) and *condition* (steady-state rest or exercise vs. CPT), while *sex* (men vs. women) was the between-subject factor. Two-way repeated-measures ANOVA analysed sex-differences between rest and each handgrip workload (e.g. magnitude of CPT-mediated vasoconstriction).

8.3 Results

8.3.1 Peripheral responses at rest and during exercise with and without CPT

Table 8.2 and 8.3 show the cardiovascular and hemodynamic variables measured at rest and during each exercise workload with and without CPT. Rhythmic handgrip exercise at 10, 25 and 40% MVC evoked only small increases in HR and profound elevations in FBF and FVC (Table and Figure 8.3; $P<0.05$), closely related to the exercise intensity. Mean BP remained unchanged (Table 8.2). There were no differences in mean BP, HR, FBF and FVC at rest and during exercise between men and women. RPE increased with increasing handgrip exercise intensity similarly in men (8±1, 9±1

and 11±1 au for 10, 25 and 40% MVC trials, respectively) and women (9±1, 10±0.4 and 12±1 au for 10, 25 and 40% MVC trials, respectively).

Sympathetic activation elicited by the CPT at rest evoked significant increases in mean BP (+9±2 mmHg in men and +8±2 mmHg and women; $P<0.05$) and HR (+2±1 beats·min^{-1} in men and +7±2 beats·min^{-1} and women; $P<0.05$; Table 8.2). The CPT-induced increases in mean BP and HR at each workload of handgrip exercise were similar to the responses at rest without differences between men and women. At rest, the CPT evoked a similar reduction in FVC in men (−44±6% ΔFVC) and women (−32±5% ΔFVC). During rhythmic handgrip exercise, sympathetic vasoconstrictor responses were attenuated in both men (−29±8, −23±9 and −20±5% for 10, 25 and 40% MVC, respectively) and women (−13±8 and −20±5% for 25 and 40% MVC trials, respectively; Table 8.3; Figure 8.4). However, only the decreases during 25 and 40% MVC trials were significantly different from rest. No sex-differences were found in the vasoconstrictor responses at any measuring time point.

8.3.2 Cerebrovascular responses at rest and during exercise with and without CPT

Rhythmic handgrip exercise at 10 and 25% MVC evoked an increase in MCA V_{mean} above resting values in men (Table 8.4; Figure 8.5; $P<0.05$). The rise in MCA V_{mean} during the 40% MVC trial did not reach statistical difference. In women, MCA V_{mean} remained at resting levels during each exercise workload. CVCi was unchanged from rest during all exercise trials, in men and women (Table 8.4; Figure 8.5). $P_{ET}CO_2$ and CO were lower in women compared to men. $P_{ET}CO_2$ decreased slightly during exercise (Table 8.4).

At rest, the CPT-induced sympathetic activation evoked a small reduction in CVCi that was similar in men and women (-5±3% in men and -6±3% ΔCVCi in

women; Table 8.4; Figure. 8.6). The decreases in CVCi that occurred with the CPT remained unchanged during each exercise workload. Sympathetic activation induced by the CPT evoked a slight but significant elevation MCA V_{mean} and CO in both groups (Table 8.4; $P<0.05$). However, no sex-differences were observed in the cerebrovascular responses to the CPT.

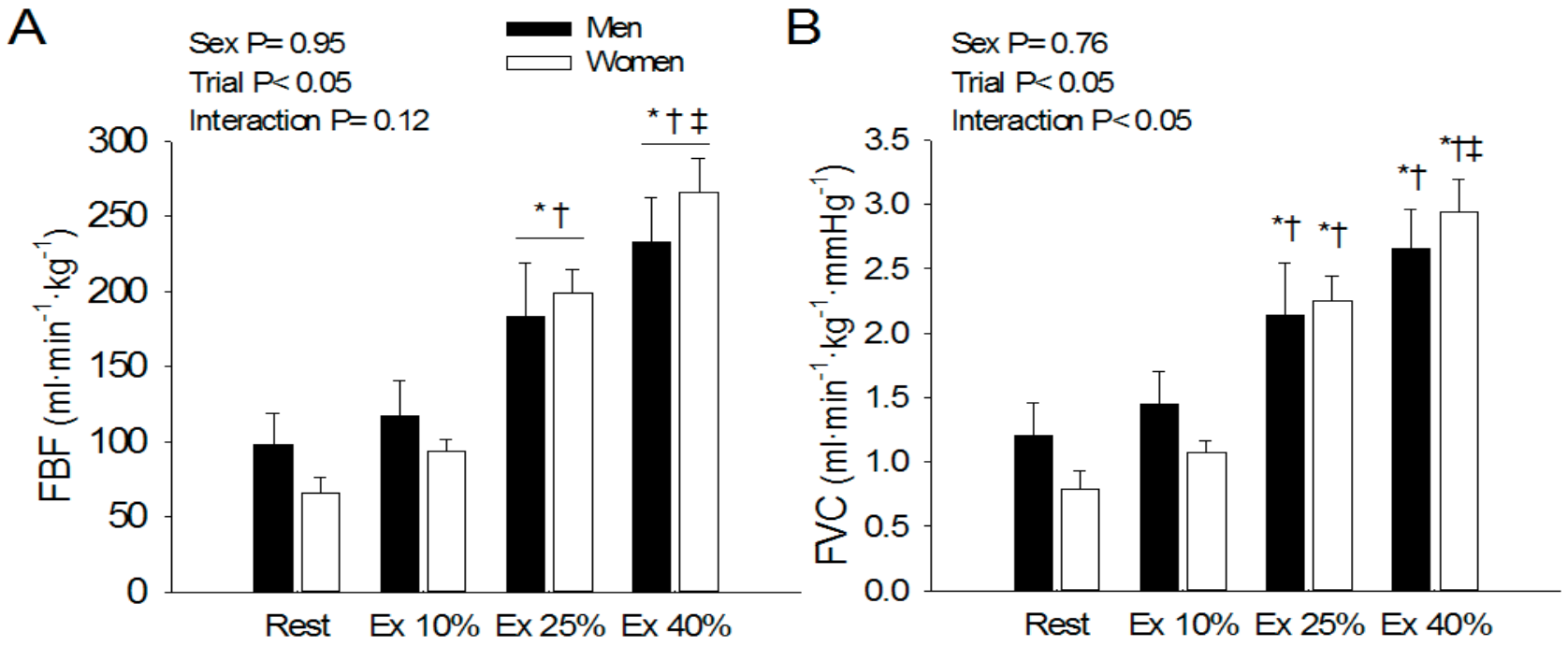

Figure 8.3. Forearm blood flow (FBF; Panel A), forearm vascular conductance (FVC; Panel B) in men (black bars) and women (white bars) at rest and during rhythmic handgrip exercise at 10, 25 and 40% maximum voluntary contraction. * P<0.05 vs. Rest, † P<0.05 vs. Ex 15%, ‡ P<0.05 vs. Ex 25%.

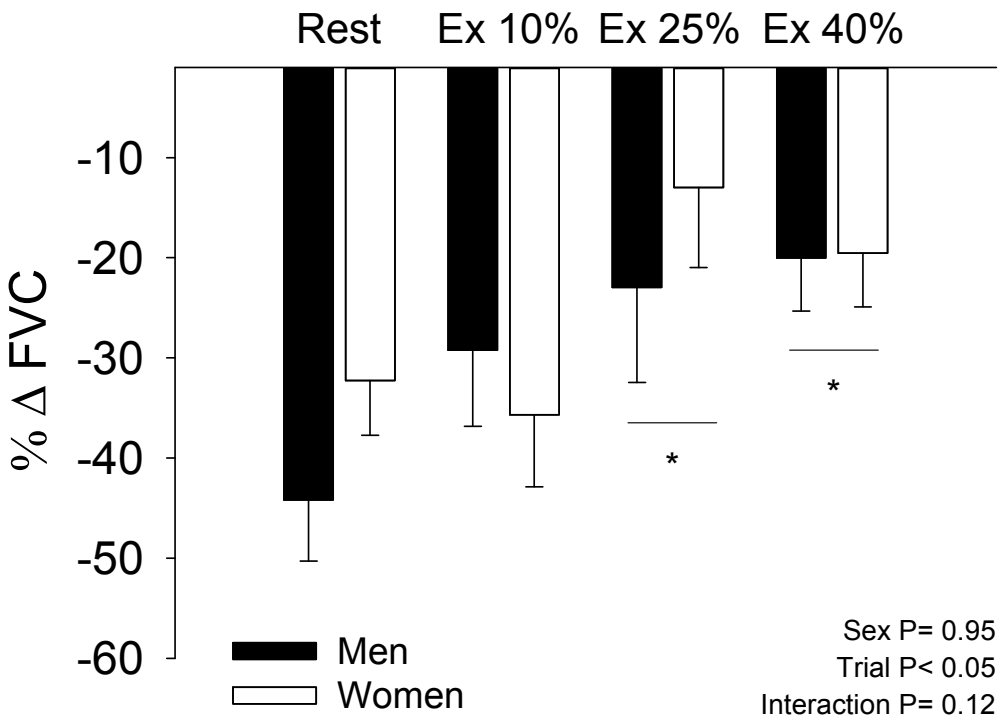

Figure 8.4. Vasoconstrictor responses to cold pressor test in men (black bars) and women (white bars) at rest and during three levels of rhythmic exercise (10, 25 and 40% maximum voluntary contraction) expressed as the percentage change in forearm vascular conductance (FVC). * P<0.05 vs. Rest.

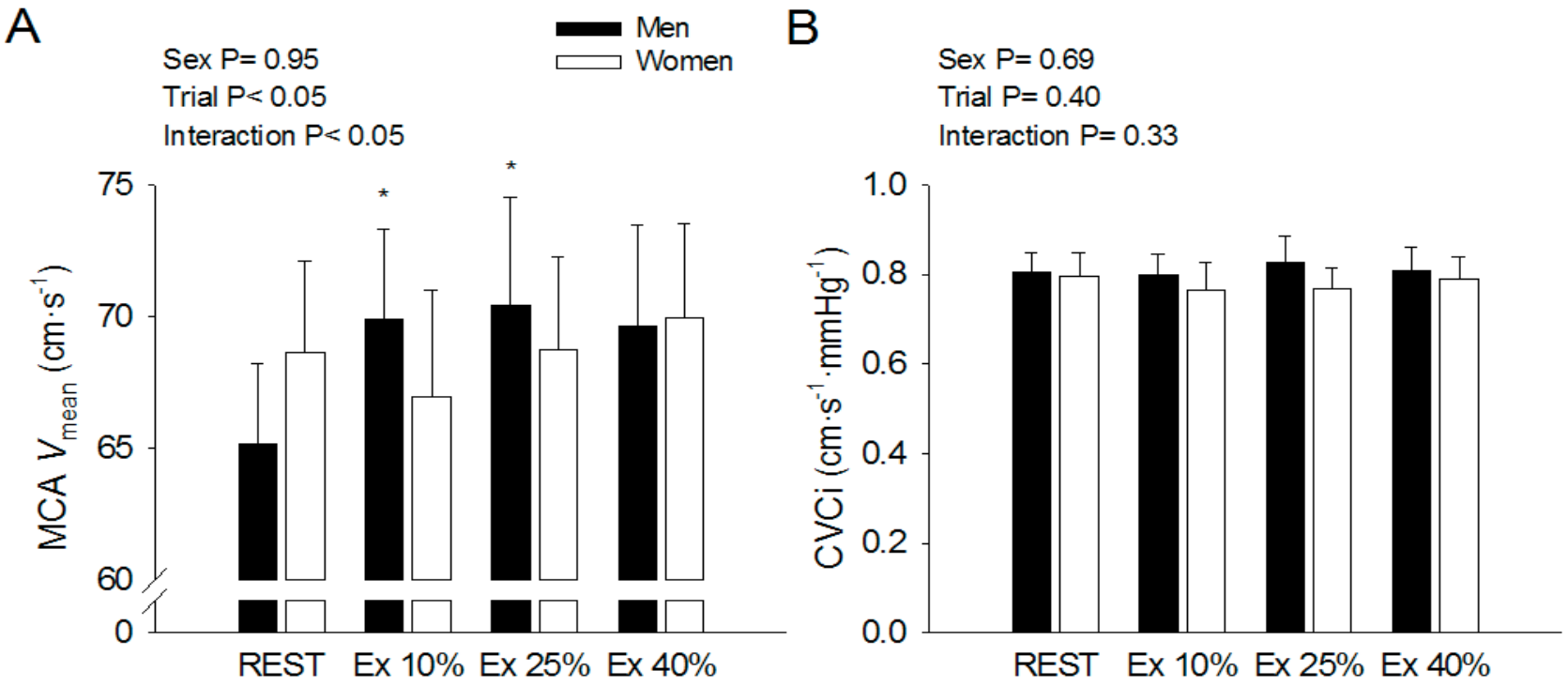

Figure 8.5. Middle cerebral artery mean blood flow velocity (MCA V_{mean}; Panel A) and cerebrovascular conductance index (CVCi; Panel B) in men (black bars) and women (white bars) at rest and during rhythmic handgrip exercise at 10, 25 and 40% maximum voluntary contraction. * P<0.05 vs. Rest.

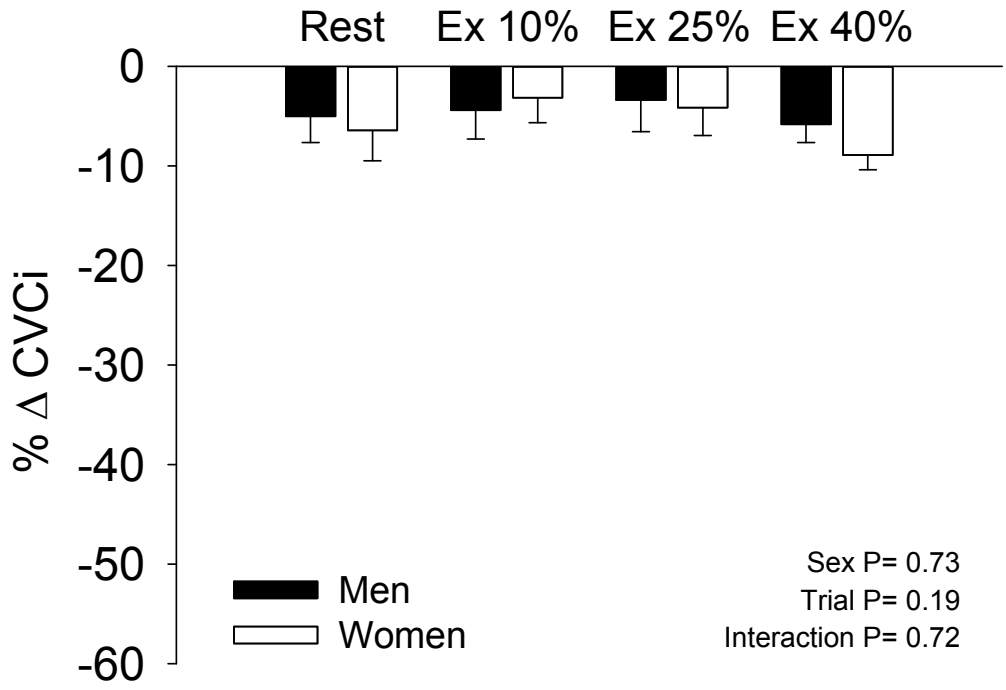

Figure 8.6. Vasoconstrictor responses to cold pressor test in men (black bars) and women (white bars) at rest and during three levels of rhythmic exercise (10, 25 and 40% maximum voluntary contraction) expressed as the percentage change in cerebrovascular conductance (CVCi).

Table 8.2. Cardiovascular parameters at rest and during rhythmic handgrip exercise with and without cold pressor test (men n=10, women n=9).

		Rest		Ex at 10% MVC		Ex at 25% MVC		Ex at 40% MVC	
		Without CPT	CPT	Without CPT	CPT	Without CPT	CPT	Without CPT	CPT
Mean BP (mmHg) *†									
	Men	82 ± 2	91 ± 2	88 ± 2	94 ± 3	85 ± 2	93 ± 3	86 ± 2	95 ± 3
	Women	85 ± 2	93 ± 2	89 ± 2	95 ± 2	90 ± 2	98 ± 3	90 ± 2	99 ± 3
Δ Mean BP (mmHg)									
	Men		9 ± 2		6 ± 2		8 ± 3		9 ± 2
	Women		8 ± 2		6 ± 1		9 ± 2		8 ± 1
HR (beats·min^{-1}) *†									
	Men	64 ± 4	66 ± 4	66 ± 5	68 ± 4	66 ± 5	70 ± 5	67 ± 5	70 ± 5
	Women	70 ± 4	77 ± 5	76 ± 5	81 ± 4	75 ± 4	79 ± 3	76 ± 4	83 ± 5
Δ HR (beats·min^{-1})									
	Men		2 ± 1		2 ± 2		5 ± 2		3 ± 2
	Women		7 ± 2		5 ± 2		4 ± 2		7 ± 2

Values are mean±SEM. CPT, cold pressor test; Ex, exercise; MVC, maximum voluntary contraction; BP, blood pressure; HR, heart rate; * P<0.05 main effect of rhythmic handgrip exercise; † P<0.05 main effect of CPT.

Table 8.3. Forearm haemodynamic parameters at rest and during rhythmic handgrip exercise with and without cold pressor test (men n=10, women n=9).

		Rest		Ex at 10% MVC		Ex at 25% MVC		Ex at 40% MVC	
		Without CPT	CPT	Without CPT	CPT	Without CPT	CPT	Without CPT	CPT
FBF ($ml \cdot min^{-1} \cdot kg^{-1}$) *†									
	Men	98 ± 20	72 ± 20	117 ± 24	95 ± 21	183 ± 36	162 ± 35	233 ± 29	210 ± 33
	Women	66 ± 11	50 ± 8	94 ± 7	66 ± 9	199 ± 16	183 ± 17	266 ± 23	235 ± 31
FVC ($ml \cdot min^{-1} \cdot kg^{-1} \cdot mmHg^{-1}$) *†									
	Men	1.2 ± 0.3	0.7 ± 0.2	1.5 ± 0.2	1.1 ± 0.2	2.1 ± 0.4	1.5 ± 0.2	2.7 ± 0.3	2.2 ± 0.3
	Women	0.8 ± 0.1	0.5 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	2.2 ± 0.2	1.9 ± 0.2	2.9 ± 0.3	2.4 ± 0.3
Diameter (cm) *†‡									
	Men	0.47 ± 0.01	0.47 ±0.01	0.48 ± 0.01	0.47 ± 0.01	0.48 ± 0.01	0.47 ± 0.01	0.48 ± 0.01	0.48 ± 0.01
	Women	0.39 ± 0.01	0.39 ±0.01	0.39 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.39 ± 0.01
% Δ FVC *									
	Men	-44 ± 6		-29 ± 8		-23 ± 9		-20 ± 5	
	Women	-32 ± 5		-36 ± 7		-13 ± 8		-20 ± 5	

Values are mean±SEM. CPT, cold pressor test; Ex, exercise; FBF, forearm blood flow; FVC, forearm vascular conductance; * $P<0.05$ main effect of rhythmic handgrip exercise; † $P<0.05$ main effect of CPT; ‡ $P<0.05$ main effect of sex.

Table 8.4. Cerebrovascular, haemodynamic and respiratory parameters at rest and during rhythmic handgrip exercise with and without cold pressor test (men n=8, women n=7).

	Rest		Ex at 10% MVC		Ex at 25% MVC		Ex at 40% MVC	
	Without CPT	CPT	Without CPT	CPT	Without CPT	CPT	Without CPT	CPT
MCA V_{mean} ($cm \cdot s^{-1}$) *†								
Men	65 ± 3	68 ± 3	70 ± 3	72 ± 4	70 ± 4	72 ± 4	70 ± 4	71 ± 4
Women	69 ± 3	69 ± 3	67 ± 4	69 ± 4	69 ± 4	72 ± 4	69 ± 4	70 ± 4
CVCi ($cm \cdot s^{-1} \cdot mmHg^{-1}$) †								
Men	0.81 ± 0.04	0.77 ±0.05	0.80 ± 0.05	0.76 ± 0.05	0.83 ± 0.06	0.79 ± 0.05	0.81 ± 0.05	0.76 ± 0.05
Women	0.80 ± 0.05	0.74 ±0.04	0.77 ± 0.06	0.74 ± 0.06	0.77 ± 0.05	0.74 ± 0.05	0.79 ± 0.05	0.72 ± 0.04
$P_{ET}CO_2$ (mmHg) *†‡								
Men	42 ± 0.5	42 ± 0.2	41 ± 0.4	41 ± 0.4	41 ± 0.3	41 ± 0.6	41 ± 0.3	41 ± 0.4
Women	40 ± 1.0	40 ± 0.7	40 ± 0.7	39 ± 0.9	39 ± 0.8	39 ± 0.7	39 ± 0.9	39 ± 0.7
CO ($l \cdot min^{-1}$) †‡								
Men	7.2 ± 0.4	7.6 ± 0.4	6.8 ± 0.4	7.4 ± 0.5	7.5 ± 0.6	7.7 ± 0.5	7.5 ± 0.6	7.6 ± 0.5
Women	5.5 ± 0.4	6.0 ± 0.4	5.8 ± 6.3	6.3 ± 0.3	5.6 ± 0.4	6.1 ± 0.4	5.8 ± 0.5	6.4 ± 0.6
% Δ CVCi								
Men	-5 ± 3		-4 ± 3		-3 ± 3		-6 ± 2	
Women	-6 ± 3		-3 ± 3		-4 ± 3		-9 ± 1	

Values are mean±SEM. CPT, cold pressor test; Ex, exercise; MCA Vmean, middle cerebral artery mean blood velocity; CVCi, cerebrovascular conductance index; PETCO2, end-tidal partial pressure of carbon dioxide; CO, cardiac output; * P<0.05 main effect of rhythmic handgrip exercise; † P<0.05 main effect of CPT; ‡ P<0.05 main effect of sex.

8.4 Discussion

The aim of the present study was twofold. Firstly, determine whether there are sex-differences in the sympathetic vasoconstrictor responses during rhythmic handgrip exercise in the brachial circulation. Secondly, identify potential sex-differences in the cerebrovascular responsiveness to an 'endogenous' sympatho-excitatory stimulus at rest and during exercise. The main findings of the current study are as follows: (i) in contrast to the initial hypothesis men and women exhibited a similar magnitude of sympathetic vasoconstriction in the peripheral circulation at rest and during three levels of rhythmic handgrip exercise; (ii) only a modest cerebrovascular constriction was observed at rest, which was sustained during exercise and not different in men and women. Taken together, the findings from the present study indicate that the magnitude of functional sympatholysis in the peripheral circulation is similar in men and women. In contrast to the peripheral circulation, the cerebral vasculature seems only modestly responsive to endogenous sympathetic stimulation at rest and during handgrip exercise, which was the same in young men and women.

8.4.1 Peripheral vascular responses to exercise

As expected a marked increase in FBF was observed during rhythmic handgrip exercise that was in proportion to the exercise intensity. Exercise hyperaemia was comparable in men and women. It is still controversial whether there are sex-differences in the dilator response during exercise. Studies have reported either greater (Koch et al., 2005, Parker et al., 2007) or similar (Gonzales et al., 2007, Limberg et al., 2010) exercise-induced increases in skeletal muscle perfusion in women compared to men. These discrepancies might stem from the application of different exercise protocols

(incremental versus steady-state exercise), limb-specificity in the hemodynamic responses, varying exercise intensities and/or the use of absolute versus relative workloads (Wray et al., 2009). Moreover, differential results might also derive from the definition of exercise hyperaemia. For example, it might be expressed as blood flow or as conductance. Therefore, the sex-specific exercise-induced increases in BP (e.g. attenuated pressor response in women) would lead to differential results, such as a greater increase in BP in men would result in a lower vascular conductance for a given increase in FBF. Accordingly, in the present study FVC was calculated to account for potential sex-differences in the exercise pressor response. Furthermore, ignoring the contribution of muscle mass to the exercise hyperaemia would lead to misinterpretations. Therefore, FBF was corrected for differences in lean forearm mass that was significantly greater in men compared to women. However, no sex-differences were observed in forearm exercise hyperaemia when expressed as either FBF or FVC.

In the present study, mild, low and moderate workloads of rhythmic handgrip exercise were chosen to avoid exercise-induced increases in sympathetic activity *per se*. These have been shown to occur only upon higher workloads (i.e. > 60% MVC (Victor and Seals, 1989)). In addition, the relatively low exercise intensities used (RPE: fairly light at 40% MVC) allowed simultaneous FBV and brachial artery diameter measurements, to account for changes in diameter during exercise. A previous (Parker et al., 2007) and the present study have shown that arterial diameter increases during exercise, although this is not a universal finding (Rådegran, 1997).

8.4.2 Cerebrovascular responses to exercise

The present study is the first to investigate sex-differences in the cerebral perfusion during exercise. Transcranial Doppler was used to assess MCA V_{mean}, because

the MCA supplies the brain region associated with the cortical representation of the forearm muscles and has previously been shown to increase particularly during contralateral handgrip exercise (Linkis et al., 1995). While exercise-induced increases in MCA V_{mean} from rest were observed in men, cerebral perfusion remained unchanged in women during handgrip exercise. Although the reason for these differential cerebrovascular responses to exercise in men and women are unclear, it is noteworthy that the cerebral perfusion is affected by a range of different factors (e.g. neural, humoral, metabolic and cardiovascular) (Querido and Sheel, 2007). The contribution of these factors will be discussed below with regards to their potential to explain the lack of exercise-induced increases in cerebral perfusion in women.

The magnitude of the increase in MCA V_{mean} during exercise in men here was similar to that previously observed during handgrip (Ide et al., 1998, Pott et al., 1997) and may be at least partly stimulated by exercise-induced elevations in cerebral neural activity (Secher et al., 2008, Vianna et al., 2009). One potential explanation for the unchanged MCA V_{mean} during mild to moderate rhythmic handgrip exercise in women derives perhaps from a differential neural regulation of the cerebral circulation during exercise. Amongst other factors, the local metabolism in brain regions associated with forearm exercise may modulate exercise-induced alterations in cerebral perfusion (Ide et al., 2000). A recent study has compared the cerebrovascular responses elicited by voluntary exercise to those elicited by electrically evoked exercise where the parallel activation of central motor and cardiovascular centres was absent (i.e. central command), but peripheral feedback remained intact (skeletal muscle afferents) (Vianna et al., 2009). Of note, while cerebral perfusion increased and CVCi was unchanged during volitional calf exercise, cerebral blood flow velocity was unchanged and CVCi fell during electrically evoked exercise. These results suggest that increases in cerebral

metabolism possibly related to central command during voluntary exercise are important modulators of the cerebrovascular response to exercise. However, in the present study, the only measurement allowing assumptions regarding differential central command activation was the RPE that was found no different between men and women.

Another potential factor which contributes to exercise-induced increases in MCA V_{mean} is CO (Ogoh et al., 2005a). Indeed, in the present study, CO was persistently higher in men compared to women in the present study. However, CO did not significantly increase during exercise in both groups. As such it seems unlikely that CO has affected the present results.

In contrast to the peripheral circulation, cerebral vascular tone has been shown to be strongly controlled by arterial PCO_2 levels (e.g. increased PCO_2 evokes vasodilation in the cerebral vasculature) (Ainslie et al., 2005). In the present study, $P_{ET}CO_2$ decreased slightly during handgrip in both men and women. Therefore, elevations in MCA V_{mean} may actually have been slightly underestimated. In women $P_{ET}CO_2$ was slightly lower compared to men at rest and during exercise. However, the magnitude of the exercise-induced decrease in $P_{ET}CO_2$ was similar in men and women. Nevertheless, it remains incompletely understood whether sex affects $P_{ET}CO_2$, and therefore the MCA V_{mean} responses during exercise.

Alternatively, rhythmic handgrip exercise might have also represented a differential 'central stimulus' for men and women (e.g. keeping the rhythm and matching expected with the actual force produced). Indeed, increases in regional perfusion during the same stress tasks have been shown to occur in different brain region in men and women indicating a very distinct cerebro-anatomical stress response between sexes (Wang et al., 2007). As such, using the MCA V_{mean} might have provided a true estimate of the exercise-induced increases in cerebral perfusion in men, but not in

women. Further studies investigating potential cerebro-regional differences between men and women during various exercise paradigms including different exercise modalities, intensities and muscle mass contribution are needed to fully explore this possibility.

Although previous reports have suggested that resting cerebral perfusion is higher in women compared to men (Parkes et al., 2004, Slopien et al., 2003), no sex-differences were found in the present study. The methodology of cerebral perfusion measures is a likely explanation for these contrasting results. As such regional cerebral blood flow velocity has been measured in the current study, while others have used magnetic resonance imaging or single photon emission computed tomography (Parkes et al., 2004, Slopien et al., 2003).

8.4.3 Peripheral vascular responses to sympatho-excitation

In animal and human experiments, it has previously been shown that sympathetic activation elicits a marked reduction in blood flow under resting conditions, which is blunted in the contracting muscles in proportion to exercise intensity. This phenomenon is termed functional sympatholysis (Hansen et al., 2000, Remensnyder et al., 1962, Thomas et al., 1994, Tschakovsky et al., 2002, Wray et al., 2004b). Since previous studies investigating the sympathetic vasoconstriction in the exercising muscles were conducted predominantly in men and/or were not designed to determine sex-differences, it remained unclear how sex might influence this phenomenon. In women, adrenergic control of BP at rest has been shown to be reduced (Coulson and Cockcroft, 2011, Schmitt et al., 2010). Also, women have been demonstrated to exhibit attenuated sympathetic vasoconstrictor responses at rest compared to men (Kneale et al., 1997). The CPT has been shown to evoke sympatho-

excitation via α-adrenergic pathways. This is evidenced by the abolished increases in MSNA and BP after administration of clonidine that blocks the release of noradrenaline (Muzi et al., 1992). However, in the present study, the CPT-induced vasoconstrictor responses at rest were not different in women and men. A potential reason for the seemingly contrasting finding might be that the attenuated sympathetic vasoconstriction in women was not based on an attenuated α-adrenergic responsiveness, but rather on enhanced β-adrenergic stimulation (e.g. greater vasodilator responsiveness in women) (Coulson and Cockcroft, 2011, Kneale et al., 2000). Moreover, Dikshit et al. (1986) have found that the pressor response elicited by the CPT was not affected by β-adrenergic blockade with propranolol. Therefore, the present results appear to be in agreement with the current literature confirming that vasoconstriction resulting from α-adrenergic stimulation is not different in men and women.

The recent study provides evidence that sympathetically-mediated vasoconstriction is similarly blunted in men and women (e.g. magnitude of functional sympatholysis). Previously, attenuated sympathetic vasoconstriction during exercise has been attributed to a metabolically-mediated blunting of post-junctional α-adrenergic receptors (Rosenmeier et al., 2003, Thomas et al., 1994, Wray et al., 2004b). Given the similar vascular responses to α-adrenergic receptor stimulation in men and women at rest (Kneale et al., 2000), the present findings indicate that the lack of a sex-difference persists during exercise. In support of this, a recent study, published after the present study was conducted, have found no differences in the sympathetic vasoconstrictor responses evoked by either noradrenaline (α_1-adrenergic agonist) or clonidine (α_2-adrenergic agonist) infusion at rest and during mild and low intensity rhythmic handgrip exercise in men and women (Limberg et al., 2010). Taken together, these present results

indicate that the magnitude of 'functional sympatholysis' during rhythmic handgrip exercise is not affected by sex.

8.4.4 Cerebrovascular responses to sympatho-excitation

Sympatho-excitatory manoeuvres (e.g. CPT, whole-body heating, lower body negative pressure) have been reported to evoke reductions in cerebral perfusion and/or increases in cerebrovascular resistance (Low et al., 2009, Micieli et al., 1994, Roatta et al., 1998, Wilson et al., 2005). In the contrast to the peripheral circulation, sympathetic activation via CPT evoked only a small reduction in CVCi in the present study. This is indicative of a modest sympathetic vasoconstriction in the cerebral vasculature, which is unchanged during exercise. Although, the present results seem to be in contrast with previous findings in dogs (Gross et al., 1980) and humans (Brassard et al., 2010) it is questionable, whether results in animals are directly translatable to humans. It needs to be further acknowledged that noradrenaline, utilized in the study by Brassard and colleagues, might not cross the blood brain barrier (Hardebo and Owman, 1980, Olesen, 1972). Moreover, the potentially important indirect effects of noradrenaline on the systemic haemodynamics (e.g. transient hypertension and reduction in CO) seem to be inconsistent from rest to exercise (Ainslie and Tzeng, 2010).

Similar to the peripheral circulation, no sex-differences were observed in the cerebrovascular response to sympathetic activation at rest or during handgrip exercise. Indeed, cerebrovascular responses to the CPT have been shown to be attenuated following administration of clonidine, thus the central actions of noradrenalin were blocked (Micieli et al., 1994). This suggests that the CPT elicits intracerebral vascular changes via a central noradrenergic mechanism. Thus, the results of the present study may indicate that the regulation of the cerebral perfusion via α-adrenergic-mechanisms

during exercise is not affected by sex. However, given the CO_2 sensitivity of the cerebral circulation, the sympathetic vasoconstrictor responses during exercise might have been confounded by changes in $P_{ET}CO_2$ (Ainslie et al., 2005). However, the small reductions in $P_{ET}CO_2$ were consistent between handgrip bouts and between sexes suggesting that any effect was similar between trials.

8.4.5 Limitations

Given that the CPT has been shown to evoke increases in sympathetic vasoconstrictor outflow resulting in a marked rise in BP (Victor et al., 1987a), it was utilised to investigate sex-differences in the differential sympathetic regulation of the peripheral and cerebral vasculature. Sex-differences in the pressor response to the cold stimulus *per se* might have influenced the results. However, in agreement with previous studies (Dishman et al., 2003, Jones et al., 1996), BP and HR responses were similar in men and women in the present study (Table 8.2). In addition, the majority of studies directly measuring the efferent sympathetic neural response have shown no differences in the CPT-induced increases in MSNA in men and women (Dishman et al., 2003, Ettinger et al., 1996, Jarvis et al., 2011, Jones et al., 1996).

The consistency of the cardiovascular responses during repeated CPT has to be considered, as a habituation might have occurred (Zbrozyna and Westwood, 1988). In consensus with Schrage et al. (2005) who have performed five consecutive CPT in their study, the first cold stimulus evoked exaggerated cardiovascular and haemodynamic responses and was therefore discarded from the analysis. In order to further minimize any effect of habituation in the present study, exercise trials were performed in a controlled randomized order. Indeed, the magnitude of the CPT-induced increases in HR and BP was similar in both groups. This suggests that any effect was similar

between trials. Thus, an attenuated sympathetic nervous outflow during repeated CPT is unlikely to explain the graded sympatholytic responses in the peripheral circulation in men and women. However, it is acknowledged that only direct measurements of efferent sympathetic outflow (e.g. MSNA) would have provided a definite prove of the comparable repeated sympathetic responses.

To minimize the confounding effects of fluctuating female reproductive hormones, women were tested within the first five days of the ovarian cycle. However, it is noteworthy that the study sample consisted of six women taking oral contraceptives (e.g. Microgynon or Yasmin). Of note Minson et al. (2000a, 2000b) have observed no ovarian cycle effect on the resting MSNA in oral contraceptive users, but not in women not taking oral contraceptives. In addition, in non-contraceptive users vascular transduction was not affected. However, the exact reasons for those differences remain unclear. Since no direct comparison between endogenous and exogenous hormone users has been performed in the present study and Minson et al. have not assessed neurovascular transduction in the oral contraceptive users, it cannot be excluded that the non-homogenous study sample may have affected the study results.

Limitations may also derive from the use of MCA V_{mean} as an indicator of cerebral perfusion. It is acknowledged that the transcranial Doppler method only allows velocity measurements. Therefore, it is only reflective of elevations in regional cerebral blood flow if diameter is of the insonated vessel remains unchanged. However, MCA V_{mean} and MCA blood flow appear to be closely related (Kirkham et al., 1986) and MCA diameter has been shown to remain relatively constant during a variety of physiological stimuli (Wilson et al., 2011). In addition, it cannot be fully excluded that myogenically-induced increases in cerebrovascular tone might have caused the decreases in CVCi during sympathetic activation. There is potential that the CPT-

induced rise in BP evoked the increase in MCA V_{mean} via action on the smooth muscle wall.

In the present study no effect of sex was found in the hyperaemic responses during three levels of rhythmic handgrip exercise. Hence, the performed workloads represent 10, 25 and 40% of the individual's MVC it cannot be excluded that sex-differences might exist when men and women exercise at the same absolute workload. However, a recent study has suggested that even if women and men exercised at the same absolute exercise workload, the vasodilatory responses (i.e. exercise hyperaemia) would be comparable between sexes (Limberg et al., 2010).

8.4.6 Alternative approaches

The sympathetic vasoconstrictor response in women has often been suggested to be related to the vascular effects of estrogen (Hogarth et al., 2007, Kneale et al., 1997). For example, estrogen has been found to attenuate α-adrenergic vasoconstrictor responses at rest in rats (Sudhir et al., 1997) and in humans (Fadel et al., 2004b). Moreover, in both ovariectomized rats and postmenopausal women when female hormone production is abolished functional sympatholysis was impaired (e.g. profound sympathetic vasoconstriction) (Fadel et al., 2004b, Fadel et al., 2003c). Following estrogen supplementation, the sympathetic vasoconstrictor response during exercise was blunted. This suggests a sympatholytic effect of this hormone. Therefore, women at different phases of the ovarian cycle, ideally during the EF (low estrogen and progesterone), LF (high estrogen, low progesterone) and luteal phases (high estrogen and progesterone) of the ovarian cycle should be tested to investigate the effect of estrogen on sympathetic vasoconstriction during exercise. Since completion of the present study this question has been addressed elsewhere. Limberg et al. (2010) have

investigated separate α_1- and α_2-adrenergic (i.e. infusion of noradrenaline and clonidine) vasoconstrictor responses in men and women during the EF and luteal phases of the ovarian cycle at rest and during very low and low-level rhythmic handgrip exercise. They have found no differences in the sympathetic vasoconstrictor responses in men and EF women for both adrenergic receptor subtypes. However, the α_2-adrenergic vasoconstrictor response was reduced at very low handgrip exercise in women during the luteal compared to the EF phases. In accordance with the results from the present study, men and women seem to obtain changes in forearm blood flow via similar adrenergic mechanisms. However, the role played by estrogen is still incompletely understood, since Limberg and colleagues (2010) have studied women during the luteal phase of the ovarian cycle when estrogen and progesterone are both elevated. This seems to be of importance, because estrogen and progesterone have been suggested to exert opposing vascular effects. As such, vasoconstriction has been shown to be attenuated in estrogen-treated and enhanced in progesterone-treated animals in response to sympathetic nerve stimulation (Ford et al., 1977). Therefore, further research is warranted to fully determine the cardiovascular actions and interactions of female reproductive via the sympathetic nervous system during exercise.

Although no sex-differences were found in MCA V_{mean} or CVCi in the present study, an area for further research might be the investigation of the effects of estrogen on the cerebral perfusion particularly during exercise. For example, estrogen deficiency after menopause has been reported to be associated with reductions in cerebral blood flow, and hormone replacement therapy in postmenopausal women has been shown to increase cerebral blood flow to premenopausal levels (Slopien et al., 2003). Furthermore, MCA V_{mean} is reportedly higher in young women during the luteal phase of the ovarian cycle, compared to EF phase (Brackley et al., 1999). Taken together,

these observations support the contention that sex hormones might play an important role in the modulation of cerebral perfusion (Krause et al., 2006). However, it is presently unknown whether fluctuations in ovarian hormone concentration might also influence the cerebral perfusion during exercise. An alternative study design to answer these questions might want to include pre- and postmenopausal women with or without estrogen supplementation performing measurements of regional cerebral blood flow (e.g. MCA V_{mean}). Additional assessment of blood flow in the internal carotid artery during exercise would further allow to obtain a measurement of global cerebral blood flow (Sato et al., 2011).

8.5 Conclusion

In summary, the results of the current study indicate that there are no sex-differences in the sympathetic vasoconstrictor responses at rest. Moreover, the vasodilator responses during exercise are similar in men and women. The sympathetically-mediated reductions in peripheral perfusion were blunted in an exercise-intensity dependent manner in both groups. There was no effect of sex on the magnitude of reduction in peripheral perfusion. In contrast to previous studies in dogs (Gross et al., 1980) and humans (Brassard et al., 2010), sympatho-excitation via the CPT evoked only a modest reduction in cerebral vascular conductance. This was not modulated during exercise of different intensities in men and women. Conclusively, these data suggest an absence of 'functional sympatholysis' in the cerebral vasculature of young men and women.

CHAPTER 9: GENERAL CONCLUSION

At rest and during exercise, the arterial baroreflex plays an important role in the beat-to-beat regulation of arterial BP and HR, by modulating sympathetic and parasympathetic activity to the heart and the peripheral vasculature. During exercise the arterial baroreflex persists its functioning by resetting to operate around higher pressures, allowing HR and BP to increase concomitantly. This resetting depends on input from central command and feedback from skeletal muscle afferents. Compared to young men, premenopausal women show attenuated pressor responses to static and dynamic exercise. Intriguingly, the exercise-induced increases in BP are exaggerated in older postmenopausal women compared to older men. However, the reason for the attenuated pressor response in young healthy women compared to men is unknown.

In the first study of this thesis (Chapter 4), a non-invasive experimental model was developed to examine the effects of augmented muscle metaboreflex activation on baroreflex function during dynamic exercise. It was demonstrated that (i) activation of metabolically sensitive skeletal muscle afferents (e.g. muscle metaboreflex) by means of partially restricting blood flow to the exercising skeletal muscle via rapid inflation of a pressure cuff around the exercising thighs during leg cycling causes a reduction in spontaneous cBRS; and (ii) pronounced reductions in spontaneous cBRS occur during isolated activation of the muscle metaboreflex during PEI following leg cycling exercise, while cBRS was unchanged during rhythmic handgrip exercise and the following PEI. This is the first time in humans that spontaneous cBRS has been assessed during augmented muscle metaboreflex activation during dynamic leg cycling exercise. The findings are consistent with evidence from studies in dogs running on a treadmill that used a pneumatic occluder around the terminal aorta to reduce blood flow to the working hind limb thus engage the muscle metaboreflex. Since cBRS presents a measurement of vagal activity (Parlow et al., 1995), it is tempting to speculate that the

muscle metaboreflex is able to withdrawal parasympathetic nerve activity and thus contributes to the exercise-induced tachycardia. It might elicit these HR increases either directly or indirectly via augmented engagement of central command. This is a novel approach, as the muscle metaboreflex is traditionally thought to evoke exercise-tachycardia via increases cardiac sympathetic nerve activity (Fisher et al., 2010, O'Leary, 1993). The reduced spontaneous cBRS during PEI after leg cycling but not after forearm exercise suggests that the effect of the muscle metaboreflex on cBRS is specific to the exercise modality, particularly to the involvement of a large muscle mass during dynamic exercise.

The second experimental study (Chapter 5) examined, for the first time in humans, the influences of sex and/or ovarian hormone concentration on the metaboreflex-induced reduction in cBRS during dynamic leg cycling followed by PEI. As expected, the BP responses during dynamic leg cycling were greater in men compared to women. However, the augmented activation of the muscle metaboreflex during leg cycling evoked comparable increases in HR and BP and a similar reduction in cBRS in men and women during the EF and LF phases of the ovarian cycle. These results imply that muscle metaboreflex control of cardiac baroreflex function during dynamic exercise is not affected by sex. However, since metaboreflex activation via partial flow-restriction was not quantified by measurements of leg blood flow, the reductions in blood flow might have differed between men and women and during the two ovarian phases. Thus non-standardized blood flow stimulus might have resulted in a differential activation of metabolically sensitive skeletal muscle afferents during leg cycling in men and women.

The third experimental study (Chapter 6) was designed to include blood flow measurements when determining sex-differences during exercise with augmented muscle metaboreflex activation. Moreover, the relationship between the magnitude of blood flow restriction (i.e. metaboreflex activation) and the systemic cardiovascular responses and spontaneous cBRS during exercise was determined. Young men and women during the EF and LF phases of the ovarian cycle performed rhythmic handgrip exercise and the muscle metaboreflex was engaged by means of stepwise reductions of forearm perfusion during rhythmic handgrip exercise. It was demonstrated that if FBF is reduced to a similar extent (e.g. standardized muscle metaboreflex activation) the magnitude of the metaboreflex-induced pressor response is comparable in men and women and not different between the two ovarian phases. Similarly to the observations in men cBRS was unchanged from rest during handgrip exercise and augmented muscle metaboreflex activation. This indicates that muscle metaboreflex sensitivity does not differ between men and women during the EF and LF phases of the ovarian cycle. From this it is tempting to speculate that any sex-differences in the pressor response during exercise may be related to a differential perfusion of the exercising skeletal muscle and thus altered muscle metaboreflex activation. Overall, muscle metaboreflex control of cardiac baroreflex responsiveness appears not to be affected by sex or the hormonal fluctuations between the EF and LF phases of the ovarian cycle during dynamic exercise with both small and large muscle mass contribution in young healthy humans.

The forth experimental study of this thesis (Chapter 7) examined for the first time potential sex-differences in the dynamic baroreflex control of BP during exercise. In young men and women NP and NS (i.e. simulated hypo- and hypertensive stimuli, respectively) were applied in an oscillatory manner to the carotid sinus at rest and during rhythmic handgrip exercise. The cardiovascular responses to dynamic

baroreceptors perturbation were similar at rest and during exercise, in men and women with NP and NS. These data indicate that in men and women the carotid baroreflex maintains to control HR and BP during exercise and supports the findings from the previous two studies in that cBRS is not affected by sex. As such, attenuated baroreflex sensitivity appears unlikely to explain the attenuated pressor response during exercise in women.

The final experimental study (Chapter 8) of this thesis investigated sex-differences in the responsiveness of the peripheral and the cerebral circulation to sympathetic stimulation at rest and during exercise. Sympathetic activation was evoked by a cold stimulus (i.e. α-adrenergic receptor stimulation) conducted at rest and during rhythmic handgrip exercise of varying intensity. At rest forearm perfusion (e.g. FVC) was decreased during sympathetic activation, while the vasoconstrictor responses during exercise were inversely related to the exercise intensity (i.e. functional sympatholysis) in both men and women. In contrast, only a modest decrease in cerebral perfusion was observed at rest that was maintained during exercise in both sexes. While indicative of a differential role of the sympathetic nervous system in the peripheral and cerebral vasculature during exercise, these results also suggest that men and women are similarly responsive to α-adrenergic receptor stimulation at rest and during exercise. Given that efferent sympathetic outflow was similar in men and women during the CPT, this would imply that the neural vascular conductance via α-adrenergic receptor mechanisms is not affected by sex. This has been confirmed by Limberg et al. (Limberg et al., 2010), whose study was published after the this study was conducted. Therefore, an altered α-adrenergic transduction of sympathetic efferent activity to the vascular smooth muscle cell is unlikely to contribute to the attenuated pressor response to exercise in women.

The work in this thesis has enhanced the current knowledge regarding the differences and similarities in neural cardiovascular control during dynamic exercise in men and women. For the first time the effects of sex and/or ovarian hormone concentrations were examined during dynamic exercise, including influences on baroreflex control of HR and BP, muscle metaboreflex sensitivity and sympathetic vasoconstrictor responsiveness. First, baroreflex responsiveness in control of HR and BP are not different in men and women. Second, muscle metaboreflex sensitivity is comparable between both groups, when blood flow is standardized. Third, the magnitude of functional sympatholysis in the peripheral circulation is similar in men and women, suggesting the sex does not affect α-adrenergic receptor responsiveness during exercise.

Nevertheless, there are further questions arising from the work of this thesis. Due to the utilized experimental approaches no conclusion can be drawn about the effects of sex and/or ovarian hormone concentration on selective activation of mechanically sensitive skeletal muscle afferents (i.e. muscle mechanoreflex) and/or central command (Figure 2.2). In this regard, the influence of sex and/or female reproductive hormones on muscle mechanoreflex-mediated reductions in cardiac baroreflex responsiveness warrants further investigation. For example, the application of muscle stretch has been shown to selectively activate mechanically sensitive skeletal muscle afferents (no central command, muscle metaboreflex) (Iellamo et al., 1999a, Kaufman and Rybicki, 1987), which therefore present a promising approach to examine potential influences of sex- and/or female hormones on the cardiovascular and haemodynamic effects of the isolated muscle mechanoreflex.

In addition, the present findings do not allow direct conclusions about the effect of sex and/or ovarian hormones on central command or the isolated central command-mediated exercise pressor response (increases in HR, BP, CO, MSNA and TPR) and its effect on baroreflex function during exercise (Krogh and Lindhard, 1917, Mark et al., 1985, Secher, 1985, Victor et al., 1995). However, the comparison of the pressor response and baroreflex responsiveness during volitional (mechanoreflex, metaboreflex and central command) and electrically stimulated (mechanoreflex, metaboreflex, but no central command) muscle contractions (Iellamo et al., 1997) in men and women plus the determination of female reproductive hormone influences could further our present understanding about influences of sex and/or ovarian hormones on cardiovascular control mechanisms during exercise.

Since the application of the sequence technique and oscillatory NP and NS only provide a measure of baroreflex sensitivity (Wray et al., 2004a), it remains to be determined whether other physiological parameters of the arterial baroreflex, such as the response range, saturation or threshold in control of HR, BP, MSNA and perfusion might be different between men and women. Thus, the application of short pulses of NP and NS (e.g. 5seconds) to the carotid sinuses at rest and during exercise in men and women would allow an estimation of these parameters. In addition, the full baroreflex function curve could be determined, while selectively activating the muscle metaboreflex (e.g. PEI vs. free-flow recovery after leg cycling) or the muscle mechanoreflex (e.g. passive calf muscle stretch vs. volitional calf muscle contraction) or in the absence and presence of central command (e.g. electrically evoked vs. volitional calf contraction) (Iellamo et al., 1997).

Although the advantages of the isolation of muscular and central neural mechanisms of the exercise pressor response are established even these approaches are limited due to the 'redundancy' argument that a combination of several factors contributes to the cardiovascular responses to exercise (Rowell, 1993b). In line with this, it is also acknowledged that in a closed-loop approach, such as the partial flow-restriction experiments (chapter 4-6) to augment the effects of the metabolically sensitive skeletal muscle afferents, the results of the present study might be affected by redundancy mechanisms. Thus, the differential exercise pressor response in men and women might be a result of sex- and/or ovarian hormone-specific alteration of more than one mechanism, known to contribute in a synergistic manner. However, on the basis of the results from this thesis these questions and possibilities remain to be examined.

REFERENCES

Abdel-Rahman, AR, Merrill, RH & Wooles, WR 1994. Gender-related differences in the baroreceptor reflex control of heart rate in normotensive humans. *J Appl Physiol,* 77**,** 606-613.

Adreani, CM & Kaufman, MP 1998. Effect of arterial occlusion on responses of group iii and iv afferents to dynamic exercise. *J Appl Physiol,* 84**,** 1827-1833.

Ainslie, PN, Ashmead, JC, Ide, K*, et al.* 2005. Differential responses to co2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol,* 566**,** 613-624.

Ainslie, PN & Tzeng, YC 2010. On the regulation of the blood supply to the brain: Old age concepts and new age ideas. *J Appl Physiol.*

Alam, M & Smirk, FH 1937. Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *J Physiol,* 89**,** 372-383.

Alam, M & Smirk, FH 1938. Observations in man on a pulse-accelerating reflex from the voluntary muscles of the legs. *J Physiol,* 92**,** 167-177.

Amann, M, Proctor, LT, Sebranek, JJ*, et al.* 2009. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol,* 587**,** 271-283.

Amann, M, Runnels, S, Morgan, DE*, et al.* 2011. On the contribution of group iii and iv muscle afferents to the circulatory response to rhythmic exercise in humans. *J Physiol,* 589**,** 3855-3866.

Andersen, P, Adams, RP, Sjogaard, G*, et al.* 1985. Dynamic knee extension as model for study of isolated exercising muscle in humans. *J Appl Physiol,* 59**,** 1647-1653.

Andres, KH, von During, M & Schmidt, RF 1985. Sensory innervation of the achilles tendon by group iii and iv afferent fibers. *Anat Embryol (Berl),* 172**,** 145-156.

Asarian, L & Geary, N 2007. Estradiol enhances cholecystokinin-dependent lipid-induced satiation and activates estrogen receptor-alpha-expressing cells in the nucleus tractus solitarius of ovariectomized rats. *Endocrinology,* 148**,** 5656-5666.

Asmussen, E, Johansen, SH, Jorgense.M*, et al.* 1965. On nervous factors controlling respiration and circulation during exercise experiments with curarization. *Acta Physiol Scand,* 63**,** 343-350.

Astrand, PO 1956. Human physical fitness with special reference to sex and age. *Physiol Rev,* 36**,** 307-335.

Auer, LM, Edvinsson, L & Johansson, BB 1983. Effect of sympathetic nerve stimulation and adrenoceptor blockade on pial arterial and venous calibre and on intracranial pressure in the cat. *Acta Physiol Scand,* 119**,** 213-217.

Bakker, SLM, de Leeuw, FE, den Heijer, T*, et al.* 2004. Cerebral haemodynamics in the elderly: The rotterdam study. *Neuroepidemiology,* 23**,** 178-184.

Barcroft, H & Millen, JL 1939. The blood flow through muscle during sustained contraction. *J Physiol,* 97**,** 17-31.

Barnett, SR, Morin, RJ, Kiely, DK*, et al.* 1999. Effects of age and gender on autonomic control of blood pressure dynamics. *Hypertension,* 33**,** 1195-1200.

Bath, E, Lindblad, LE & Wallin, BG 1981. Effects of dynamic and static neck suction on muscle nerve sympathetic activity, heart rate and blood pressure in man. *J Physiol,* 311**,** 551-564.

Bell, MP & White, MJ 2005. Cardiovascular responses to external compression of human calf muscle vary during graded metaboreflex stimulation. *Exp Physiol,* 90**,** 383-391.

Bernardi, L, Hayoz, D, Wenzel, R*, et al.* 1997. Synchronous and baroceptor-sensitive oscillations in skin microcirculation: Evidence for central autonomic control. *Am J Physiol,* 273**,** H1867-1878.

Beske, SD, Alvarez, GE, Ballard, TP*, et al.* 2001. Gender difference in cardiovagal baroreflex gain in humans. *J Appl Physiol,* 91**,** 2088-2092.

Bevegard, BS & Shepherd, JT 1966. Circulatory effects of stimulating the carotid arterial stretch receptors in man at rest and during exercise. *J Clin Invest,* 45**,** 132-142.

Bigland, B & Lippold, OC 1954. The relation between force, velocity and integrated electrical activity in human muscles. *J Physiol,* 123**,** 214-224.

Billman, GE 2006. A comprehensive review and analysis of 25 years of data from an in vivo canine model of sudden cardiac death: Implications for future anti-arrhythmic drug development. *Pharmacol & Ther,* 111**,** 808-835.

Bogert, LW & van Lieshout, JJ 2005. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Exp Physiol,* 90**,** 437-446.

Bonde-Petersen, F, Rowell, LB, Murray, RG*, et al.* 1978. Role of cardiac output in the pressor responses to graded muscle ischemia in man. *J Appl Physiol,* 45**,** 574-580.

Borg, GA 1998. Borg's perceived exertion and pain scales. Champaign, IL: Human Kinetics.

Boushel, R, Langberg, H, Gemmer, C*, et al.* 2002. Combined inhibition of nitric oxide and prostaglandins reduces human skeletal muscle blood flow during exercise. *J Physiol,* 543**,** 691-698.

Boushel, R, Madsen, P, Nielsen, HB*, et al.* 1998. Contribution of ph, diprotonated phosphate and potassium for the reflex increase in blood pressure during handgrip. *Acta Physiol Scand,* 164**,** 269-275.

Brackley, KJ, Ramsay, MM, Broughton Pipkin, F*, et al.* 1999. The effect of the menstrual cycle on human cerebral blood flow: Studies using doppler ultrasound. *Ultrasound Obstet Gynecol,* 14**,** 52-57.

Brassard, P, Seifert, T, Wissenberg, M*, et al.* 2010. Phenylephrine decreases frontal lobe oxygenation at rest but not during moderately intense exercise. *J Appl Physiol,* 108**,** 1472-1478.

Bristow, JD, Brown, EB, Cunningham, DJ*, et al.* 1969. Changes in the baroreceptor-cardiac reflex in exercise. *J Physiol,* 201**,** 106P-107P.

Brown, CM, Hecht, MJ, Weih, A*, et al.* 2003. Effects of age on the cardiac and vascular limbs of the arterial baroreflex. *Eur J Clin Invest,* 33**,** 10-16.

Buckwalter, JB & Clifford, PS 2001. The paradox of sympathetic vasoconstriction in exercising skeletal muscle. *Exerc Sport Sci Rev,* 29**,** 159-163.

Bull, RK, Davies, CT, Lind, AR*, et al.* 1989. The human pressor response during and following voluntary and evoked isometric contraction with occluded local blood supply. *J Physiol,* 411**,** 63-70.

Cameron, JD, Stevenson, I, Reed, E*, et al.* 2004. Accuracy of automated auscultatory blood pressure measurement during supine exercise and treadmill stress electrocardiogram-testing. *Blood Press Monit,* 9**,** 269-275.

Carrington, CA, White, MJ, Harridge, SD*, et al.* 1995. The relationship between the pressor response to involuntary isometric exercise and the contractile protein profile of the active muscle in man. *Eur J Appl Physiol Occup Physiol,* 72**,** 81-85.

Casey, DP & Joyner, MJ 2009. Skeletal muscle blood flow responses to hypoperfusion at rest and during rhythmic exercise in humans. *Journal of Applied Physiology,* 107**,** 429-437.

Charkoudian, N 2001. Influences of female reproductive hormones on sympathetic control of the circulation in humans. *Clin Auton Res,* 11**,** 295-301.

Charkoudian, N & Joyner, MJ 2004. Physiologic considerations for exercise performance in women. *Clin Chest Med,* 25**,** 247-255.

Charkoudian, N, Joyner, MJ, Johnson, CP*, et al.* 2005. Balance between cardiac output and sympathetic nerve activity in resting humans: Role in arterial pressure regulation. *J Physiol,* 568**,** 315-321.

Chen, CY & DiCarlo, SE 1996. Daily exercise and gender influence arterial baroreflex regulation of heart rate and nerve activity. *Am J Physiol,* 271**,** H1840-1848.

Cheng, DY & Gruetter, CA 1992. Chronic estrogen alters contractile responsiveness to angiotensin-ii and norepinephrine in female rat aorta. *Eur J Pharmacol,* 215**,** 171-176.

Chess, GF, Tam, RM & Calaresu, FR 1975. Influence of cardiac neural inputs on rhythmic variations of heart period in the cat. *Am J Physiol,* 228**,** 775-780.

Christ, M, Seyffart, K, Tillmann, HC*, et al.* 2002. Hormone replacement in postmenopausal women: Impact of progestogens on autonomic tone and blood pressure regulation. *Menopause,* 9**,** 127-136.

Christou, DD, Jones, PP, Jordan, J*, et al.* 2005. Women have lower tonic autonomic support of arterial blood pressure and less effective baroreflex buffering than men. *Circulation,* 111**,** 494-498.

Collins, HL, Augustyniak, RA, Ansorge, EJ*, et al.* 2001. Carotid baroreflex pressor responses at rest and during exercise: Cardiac output vs. Regional vasoconstriction. *Am J Physiol Heart Circ Physiol,* 280**,** H642-H648.

Convertino, VA 1998. Gender differences in autonomic functions associated with blood pressure regulation. *Am J Physiol,* 275**,** R1909-1920.

Cooke, WH, Hoag, JB, Crossman, AA*, et al.* 1999. Human responses to upright tilt: A window on central autonomic integration. *J Physiol,* 517 (Pt 2)**,** 617-628.

Coote, JH 2010. Recovery of heart rate following intense dynamic exercise. *Exp Physiol,* 95**,** 431-440.

Coote, JH & Dodds, WN 1976. The baroreceptor reflex and the cardiovascular changes associated with sustained muscular contraction in the cat. *Pflugers Arch,* 363**,** 167-173.

Coote, JH, Hilton, SM & Perez-Gonzalez, JF 1971. The reflex nature of the pressor response to muscular exercise. *J Physiol,* 215**,** 789-804.

Costa, F, Diedrich, A, Johnson, B*, et al.* 2001. Adenosine, a metabolic trigger of the exercise pressor reflex in humans. *Hypertension,* 37**,** 917-922.

Coulson, JM & Cockcroft, JR 2011. Sex differences in the systemic response to adrenoreceptor antagonists during sympathetic activation. *Eur J Clin Invest,* 41**,** 1129-1132.

Craig, AD & Mense, S 1983. The distribution of afferent-fibers from the gastrocnemius-soleus muscle in the dorsal horn of the cat, as revealed by the transport of horseradish-peroxidase. *Neurosci Lett,* 41**,** 233-238.

Critchley, HD, Corfield, DR, Chandler, MP*, et al.* 2000. Cerebral correlates of autonomic cardiovascular arousal: A functional neuroimaging investigation in humans. *J Physiol,* 523 Pt 1**,** 259-270.

Cui, J, Mascarenhas, V, Moradkhan, R*, et al.* 2008. Effects of muscle metabolites on responses of muscle sympathetic nerve activity to mechanoreceptor(s) stimulation in healthy humans. *Am J Physiol Regul Integr Comp Physiol,* 294**,** R458-R466.

Cui, J, McQuillan, P, Moradkhan, R*, et al.* 2009. Sympathetic responses during saline infusion into the veins of an occluded limb. *J Physiol,* 587**,** 3619-3628.

Cui, J, Wilson, TE & Crandall, CG 2002. Baroreflex modulation of muscle sympathetic nerve activity during cold pressor test in humans. *Am J Physiol Heart Circ Physiol,* 282**,** H1717-1723.

Cui, J, Wilson, TE, Shibasaki, M*, et al.* 2001. Baroreflex modulation of muscle sympathetic nerve activity during posthandgrip muscle ischemia in humans. *J Appl Physiol,* 91**,** 1679-1686.

Daley, JC, 3rd, Khan, MH, Hogeman, CS*, et al.* 2003. Autonomic and vascular responses to reduced limb perfusion. *J Appl Physiol,* 95**,** 1493-1498.

Dampney, RA, Horiuchi, J, Tagawa, T*, et al.* 2003. Medullary and supramedullary mechanisms regulating sympathetic vasomotor tone. *Acta Physiol Scand,* 177**,** 209-218.

Dart, AM, Du, XJ & Kingwell, BA 2002. Gender, sex hormones and autonomic nervous control of the cardiovascular system. *Cardiovasc Res,* 53**,** 678-687.

Davies, CT & Starkie, DW 1985. The pressor response to voluntary and electrically evoked isometric contractions in man. *Eur J Appl Physiol Occup Physiol,* 53**,** 359-363.

de Simone, G, Devereux, RB, Roman, MJ*, et al.* 1991. Gender differences in left ventricular anatomy, blood viscosity and volume regulatory hormones in normal adults. *Am J Cardiol,* 68**,** 1704-1708.

Degtyarenko, AM & Kaufman, MP 2000. Stimulation of the mlr inhibits the discharge of dorsal horn neurons responsive to muscular contraction. *Brain Res,* 880**,** 178-182.

Diaz, T & Taylor, JA 2006. Probing the arterial baroreflex: Is there a 'spontaneous' baroreflex? *Clinical Autonomic Research,* 16**,** 256-261.

Dikshit, MB & Patrick, JM 1986. Beta-adrenoceptor blockade and cardiovascular response to the cold pressor test. *Indian J Physiol Pharmacol,* 30**,** 1-10.

Dinenno, FA & Joyner, MJ 2003. Blunted sympathetic vasoconstriction in contracting skeletal muscle of healthy humans: Is nitric oxide obligatory? *J Physiol,* 553**,** 281-292.

Dishman, RK, Nakamura, Y, Jackson, EM*, et al.* 2003. Blood pressure and muscle sympathetic nerve activity during cold pressor stress: Fitness and gender. *Psychophysiology,* 40**,** 370-380.

Donato, AJ, Uberoi, A, Wray, DW*, et al.* 2006. Differential effects of aging on limb blood flow in humans. *Am J Physiol Heart Circ Physiol,* 290**,** H272-278.

Drew, RC, Bell, MP & White, MJ 2008a. Modulation of spontaneous baroreflex control of heart rate and indexes of vagal tone by passive calf muscle stretch during graded metaboreflex activation in humans. *J Appl Physiol,* 104**,** 716-723.

Drew, RC, McIntyre, DB, Ring, C*, et al.* 2008b. Local metabolite accumulation augments passive muscle stretch-induced modulation of carotid-cardiac but not carotid-vasomotor baroreflex sensitivity in man. *Exp Physiol,* 93**,** 1044-1057.

Du, XJ, Dart, AM & Riemersma, RA 1994. Sex differences in the parasympathetic nerve control of rat heart. *Clin Exp Pharmacol Physiol,* 21**,** 485-493.

Dyke, CK, Proctor, DN, Dietz, NM*, et al.* 1995. Role of nitric-oxide in exercise hyperemia during prolonged rhythmic handgripping in humans. *J Physiol,* 488**,** 259-265.

Eckberg, DL, Kifle, YT & Roberts, VL 1980. Phase relationship between normal human respiration and baroreflex responsiveness. *J Physiol,* 304**,** 489-502.

Eckberg, DL, Nerhed, C & Wallin, BG 1985. Respiratory modulation of muscle sympathetic and vagal cardiac outflow in man. *J Physiol,* 365**,** 181-196.

Eckberg, DL, Rea, RF, Andersson, OK*, et al.* 1988. Baroreflex modulation of sympathetic activity and sympathetic neurotransmitters in humans. *Acta Physiol Scand,* 133**,** 221-231.

Eckberg, DL & Wallin, BG 1987. Isometric exercise modifies autonomic baroreflex responses in humans. *J Appl Physiol,* 63**,** 2325-2330.

Edvinsson, L, Owman, C & Siesjo, B 1976. Physiological role of cerebrovascular sympathetic nerves in the autoregulation of cerebral blood flow. *Brain Res,* 117**,** 519-523.

Eiken, O & Bjurstedt, H 1987. Dynamic exercise in man as influenced by experimental restriction of blood flow in the working muscles. *Acta Physiol Scand,* 131**,** 339-345.

Eiken, O, Convertino, VA, Doerr, DF*, et al.* 1992. Characteristics of the carotid baroreflex in man during normal and flow-restricted exercise. *Acta Physiol Scand,* 144**,** 325-331.

el-Mas, MM & Abdel-Rahman, AA 1998. Estrogen enhances baroreflex control of heart rate in conscious ovariectomized rats. *Can J Physiol Pharmacol,* 76**,** 381-386.

Eldridge, FL, Millhorn, DE, Kiley, JP, *et al.* 1985. Stimulation by central command of locomotion, respiration and circulation during exercise. *Respir Physiol,* 59**,** 313-337.

Eldridge, FL, Millhorn, DE & Waldrop, TG 1981. Exercise hyperpnea and locomotion: Parallel activation from the hypothalamus. *Science,* 211**,** 844-846.

Ellsworth, ML, Forrester, T, Ellis, CG, *et al.* 1995. The erythrocyte as a regulator of vascular tone. *Am J Physiol Heart Circ Physiol,* 269**,** H2155-H2161.

Ernsting, J & Parry, DJ. Some observations on the effects of stimulating the stretch receptors in the carotid artery of man. Proc Physiol Soc, 1957. J Physiol, 45-46.

Ettinger, SM, Silber, DH, Collins, BG, *et al.* 1996. Influences of gender on sympathetic nerve responses to static exercise. *J Appl Physiol,* 80**,** 245-251.

Ettinger, SM, Silber, DH, Gray, KS, *et al.* 1998. Effects of the ovarian cycle on sympathetic neural outflow during static exercise. *J Appl Physiol,* 85**,** 2075-2081.

Evans, JM, Ziegler, MG, Patwardhan, AR, *et al.* 2001. Gender differences in autonomic cardiovascular regulation: Spectral, hormonal, and hemodynamic indexes. *J Appl Physiol,* 91**,** 2611-2618.

Fadel, PJ, Ogoh, S, Keller, DM, *et al.* 2003a. Recent insights into carotid baroreflex function in humans using the variable pressure neck chamber. *Exp Physiol,* 88**,** 671-680.

Fadel, PJ, Ogoh, S, Watenpaugh, DE, *et al.* 2001. Carotid baroreflex regulation of sympathetic nerve activity during dynamic exercise in humans. *Am J Physiol Heart Circ Physiol,* 280**,** H1383-1390.

Fadel, PJ, Wang, Z & Thomas, GD 2004a. Exercise-induced sympathoexcitation is attenuated in healthy postmenopausal women by short-term estrogen replacement therapy. *The FASEB Journal,* 18.

Fadel, PJ, Wang, Z, Watanabe, H, *et al.* 2004b. Augmented sympathetic vasoconstriction in exercising forearms of postmenopausal women is reversed by oestrogen therapy. *J Physiol,* 561**,** 893-901.

Fadel, PJ, Wang, ZY, Tuncel, M, *et al.* 2003b. Reflex sympathetic activation during static exercise is severely impaired in patients with myophosphorylase deficiency. *J Physiol,* 548**,** 983-993.

Fadel, PJ, Zhao, W & Thomas, GD 2003c. Impaired vasomodulation is associated with reduced neuronal nitric oxide synthase in skeletal muscle of ovariectomized rats. *J Physiol,* 549**,** 243-253.

Fallentin, N, Jensen, BR, Bystrom, S, *et al.* 1992. Role of potassium in the reflex regulation of blood pressure during static exercise in man. *J Physiol,* 451**,** 643-651.

Fallentin, N, Sidenius, B & Jorgensen, K 1985. Blood pressure, heart rate and emg in low level static contractions. *Acta Physiol Scand,* 125**,** 265-275.

Fisher, JP, Ogoh, S, Ahmed, A, *et al.* 2007. Influence of age on cardiac baroreflex function during dynamic exercise in humans. *Am J Physiol Heart Circ Physiol,* 293**,** H777-783.

Fisher, JP, Ogoh, S, Dawson, EA, *et al.* 2006. Cardiac and vasomotor components of the carotid baroreflex control of arterial blood pressure during isometric exercise in humans. *J Physiol,* 572**,** 869-880.

Fisher, JP, Ogoh, S, Junor, C, *et al.* 2009. Spontaneous baroreflex measures are unable to detect age-related impairments in cardiac baroreflex function during dynamic exercise in humans. *Exp Physiol,* 94**,** 447-458.

Fisher, JP, Sander, M, MacDonald, I, *et al.* 2005. Decreased muscle sympathetic nerve activity does not explain increased vascular conductance during contralateral isometric exercise in humans. *Exp Physiol,* 90**,** 377-382.

Fisher, JP, Seifert, T, Hartwich, D, *et al.* 2010. Autonomic control of heart rate by metabolically sensitive skeletal muscle afferents in humans. *J Physiol,* 588**,** 1117-1127.

Fisher, JP, Young, CN & Fadel, PJ 2008. Effect of muscle metaboreflex activation on carotid-cardiac baroreflex function in humans. *Am J Physiol Heart Circ Physiol,* 294**,** H2296-2304.

Fleg, JL, O'Connor, F, Gerstenblith, G, *et al.* 1995. Impact of age on the cardiovascular response to dynamic upright exercise in healthy men and women. *J Appl Physiol,* 78**,** 890-900.

Ford, SP, Weber, LJ & Stormshak, F 1977. Role of estradiol-17 beta and progesterone in regulating constriction of ovine uterine arteries. *Biol Reprod,* 17**,** 480-483.

Freund, PR, Hobbs, SF & Rowell, LB 1978. Cardiovascular responses to muscle ischemia in man--dependency on muscle mass. *J Appl Physiol,* 45**,** 762-767.

Freyschuss, U 1970. Cardiovascular adjustment to somatomotor activation. The elicitation of increments in heart rate, aortic pressure and venomotor tone with the initiation of muscle contraction. *Acta Physiol Scand Suppl,* 342**,** 1-63.

Gallagher, KM, Fadel, PJ, Smith, SA, *et al.* 2001a. Increases in intramuscular pressure raise arterial blood pressure during dynamic exercise. *J Appl Physiol,* 91**,** 2351-2358.

Gallagher, KM, Fadel, PJ, Smith, SA, *et al.* 2006. The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in humans. *Exp Physiol,* 91**,** 79-87.

Gallagher, KM, Fadel, PJ, Stromstad, M, *et al.* 2001b. Effects of exercise pressor reflex activation on carotid baroreflex function during exercise in humans. *J Physiol,* 533**,** 871-880.

Gallagher, KM, Fadel, PJ, Stromstad, M, *et al.* 2001c. Effects of partial neuromuscular blockade on carotid baroreflex function during exercise in humans. *J Physiol,* 533**,** 861-870.

Gandevia, SC 2001. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev,* 81**,** 1725-1789.

Gandevia, SC, Macefield, VG, Bigland-Ritchie, B, *et al.* 1993. Motoneuronal output and gradation of effort in attempts to contract acutely paralysed leg muscles in man. *J Physiol,* 471**,** 411-427.

Gilligan, DM, Panza, JA, Kilcoyne, CM, *et al.* 1994. Contribution of endothelium-derived nitric-oxide to exercise-induced vasodilation. *Circulation,* 90**,** 2853-2858.

Gladwell, VF & Coote, JH 2002. Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: A role for mechanoreceptors. *J Physiol,* 540**,** 1095-1102.

Gladwell, VF, Fletcher, J, Patel, N, *et al.* 2005. The influence of small fibre muscle mechanoreceptors on the cardiac vagus in humans. *J Physiol,* 567**,** 713-721.

Gleim, GW, Stachenfeld, NS, Coplan, NL, *et al.* 1991. Gender differences in the systolic blood pressure response to exercise. *Am Heart J,* 121**,** 524-530.

Gonzales, JU, Thompson, BC, Thistlethwaite, JR, *et al.* 2007. Forearm blood flow follows work rate during submaximal dynamic forearm exercise independent of sex. *J Appl Physiol,* 103**,** 1950-1957.

Gonzalez-Alonso, J, Olsen, DB & Saltin, B 2002. Erythrocyte and the regulation of human skeletal muscle blood flow and oxygen delivery - role of circulating atp. *Circ Res,* 91**,** 1046-1055.

Goodwin, GM, McCloskey, DI & Mitchell, JH 1972. Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. *J Physiol,* 226**,** 173-190.

Gorski, J, Stankiewicz, B, Brycka, R, *et al.* 1976. The effect of estradiol on carbohydrate utilization during prolonged exercise in rats. *Acta Physiol Pol,* 27**,** 361-367.

Grassi, G, Seravalle, G, Cattaneo, BM, *et al.* 1995. Sympathetic activation and loss of reflex sympathetic control in mild congestive-heart-failure. *Circulation,* 92**,** 3206-3211.

Green, AL, Wang, S, Purvis, S, *et al.* 2007. Identifying cardiorespiratory neurocircuitry involved in central command during exercise in humans. *J Physiol,* 578**,** 605-612.

Green, JS, Stanforth, PR, Gagnon, J, *et al.* 2002. Menopause, estrogen, and training effects on exercise hemodynamics: The heritage study. *Med Sci Sports Exerc,* 34**,** 74-82.

Gross, PM, Marcus, ML & Heistad, DD 1980. Regional distribution of cerebral blood-flow during exercise in dogs. *J Appl Physiol,* 48**,** 213-217.

Gur, RC, Gur, RE, Obrist, WD, *et al.* 1982. Sex and handedness differences in cerebral blood-flow during rest and cognitive activity. *Science,* 217**,** 659-661.

Hanna, RL, Hayes, SG & Kaufman, MP 2002. Alpha,beta-methylene atp elicits a reflex pressor response arising from muscle in decerebrate cats. *J Appl Physiol,* 93**,** 834-841.

Hanna, RL & Kaufman, MP 2004. Activation of thin-fiber muscle afferents by a p2x agonist in cats. *J Appl Physiol,* 96**,** 1166-1169.

Hansen, J, Sander, M & Thomas, GD 2000. Metabolic modulation of sympathetic vasoconstriction in exercising skeletal muscle. *Acta Physiol Scand,* 168**,** 489-503.

Hansen, J, Thomas, GD, Harris, SA, *et al.* 1996. Differential sympathetic neural control of oxygenation in resting and exercising human skeletal muscle. *J Clin Invest,* 98**,** 584-596.

Hansen, J, Thomas, GD, Jacobsen, TN, *et al.* 1994. Muscle metaboreflex triggers parallel sympathetic activation in exercising and resting human skeletal muscle. *Am J Physiol,* 266**,** H2508-2514.

Haouzi, P, Hill, JM, Lewis, BK, *et al.* 1999. Responses of group iii and iv muscle afferents to distension of the peripheral vascular bed. *J Appl Physiol,* 87**,** 545-553.

Hardebo, JE & Owman, C 1980. Barrier mechanisms for neurotransmitter monoamines and their precursors at the blood-brain interface. *Ann Neurol,* 8**,** 1-31.

Harms, CA & Rosenkranz, S 2008. Sex differences in pulmonary function during exercise. *Med Sci Sports Exerc,* 40**,** 664-668.

Hart, EC, Charkoudian, N, Wallin, BG, *et al.* 2009. Sex differences in sympathetic neural-hemodynamic balance: Implications for human blood pressure regulation. *Hypertension,* 53**,** 571-576.

Hartwich, D, Fowler, KL, Wynn, LJ, *et al.* 2010. Differential responses to sympathetic stimulation in the cerebral and brachial circulations during rhythmic handgrip exercise in humans. *Exp Physiol,* 95**,** 1089-1097.

Hashimoto, M, Akishita, M, Eto, M, *et al.* 1995. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation,* 92**,** 3431-3435.

Hayes, SG, Kindig, AE & Kaufman, MP 2005. Comparison between the effect of static contraction and tendon stretch on the discharge of group iii and iv muscle afferents. *J Appl Physiol,* 99**,** 1891-1896.

Hayes, SG, Moya Del Pino, NB & Kaufman, MP 2002. Estrogen attenuates the cardiovascular and ventilatory responses to central command in cats. *J Appl Physiol,* 92**,** 1635-1641.

He, XR, Wang, W, Crofton, JT*, et al.* 1998. Effects of 17beta-estradiol on sympathetic activity and pressor response to phenylephrine in ovariectomized rats. *Am J Physiol,* 275**,** R1202-1208.

Heesch, CM & Foley, CM 2001. Cns effects of ovarian hormones and metabolites on neural control of circulation. *Ann N Y Acad Sci,* 940**,** 348-360.

Heesch, CM & Rogers, RC 1995. Effects of pregnancy and progesterone metabolites on regulation of sympathetic outflow. *Clin Exp Pharmacol Physiol,* 22**,** 136-142.

Henneman, E, Somjen, G & Carpenter, DO 1965. Excitability and inhibitability of motoneurons of different sizes. *J Neurophysiol,* 28**,** 599-620.

Higginbotham, MB, Morris, KG, Coleman, RE*, et al.* 1984. Sex-related differences in the normal cardiac response to upright exercise. *Circulation,* 70**,** 357-366.

Hillig, T, Krustrup, P, Fleming, I*, et al.* 2003. Cytochrome p450 2c9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. *J Physiol,* 546**,** 307-314.

Hines, EA & Brown, G 1933. A standard test measuring the variability of blood pressure. Its significance as an index of the prehypertensive state. *Ann Intern Med***,** 209-217.

Hnik, P, Holas, M, Krekule, I*, et al.* 1976. Work-induced potassium changes in skeletal-muscle and effluent venous-blood assessed by liquid ion-exchanger microelectrodes. *Pflugers Arch,* 362**,** 85-94.

Hobbs, SF & Gandevia, SC 1985. Cardiovascular responses and the sense of effort during attempts to contract paralysed muscles: Role of the spinal cord. *Neurosci Lett,* 57**,** 85-90.

Hogarth, AJ, Mackintosh, AF & Mary, DA 2007. Gender-related differences in the sympathetic vasoconstrictor drive of normal subjects. *Clin Sci,* 112**,** 353-361.

Hollander, AP & Bouman, LN 1975. Cardiac acceleration in man elicited by a muscle-heart reflex. *J Appl Physiol,* 38**,** 272-278.

Hossack, KF & Bruce, RA 1982. Maximal cardiac function in sedentary normal men and women: Comparison of age-related changes. *J Appl Physiol,* 53**,** 799-804.

Huang, A, Sun, D, Koller, A*, et al.* 1998. Gender difference in flow-induced dilation and regulation of shear stress: Role of estrogen and nitric oxide. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 275**,** R1571-R1577.

Huikuri, HV, Pikkujamsa, SM, Airaksinen, KE*, et al.* 1996. Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. *Circulation,* 94**,** 122-125.

Hultman, E & Sjoholm, H 1982. Blood pressure and heart rate response to voluntary and nonvoluntary static exercise in man. *Acta Physiol Scand,* 115**,** 499-501.

Humphreys, PW & Lind, AR 1963. The blood flow through active and inactive muscles of the forearm during sustained hand-grip contractions. *J Physiol,* 166**,** 120-135.

Hunt, BE, Taylor, JA, Hamner, JW*, et al.* 2001. Estrogen replacement therapy improves baroreflex regulation of vascular sympathetic outflow in postmenopausal women. *Circulation,* 103**,** 2909-2914.

Ichinose, M, Delliaux, S, Watanabe, K*, et al.* 2011. Evaluation of muscle metaboreflex function through graded reduction in forearm blood flow during rhythmic handgrip exercise in humans. *Am J Physiol Heart Circ Physiol,* 301**,** H609-616.

Ichinose, M, Saito, M, Fujii, N*, et al.* 2008. Modulation of the control of muscle sympathetic nerve activity during incremental leg cycling. *J Physiol,* 586**,** 2753-2766.

Ichinose, M, Saito, M, Kondo, N*, et al.* 2006. Time-dependent modulation of arterial baroreflex control of muscle sympathetic nerve activity during isometric exercise in humans. *Am J Physiol Heart Circ Physiol,* 290**,** H1419-1426.

Ichinose, M, Saito, M, Wada, H*, et al.* 2002. Modulation of arterial baroreflex dynamic response during muscle metaboreflex activation in humans. *J Physiol,* 544**,** 939-948.

Ide, K, Boushel, R, Sorensen, HM*, et al.* 2000. Middle cerebral artery blood velocity during exercise with beta-1 adrenergic and unilateral stellate ganglion blockade in humans. *Acta Physiol Scand,* 170**,** 33-38.

Ide, K, Pott, F, Van Lieshout, JJ*, et al.* 1998. Middle cerebral artery blood velocity depends on cardiac output during exercise with a large muscle mass. *Acta Physiol Scand,* 162**,** 13-20.

Iellamo, F, Di Rienzo, M, Lucini, D*, et al.* 2006. Muscle metaboreflex contribution to cardiovascular regulation during dynamic exercise in microgravity: Insights from mission sts-107 of the space shuttle columbia. *J Physiol,* 572**,** 829-838.

Iellamo, F, Legramante, JM, Raimondi, G*, et al.* 1997. Baroreflex control of sinus node during dynamic exercise in humans: Effects of central command and muscle reflexes. *Am J Physiol,* 272**,** H1157-1164.

Iellamo, F, Massaro, M, Legramante, JM*, et al.* 1998. Spontaneous baroreflex modulation of heart rate during incremental exercise test in humans. *The FASEB Journal,* 12**,** 4012.

Iellamo, F, Massaro, M, Raimondi, G*, et al.* 1999a. Role of muscular factors in cardiorespiratory responses to static exercise: Contribution of reflex mechanisms. *J Appl Physiol,* 86**,** 174-180.

Iellamo, F, Pizzinelli, P, Massaro, M*, et al.* 1999b. Muscle metaboreflex contribution to sinus node regulation during static exercise: Insights from spectral analysis of heart rate variability. *Circulation,* 100**,** 27-32.

Imholz, BP 1996. Automated blood pressure measurement during ergometric stress testing: Possibilities of finapres. *Z Kardiol,* 85 Suppl 3**,** 76-80.

Iwamoto, GA & Kaufman, MP 1987. Caudal ventrolateral medullary cells responsive to muscular contraction. *J Appl Physiol,* 62**,** 149-157.

Iwamoto, GA, Mitchell, JH, Mizuno, M*, et al.* 1987. Cardiovascular responses at the onset of exercise with partial neuromuscular blockade in cat and man. *J Physiol,* 384**,** 39-47.

Iwamoto, GA, Wappel, SM, Fox, GM*, et al.* 1996. Identification of diencephalic and brainstem cardiorespiratory areas activated during exercise. *Brain Res,* 726**,** 109-122.

Jacob, G, Costa, F, Shannon, J*, et al.* 2000. Dissociation between neural and vascular responses to sympathetic stimulation : Contribution of local adrenergic receptor function. *Hypertension,* 35**,** 76-81.

Jansen, JR, Schreuder, JJ, Mulier, JP*, et al.* 2001. A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *Br J Anaesth,* 87**,** 212-222.

Jarvis, SS, Vangundy, TB, Galbreath, MM*, et al.* 2011. Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans. *Am J Physiol Regul Integr Comp Physiol,* 301**,** R193-200.

Johansson, B 1962. Circulatory responses to stimulation of somatic afferents with special reference to depressor effects from muscle nerves. *Acta Physiol Scand Suppl,* 198**,** 1-91.

Johansson, JE 1893. Uber die einwirkung der muskelthatigkeit auf die athmung und die herzthatigkeit. *Scand Arch Physiol,* 5**,** 20-66.

Jones, PP, Davy, KP & Seals, DR 1999. Influence of gender on the sympathetic neural adjustments to alterations in systemic oxygen levels in humans. *Clin Physiol,* 19**,** 153-160.

Jones, PP, Spraul, M, Matt, KS*, et al.* 1996. Gender does not influence sympathetic neural reactivity to stress in healthy humans. *Am J Physiol,* 270**,** H350-357.

Jones, PR & Pearson, J 1969. Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *J Physiol,* 204**,** 63P-66P.

Joyner, MJ 1991. Does the pressor response to ischemic exercise improve blood flow to contracting muscles in humans? *J Appl Physiol,* 71**,** 1496-1501.

Joyner, MJ 2011. Giant sucking sound: Can physiology fill the intellectual void left by the reductionists? *J Appl Physiol,* 111**,** 335-342.

Joyner, MJ, Nauss, LA, Warner, MA*, et al.* 1992. Sympathetic modulation of blood flow and o2 uptake in rhythmically contracting human forearm muscles. *Am J Physiol,* 263**,** H1078-1083.

Jurkowski, JE, Jones, NL, Toews, CJ*, et al.* 1981. Effects of menstrual cycle on blood lactate, o2 delivery, and performance during exercise. *J Appl Physiol,* 51**,** 1493-1499.

Kamiya, A, Michikami, D, Fu, Q*, et al.* 2001. Static handgrip exercise modifies arterial baroreflex control of vascular sympathetic outflow in humans. *Am J Physiol Regul Integr Comp Physiol,* 281**,** R1134-1139.

Katona, PG & Jih, F 1975. Respiratory sinus arrhythmia: Noninvasive measure of parasympathetic cardiac control. *J Appl Physiol,* 39**,** 801-805.

Kaufman, MP, Iwamoto, GA, Longhurst, JC*, et al.* 1982. Effects of capsaicin and bradykinin on afferent-fibers with endings in skeletal-muscle. *Circ Res,* 50**,** 133-139.

Kaufman, MP, Longhurst, JC, Rybicki, KJ*, et al.* 1983. Effects of static muscular contraction on impulse activity of groups iii and iv afferents in cats. *J Appl Physiol,* 55**,** 105-112.

Kaufman, MP & Rybicki, KJ 1987. Discharge properties of group iii and iv muscle afferents: Their responses to mechanical and metabolic stimuli. *Circ Res,* 61**,** I60-65.

Kaufman, MP, Waldrop, TG, Rybicki, KJ*, et al.* 1984. Effects of static and rhythmic twitch contractions on the discharge of group iii and iv muscle afferents. *Cardiovasc Res,* 18**,** 663-668.

Keller, DM, Fadel, PJ, Ogoh, S*, et al.* 2004. Carotid baroreflex control of leg vasculature in exercising and non-exercising skeletal muscle in humans. *J Physiol,* 561**,** 283-293.

Keller, DM, Wasmund, WL, Wray, DW*, et al.* 2003. Carotid baroreflex control of leg vascular conductance at rest and during exercise. *J Appl Physiol,* 94**,** 542-548.

Kendrick, ZV & Ellis, GS 1991. Effect of estradiol on tissue glycogen metabolism and lipid availability in exercised male rats. *J Appl Physiol,* 71**,** 1694-1699.

Kent, BB, Drane, JW, Blumenst.B*, et al.* 1972. Mathematical-model to assess changes in baroreceptor reflex. *Cardiology,* 57**,** 295-310.

Kim, A, Deo, SH, Fisher, JP*, et al.* 2010. Alterations in carotid baroreflex control of arterial blood pressure during the menstrual cycle in young women. *The FASEB Journal,* 24**,** 10204.

Kim, A, Deo, SH, Vianna, LC*, et al.* 2011. Sex differences in carotid baroreflex control of arterial blood pressure in humans: Relative contribution of cardiac output and total vascular conductance. *Am J Physiol Heart Circ Physiol*.

Kim, JK, Sala-Mercado, JA, Rodriguez, J*, et al.* 2005. Arterial baroreflex alters strength and mechanisms of muscle metaboreflex during dynamic exercise. *Am J Physiol Heart Circ Physiol,* 288**,** H1374-1380.

Kingwell, BA, Thompson, JM, Kaye, DM*, et al.* 1994. Heart rate spectral analysis, cardiac norepinephrine spillover, and muscle sympathetic nerve activity during human sympathetic nervous activation and failure. *Circulation,* 90**,** 234-240.

Kirkham, FJ, Padayachee, TS, Parsons, S*, et al.* 1986. Transcranial measurement of blood velocities in the basal cerebral arteries using pulsed doppler ultrasound: Velocity as an index of flow. *Ultrasound Med Biol,* 12**,** 15-21.

Knaflitz, M, Merletti, R & De Luca, CJ 1990. Inference of motor unit recruitment order in voluntary and electrically elicited contractions. *J Appl Physiol,* 68**,** 1657-1667.

Kneale, BJ, Chowienczyk, PJ, Brett, SE*, et al.* 2000. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *J Am Coll Cardiol,* 36**,** 1233-1238.

Kneale, BJ, Chowienczyk, PJ, Cockcroft, JR*, et al.* 1997. Vasoconstrictor sensitivity to noradrenaline and ng-monomethyl-l-arginine in men and women. *Clin Sci,* 93**,** 513-518.

Koch, DW, Newcomer, SC & Proctor, DN 2005. Blood flow to exercising limbs varies with age, gender, and training status. *Can J Appl Physiol,* 30**,** 554-575.

Koizumi, K, Ushiyama, J & Brooks, CM 1961. Muscle afferents and activity of respiratory neurons. *Am J Physiol,* 200**,** 679-684.

Komi, PV & Karlsson, J 1978. Skeletal muscle fibre types, enzyme activities and physical performance in young males and females. *Acta Physiol Scand,* 103**,** 210-218.

Kozelka, JW, Christy, GW & Wurster, RD 1987. Ascending pathways mediating somatoautonomic reflexes in exercising dogs. *J Appl Physiol,* 62**,** 1186-1191.

Krause, DN, Duckles, SP & Pelligrino, DA 2006. Influence of sex steroid hormones on cerebrovascular function. *J Appl Physiol,* 101**,** 1252-1261.

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

Krogh, A & Lindhard, J 1917. A comparison between voluntary and electrically induced muscular work in man. *J Physiol,* 51**,** 182-201.

Kucher, N, Lipp, E, Schwerzmann, M*, et al.* 2001. Gender differences in coronary artery size per 100 g of left ventricular mass in a population without cardiac disease. *Swiss Med Wkly,* 131**,** 610-615.

Kuo, TB, Lin, T, Yang, CC*, et al.* 1999. Effect of aging on gender differences in neural control of heart rate. *Am J Physiol,* 277**,** H2233-2239.

Kuwahara, T, Inoue, Y, Abe, M*, et al.* 2005. Effects of menstrual cycle and physical training on heat loss responses during dynamic exercise at moderate intensity in a temperate environment. *Am J Physiol Regul Integr Comp Physiol,* 288**,** R1347-R1353.

Laitinen, T, Hartikainen, J, Vanninen, E*, et al.* 1998. Age and gender dependency of baroreflex sensitivity in healthy subjects. *J Appl Physiol,* 84**,** 576-583.

Lambert, E, Straznicky, N, Eikelis, N*, et al.* 2007. Gender differences in sympathetic nervous activity: Influence of body mass and blood pressure. *J Hypertens,* 25**,** 1411-1419.

Laprad, SL, Augustyniak, RA, Hammond, RL*, et al.* 1999. Does gender influence the strength and mechanisms of the muscle metaboreflex during dynamic exercise in dogs? *Am J Physiol,* 276**,** R1203-1208.

Leonard, B, Mitchell, JH, Mizuno, M*, et al.* 1985. Partial neuromuscular blockade and cardiovascular-responses to static exercise in man. *J Physiol,* 359**,** 365-379.

Lerner, DJ & Kannel, WB 1986. Patterns of coronary heart disease morbidity and mortality in the sexes: A 26-year follow-up of the framingham population. *Am Heart J,* 111**,** 383-390.

Levine, BD & Zhang, R 2008. Comments on point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Autonomic control of the cerebral circulation is most important for dynamic cerebral autoregulation. *J Appl Physiol,* 105**,** 1369-1373.

Lewandowski, J, Pruszczyk, P, Elaffi, M*, et al.* 1998. Blood pressure, plasma npy and catecholamines during physical exercise in relation to menstrual cycle, ovariectomy, and estrogen replacement. *Regul Pept,* 75-76**,** 239-245.

Limberg, JK, Eldridge, MW, Proctor, LT*, et al.* 2010. Alpha-adrenergic control of blood flow during exercise: Effect of sex and menstrual phase. *J Appl Physiol,* 109**,** 1360-1368.

Lind, AR & McNicol, GW 1967. Muscular factors which determine the cardiovascular responses to sustained and rhythmic exercise. *Can Med Assoc J,* 96**,** 706-715.

Linkis, P, Jorgensen, LG, Olesen, HL*, et al.* 1995. Dynamic exercise enhances regional cerebral artery mean flow velocity. *J Appl Physiol,* 78**,** 12-16.

Liu, CC, Kuo, TB & Yang, CC 2003. Effects of estrogen on gender-related autonomic differences in humans. *Am J Physiol Heart Circ Physiol,* 285**,** H2188-2193.

Low, DA, Wingo, JE, Keller, DM*, et al.* 2009. Dynamic cerebral autoregulation during passive heat stress in humans. *Am J Physiol Regul Integr Comp Physiol,* 296**,** R1598-1605.

Lowe, RF & Gilboe, DD 1971. Demonstration of alpha and beta adrenergic receptors in canine cerebral vasculature. *Stroke,* 2**,** 193-200.

Lucas, SJ, Tzeng, YC, Galvin, SD*, et al.* 2010. Influence of changes in blood pressure on cerebral perfusion and oxygenation. *Hypertension,* 55**,** 698-705.

Ludbrook, J, Mancia, G, Ferrari, A*, et al.* 1977. The variable-pressure neck-chamber method for studying the carotid baroreflex in man. *Clin Sci Mol Med,* 53**,** 165-171.

Luzier, AB, Nawarskas, JJ, Anonuevo, J*, et al.* 1998. The effects of gender on adrenergic receptor responsiveness. *J Clin Pharmacol,* 38**,** 618-624.

Malliani, A, Pagani, M, Lombardi, F*, et al.* 1991. Cardiovascular neural regulation explored in the frequency domain. *Circulation,* 84**,** 482-492.

Mark, AL, Victor, RG, Nerhed, C*, et al.* 1985. Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circ Res,* 57**,** 461-469.

Martin, WH, 3rd, Ogawa, T, Kohrt, WM*, et al.* 1991. Effects of aging, gender, and physical training on peripheral vascular function. *Circulation,* 84**,** 654-664.

McClain, J, Hardy, C, Enders, B*, et al.* 1993. Limb congestion and sympathoexcitation during exercise. Implications for congestive heart failure. *J Clin Invest,* 92**,** 2353-2359.

McCloskey, DI & Mitchell, JH 1972. The use of differential nerve blocking techniques to show that the cardiovascular and respiratory reflexes originating in exercising muscle are not mediated by large myelinated afferents. *J Physiol,* 222**,** 50P-51P.

McCord, JL & Kaufman, MP 2010. Reflex autonomic responses evoked by group iii and iv muscle afferents. *In:* Kruger, L. & Light, A. R. (eds.) *Translational pain research: From mouse to man. Chapter 12.* Boca Raton, FL: CRC Press.

McIlveen, SA, Hayes, SG & Kaufman, MP 2001. Both central command and exercise pressor reflex reset carotid sinus baroreflex. *Am J Physiol Heart Circ Physiol,* 280**,** H1454-1463.

McMahon, SE & McWilliam, PN 1992. Changes in r-r interval at the start of muscle contraction in the decerebrate cat. *J Physiol,* 447**,** 549-562.

McWilliam, PN & Yang, T 1991. Inhibition of cardiac vagal component of baroreflex by group iii and iv afferents. *Am J Physiol,* 260**,** H730-734.

Mense, S & Stahnke, M 1983. Responses in muscle afferent fibres of slow conduction velocity to contractions and ischaemia in the cat. *J Physiol,* 342**,** 383-397.

Micieli, G, Tassorelli, C, Bosone, D*, et al.* 1994. Intracerebral vascular changes induced by cold pressor test: A model of sympathetic activation. *Neurol Res,* 16**,** 163-167.

Middlekauff, HR & Chiu, J 2004. Cyclooxygenase products sensitize muscle mechanoreceptors in healthy humans. *Am J Physiol Heart Circ Physiol,* 287**,** H1944-H1949.

Minson, CT, Halliwill, JR, Young, TM*, et al.* 2000a. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation,* 101**,** 862-868.

Minson, CT, Halliwill, JR, Young, TM*, et al.* 2000b. Sympathetic activity and baroreflex sensitivity in young women taking oral contraceptives. *Circulation,* 102**,** 1473-1476.

Misteli, M, Duschek, S, Richter, A*, et al.* 2011. Gender characteristics of cerebral hemodynamics during complex cognitive functioning. *Brain Cogn,* 76**,** 123-130.

Mitchell, JH 1990. Neural control of the circulation during exercise. *Med Sci Sports Exerc,* 22**,** 141-154.

Mitchell, JH, Payne, FC, Saltin, B*, et al.* 1980. The role of muscle mass in the cardiovascular response to static contractions. *J Physiol,* 309**,** 45-54.

Mitchell, JH, Reeves, DR, Jr., Rogers, HB*, et al.* 1989a. Epidural anaesthesia and cardiovascular responses to static exercise in man. *J Physiol,* 417**,** 13-24.

Mitchell, JH, Reeves, DR, Jr., Rogers, HB*, et al.* 1989b. Autonomic blockade and cardiovascular responses to static exercise in partially curarized man. *J Physiol,* 413**,** 433-445.

Mitchell, JH, Schibye, B, Payne, FC, 3rd*, et al.* 1981. Response of arterial blood pressure to static exercise in relation to muscle mass, force development, and electromyographic activity. *Circ Res,* 48**,** I70-75.

Miyamoto, T, Kawada, T, Takaki, H*, et al.* 2003. High plasma norepinephrine attenuates the dynamic heart rate response to vagal stimulation. *Am J Physiol Heart Circ Physiol,* 284**,** H2412-2418.

Monahan, KD & Ray, CA 2004. Gender affects calf venous compliance at rest and during baroreceptor unloading in humans. *Am J Physiol Heart Circ Physiol,* 286**,** H895-901.

Mortensen, SP, Gonzalez-Alonso, J, Damsgaard, R*, et al.* 2007. Inhibition of nitric oxide and prostaglandins, but not endothelial-derived hyperpolarizing factors, reduces blood flow and aerobic energy turnover in the exercising human leg. *J Physiol,* 581**,** 853-861.

Mortensen, SP, Nyberg, M, Thaning, P*, et al.* 2009. Adenosine contributes to blood flow regulation in the exercising human leg by increasing prostaglandin and nitric oxide formation. *Hypertension,* 53**,** 993-U177.

Muzi, M, Goff, DR, Kampine, JP*, et al.* 1992. Clonidine reduces sympathetic activity but maintains baroreflex responses in normotensive humans. *Anesthesiology,* 77**,** 864-871.

Ng, AV, Callister, R, Johnson, DG*, et al.* 1993. Age and gender influence muscle sympathetic-nerve activity at rest in healthy humans. *Hypertension,* 21**,** 498-503.

Nielsen, KC & Owman, C 1967. Adrenergic innervation of pial arteries related to the cirele of willis in the cat [fluorescent microscopy]. *Brain Res,* 6**,** 773-776.

Nielsen, MS, Barton, SD, Hatasaka, HH*, et al.* 2001. Comparison of several one-step home urinary luteinizing hormone detection test kits to ovuquick. *Fertil Steril,* 76**,** 384-387.

Nobrega, AC & Araujo, CG 1993. Heart rate transient at the onset of active and passive dynamic exercise. *Med Sci Sports Exerc,* 25**,** 37-41.

Norton, KH, Boushel, R, Strange, S*, et al.* 1999. Resetting of the carotid arterial baroreflex during dynamic exercise in humans. *J Appl Physiol,* 87**,** 332-338.

Nowak, M, Olsen, KS, Law, I*, et al.* 1999. Command-related distribution of regional cerebral blood flow during attempted handgrip. *J Appl Physiol,* 86**,** 819-824.

O'Leary, DS 1993. Autonomic mechanisms of muscle metaboreflex control of heart rate. *J Appl Physiol,* 74**,** 1748-1754.

O'Leary, DS, Robinson, ED & Butler, JL 1997. Is active skeletal muscle functionally vasoconstricted during dynamic exercise in conscious dogs? *Am J Physiol,* 272**,** R386-391.

O'Leary, DS, Sala-Mercado, JA, Hammond, RL*, et al.* 2007. Muscle metaboreflex-induced increases in cardiac sympathetic activity vasoconstrict the coronary vasculature. *J Appl Physiol,* 103**,** 190-194.

Ogawa, T, Spina, RJ, Martin, WH, 3rd*, et al.* 1992. Effects of aging, sex, and physical training on cardiovascular responses to exercise. *Circulation,* 86**,** 494-503.

Ogoh, S & Ainslie, PN 2009. Cerebral blood flow during exercise: Mechanisms of regulation. *J Appl Physiol,* 107**,** 1370-1380.

Ogoh, S, Brothers, RM, Barnes, Q*, et al.* 2005a. The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol,* 569**,** 697-704.

Ogoh, S, Fadel, PJ, Hardisty, JM*, et al.* 2003a. Does pulsatile and sustained neck pressure or neck suction produce differential cardiovascular and sympathetic responses in humans? *Exp Physiol,* 88**,** 595-601.

Ogoh, S, Fadel, PJ, Monteiro, F*, et al.* 2002a. Haemodynamic changes during neck pressure and suction in seated and supine positions. *J Physiol,* 540**,** 707-716.

Ogoh, S, Fadel, PJ, Nissen, P*, et al.* 2003b. Baroreflex-mediated changes in cardiac output and vascular conductance in response to alterations in carotid sinus pressure during exercise in humans. *J Physiol,* 550**,** 317-324.

Ogoh, S, Fisher, JP, Dawson, EA*, et al.* 2005b. Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans. *J Physiol,* 566**,** 599-611.

Ogoh, S, Fisher, JP, Raven, PB*, et al.* 2007. Arterial baroreflex control of muscle sympathetic nerve activity in the transition from rest to steady-state dynamic exercise in humans. *Am J Physiol Heart Circ Physiol,* 293**,** H2202-2209.

Ogoh, S, Fisher, JP, Young, CN, *et al.* 2009. Transfer function characteristics of the neural and peripheral arterial baroreflex arcs at rest and during postexercise muscle ischemia in humans. *Am J Physiol Heart Circ Physiol,* 296**,** H1416-1424.

Ogoh, S, Wasmund, WL, Keller, DM, *et al.* 2002b. Role of central command in carotid baroreflex resetting in humans during static exercise. *J Physiol,* 543**,** 349-364.

Olesen, J 1972. The effect of intracarotid epinephrine, norepinephrine, and angiotensin on the regional cerebral blood flow in man. *Neurology,* 22**,** 978-987.

Olivetti, G, Giordano, G, Corradi, D, *et al.* 1995. Gender differences and aging: Effects on the human heart. *J Am Coll Cardiol,* 26**,** 1068-1079.

Oppenheimer, SM & Cechetto, DF 1990. Cardiac chronotropic organization of the rat insular cortex. *Brain Res,* 533**,** 66-72.

Pagani, M, Montano, N, Porta, A, *et al.* 1997. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation,* 95**,** 1441-1448.

Paintal, AS 1960. Functional analysis of group iii afferent fibres of mammalian muscles. *J Physiol,* 152**,** 250-270.

Papelier, Y, Escourrou, P, Gauthier, JP, *et al.* 1994. Carotid baroreflex control of blood pressure and heart rate in men during dynamic exercise. *J Appl Physiol,* 77**,** 502-506.

Papelier, Y, Escourrou, P, Helloco, F, *et al.* 1997. Muscle chemoreflex alters carotid sinus baroreflex response in humans. *J Appl Physiol,* 82**,** 577-583.

Parati, G, Di Rienzo, M & Mancia, G 2000. How to measure baroreflex sensitivity: From the cardiovascular laboratory to daily life. *J Hypertens,* 18**,** 7-19.

Parker, BA, Smithmyer, SL, Pelberg, JA, *et al.* 2007. Sex differences in leg vasodilation during graded knee extensor exercise in young adults. *J Appl Physiol,* 103**,** 1583-1591.

Parker, P, Celler, BG, Potter, EK, *et al.* 1984. Vagal-stimulation and cardiac slowing. *J Auton Nerv Syst,* 11**,** 226-231.

Parkes, LM, Rashid, W, Chard, DT, *et al.* 2004. Normal cerebral perfusion measurements using arterial spin labeling: Reproducibility, stability, and age and gender effects. *Magn Reson Med,* 51**,** 736-743.

Parlow, J, Viale, JP, Annat, G, *et al.* 1995. Spontaneous cardiac baroreflex in humans - comparison with drug-induced responses. *Hypertension,* 25**,** 1058-1068.

Petrofsky, JS, LeDonne, DM, Rinehart, JS, *et al.* 1976. Isometric strength and endurance during the menstrual cycle. *Eur J Appl Physiol Occup Physiol,* 35**,** 1-10.

Petrofsky, JS & Lind, AR 1975. Isometric strength, endurance, and the blood pressure and heart rate responses during isometric exercise in healthy men and women, with special reference to age and body fat content. *Pflugers Arch,* 360**,** 49-61.

Petrofsky, JS, Phillips, CA, Sawka, MN, *et al.* 1981. Muscle fiber recruitment and blood pressure response to isometric exercise. *J Appl Physiol,* 50**,** 32-37.

Pfaff, D & Keiner, M 1973. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *J Comp Neurol,* 151**,** 121-158.

Pickering, TG, Gribbin, B, Petersen, ES, *et al.* 1972. Effects of autonomic blockade on the baroreflex in man at rest and during exercise. *Circ Res,* 30**,** 177-185.

Piepoli, M, Clark, AL, Volterrani, M, *et al.* 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.

Pikkujamsa, SM, Makikallio, TH, Airaksinen, KE, *et al.* 2001. Determinants and interindividual variation of r-r interval dynamics in healthy middle-aged subjects. *Am J Physiol Heart Circ Physiol,* 280**,** H1400-1406.

Pines, A, Fisman, EZ, Drory, Y, *et al.* 1998. The effects of sublingual estradiol on left ventricular function at rest and exercise in postmenopausal women: An echocardiographic assessment. *Menopause,* 5**,** 79-85.

Pomeranz, B, Macaulay, RJ, Caudill, MA, *et al.* 1985. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol,* 248**,** H151-153.

Pomeroy, G, Ardell, JL & Wurster, RD 1986. Spinal opiate modulation of cardiovascular reflexes in the exercising dog. *Brain Res,* 381**,** 385-389.

Pott, F, Ray, CA, Olesen, HL, *et al.* 1997. Middle cerebral artery blood velocity, arterial diameter and muscle sympathetic nerve activity during post-exercise muscle ischaemia. *Acta Physiol Scand,* 160**,** 43-47.

Potts, JT 2006. Inhibitory neurotransmission in the nucleus tractus solitarii: Implications for baroreflex resetting during exercise. *Exp Physiol,* 91**,** 59-72.

Potts, JT & Mitchell, JH 1998. Rapid resetting of carotid baroreceptor reflex by afferent input from skeletal muscle receptors. *Am J Physiol,* 275**,** H2000-2008.

Potts, JT, Shi, XR & Raven, PB 1993. Carotid baroreflex responsiveness during dynamic exercise in humans. *Am J Physiol,* 265**,** H1928-1938.

Proctor, DN, Koch, DW, Newcomer, SC*, et al.* 2004. Leg blood flow and vo2 during peak cycle exercise in younger and older women. *Med Sci Sports Exerc,* 36**,** 623-631.

Pryor, SL, Lewis, SF, Haller, RG*, et al.* 1990. Impairment of sympathetic activation during static exercise in patients with muscle phosphorylase deficiency (mcardle's disease). *J Clin Invest,* 85**,** 1444-1449.

Querido, JS & Sheel, AW 2007. Regulation of cerebral blood flow during exercise. *Sports Med,* 37**,** 765-782.

Querry, RG, Smith, SA, Stromstad, M*, et al.* 2001. Neural blockade during exercise augments central command's contribution to carotid baroreflex resetting. *Am J Physiol Heart Circ Physiol,* 280**,** H1635-1644.

Rådegran, G 1997. Ultrasound doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *J Appl Physiol,* 83**,** 1383-1388.

Radegran, G & Calbet, JAL 2001. Role of adenosine in exercise-induced human skeletal muscle vasodilatation. *Acta Physiol Scand,* 171**,** 177-185.

Ramaekers, D, Ector, H, Aubert, AE*, et al.* 1998. Heart rate variability and heart rate in healthy volunteers. Is the female autonomic nervous system cardioprotective? *Eur Heart J,* 19**,** 1334-1341.

Raven, PB, Fadel, PJ & Ogoh, S 2006. Arterial baroreflex resetting during exercise: A current perspective. *Exp Physiol,* 91**,** 37-49.

Raven, PB, Fadel, PJ & Smith, SA 2002. The influence of central command on baroreflex resetting during exercise. *Exerc Sport Sci Rev,* 30**,** 39-44.

Remensnyder, JP, Mitchell, JH & Sarnoff, SJ 1962. Functional sympatholysis during muscular activity - observations on influence of carotid sinus on oxygen uptake. *Circ Res,* 11**,** 370-380.

Roatta, S, Micieli, G, Bosone, D*, et al.* 1998. Effect of generalised sympathetic activation by cold pressor test on cerebral haemodynamics in healthy humans. *J Auton Nerv Syst,* 71**,** 159-166.

Roberts, CS & Roberts, WC 1980. Cross-sectional area of the proximal portions of the three major epicardial coronary arteries in 98 necropsy patients with different coronary events. Relationship to heart weight, age and sex. *Circulation,* 62**,** 953-959.

Robinson, BF, Epstein, SE, Beiser, GD*, et al.* 1966. Control of heart rate by autonomic nervous system - studies in man on interrelation between baroreceptor mechanisms and exercise. *Circ Res,* 19**,** 400-411.

Rodriguez, G, Warkentin, S, Risberg, J*, et al.* 1988. Sex-differences in regional cerebral blood-flow. *J Cereb Blood Flow Metab,* 8**,** 783-789.

Rogers, J & Sheriff, DD 2004. Role of estrogen in nitric oxide- and prostaglandin-dependent modulation of vascular conductance during treadmill locomotion in rats. *J Appl Physiol,* 97**,** 756-763.

Rosenmeier, JB, Dinenno, FA, Fritzlar, SJ*, et al.* 2003. Alpha(1)- and alpha(2)-adrenergic vasoconstriction is blunted in contracting human muscle. *J Physiol,* 547**,** 971-976.

Rotto, DM & Kaufman, MP 1988. Effect of metabolic products of muscular contraction on discharge of group iii and iv afferents. *J Appl Physiol,* 64**,** 2306-2313.

Rotto, DM, Massey, KD, Burton, KP*, et al.* 1989. Static contraction increases arachidonic acid levels in gastrocnemius muscles of cats. *J Appl Physiol,* 66**,** 2721-2724.

Rowell, LB 1993a. Control of regional blood flow during dynamic exercise. *Human cardiovascular control 6.* New York: Oxford University Press.

Rowell, LB 1993b. What signals govern the cardiovascular responses to exercise? Reflexes from active muscles. *Human cardiovascular control 11.* New York: Oxford University Press.

Rowell, LB, Savage, MV, Chambers, J*, et al.* 1991. Cardiovascular responses to graded reductions in leg perfusion in exercising humans. *Am J Physiol,* 261**,** H1545-1553.

Rushmer, RF & Smith, OA, Jr. 1959. Cardiac control. *Physiol Rev,* 39**,** 41-68.

Ryan, SM, Goldberger, AL, Pincus, SM*, et al.* 1994. Gender-related and age-related differences in heart-rate dynamics - are women more complex than men. *J Am Coll Cardiol,* 24**,** 1700-1707.

Rybicki, KJ, Kaufman, MP, Kenyon, JL*, et al.* 1984. Arterial pressure responses to increasing interstitial potassium in hindlimb muscle of dogs. *Am J Physiol,* 247**,** R717-721.

Sala-Mercado, JA, Ichinose, M, Coutsos, M*, et al.* 2010. Progressive muscle metaboreflex activation gradually decreases spontaneous heart rate baroreflex sensitivity during dynamic exercise. *Am J Physiol Heart Circ Physiol,* 298**,** H594-600.

Sala-Mercado, JA, Ichinose, M, Hammond, RL*, et al.* 2007. Muscle metaboreflex attenuates spontaneous heart rate baroreflex sensitivity during dynamic exercise. *Am J Physiol Heart Circ Physiol,* 292**,** H2867-2873.

Saleh, MC, Connell, BJ & Saleh, TM 2000a. Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. *Brain Res,* 879**,** 105-114.

Saleh, MC, Connell, BJ & Saleh, TM 2000b. Medullary and intrathecal injections of 17 beta-estradiol in male rats. *Brain Res,* 867**,** 200-209.

Saleh, TM & Connell, BJ 2000. 17beta-estradiol modulates baroreflex sensitivity and autonomic tone of female rats. *J Auton Nerv Syst,* 80**,** 148-161.

Saleh, TM, Connell, BJ & Saleh, MC 2000c. Acute injection of 17beta-estradiol enhances cardiovascular reflexes and autonomic tone in ovariectomized female rats. *Auton Neurosci,* 84**,** 78-88.

Saltin, B & Gollnick, PD 1983. Skeletal muscle adaptibility:Significance for metabolism and performance. *In:* LD, P. (ed.) *Handbook of physiology. 19.* Bethesda, MD, USA: American Physiological Society.

Saltin, B, Radegran, G, Koskolou, MD*, et al.* 1998. Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol Scand,* 162**,** 421-436.

Sato, K, Ogoh, S, Hirasawa, A*, et al.* 2011. The distribution of blood flow in the carotid and vertebral arteries during dynamic exercise in humans. *J Physiol,* 589**,** 2847-2856.

Saul, JP, Berger, RD, Albrecht, P*, et al.* 1991. Transfer function analysis of the circulation: Unique insights into cardiovascular regulation. *Am J Physiol,* 261**,** H1231-1245.

Saul, JP, Rea, RF, Eckberg, DL*, et al.* 1990. Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. *Am J Physiol,* 258**,** H713-721.

Schenck-Gustafsson, K 1996. Risk factors for cardiovascular disease in women: Assessment and management. *Eur Heart J,* 17 Suppl D**,** 2-8.

Scherrer, U, Pryor, SL, Bertocci, LA*, et al.* 1990. Arterial baroreflex buffering of sympathetic activation during exercise-induced elevations in arterial pressure. *J Clin Invest,* 86**,** 1855-1861.

Schmitt, JA, Joyner, MJ, Charkoudian, N*, et al.* 2010. Sex differences in alpha-adrenergic support of blood pressure. *Clin Auton Res,* 20**,** 271-275.

Schmitt, PM & Kaufman, MP 2003a. Estrogen attenuates the exercise pressor reflex in female cats. *J Appl Physiol,* 95**,** 1418-1424.

Schmitt, PM & Kaufman, MP 2003b. High concentrations of 17beta -estradiol attenuate the exercise pressor reflex in male cats. *J Appl Physiol,* 94**,** 1431-1436.

Schrage, WG, Joyner, MJ & Dinenno, FA 2004. Local inhibition of nitric oxide and prostaglandins independently reduces forearm exercise hyperaemia in humans. *J Physiol,* 557**,** 599-611.

Schrage, WG, Wilkins, BW, Dean, VL*, et al.* 2005. Exercise hyperemia and vasoconstrictor responses in humans with cystic fibrosis. *J Appl Physiol,* 99**,** 1866-1871.

Scott, AC, Davies, LC, Coats, AJS*, et al.* 2002. Relationship of skeletal muscle metaboreceptors in the upper and lower limbs with the respiratory control in patients with heart failure. *Clin Sci,* 102**,** 23-30.

Seals, DR 1989a. Influence of muscle mass on sympathetic neural activation during isometric exercise. *J Appl Physiol,* 67**,** 1801-1806.

Seals, DR 1989b. Sympathetic neural discharge and vascular resistance during exercise in humans. *J Appl Physiol,* 66**,** 2472-2478.

Secher, NH 1985. Heart rate at the onset of static exercise in man with partial neuromuscular blockade. *J Physiol,* 368**,** 481-490.

Secher, NH, Seifert, T & Van Lieshout, JJ 2008. Cerebral blood flow and metabolism during exercise: Implications for fatigue. *J Appl Physiol,* 104**,** 306-314.

Sevre, K, Lefrandt, JD, Nordby, G*, et al.* 2001. Autonomic function in hypertensive and normotensive subjects: The importance of gender. *Hypertension,* 37**,** 1351-1356.

Sheriff, DD, O'Leary, DS, Scher, AM*, et al.* 1990. Baroreflex attenuates pressor response to graded muscle ischemia in exercising dogs. *Am J Physiol,* 258**,** H305-310.

Sheriff, DD, Rowell, LB & Scher, AM 1993. Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *Am J Physiol,* 265**,** H1227-1234.

Sheriff, DD, Wyss, CR, Rowell, LB*, et al.* 1987. Does inadequate oxygen delivery trigger pressor response to muscle hypoperfusion during exercise? *Am J Physiol,* 253**,** H1199-1207.

Shoemaker, JK, Halliwill, JR, Hughson, RL*, et al.* 1997. Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery. *Am J Physiol Heart Circ Physiol,* 273**,** H2388-H2395.

Shoemaker, JK, Hogeman, CS, Khan, M*, et al.* 2001. Gender affects sympathetic and hemodynamic response to postural stress. *Am J Physiol Heart Circ Physiol,* 281**,** H2028-H2035.

Simoneau, JA & Bouchard, C 1989. Human variation in skeletal muscle fiber-type proportion and enzyme activities. *Am J Physiol,* 257**,** E567-572.

Sinnreich, R, Kark, JD, Friedlander, Y*, et al.* 1998. Five minute recordings of heart rate variability for population studies: Repeatability and age-sex characteristics. *Heart,* 80**,** 156-162.

Sinoway, LI, Smith, MB, Enders, B*, et al.* 1994. Role of diprotonated phosphate in evoking muscle-reflex responses in cats and humans. *Am J Physiol,* 267**,** H770-H778.

Slopien, R, Junik, R, Meczekalski, B*, et al.* 2003. Influence of hormonal replacement therapy on the regional cerebral blood flow in postmenopausal women. *Maturitas,* 46**,** 255-262.

Smith, SA, Mitchell, JH & Garry, MG 2006. The mammalian exercise pressor reflex in health and disease. *Exp Physiol,* 91**,** 89-102.

Smith, SA, Querry, RG, Fadel, PJ*, et al.* 2003. Partial blockade of skeletal muscle somatosensory afferents attenuates baroreflex resetting during exercise in humans. *J Physiol,* 551**,** 1013-1021.

Spaak, J, Sundblad, P & Linnarsson, D 1998. Human carotid baroreflex during isometric lower arm contraction and ischemia. *Am J Physiol Heart Circ Physiol,* 275**,** H940-H945.

Spadacini, G, Passino, C, Leuzzi, S*, et al.* 2006. Frequency-dependent baroreflex control of blood pressure and heart rate during physical exercise. *Int J Cardiol,* 107**,** 171-179.

Spary, EJ, Maqbool, A & Batten, TFC 2009. Oestrogen receptors in the central nervous system and evidence for their role in the control of cardiovascular function. *J Chem Neuroanat,* 38**,** 185-196.

Stachenfeld, NS 2008. Sex hormone effects on body fluid regulation. *Exerc Sport Sci Rev,* 36**,** 152-159.

Stachenfeld, NS, Taylor, HS, Leone, CA*, et al.* 2003. Oestrogen effects on urine concentrating response in young women. *J Physiol,* 552**,** 869-880.

Stebbins, CL, Brown, B, Levin, D*, et al.* 1988. Reflex effect of skeletal muscle mechanoreceptor stimulation on the cardiovascular system. *J Appl Physiol,* 65**,** 1539-1547.

Stebbins, CL & Longhurst, JC 1986. Bradykinin in reflex cardiovascular-responses to static muscular-contraction. *J Appl Physiol,* 61**,** 271-279.

Stoney, CM, Davis, MC & Matthews, KA 1987. Sex differences in physiological responses to stress and in coronary heart disease: A causal link? *Psychophysiology,* 24**,** 127-131.

Strange, S, Secher, NH, Pawelczyk, JA*, et al.* 1993. Neural control of cardiovascular responses and of ventilation during dynamic exercise in man. *J Physiol,* 470**,** 693-704.

Sudhir, K, Elser, MD, Jennings, GL*, et al.* 1997. Estrogen supplementation decreases norepinephrine-induced vasoconstriction and total body norepinephrine spillover in perimenopausal women. *Hypertension,* 30**,** 1538-1543.

Sudhir, K, Jennings, GL, Funder, JW*, et al.* 1996. Estrogen enhances basal nitric oxide release in the forearm vasculature in perimenopausal women. *Hypertension,* 28**,** 330-334.

Sullivan, MJ, Cobb, FR & Higginbotham, MB 1991. Stroke volume increases by similar mechanisms during upright exercise in normal men and women. *Am J Cardiol,* 67**,** 1405-1412.

Sun, JC, Eiken, O & Mekjavic, IB 1993. Autonomic nervous control of heart rate during blood-flow restricted exercise in man. *Eur J Appl Physiol Occup Physiol,* 66**,** 202-206.

Sundberg, CJ & Kaijser, L 1992. Effects of graded restriction of perfusion on circulation and metabolism in the working leg; quantification of a human ischaemia-model. *Acta Physiol Scand,* 146**,** 1-9.

Tanaka, M, Sato, M, Umehara, S*, et al.* 2003. Influence of menstrual cycle on baroreflex control of heart rate: Comparison with male volunteers. *Am J Physiol Regul Integr Comp Physiol,* 285**,** R1091-1097.

Tank, J, Diedrich, A, Szczech, E*, et al.* 2005. Baroreflex regulation of heart rate and sympathetic vasomotor tone in women and men. *Hypertension,* 45**,** 1159-1164.

Task, F 1996. Heart rate variability: Standards of measurement, physiological interpretation and clinical use. Task force of the european society of cardiology and the north american society of pacing and electrophysiology. *Circulation,* 93**,** 1043-1065.

Taylor, JA, Myers, CW, Halliwill, JR*, et al.* 2001. Sympathetic restraint of respiratory sinus arrhythmia: Implications for vagal-cardiac tone assessment in humans. *Am J Physiol Heart Circ Physiol,* 280**,** H2804-2814.

Taylor, JA, Williams, TD, Seals, DR*, et al.* 1998. Low-frequency arterial pressure fluctuations do not reflect sympathetic outflow: Gender and age differences. *Am J Physiol Heart Circ Physiol,* 274**,** H1194-H1201.

Tersman, Z, Collins, A & Eneroth, P 1991. Cardiovascular responses to psychological and physiological stressors during the menstrual cycle. *Psychosom Med,* 53**,** 185-197.

Thijssen, DH, de Groot, P, Kooijman, M*, et al.* 2006. Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *Am J Physiol Heart Circ Physiol,* 291**,** H3122-3129.

Thimm, F, Carvalho, M, Babka, M, *et al.* 1984. Reflex increases in heart-rate induced by perfusing the hind leg of the rat with solutions containing lactic-acid. *Pflugers Arch,* 400**,** 286-293.

Thomas, GD, Hansen, J & Victor, RG 1994. Inhibition of alpha 2-adrenergic vasoconstriction during contraction of glycolytic, not oxidative, rat hindlimb muscle. *Am J Physiol,* 266**,** H920-929.

Thomas, GD, Hansen, J & Victor, RG 1997. Atp-sensitive potassium channels mediate contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle. *J Clin Invest,* 99**,** 2602-2609.

Thornton, JM, Aziz, T, Schlugman, D, *et al.* 2002. Electrical stimulation of the midbrain increases heart rate and arterial blood pressure in awake humans. *J Physiol,* 539**,** 615-621.

Tschakovsky, ME, Shoemaker, JK & Hughson, RL 1996. Vasodilation and muscle pump contribution to immediate exercise hyperemia. *Am J Physiol Heart Circ Physiol,* 271**,** H1697-H1701.

Tschakovsky, ME, Sujirattanawimol, K, Ruble, SB, *et al.* 2002. Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? *J Physiol,* 541**,** 623-635.

Vanderhorst, VG, Gustafsson, JA & Ulfhake, B 2005. Estrogen receptor-alpha and -beta immunoreactive neurons in the brainstem and spinal cord of male and female mice: Relationships to monoaminergic, cholinergic, and spinal projection systems. *J Comp Neurol,* 488**,** 152-179.

VanTeeffelen, JWGE & Segal, SS 2003. Interaction between sympathetic nerve activation and muscle fibre contraction in resistance vessels of hamster retractor muscle. *J Physiol,* 550**,** 563-574.

Vianna, LC, Araujo, CGS & Fisher, JP 2009. Influence of central command and muscle afferent activation on anterior cerebral artery blood velocity responses to calf exercise in humans. *J Appl Physiol,* 107**,** 1113-1120.

Victor, RG, Bertocci, LA, Pryor, SL, *et al.* 1988. Sympathetic-nerve discharge is coupled to muscle-cell ph during exercise in humans. *J Clin Invest,* 82**,** 1301-1305.

Victor, RG, Leimbach, WN, Jr., Seals, DR, *et al.* 1987a. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension,* 9**,** 429-436.

Victor, RG, Pryor, SL, Secher, NH, *et al.* 1989a. Effects of partial neuromuscular blockade on sympathetic nerve responses to static exercise in humans. *Circ Res,* 65**,** 468-476.

Victor, RG, Rotto, DM, Pryor, SL, *et al.* 1989b. Stimulation of renal sympathetic activity by static contraction: Evidence for mechanoreceptor-induced reflexes from skeletal muscle. *Circ Res,* 64**,** 592-599.

Victor, RG & Seals, DR 1989. Reflex stimulation of sympathetic outflow during rhythmic exercise in humans. *Am J Physiol,* 257**,** H2017-2024.

Victor, RG, Seals, DR & Mark, AL 1987b. Differential control of heart rate and sympathetic nerve activity during dynamic exercise. Insight from intraneural recordings in humans. *J Clin Invest,* 79**,** 508-516.

Victor, RG, Secher, NH, Lyson, T, *et al.* 1995. Central command increases muscle sympathetic nerve activity during intense intermittent isometric exercise in humans. *Circ Res,* 76**,** 127-131.

Virtanen, I, Polo, O, Polo-Kantola, P, *et al.* 2000. The effect of estrogen replacement therapy on cardiac autonomic regulation. *Maturitas,* 37**,** 45-51.

Vissing, J, MacLean, DA, Vissing, SF, *et al.* 2001. The exercise metaboreflex is maintained in the absence of muscle acidosis: Insights from muscle microdialysis in humans with mcardle's disease. *J Physiol,* 537**,** 641-649.

Wagerle, LC, Heffernan, TM, Sacks, LM, *et al.* 1983. Sympathetic effect on cerebral blood flow regulation in hypoxic newborn lambs. *Am J Physiol,* 245**,** H487-494.

Waki, H, Kasparov, S, Katahira, K, *et al.* 2003. Dynamic exercise attenuates spontaneous baroreceptor reflex sensitivity in conscious rats. *Exp Physiol,* 88**,** 517-526.

Waldrop, TG & Mitchell, JH 1985. Effects of barodenervation on cardiovascular-responses to static muscular-contraction. *Am J Physiol,* 249**,** H710-H714.

Waldrop, TG, Rybicki, KJ & Kaufman, MP 1984. Chemical activation of group i and ii muscle afferents has no cardiorespiratory effects. *J Appl Physiol,* 56**,** 1223-1228.

Waldrop, TG & Stremel, RW 1989. Muscular-contraction stimulates posterior hypothalamic neurons. *Am J Physiol,* 256**,** R348-R356.

Walgenbach, SC & Donald, DE 1983. Inhibition by carotid baroreflex of exercise-induced increases in arterial pressure. *Circ Res,* 52**,** 253-262.

Wang, J, Korczykowski, M, Rao, H, *et al.* 2007. Gender difference in neural response to psychological stress. *Soc Cogn Affect Neurosci,* 2**,** 227-239.

Warner, HR & Cox, A 1962. A mathematical model of heart rate control by sympathetic and vagus efferent information. *J Appl Physiol,* 17**,** 349-355.

Watanabe, H, Watanabe, K, Wadazumi, T, *et al.* 2007. Effect of exercise intensity on mild rhythmic-handgrip-exercise-induced functional sympatholysis. *J Physiol Anthropol,* 26**,** 593-597.

Williamson, JW 2010. The relevance of central command for the neural cardiovascular control of exercise. *Exp Physiol,* 95**,** 1043-1048.

Williamson, JW, McColl, R, Mathews, D*, et al.* 1999. Activation of the insular cortex is affected by the intensity of exercise. *J Appl Physiol,* 87**,** 1213-1219.

Williamson, JW, McColl, R, Mathews, D*, et al.* 2001. Hypnotic manipulation of effort sense during dynamic exercise: Cardiovascular responses and brain activation. *J Appl Physiol,* 90**,** 1392-1399.

Williamson, JW, McColl, R, Mathews, D*, et al.* 2002. Brain activation by central command during actual and imagined handgrip under hypnosis. *J Appl Physiol,* 92**,** 1317-1324.

Wilson, MH, Edsell, ME, Davagnanam, I*, et al.* 2011. Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia-an ultrasound and mri study. *J Cereb Blood Flow Metab***,** 1–11.

Wilson, TD, Shoemaker, JK, Kozak, R*, et al.* 2005. Reflex-mediated reduction in human cerebral blood volume. *J Cereb Blood Flow Metab,* 25**,** 136-143.

Wray, DW, Donato, AJ, Uberoi, A*, et al.* 2005. Onset exercise hyperaemia in humans: Partitioning the contributors. *J Physiol,* 565**,** 1053-1060.

Wray, DW, Fadel, PJ, Keller, DM*, et al.* 2004a. Dynamic carotid baroreflex control of the peripheral circulation during exercise in humans. *J Physiol,* 559**,** 675-684.

Wray, DW, Fadel, PJ, Smith, ML*, et al.* 2004b. Inhibition of alpha-adrenergic vasoconstriction in exercising human thigh muscles. *J Physiol,* 555**,** 545-563.

Wray, DW, Nishiyama, SK & Richardson, RS 2009. Role of alpha(1)-adrenergic vasoconstriction in the regulation of skeletal muscle blood flow with advancing age. *Am J Physiol Heart Circ Physiol,* 296**,** H497-H504.

Wyss, CR, Ardell, JL, Scher, AM*, et al.* 1983. Cardiovascular-responses to graded reductions in hindlimb perfusion in exercising dogs. *Am J Physiol,* 245**,** H481-H486.

Yasui, Y, Breder, CD, Saper, CB*, et al.* 1991. Autonomic responses and efferent pathways from the insular cortex in the rat. *J Comp Neurol,* 303**,** 355-374.

Young, CN, Fisher, JP & Fadel, PJ 2008. The ups and downs of assessing baroreflex function. *J Physiol,* 586**,** 1209-1211.

Younis, LT, Melin, JA, Robert, AR*, et al.* 1990. Influence of age and sex on left ventricular volumes and ejection fraction during upright exercise in normal subjects. *Eur Heart J,* 11**,** 916-924.

Zbrozyna, AW & Westwood, DM 1988. Habituation of vasodilatation in the calf elicited by repeated sensory stimulation in man. *Eur J Appl Physiol Occup Physiol,* 58**,** 284-290.