The contribution of somatosensory afferent inputs from the neck to autonomic regulation of cardiovascular function in humans

A thesis submitted in fulfilment of the requirements for

the degree of Doctor of Philosophy

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March 2008

Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; ethics procedures and guidelines have been followed.

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Doctoral Research Publications

The following research papers have resulted from the work undertaken in this thesis.

Watanabe, N & Polus, BI 2007, 'A single mechanical impulse to the neck: does it influence autonomic regulation of cardiovascular function?' *Chiro J Aust*, vol. 37, no. 2, pp. 42-8.

Watanabe, N, Reece, J & Polus, BI 2007, 'Effects of body position on autonomic regulation of cardiovascular function in young, healthy adults', *Chiropr Osteopat*, vol. 15, no. 1, p. 19.

Conference Abstract

Watanabe, N & Polus BI 2005, 'The effects of different body positions on autonomic regulation of cardiovascular function', paper presented at the First Research Conference of the School of Health Sciences, Melbourne, Australia.

Watanabe, N & Polus BI 2005, 'The effects of different body positions on autonomic regulation of cardiovascular function', poster presented at The 8th Biennial Congress of the World Federation of Chiropractic, Sydney, Australia.

Watanabe, N & Polus BI 2005, 'The effects of different body positions on autonomic regulation of cardiovascular function', poster presented at The 4th Congress of International Society for Autonomic Neuroscience, Marseille, France.

Watanabe, N & Polus BI 2006, 'Mechanical perturbation of the neck: Does it influence cardiovascular function?', poster presented at The 26th annual meeting of the Australian Neuroscience Society, Sydney, Australia.

Watanabe, N & Polus BI 2006, 'Mechanical perturbation of the neck; Does it influence cardiovascular function?', poster presented at the Second Research Conference of the School of Health Sciences, Melbourne, Australia.

Watanabe, N & Polus BI 2006, 'A single mechanical impulse to the neck; Does it influence autonomic regulation of cardiovascular function?', paper presented at the Scientific symposium and policy forum of Chiropractors' Association of Australia, Sydney, Australia.

Watanabe, N, Potocnik, S & Polus BI 2007, 'The role of proprioceptive inputs from the dorsal neck to cardiovascular regulation during orthostasis in humans', paper presented at the Third Research Conference of the School of Health Sciences, Melbourne, Australia.

Watanabe, N, Reece, J & Polus BI 2007, 'The effects of mechanical impulse to the neck on cardiac autonomic and cardiovascular functions in young healthy adults: Is it an artefact or a genuine response?', poster presented at the Third Research Conference of the School of Health Sciences, Melbourne, Australia.

Watanabe, N, Potocnik, S, Reece, J & Polus BI 2007, 'The contribution of proprioceptive inputs from the neck to autonomic regulation of cardiovascular function in humans', poster presented at the Third Research Conference of the School of Health Sciences, Melbourne, Australia.

Watanabe, N, Reece, J & Polus BI 2007, 'The Effects of Phasic Mechanical Stimulation to the Neck on Autonomic Regulation of Cardiovascular Function in Healthy Young Adults; Is it Arousal or Genuine Response?', poster presented at the The 7th International Brain Research Organization World Congress of Neuroscience, Melbourne, Australia.

Watanabe, N, Potocnik, S, Reece, J & Polus BI 2007, 'The contribution of proprioceptive inputs from the neck to autonomic regulation of cardiovascular function in humans', poster presented at

the The 7th International Brain Research Organization World Congress of Neuroscience, Melbourne, Australia.

Watanabe, N, Potocnik, S & Polus BI 2007, 'The contribution of neck sensory inputs to cardiovascular regulation during moderate head-up tilt in humans', paper presented at the 5th International Society for Autonomic Neuroscience congress, Kyoto, Japan.

Watanabe, N, Potocnik, S & Polus BI 2008, 'The contribution of neck afferents to cardiovascular function in sitting humans', poster presented at the 28th annual meeting of the Australian Neuroscience Society, Hobart, Australia.

Acknowledgements

My four-year journey in Australia is exceptionally invaluable. To complete this work, I have always kept one phase in my mind throughout my challenge, “Never give up!” However, needless to say, without many many people’s support, it would never be possible to achieve the completion of this thesis. Especially, I would like to express my special thanks towards the following people.

To my senior supervisor, Associate Professor Barbara Polus, thank you for your warm welcome me into the Clinical Neuroscience Research Group, for kind and patient communication, and for taking me through precious research training. Your critical comments are always helpful and constructive.

To my supervisor, Associate Professor Brian Budgell, thank you for your expert knowledge and for long-distance but warm continuous support through my research project.

Dr. Simon Potocnik, thank you for your professional support for preliminary experiments, brilliant opinions, and great encouragement.

Dr. Ken Kamei, thank you for your unquestioning help (especially your translation!) and friendship. Your voluntary support has made my life in Australia easier and more enjoyable.

Dr. Hiroshi Sasaki, thank you for your great help for developing my knowledge on physiology and friendship. Your remarkable drawing skill always helps my presentation and this thesis.

Dr. Adrian Repka, thank you for your unquestioning help and great discussion on neck sensory-vestibular physiology.

Dr. Karen Lucus, thank you for proof reading of some thesis chapters and your great advice on research student life. Your guidance has made relaxed me and make my path clearer.

Associate Professor Clifford Da Costa and Dr. John Reece, thank you for your professional advice and support of statistics.

Ms. Julie Barnett, thank you for your help on all my documentation submissions such as ethics, enrolment, program extension, and even student visa.

Dr. Alan Fuhr, thank you for a generous donation and very kind communication regarding an Activator® Instrument.

Dr. Harumi Hotta, thank you for your kind communication and a precious gift and for giving me an opportunity to visit the prestigious laboratory of somato-autonomic reflex research.

Red Lettuce Communications, thank you for your professional editing service associated with a part of the studies in this thesis.

RMIT University, thank you for supporting my research life in Australia with a scholarship.

School of Health Sciences and Division of Chiropractic, thank you for supporting my research life at RMIT University with continuous encouragement and finance supports for experiments and congress attendance.

RMIT Chiropractic Teaching Clinic, thank you for your continuous great support for over four years. Without healthy body, it would be difficult to focus on my work.

RMIT Japan, thank you for your kind communication and continuous encouragement.

Research volunteers involved in the studies of this thesis, thank you for your kind support. Without your participation, any of the studies would not have been done.

To Ms. Hyou Ju Jin, thank you for special friendship, your kindness, and taking much load in order to focus on this big challenge.

Finally, my special thanks are forwarded especially to my family members. To mother, father, and grandparents, thank you for allowing me to study in Australia for five years in total without any questions. Without your physical, emitional, and financial support, I would not have completed this thesis.

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Abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATP	adenosine triphosphate
BP	blood pressure
bpm	beats per minute
BMI	body mass index
Ca^{2+}	calcium ion
CNS	central nervous system
DBP	diastolic blood pressure
ECG	electrocardiogram
EMG	electromyography, electromyograph, electromyographic, electromyogram
FIN	placing the finger only on the neck without any mechanical impulse delivered
FiBF	finger blood flow
FoBF	forearm blood flow
HDNF	head down neck flexion
HF	high frequency
HR	heart rate
HRV	heart rate variability
HUT	head-up tilt
ICC	intraclass correlation coefficient
K^{+}	potassium ion
LF	low frequency
LF/HF	the ratio of low frequency to high frequency

MAP	mean arterial pressure
MID	a mechanical impulse delivered directly to the neck
MIF	a single mechanical impulse to the neck over the examiner's finger placed on the stimulus point
MIH	a mechanical impulse to the examiner's dorsal hand
ml/100ml tissue/min	millilitres per 100 millilitre tissue per minute
mmHg	millimetres of mercury
ms^2	millisecond squared
nu	normalised unit
Ns^{-1}	newton per second
PPG	photoplethysmogram
RMSSD	the square root of the mean of the sum of the squares of differences between adjacent R-R intervals
SD	standard deviation
SDNN	the standard deviation of all R-R intervals
SEM	standard error of the mean
sEMG	surface electromyography
SIAU	authentic manipulation in sitting posture
SISH	sham manipulation in sitting posture
SBP	systolic blood pressure
SGP	strain-gauge plethysmograph, strain-gauge plethysmography, strain-gauge plethysmogram
SUAU	authentic manipulation in supine posture,
SUSH	sham manipulation in supine posture
TP	total power
VAS	visual analogue scale

VLF	very low frequency
Δ_{flex}	values obtained following hold-intermediate conditioning were subtracted from values recorded after hold-flexion conditioning
Δ_{ext}	values obtained following hold-intermediate conditioning were subtracted from values recorded after hold-extension conditioning

Thesis Structure

An outline of the structure of this thesis is as follows;

- Chapter 1 is a summary of the current state of knowledge related to autonomic regulation of cardiovascular function and the somato-autonomic and vestibulo-autonomic reflexes.

- Chapter 2 describes experimental techniques used in this thesis, including the basic mechanisms of operation of equipment and analytical methods that were used to prepare raw data for statistical analysis. Also, this chapter provides a brief review of the advantages and disadvantages associated with measurement technique used in this thesis.

- Chapter 3 examined the effects of body position on autonomic regulation of cardiovascular function. Results from this chapter informed the design of subsequent studies.

- Chapter 4 examined the effects of mechanical stimulation to the neck on autonomic regulation of cardiovascular function. In order to simulate cervical manipulation, a therapeutic instrument was employed to deliver a mechanical stimulus without/minimising head displacement. This chapter consists of two parts. The first parts employed two different forms of mechanical stimuli during the supine or sitting posture (therefore, four conditions). The latter part employed two additional different forms of mechanical stimuli during the supine posture. A discussion section is provided at the end of the first part of this chapter and an overall discussion associated with this chapter is provided at the end of this chapter.

- Chapter 5 examined the contribution of neck proprioceptive inputs to autonomic regulation of cardiovascular function in sitting humans. This chapter also consists of two parts. The first part includes experiments using the muscle conditioning manoeuvre and a vibratory stimulus to the neck. The latter part includes a follow-up experiment of the study that used a vibratory stimulus in the first part of Chapter 5.

- Chapter 6 examined the contribution of proprioceptive inputs from the dorsal neck muscles to autonomic regulation of cardiovascular function in the supine posture. The muscle conditioning manoeuvre was applied to the dorsal neck and performed in head-and-neck-flexion, -intermediate, and -extension position during the supine posture.

- Chapter 7 examined the contribution of proprioceptive inputs from the dorsal neck muscles to autonomic regulation of cardiovascular function during a mild orthostatic stress. Following the application of the muscle conditioning manoeuvre to the dorsal neck, 20° head-up tilt was applied. This chapter investigated the characteristics of the interaction between neck proprioceptive and vestibular inputs in autonomic regulation of cardiovascular function.

- Chapter 8 presents general conclusion of this thesis, limitations, and recommendation for future investigations.

Summary

There have been reports that manually-delivered therapeutic interventions to the neck may normalise cardiovascular dysfunctions associated with such conditions as arrhythmia and hypertension. This suggests that neck sensory afferent inputs may be capable of influencing cardiovascular function. In addition, it has been documented that manipulative procedures to the neck may influence cardiovascular function and cardiac sympathovagal balance in asymptomatic groups. However, as these studies did not restrict head motion during the application of the intervention, vestibular stimulation may have potentially affected these results. Therefore, the aims of this thesis are as follows: 1) to examine the effects of simulated cervical manipulation while controlling head motion on autonomic regulation of cardiovascular function in humans and 2) to examine whether muscle proprioceptors in the neck are related to autonomic regulation of cardiovascular function in humans.

Chapter 3 aimed to characterise the effect of body position on autonomic regulation of cardiovascular function. In this chapter, cardiac autonomic nervous (heart rate variability; HRV) and cardiovascular (heart rate; HR, and blood pressure; BP) parameters were compared between different postures (prone vs. supine and prone vs. sitting). Compared with supine, BP and HR were significantly higher in the prone posture while HRV parameters did not differ between these postures. Higher BP and lower HR were found in the prone posture and some components of HRV significantly differed between prone and sitting postures. In addition to these significant posture effects, BP and HR recorded in the prone posture exhibited lability compared to the other postures. Thus, the prone posture was found to be inappropriate for the planned experiments and accordingly, this posture was not used in subsequent experimental work.

In Chapter 4, to simulate spinal manipulation, an Activator® Instrument was used to deliver a mechanical stimulus to the upper cervical region. Chapter 4 consists of two parts. The first part employed two forms of mechanical stimuli in both supine and sitting postures. Both interventions induced similar BP responses while HR did not change. Because the observed cardiovascular responses were similar to arousal (startle) responses, a subsequent experiment presented in the second part of this chapter aimed to address whether cardiovascular responses to a mechanical stimulus to the neck were due to an arousal reaction. The experiments in this part were conducted with two additional interventions (a mechanical impulse delivered directly to the neck (MID) and placing the finger on the neck only) and two further outcome measurements (finger blood flow (FiBF) and a change in skin potential). The MID intervention induced a significant reduction in BP and changes in HRV parameters as well as skin potential change. The BP reduction remained despite the removal of the influence of FiBF (which is documented to occur in arousal reactions) in an analysis of covariance. Therefore, genuine effects of the mechanical stimulus and presumably an arousal effect may constitute the observed cardiac autonomic and cardiovascular responses.

Chapter 5 focussed on the contribution of more specific types of neck sensory receptors by employing the muscle conditioning manoeuvre and a vibratory stimulus. Muscle spindles are known to be sensitive to a vibratory stimulus and the muscle conditioning manoeuvre alters the quantity of muscle proprioceptive inputs in a systematic way. Neck muscle proprioceptive inputs are known to be involved in posture control, but their role in cardiovascular regulation is unclear. Hence, to investigate this contribution to cardiovascular regulation, the first part of this chapter employed these two techniques. Data were recorded during sitting with the neck rotated to 20° after the muscle conditioning manoeuvre was performed. No consistent cardiovascular changes were revealed by the manoeuvre. In contrast, during one-minute vibration on the right dorsal neck, forearm blood flow (FoBF) decreased. Furthermore, soon

after the vibratory stimulus was terminated, systolic BP slightly decreased and FiBF increased by approximately 20% compared with pre-vibration values. Also, the reduction in FoBF persisted. In the second part of this chapter, vibration was applied to the left dorsal neck or right leg in order to determine factors which may have accounted for the results above such as an arousal effect or laterality of the response. Left neck vibration revealed similar cardiovascular responses to right neck vibration (i.e., the first part of Chapter 5). On the other hand, leg vibration induced different cardiovascular responses. Thus, the effects of neck vibration were presumed to be genuine while an arousal effect may superimpose. Hence, the combined findings of these experiments may indicate that neck sensory inputs, including muscle proprioceptive inputs, induce cardiovascular responses, regardless of which side of the neck the vibratory stimulus is applied.

The effect of the muscle conditioning manoeuvre on autonomic regulation of cardiovascular function was negligible in Chapter 5. One potential reason for this finding was the likelihood that the neck muscles were somewhat active during data collection in the sitting position. Muscle relaxation at the appropriate times is critical for the success of the manoeuvre. Therefore, Chapter 6 applied dorsal neck muscle conditioning in the supine posture in either neck-flexion (hold-long), -intermediate (hold-intermediate), or -extension (hold-short) positions. There were no significant effects of the manoeuvre on HRV and cardiovascular parameters aside from a difference in systolic BP between hold-flexion and -extension conditioning at the first minute.

In Chapter 7, 20° head-up tilt (HUT) was applied following the muscle conditioning manoeuvre used in Chapter 6, in order to examine the contribution of neck muscle proprioceptive inputs to autonomic regulation of cardiovascular function during a mild orthostatic stress. In response to HUT following hold-flexion conditioning, HR did not change

while BP significantly decreased by 10%. Following hold-extension conditioning, HR increased by 10% and BP did not change. Additionally, HRV parameters indicated a significant cardiac vagal withdrawal following hold-extension conditioning. These results indicate that changes in both autonomic and cardiovascular responses to 20° HUT were dependent on the form of muscle conditioning. This suggests that muscle proprioceptive inputs from the neck may play a significant role in autonomic regulation of cardiovascular function especially during orthostasis.

Chapter 1

Review of Literature

1.1. Introduction

This chapter provides a descriptive review of the contribution of sensory inputs (particularly, vestibular and somatosensory inputs) to autonomic regulation of cardiovascular function. Prior to describing this topic, short-term cardiovascular regulation will briefly be reviewed. To conclude this chapter, the current state of research and the needs for improving the body of knowledge on this topic will be outlined.

1.2. Autonomic regulation of cardiovascular function

Adequate control of circulation is essential for maintaining function of the vital organs. The regulation of blood pressure (BP) is achieved by not only one system but multiple systems as illustrated in Figure 1.1 (Guyton & Hall 2006).

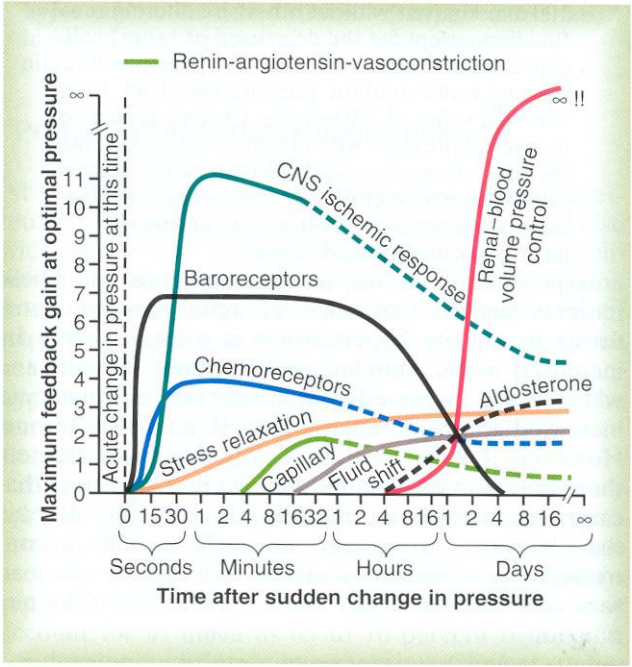

Figure 1.1. Multiple cardiovascular regulatory systems.
Each system contributes to blood pressure control at different periods following blood pressure change. Adapted from Guyton & Hall (2006).

In response to a sudden change in BP, baroreceptors are activated and their signal initiates events that modify cardiovascular function within seconds to minutes with the aim of restoring adequate BP. Within hours, the renin-angiotensin system reaches peak activation to

participate in BP regulation. For longer-term BP control, the kidney contributes by controlling blood volume.

The groups of nuclei in the medulla oblongata, collectively called the cardiovascular centre, coordinate cardiovascular control, while the cardiovascular centre is influenced by various regions of the central nervous system (CNS) such as the cerebral cortex and cingulate gyrus (Guyton & Hall 2006). In particular, the neurons in the rostral and caudal (and intermediate) ventrolateral medulla and nucleus tractus solitarius play a crucial role in the neural process of baroreceptor-mediated cardiovascular responses (Guyenet 1990; Dampney 1994). The focus of this section of the thesis is neurally-mediated short-term control of cardiovascular function.

1.2.1. Baroreflex

One of the most common cardiovascular perturbations in daily life is posture change. Cardiovascular function needs to be adequately regulated in response to gravitational stresses such as moving from a horizontal posture to sitting and to standing. If cardiovascular regulation fails, orthostatic hypotension may occur and consequently, blood supply to the vital organs is compromised, particularly that to the brain. In response to the dynamic body fluid shift towards the feet, feedback mechanisms significantly contribute to cardiovascular function, one of these is the “baroreflex”.

Arterial pressure is continuously monitored by the baroreceptors, which are located in the aortic arch and carotid sinus (Boron & Boulpaep 2003). The baroreceptor is activated by stretching the wall of the blood vessel and its afferent inputs are projected via the glossopharyngeal and vagus nerves and processed in the cardiovascular centre. Afferent inputs (impulses) from the baroreceptors in the carotid sinus progressively increase as BP rises but are sensitive particularly between approximately 50 and 150 mmHg of BP

(Dampney et al. 2002). Additionally, baroreceptors in the aortic arch respond to BP approximately 30 mmHg higher than that in the carotid sinus (Guyton & Hall 2006).

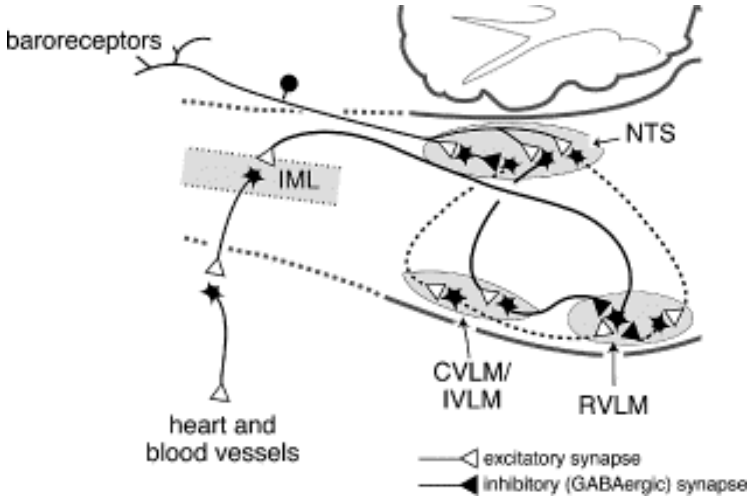

Figure 1.2. Central neural circuit of baroreflex control of sympathetic nervous efferent.
The solid line indicates a well-established neural pathway and the dashed line represents a presumed pathway. CVLM; the caudal ventrolateral medulla, IML; the intermediolateral cell column, IVLM; the intermediate ventrolateral medulla, NTS; the nucleus tractus solitarius, RVLM; the rostral ventrolateral medulla. Adapted from Dampney & Horiuchi (2003).

Afferent inputs from the baroreceptors project to the nucleus tractus solitarius and second-order (excitatory) neurons terminate in the rostral and caudal (and intermediate) ventrolateral medulla (Dampney et al. 2002; Dampney & Horiuchi 2003). While inhibitory neural connections between the rostral and caudal (and intermediate) ventrolateral medulla have been established, it is yet unclear whether excitatory connections also exist. (Dampney et al. 2002; Dampney & Horiuchi 2003; Horiuchi et al. 2004). Subsequently, sympathetic excitatory outputs from the rostral ventrolateral medulla project directly to pre-ganglionic sympathetic motorneurons located in the intermediolateral cell column of the spinal cord at the T1-L3 levels (Dampney et al. 2002; Boron & Boulpaep 2003). The sympathetic outflow then influences the function of the heart and blood vessels after synapsing with post-ganglionic fibres located in the paravertebral ganglia. Other visceral organs (including the kidney) are innervated by post-ganglionic fibres after the pre-ganglionic fibres synapse with post-ganglionic fibres in the prevertebral ganglia (Boron & Boulpaep 2003). Hence, it appears

that the neurons in the caudal (and intermediate) ventrolateral medulla contribute to the negative feedback loop of the baroreflex.

In addition to sympathetic excitatory outputs, vagal outflow to the heart is also involved in the baroreflex. Some of the afferent inputs from the baroreceptors to the nucleus tractus solitarius project to the nucleus ambiguus and the dorsal motor nucleus of the vagus (predominantly the former nucleus) (Boron & Boulpaep 2003; Mohrman & Heller 2006). Post-ganglionic vagal outputs then reach the sinoatrial node of the heart after synapsing on the atrial wall (Boron & Boulpaep 2003). Thus, when BP increases, the walls of the carotid artery and aortic arch are stretched and in turn impulses are generated by the baroreceptors. As a result, sympatho-inhibitory and vagal-excitatory mechanisms are activated and BP is lowered. Conversely, when BP decreases, the opposite responses occur; that is inhibition of sympatho-inhibitory and vagal-excitatory mechanisms. Therefore, BP increases as a consequence of increases in cardiac output and peripheral vascular resistance.

1.2.2. Cardiopulmonary baroreceptor-related reflexes

According to Boron and Boulpaep (2003), the cardiovascular reflexes related to the cardiopulmonary baroreceptors are initiated by a blood volume change, which stimulates stretch receptors located in the atria and the pulmonary arteries, so called the low pressure baroreceptors (Guyton & Hall 2006). Afferent inputs from these receptors project to the medulla via the vagus nerve and intramedullary pathways of these reflexes are similar to those of the baroreflex (Boron & Boulpaep 2003). Efferent outputs from both the sympathetic and vagus nerves project to the heart and blood vessels (Berne & Levy 2001; Guyton & Hall 2006), and especially to the kidney (Boron & Boulpaep 2003). Thus, an increase in central venous volume activates the low pressure receptors and subsequently an increase in heart rate (HR) (the Bainbridge reflex) and decreases sympathetic outflow to the kidneys (Berne &

Levy 2001; Boron & Boulpaep 2003). As a result of renal sympathetic outflow changes, blood flow in the kidney is increased, activity of the renin-angiotensin system is inhibited, and consequently diuresis is increased (thus, fluid volume in the body decreases) (Boron & Boulpaep 2003). Conversely, a reduction in blood volume in the atria results in an increase in renal sympathetic nerve activity and in turn a reduction in renal blood flow (Berne & Levy 2001). However, the atrial blood flow decrease seldom influences HR and a reduction in stroke volume results in the initiation of the baroreflex so as to maintain cardiac output (Figure 1.3) (Boron & Boulpaep 2003).

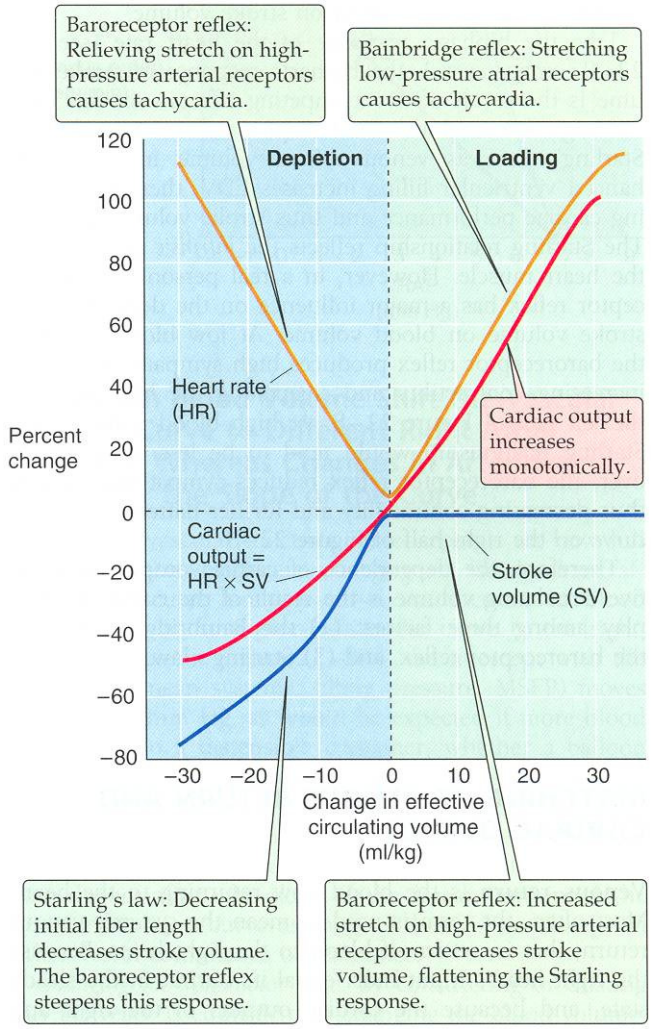

Figure 1.3. The influence of blood volume on cardiac output.
The horizontal axis indicates blood volume and the vertical axis indicates percent changes in cardiac output, heart rate, and stroke volume. Adapted from Boron & Boulpaep (2003).

In addition to the body fluid control mechanism summarised above, since the afferent fibres of the low-pressure receptors project not only to the medulla but also the hypothalamus

(Boron & Boulpaep 2003; Klabunde 2005) (which is known to regulate pituitary function), arginine vasopressin (i.e., antidiuretic hormone) is also involved in cardiopulmonary baroreceptor-related reflexes. Arginine vasopressin is released from the posterior pituitary into blood and affects the distal convoluted tubules and collecting ducts in the renal medulla, resulting in the reabsorption of water (Berne 2004; Klabunde 2005). Hence, arginine vasopressin increases BP by increasing blood volume as well as affecting the smooth muscle of blood vessels (i.e., vasoconstriction) (Berne 2004). It is thought that arginine vasopressin induces changes the tone of blood vessels particularly when there is severe blood loss (such as haemorrhage) (Mohrman & Heller 2006). An increase in blood volume stimulates low pressure baroreceptors and an increase in afferent inputs of these baroreceptors. The afferent inputs from the low pressure baroreceptors induces a reduction in secretion of arginine vasopressin and subsequently an increase in diuresis (Boron & Boulpaep 2003). Therefore, a decrease in blood (fluid) volume is achieved.

The quantity of afferent inputs from the low pressure baroreceptors may also be altered by posture change due to a change in central venous flow. Hence, it is important to consider the involvement of arginine vasopressin in BP control associated with posture change, since posture change is involved in some experiments of this thesis. There is evidence that a change in plasma arginine vasopressin occurs as early as 5 minutes after posture change and is still evident at 10 minutes (Geelen et al. 2002). In addition, Pump et al. (1999) observed that a change in plasma arginine vasopressin associated with posture change was stable over a 30 minute time period (data was collected every 10 minutes). Collectively, it may be assumed that the secretion of arginine vasopressin is stabilised by 10-20 minutes following the completion of posture change. However, there is a time lag between the stabilization of plasma arginine vasopressin concentration and an observable BP change. It is known that it takes as long as 15 minutes to reveal a measurable influence of fluid change on BP (see

Figure 1.1). The majority of experiments in this thesis required participants to maintain the same posture for as long as 10 minutes (except experiments in Chapter 5) after a posture change. This time frame is shorter than when the contribution of fluid change to BP control becomes evident. Therefore, it was assumed that the involvement of arginine vasopressin in BP regulation may not be a critical factor in most of the experiments in this thesis.

Another atria-related cardiovascular regulation is atrial natriuretic peptide, which also contributes to fluid volume control (in turn, cardiovascular regulation) (Boron & Boulpaep 2003). The peptide is released from myocytes of the atria when the atrial wall is stretched. By enhancing diuresis and dilating blood vessels, atrial natriuretic peptide lowers BP (Boron & Boulpaep 2003).

1.3. Vestibulo-autonomic reflexes

1.3.1. The vestibular system

The vestibular system, a tiny apparatus located within the bony labyrinth of the inner ear detects head position and its motion in space. The vestibular system consists of two interconnected apparatus – the otolith and the semicircular canals. The otolith organs detect linear acceleration such as accelerating or braking a car and being carried by an elevator, and the semicircular canals detect angular acceleration (rotatory movements) such as turning the head. These apparatus provide the CNS with sensory information as electrical signals (i.e., action potential) induced by mechanical deformation of the hair cells (Berne 2004).

1.3.1.1. Hair cells

Hair cells of the vestibular system include cilia of different lengths (stereocilia). The longest cilium on the cell body is called the kinocilium and the length of the stereocilia become shorter as the distance from the kinocilium increases.

The rate of vestibular afferent discharge depends on the state of the stereocilia in the vestibular apparatus. For example, when the stereocilia bend towards the kinocilium, potassium ion (K^+) channels open in the stereocilia and K^+ enters the hair cells (Boron & Boulpaep 2003). In turn, the hair cells depolarise and the depolarization opens voltage-gated calcium ion (Ca^{2+}) channels at the base of the hair cells. Calcium ions enter the cell and this influx initiates neurotransmitter release, and consequently vestibular afferent signals are transmitted via the vestibular nerves. Potassium ion efflux occurs to preserve the electrochemical gradient of the hair cell. Conversely, when the stereocilia bend away from the kinocilium, K^+ channels close in the stereocilia and K^+ influx to the hair cells is obstructed. Thus, the hair cells hyperpolarise and movements of Ca^{2+} and K^+ and neurotransmitter release from the base of the hair cells are limited. As a result, vestibular afferent discharge decreases. Under resting conditions, vestibular afferents arising from the hair cells tonically discharge at approximately 100 impulses per second (Guyton & Hall 2006, p. 694). Therefore, depolarization of the hair cells increases the discharge rate of the vestibular afferents which hyperpolarization of the hair cell decreases discharge rate (Boron & Boulpaep 2003).

1.3.1.2. Otolith organs

The otolith organ is the general term for the saccule and utricle and these are located near the centre of the membranous labyrinth. There is a thickening of the epithelium of the saccule and utricle to form the macula. The sensory apparatus of the otolith organs is located in the macula. The cilia of the hair cells are covered by a jelly-like layer known as the "gelatinous cap" (Boron & Boulpaep 2003). A number of tiny pieces of carbonated calcium ("otoliths" or "ear stone") cover the gelatinous layer. These structures together are called the otolithic membrane (Berne 2004). When a linear acceleration is applied to the head, for example accelerating a car, a positional gap is caused between the hair cells and the otolithic membrane due to the greater specific gravity of the otoliths (approximately 2.7) (Baloh &

Honrubia 1990). Consequently, the gap bends the cilia of the hair cells and polarization of the hair cells occurs.

As discussed above, the direction of cilia movement, either towards or away from the kinocilium, determines the change in potential inside the hair cell. The length of the cilia on hair cells is arranged towards the centre of the saccule or utricle, forming an elevated structure which is known as the striola. Therefore, the preferred direction of cilium movement in the otolith organs is separated at the striola. In the saccule, the hair cells depolarise when the cilia bend away from the striola. Conversely, in the utricle, the hair cell depolarises when the cilia bend towards the striola. Importantly, because the striola lies along at the middle of the otolith organs and is curved, the otolith organs are, in totality, able to respond to various directions of linear acceleration.

1.3.1.3. Semicircular canals

The role of the semicircular canals is to detect angular acceleration of the head; for example nodding the head or rotating the head to look behind over the shoulder. The semicircular canals in each inner ear consist of three half-circle ducts connecting with the utricle. The front side of the horizontal semicircular canal is angled 30 degrees cephalad with respect to the horizontal plane (Baloh & Honrubia 1990). The other two canals are positioned vertically and are perpendicular to each other (Baloh & Honrubia 1990).

Each semicircular canal has an ampulla where the hair cells and their cilia are located. The cilia of the hair cells are fixed in the cupula so that when there is movement of the cupula, the cilia bend as following the cupula movement. Thus, when endolymph flow occurs with head motion, the cupula is bent by the endolymph flow and this induces polarization of the hair cells. The direction of the cilia of the hair cells in the cupula is all the same within each

semicircular canal. The direction of the cilia in the horizontal semicircular canal is towards the utricle and that in the other canals is away from the utricle (Berne 2004). Therefore, when the cupula is bent in the direction of the cilia, the hair cells depolarise and vestibular nerve discharge increases (Berne 2004). Conversely, when the cupula is bent in a direction opposite to that of the cilia, the hair cells hyperpolarise and vestibular discharge decreases (Berne 2004).

In addition to the role of the vestibular system as a head position/motion detector, it is also known that this system participates in posture control (Bent et al. 2007). Of interest to this discussion is that associated with a dynamic posture change such as lying to standing, body fluids including blood are significantly shifted towards the feet. As a result of this orthostatic stress, 300-800 ml of blood shifts towards the feet (Berne & Levy 2001). This fluid movement leads to a reduction in blood perfusion to the brain and blood distribution must be reorganised. If this fails to occur, unpleasant symptoms are sometimes caused such as dizziness, nausea, and fainting (orthostatic hypotension). In order to combat cardiovascular perturbations, multiple regulatory systems are involved as summarised above (see *section 1.2*).

The baroreflex powerfully influences cardiovascular function in response to perturbations to the system (feedback mechanism). On the other hand, the vestibular system responds as soon as a posture change starts, so it may be reasonable to suggest that vestibular input contributes to not only posture control but also autonomic regulation of cardiovascular function associated with orthostasis. It has been proposed that vestibular influences on cardiovascular function act in a feedforward manner in order to minimise cardiovascular perturbation as if expecting an orthostatic hypotensive incident to occur (Bolton & Ray 2000).

1.3.2. Vestibulo-autonomic reflexes in animals

1.3.2.1. Autonomic responses to vestibular stimulation

There is no doubt that baroreceptors are one of the major contributors to cardiovascular regulation during postural change. According to Spiegel and Sommer (1944), there is evidence that stimulation of the vestibular system also induces autonomic (i.e., the vestibulo-autonomic reflex) and cardiovascular responses. Later, it was demonstrated that vestibular activation using an electrical stimulus excited not only renal sympathetic nerves (Ishikawa & Miyazawa 1980) but also other sympathetic nerves including the cardiac and splanchnic nerves (for review see Yates 1992). Furthermore, the effect of natural vestibular stimulation on autonomic dirves was examined in anaesthetised cats after dissection of cervical dorsal roots (Yates & Miller 1994). Natural vestibular stimuli used in the study included head movements in the sagittal plane (nose-up and -down), the coronal plane (ipsilateral- and contralateral-ear-down), as well as coupled head movements (Yates & Miller 1994). In response to nose-up head motion which predominantly stimulates the otolith organs, maximum splanchnic nerve responses tended to be observed (i.e., the vestibulo-sympathetic reflex) and the response was diminished by a lesion to the medial and inferior vestibular nuclei. This result suggested that the vestibulo-sympathetic reflex was induced primarily by the otolith organs and that these vestibular nuclei were important in the response (Yates & Miller 1994). More recently, Woodring et al. (1997) examined BP responses to vestibular activation using a similar study design to that of Yates and Miller (1994). In their study, nose-up head motion induced a significant increase in BP of 18 mmHg (Woodring et al. 1997). Blood pressure began to rise 1.4 seconds following the point when the head had reached the pre-determined nose-up position and reached 80% of the maximum response by 6.2 seconds, while “ear-down head motion” revealed no or only a small BP response (Woodring et al. 1997). Furthermore, the observed BP response was diminished by vestibular nerve resection (Woodring et al. 1997).

Therefore, these previous studies demonstrated that vestibular activation induced by a direct electrical stimulus of vestibular nerves or by head motion (but not whole body movement) influences autonomic drives and cardiovascular function. In particular, the vestibulo-sympathetic reflex induced by head motion appeared to be a direction-dependent reaction. That is, autonomic drive and cardiovascular responses were more profound when the head was moved in the "nose-up" direction, which is equivalent to the posture an animal assumes when climbing a tree or standing on hindlimbs as some rodents do.

1.3.2.2. The vestibulo-autonomic reflex related to orthostasis

The significance of the vestibulo-autonomic reflex is thought to minimise cardiovascular perturbations related to posture change, particularly orthostasis. This association was directly demonstrated by Doba and Reis (1974). In their study, although the baroreceptors were intact, orthostatic hypotension occurred in response to whole body tilt in nose-up direction following peripheral vestibular nerve dissection and a fastigial nucleus lesion in paralysed and anaesthetised cats.

Doba and Reis' experiment was further explored using conscious cats by Yates and colleagues. Jian et al. (1999) applied whole body tilt in nose-up direction in conscious cats under four conditions (vestibular input present and absent; visual input present and absent). Prior to vestibular nerve removal, BP remained stable following nose-up tilt (BP reduction was usually less than 10 mmHg). However, once the vestibular nerves were bilaterally transected, moderate (10-20 mmHg) and large (greater than 20 mmHg) reductions in BP became more common and these hypotensive responses were worsed when visual input was also absent (Jian et al. 1999). The contribution of regions of the cerebellum (the nodulus and uvula), which have considerable connections with the vestibular nuclei, to cardiovascular regulation have also been examined (Holmes et al. 2002). This study revealed that ablation of

the uvula attenuated HR increase in response to orthostasis, while a lesion of the nodulus significantly increased a HR response compared with the pre-lesion condition (Holmes et al. 2002). In contrast to this remarkable influence on HR, the effect of lesion of the nodulus and uvula on BP responses to whole body tilt in nose-up direction were negligible or inconsistent (Holmes et al. 2002). When the vestibular apparatus was eliminated following a uvula lesion, more severe and prolonged orthostatic hypotension occurred as demonstrated previously (Jian et al. 1999). This cardiovascular regulation failure was compensated for within a week, presumably by non-vestibular sensory inputs. Additionally, Holmes et al.'s study (2002) demonstrated more severe orthostatic hypotension occurred following both vestibular and uvula removal compared with vestibulectomised cats, indicating a possibility that the uvula may integrate vestibular and non-vestibular inputs for cardiovascular regulation associated with posture change (but not the nodulus). Furthermore, Mori et al. (2005) demonstrated that damage to the inferior and medial vestibular nuclei, which was assumed to integrate sensory inputs regarding body position, resulted in a significant increase in HR and instability in BP in response to nose-up whole body tilt in conscious cats. However, as with previous studies (Jian et al. 1999; Holmes et al. 2002), changes in cardiovascular response to tilt slowly eased over the experimental period after vestibular lesions (4 weeks) (Mori et al. 2005). The recovery of cardiovascular responses to nose-up whole body tilt was attributed to three possibilities including 1) the remaining parts of the vestibular nuclei were thought to compensate for the function of the lesioned parts of the vestibular nuclei, 2) damaged vestibular neurons recovered from the lesion, and/or 3) other parts of the brain took over the role of cardiovascular regulation (see discussion in Mori et al. 2005).

In contrast to the "nose-up tilt" form of gravitational stress, Gotoh et al. (2004) applied a centrifugal force (up to 3G) to conscious rats under four conditions of vestibular and baroreceptor inputs (both intact, both impaired, vestibular only impaired, and baroreceptor

only impaired). This study demonstrated that, in response to the centrifugal force, BP slightly increased in the "both intact" models while BP did not change in the "vestibular impaired" models. Also, BP tended to overshoot its set point in the "baroreceptor impaired" model whereas BP significantly decreased in the "both impaired" model. This study suggests that the vestibular system and baroreceptors co-operate to achieve stable cardiovascular function, and the vestibulo-sympathetic reflex particularly appears to play a pressor role in response to orthostatic stress (Gotoh et al. 2004). Similarly, cardiovascular responses to linear acceleration, which is thought to preferentially stimulate the otolith organs, were examined in conscious rats (Zhu et al. 2007). Linear acceleration was associated with immediate increases in BP and HR (latency 1.97 and 5.3 seconds, respectively). The BP increase was reduced by bilateral labyrinthectomy but the HR response was not, so the vestibular system appeared responsible for the initial increase in BP but not the subsequent HR change. In addition, the BP change was reduced following medial and inferior vestibular nuclei lesions and this is consistent with the result obtained in the conscious cat model (Mori et al. 2005).

1.3.3. Vestibulo-autonomic reflexes in humans

The study of the role of the vestibular system in autonomic regulation of cardiovascular function was extended to humans in the late 1980's. Essandoh et al. (1988) may be credited with the first study which reported that alteration of vestibular inputs resulted in vasoconstriction in the musculature.

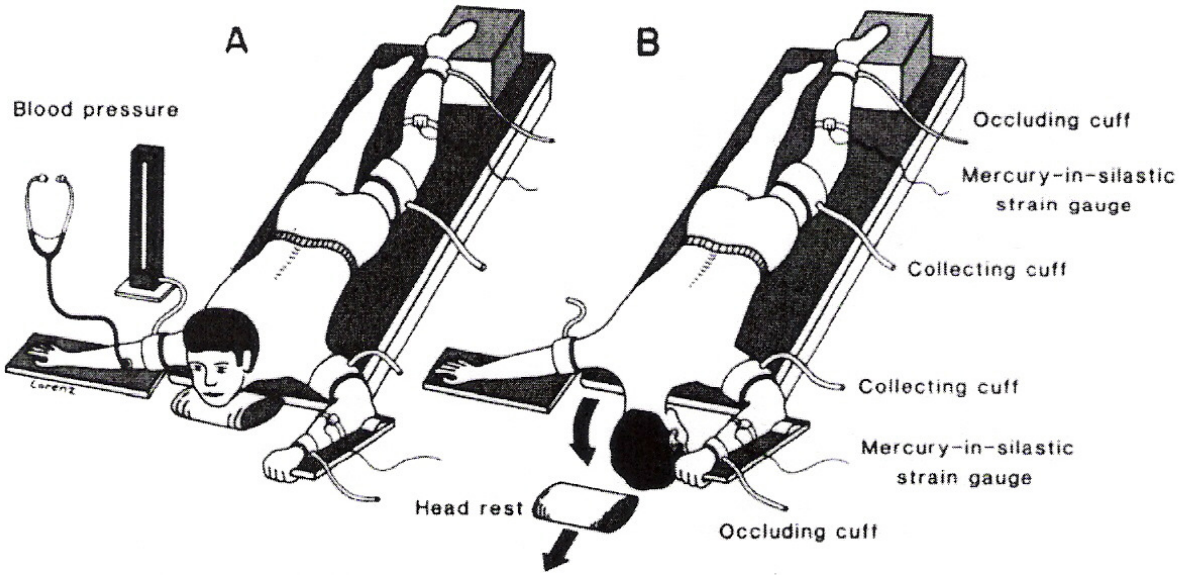

Figure 1.4. The schema of the head-down neck flexion.
Participants lay prone (A) and head position is lowered below heart level (B) to stimulate the vestibular apparatus, particularly the otolith organs. Adapted from Essandoh et al. (1988).

The technique used by Essandoh et al. (1988) to alter vestibular activity was "head-down neck flexion (HDNF)", designed to lower head position below heart level while participants lay prone (Figure 1.4). Ray and colleagues used HDNF to extensively investigate the vestibulo-sympathetic reflex in humans and demonstrated that vestibular activation resulted in increases in BP (Shortt & Ray 1997; Hume & Ray 1999), HR (Shortt & Ray 1997), and muscle sympathetic nerve activity (Shortt & Ray 1997; Ray & Hume 1998; Hume & Ray 1999; Monahan & Ray 2002) and a decrease in muscle blood flow (Ray et al. 1997; Shortt & Ray 1997; Hume & Ray 1999; Monahan & Ray 2002). Other researchers have also investigated the vestibulo-sympathetic reflex evoked as a result of HDNF, some recording similar parameters to that of Ray and colleagues (Normand et al. 1997; Tobal et al. 2002) while others measured cerebral blood flow (Wilson et al. 2003; Cooke et al. 2004) and cardiac autonomic nervous outflows (Lee et al. 2001; Cooke et al. 2004). Vestibular activation does not appear to influence sympathetic nerve outflow to the skin (Ray et al. 1997) or the velocity of cerebral blood flow (Wilson et al. 2003; Cooke et al. 2004).

The effect of HDNF on cardiac autonomic nervous drives (i.e., HRV parameters) is inconclusive (Lee et al. 2001; Cooke et al. 2004). It was shown that vestibular activation was associated with reciprocal changes in cardiac autonomic balance – that is cardiac sympathetic activation and vagal withdrawal (Lee et al. 2001) while there was no effect of the manoeuvre on HRV parameters (Cooke et al. 2004). It was suggested that the difference in study results might be attributed to whether respiratory rate was controlled during data collection (Cooke et al. 2004). Additionally, it was found that calculated effect sizes on HRV parameters were different between these two studies. The effect size in the previous study (Lee et al. 2001) was large (ranged from 0.75 to 2.46) while that in Cooke et al.'s study was small to moderate (ranged from 0.24 to 0.68). This difference in effect size between the two studies may result in the contradictory results and in turn, the HDNF form of vestibular activation is likely to increase sympathetic nerve outflow to the heart. Therefore, the effect of HDNF on cardiac autonomic nervous drives is yet to be resolved.

"Head-down neck flexion" has been commonly employed to investigate the vestibulo-sympathetic reflex, however, it has been argued that the head displacement potentially alters other sensory inputs including neck proprioceptors and (transmurally) baroreceptors (Bolton et al. 2004; Wilson et al. 2006a). To address the possible influence of these factors, Essandoh et al. (1988) carried out series of experiments. In order to examine the involvement of neck proprioceptors, the head was displaced to the flexion position (chin-to-chest) while volunteers were in the supine posture (n = 3). The head displacement did not reveal cardiovascular changes. The authors stated that the result provided evidence that that neck proprioceptors did not involve in changes in autonomic drives and cardiovascular function to "head-down neck flexion" (Essandoh et al. 1988). In contrast, later studies by these same investigators using a larger sample size demonstrated that neck flexion during the side-lying posture had a significant impact on cardiovascular function (Normand et al. 1997; Lee et al. 2001), however

Ray and Hume (1998) and Watenpaugh et al. (2002) failed to demonstrate any influence from neck receptors using the same procedures. Because the neck flexion manoeuvre used in these studies may stimulate various (sensory) receptors including transmurally baroreceptors, the contribution of neck afferent to autonomic and cardiovascular functions are not conclusive. On the other hand, neuroanatomical evidence supports that neck proprioceptive inputs may influence autonomic regulation of cardiovascular function (Yates & Stocker 1998; Gdowski & McCrea 2000).

Investigations on the vestibulo-sympathetic reflex have been conducted using methods other than HDNF in attempt to eliminate the involvement of non-vestibular receptors including neck proprioceptors (Yates et al. 1999; Radtke et al. 2000; Yamamoto et al. 2000; Cui et al. 2001; Kaufmann et al. 2002). Cui et al. (2001) employed four directions of a horizontal linear acceleration (anterior-to-posterior and right-to-left) and found that muscle sympathetic nerve activity of the common peroneal nerve was suppressed in response to the linear acceleration. This result seems to contradict eariler studies, which used HDNF (e.g., Shortt & Ray 1997; Ray & Hume 1998). However, the authors explained the differences that 1) HDNF may stimulate both utricle and saccule while the linear acceleration likely stimulates the utricle only, and 2) the duration and/or strength of vestibular stimulus were different between HDNF and linear acceleration (Cui et al. 2001).

Kaufmann et al. (2002) employed a vertical axis rotation with simultaneous ±15° whole body tilt in the sagittale plane in seated humans. The purpose of the constant vertical axis rotation was to induce a constant signal from the semicircular canals. In this way, sensory input from the otolith organs could be altered continuously during the rotation (and head orientation changes). It was found that muscle sympathetic nerve activity increased when the head was tilted towards the nose-up position while when the head orientation moved towards to the

nose-down position muscle sympathetic nerve activity decreased (Kaufmann et al. 2002). It was concluded that the responses of autonomic drives to otolith stimulation were dependent on the direction of the gravitational stimulus (Kaufmann et al. 2002), whereas direction-dependency of gravity application to the head was not observed in muscle sympathetic nerve activity (Cui et al. 2001). This contradictory result may be attributed to differences in analytical methods; for example, Cui et al. (2001) merged muscle sympathetic nerve activity obtained during one direction of linear acceleration whereas Kaufmann et al. (2002) averaged data every 0.1 seconds.

Other studies compared cardiovascular responses to vestibular stimulation between healthy controls and vestibular deficit patients (Yates et al. 1999; Radtke et al. 2000; Yamamoto et al. 2000). Yates et al. (1999) showed that vestibular activation by linear acceleration induced a significant increase by 9 mmHg in BP in seated asymptomatic controls but only a small increase by 4 mmHg in BP in bilaterally vestibulectomised patients. Radtke et al. (2000) employed an abrupt fall of the head to induce vestibular stimulation. The head was displaced backwards by 10 cm from a head lifted position while participants lay supine. The head fall resulted in shortened R-R intervals immediately following head drop only in healthy controls (not in vestibular deficit patients) although the same response with a longer latency was seen in both groups presumably due to neck sensory activation (Radtke et al. 2000). Furthermore, in order to stimulate the otolith organs, Yamamoto et al. (2000) applied a centrifugal force to seated participants while simultaneously moving them forward or to the side. A laterally applied centrifugal force increased BP in healthy participants while either type of centrifugal force did not induce any cardiovascular changes in vestibular deficit patients. Taken together, these studies provide convincing evidence that the observed cardiovascular responses resulted from vestibular stimulation.

Another technique to stimulate the vestibular apparatus, percutaneous electrical stimulation (galvanic vestibular stimulation), was recently employed in vestibulo-autonomic reflex investigations (Bolton et al. 2004; Bent et al. 2006; Voustianiouk et al. 2006). Importantly, galvanic vestibular stimulation is capable of isolating vestibular activation from other sensory inputs (Carter et al. 2005) compared with HDNF. Bolton and colleagues (2004) first documented galvanic vestibular stimulation use in investigations on the vestibulo-autonomic reflex in humans. It was demonstrated that a 1-second vestibular stimulus was associated with skin sympathetic nerve activation (Bolton et al. 2004). A follow-up study applied a sinusoidal polarity change to the vestibular apparatus (at 0.5 or 0.8 Hz, 60-100 cycles) (Bent et al. 2006). It was demonstrated that muscle sympathetic nerve activity increased in response to the sinusoidal vestibular stimulation and the extent of the increase was dependent on the time of vestibular stimulus application within a heart beat cycle (Bent et al. 2006). A similar phenomenon were observed by Voustianiouk and colleagues (2006) where train-formed galvanic vestibular stimulation produced a significant alteration in muscle sympathetic nerve activity at a certain time within the heart cycle. Therefore, these results indicate not only that vestibular stimulation is likely to influence sympathetic nerve drive to the vasculature but also that afferent inputs from the vestibular apparatus and barocereptors interact to control sympathetic outflow to the muscle vasculature (Bent et al. 2006).

The human studies discussed above employed stimulation of the otolith organs or both otolith and semicircular canals. However, there have been studies which have examined the participation of the semicircular canals only in the responses of autonomic drives. For example, Ray and colleagues investigated the effect of horizontal semicircular canal stimulation achieved by voluntary neck rotation around the horizontal plane (Ray & Hume 1998; Ray et al. 1998). These studies demonstrated that neck rotation resulted in non-significant changes in sympathetic nervous outflows to the skin and muscle although a slight

increase in BP (by 3 mmHg) was found (Ray & Hume 1998). On the other hand, Convertino et al. (1997) employed whole body rotation around a vertical axis to stimulate the horizontal semicircular canals in stead of neck rotation, which eliminates stimulation of neck structures. This study demonstrated that horizontal semicircular canal stimulation induced a suppression in HR control mediated by parasympathetic drive (Convertino et al. 1997). In addition to rotatory motion of the head or head-body, caloric vestibular stimulation (specifically stimulating the horizontal semicircular canal) has also been used as a stimulus to investigate the vestibulo-autonomic reflex. It was shown that caloric vestibular stimulation did not influence cardiovascular parameters (BP and HR) or muscle sympathetic nerve activity (Costa et al. 1995). However, more recently, Cui and colleagues demonstrated a significant enhancement in muscle sympathetic nerve activity (Cui et al. 1997b) and an inhibition in skin sympathetic nerve activity (Cui et al. 1999). It was explained that the conflicting results might be attributed to a difference in analytical method (Cui et al. 1997b); that is Cui et al. (1997b; 1999) compared data averaged over 10 second periods while Costa et al. (1995) averaged data over 30 seconds. Data averaged over a longer period might have lost sensitivity to detect a transient change in outcome measures (Cui et al. 1997b).

1.3.4. The pattern of the vestibulo-autonomic reflex in different vascular beds

As summarised above, it has been well documented that the vestibular system contributes to autonomic regulation of cardiovascular function in anaesthetised and awake animals and humans. The physiological significance of the vestibulo-autonomic reflex is thought to minimise cardiovascular perturbations that result from orthostatic stress, in a feedforward manner in contrast to the baroreflex that operates in a feedback manner (Gotoh et al. 2004). Therefore, the vestibulo-autonomic reflex may be different from global sympathetic activation, which is induced as a consequence of a “fight-or-flight response” (Rhoades & Tanner 2003).

Kerman and colleagues carried out a series of experiments (Kerman & Yates 1998; Kerman et al. 2000a). These studies found that responses of the sympathetic nerves to electrical stimulation of the vestibular nerves were dependent on the internal organs innervated by the recorded nerves. For example, the renal nerve, which regulates blood flow in the kidney, remarkably responded to the vestibular stimlation while the hypogastric nerve, which contributes to bladder constriction rather than vasculature control, responded only modestly (for review see Kerman et al. 2000b). In addition, Wilson and colleagues employed orthostatic stress (whole body tilt in a nose-up direction) to examine the effect of vestibular removal on blood perfusion to the head (Wilson et al. 2006a) and limbs (Wilson et al. 2006b) in conscious cats. It was found that in response to the orthostatic stress, blood flow in the limbs significantly decreased in vestibular-intact cats while these reductions were blunted following vestibular removal (Wilson et al. 2006b). This suggests that vestibular input makes a significant contribution to the regulation of blood vessels in the limbs. In contrast, it was found that the response of head blood perfusion to the orthostatic stress was not significantly influenced by vestibulectomy (Wilson et al. 2006a). It is important to note that the enhancement of blood perfusion to the head at rest occurs with increases in BP (Boron & Boulpaep 2003). In the vestibulectomised cats, BP recorded in the horizontal body position did not show consistent changes across individuals. Thus, it was assumed that the vestibular system might contribute to the control of the tonus of the vasculature in the head (Wilson et al. 2006a).

Hence, these studies indicate that the vestibulo-autonomic reflex influences specific sympathetic efferents in order to maintain cardiovascular homeostasis rather than non-specific global sympathetic responses. It appears that this reflex prioritises its influence on those sympathetic efferents that innervate end organs, which have more blood vessels. To preven an

orthostatic hypotensive event, this prioritization is advantageous for optimising blood re-distribution in response to orthostatic stress (Wilson et al. 2006b).

1.3.5. The vestibulo-autonomic reflex mediated by the parasympathetic component of the autonomic nervous system

Many studies discussed so far used direct recording of sympathetic nerve activation or measured end organ functions. Although neuroanatomical evidence suggests that vestibular afferents project to the dorsal motor nucleus of the vagal nerve (Ruggiero et al. 1996; for review see also Balaban & Yates 2004), fewer physiological studies have examined vestibular-related parasympathetic responses as compared with sympathetic responses. It has been demonstrated though that parasympathetic drive contributes to the vestibulo-autonomic reflex when measured in the heart (Convertino et al. 1997; Lee et al. 2001). For example, as discussed in *section 1.3.3*, Convertino et al. (1997) demonstrated that activation of the horizontal semicircular canal resulted in a suppression of HR control mediated by vagal drive. Lee et al. (2001) showed that otolith organ stimulation using the HDNF manoeuvre decreased the participation of parasympathetic drive in cardiac control using HRV analysis.

Collectively, these studies are suggestive that the vagus nerve may also contribute to the vestibular-induced responses. Because the two divisions of the autonomic nervous system often influence the internal organs reciprocally but not always (Boron & Boulpaep 2003), further investigations on the contribution of the vagus nerve to vestibular-related responses such as cardiovascular regulation are essential.

1.4. Somato-autonomic reflexes

"Somato-autonomic reflexes" are a response of the autonomic nervous system in mediating to stimulation of somatic structures such as skin and joints. Subsequently, internal organ

function is influenced (somato-visceral reflexes). According to Sato et al. (1997a), Ludwing and colleagues first demonstrated that electrical stimulation of limb afferents induced a BP response in anaesthetised animals during the 1860's and 70's. Alexander was the first to report the existence of the somato-autonomic (-sympathetic) reflex *per se* in 1946 using an electrical stimulus to excite somatic nerves and in 1964 Beacham and Perl provided the first evidence that the spinal cord is an integration centre for somato-autonomic reflexes using electrical stimulation of somatic nerves to elicit a response from the sympathetic nervous system (Sato et al. 1997a).

One of the significant contributions of somato-autonomic reflex research is to elucidate mechanisms of many types of physical therapies such as manipulation, acupuncture, and moxibustion (Sato et al. 1997a). In fact, in Japan, these kinds of physical therapies were routinely utilised to treat various organ dysfunctions from the 7^{th} to 19^{th} century (Sato & Schmidt 1987). In Western society, manipulation was used for Greek athletes as early as 400 B.C. (Brolinson 2003). Although manipulation has such a long history and is frequently used for the treatment of musculoskeletal conditions (Budgell 1999), so far little evidence supports the proposal that manipulative therapy including chiropractic may make some contribution to the treatment of visceral complaints (Budgell 1999). Reports in the literature from both clinics and laboratories are relatively few, however important evidence has been obtained that points to the plausibility of the notion that stimulation of somatic structures (such as with spinal joint manipulation or acupuncture) might be capable of influencing autonomic nervous activity and consequently, visceral function. However, psychological factors are an inescapable barrier to somato-autonomic reflex investigations in humans. Use of anaesthesia enables studies in animals to overcome these confounding factors (Sato et al. 1997a). The purpose of this section is to review literature relating to the somato-autonomic reflex before exploring human studies where these reflexes may be presumed to be manifested.

1.4.1. Somato-autonomic reflexes in animals

An enormous number of studies have been carried out in animals on somato-autonomic reflexes and such reflexes have been demonstrated in various organs including the heart, stomach, and bladder in animals. An extensive review appears in the monograph by Sato and colleagues (1997a). The following sections will focus on studies related to cardiovascular responses to somatic stimuli.

Compared with observable cardiovascular responses to somatic stimuli, sympathetic nerve responses to somatic stimulation are complicated and are the result of the integration of each component of the somato-autonomic reflex (Sato et al. 1997a). The characterization of somato-autonomic reflexes has been fully reviewed (for review see Sato et al. 1997a).

The responses of the autonomic nervous system to electric somatic stimulation has been characterised according to the type of afferents that are stimulated (myelinated or non-myelinated) and the location of the response centre (spinal or supraspinal). Firstly, sympathetic responses to stimulation of the myelinated nerves (Aβ and Aδ afferents) are observed at different latencies. When the sciatic nerve is electrically stimulated in chloralose-anaesthetised cats with denervated baroreceptor afferents, two phases of reflexive response are recorded from a lumbar white ramus. Responses are observed at 25-50 and 80-120 ms following electrical stimulation of the corresponding dorsal root using stimulus intensities that recruit the larger diameter families of nerve fibres (Sato et al. 1965, quoted in Sato et al. 1997a). The responses have been collectively called the "A" reflex, and particularly the former component is called the "early A" reflex and the latter is called the "late A" reflex. Furthermore, a longer latency (300-350 ms) sympathetic response to myelinated nerve stimulation has also been reported only in animals maintained under in lightly chloralose-anaesthetised condition (Sato 1972, quoted in Sato et al. 1997a). This reflex has been called

the “very late A” reflex. The late and very late A reflexes disappear after spinalization, suggesting that supraspinal structures are involved in these reflex components. In contrast, the early A reflex remains following spinalization, which indicates that the integration (processing) centre for this reflex lies in the spinal cord.

In addition to the A reflexes, sympathetic nerve responses have also been observed after electrical stimulation at a strength sufficient to recruit unmyelinated afferent (C fibres) fibres. This reflex response is known as the “C” reflex. The C reflex has a longer latency than the A reflexes and involves both spinal and supraspinal structures as integration centres. Afferent inputs evoked by a single electrical stimulus are capable of inducing sympathetic nerve responses (C reflex) when the electrical stimulus is evoked from a dorsal root at the same level or close to the same spinal cord level to where the efferent sympathetic response is recorded from. This C reflex has been shown to persist after spinalization, indicating that spinal cord structures are responsible for integrating the afferent signal and initiating the reflex response. In addition, sympathetic nerve responses (C reflex) have been recorded at spinal cord levels some distance away from where the stimulation was induced when multiple volleys are used (i.e., “temporal facilitation”). Because the C reflex is observed at spinal cord segments away from the level of the spinal cord where afferent fibres are stimulated and these responses disappear after spinalization, it must be concluded that supraspinal structures are involved in the organization of the sympathetic response.

1.4.1.1. Noxious stimulation from cutaneous tissues and muscles

Noxious stimuli include mechanical, thermal, and chemical stimuli and activate nociceptors. The sensory information is sent to the CNS via smaller diameter afferents (group III and IV). In general, this type of stimulus induces powerful and consistent autonomic responses (Sato et al. 1997a). For example, Kaufman et al. (1977) examined the effects of noxious mechanical

and thermal stimuli on HR in anaesthetised cats. In response to a noxious thermal stimulus, HR tended to increase progressively both below (13-19°C for cold stimulus) and above the threshold (approximately 40°C for warm stimulus) stimulus for activation of these receptors. Particularly, a thermal stimulus lower than 7°C or higher than 43°C, which was estimated to be a noxious or close-to-noxious stimulus because a flight response was observed in response to the application of both extreme temperatures (7°C or lower, and 46°C or higher), induced a HR response. In addition to thermal stimulation, both noxious and innocuous mechanical stimuli were employed in this study (Kaufman et al. 1977). In response to noxious mechanical stimulation of the skin (pinching), HR increased more than four times to that observed after innocuous mechanical and thermal stimulation.

Araki et al. (1980) demonstrated that the adrenal efferent nerve responded to a similar form of noxious mechanical stimulation (pinching) applied to various areas of the skin over the entire body of the rat. The adrenal efferent nerve response was always observed in CNS intact rats, however the amplitude of the response was different when stimulating different body areas. Following spinalization at the C_{1-2} level, significant adrenal efferent responses were observed by a noxious mechanical stimulus applied to only *limited* segments of the body (i.e., the lower chest and abdomen). More recently, Kimura et al. (1995) systematically investigated the effect of a noxious mechanical stimulus (pinching) applied to various segmental skin regions on autonomic nervous (cardiac and renal sympathetic nervous activity) and cardiovascular (HR and BP) functions in anaesthetised rats. All parameters were increased by the noxious stimulus when this was applied to any region of the body when the CNS was intact. Particularly large responses were induced by hindpaw stimulation. In contrast, when the animal was spinalised at the C_2 level, sympathetic and cardiovascular responses were induced by stimulation of limited regions of the body, specifically, thoracic-to-lumbar spinal segments (Kimura et al. 1995). This suggests that there is the integration centre of the somato-

autonomic reflexes in the spinal cord. In addition, this study also demonstrated lateralization of the somato-autonomic reflex. Ipsilateral stimulation produced larger cardiac and renal sympathetic responses and when the somatic stimulus was applied to the right side of the animal and a larger HR increase was also observed. The laterality was observed only following spinalization but was not observed in CNS intact animals. The study clearly revealed the two different integrating centres for the processing of somato-autonomic reflexes (at spinal cord and supraspinal levels). These study results also indicated that the supraspinal component, in particular, was responsible for organising segmentalised and lateralised somatosensory inputs (Kimura et al. 1995).

1.4.1.2. Innocuous stimulation of cutaneous and deeper tissues including muscles

Sensory information from innocuous stimuli is conveyed to the CNS by larger diameter afferent fibres (group I-III). Innocuous somatosensory afferents are connected to receptors such as muscle spindles, Golgi tendon organs, Pacinian corpuscles, and Ruffini organs. Each type of sensory receptor responds to specific type of stimulus including muscle stretch, vibration, pressure, and thermal stimuli.

It was assumed that all type of somatosensory inputs similarly evoked autonomic responses, however, it has been known that the assumption is not correct and somato-autonomic reflexes induced by innocuous stimuli tend to be inconsistent unlike autonomic responses to noxious stimuli (Sato et al. 1997a). However, Kurosawa et al. (1982) demonstrated that an innocuous mechanical stimulus (brushing) applied to various body areas of the skin decreased adrenal efferent nerve activity in CNS intact rats. Furthermore, when the spinal cord was transected at the C_{1}-$_{2}$ level, this same stimulus applied ipsilateral to the recording site induced an opposite response; that is, adrenal efferent activity increased. This study indicated that supraspinal structures are required to distinguish the type of stimulation (noxious or innocuous) and that

this differentiation does not occur at the spinal level (Kurosawa et al. 1982). Recently, the effect of an innocuous cutaneous mechanical stimulus (brushing) on various body areas on dorsal spinal cord blood flow was examined in anaesthetised rats (Kurosawa et al. 2006; Kurosawa et al. 2007). The blood flow response was observed when the adjacent segment of the skin to the recording site was stimulated while BP and HR were not influenced (Kurosawa et al. 2006). Blockade of α-adrenergic receptors moderately suppressed the blood flow response to the cutaneous stimulus (Kurosawa et al. 2006) and spinalization at the C_2 level abolished the suppressive effect of α-adrenergic receptor blockade (Kurosawa et al. 2007). These studies suggested that the response of dorsal spinal cord blood flow to an innocuous stimulus was regulated both spinally and supraspinally and especially, α-adrenergic receptors were involved in the supraspinal arc (Kurosawa et al. 2007).

In addition to the brushing form of innocuous cutaneous stimulus, Kurosawa and colleagues showed that a massage-like stroking stimulus, which was presumed to be a non-noxious stimulus, decreased HR and BP in anaesthetised (1995) and conscious (1999) rats. In these studies, it was noted that the stroking-like stimulus delivered to the ventral side of the body induced larger depressive cardiovascular responses than when the stroking-like stimulus was applied to the back. The depressive effects of the back stroking stimulus on cardiovascular parameters lasted up to 60 minutes while 5-minutes of abdomen stroking induced longer cardiovascular effects (< 4 hours) (Lund et al. 1999).

There has been general agreement in the literature that group I and II fibres originating from muscle spindles and Golgi tendon organs do not evoke somato-autonomic or -visceral reflexes (Coote 1975; Sato et al. 1997a). A few studies investigated the involvement of the larger diameter afferents (group I and II) in somato-autonomic and -visceral reflexes using vibration (McCloskey et al. 1972), electrical stimulation (Sato et al. 1981), and succinylcholine

injection to the saphenous artery (while the venous return from the ipsilateral hindlimb was temporarily occluded) (Sato et al. 1982). These studies showed that there was no contribution of these types of afferent nerves to cardiovascular function. However, it is important to note that these studies examined muscle afferents arising from the limbs only, and not from the paraspinal structures. Therefore, the notion that group I and II fibres supplying paraspinal tissues might contribute to somato-autonomic reflexes will be discussed separately below.

1.4.1.3. Somatic stimulation of paraspinal structures

So far, there have been only a few studies that have examined autonomic responses to spinal joint stimulation in an animal model (Sato & Swenson 1984; Bolton et al. 1998; Kang et al. 2003; Bolton et al. 2006).

Sato and Swenson (1984) examined the influence of mechanical stimulation directed to spinal structures that may have expected to include joints, intervertebral discs, and ligaments, on renal and adrenal sympathetic nerve activity and cardiovascular function in anaesthetised rats. The mechanical stimulation used in the study was a laterally applied mechanical force (0.5-3.0 kg) to the thoracic or lumbar column after the surrounding muscles were eliminated. In response to the mechanical load (> 2.0 kg), BP and renal nerve activity decreased during the application of the stimulus, and adrenal nerve activity increased above baseline levels following a transient decrease. Therefore, this study suggested that spinal afferent inputs arising from the thoracolumbar regions had an impact on sympathetic nerve activity and cardiovascular function. However, it was noted that the mechanical stimulation used in the study was presumed to be innocuous but this assumption still requires clarification (see discussion in Sato & Swenson 1984). Kang et al. (2003) carried out a series of experiments to investigate the effects of mechanical stimulation on sympathetic nervous drive and cardiovascular function in anaesthetised cats. Compared with the above-mentioned study

(Sato & Swenson 1984), Kang et al. (2003) applied a mechanical load (100% of animals' body weight), which was assumed to be innocuous, to the lumber spinous process without dissecting surrounding muscles. The study showed that this form of mechanical stimulation to the lumbar spine did not induce any significant responses. However, once the lumbar muscles were in an inflamed condition, which was experimentally induced by a mustard oil injection, the effects of the mechanical load were revealed. The effects were suppressive in nature and included reductions in HR and renal and splenic sympathetic nerve activity (Kang et al. 2003).

Bolton and colleagues investigated the influence of neck afferent stimulation on autonomic drives in cats (Bolton et al. 1998) and rats (Bolton et al. 2006). It was demonstrated that electrical stimulation of the C_2 or C_3 dorsal ramus (at the intensity to activate afferents arising from muscle spindles and tendon organs) induced splanchnic, hypoglossal, and abdominal nerve responses (Bolton et al. 1998). Also, head motion around the sagittal plane (with steady body position) induced a change in hypoglossal nerve activity but not in splanchnic and abdominal nerve activity, whereas following denervation of the neck afferents, a modulation of splanchnic and abdominal nerve activity were revealed and hypoglossal nerve activity became unstable in response to head motion around the sagittal plane. This may suggest that neck afferent inputs participate in the coordination of lingual musculature movement (the hypoglossal nerve) in a synergic manner with vestibular inputs (induced by the head motion) and sympathetic regulation (the greater splanchnic nerve) and in an antagonistic manner with respiratory control (the abdominal nerve) (Bolton et al. 1998). More recently, C_2 vertebrae movement (around the cephalic-caudal axis) was employed to stimulate cervical structures independently of whole head motion to investigate the influence of neck afferent inputs on adrenal efferent activity (Bolton et al. 2006). It was shown that the cervical rotation, regardless of whether this motion was in the noxious range, greater than 20° or innocuous range (less than 20°), induced little change in adrenal efferent activity (Bolton et al. 2006).

Collectively, although there have been relatively few studies that have investigated the role of spinal and paraspinal structures in autonomic and cardiovascular regulation, the current available evidence appears to suggest that somatic stimulation of the axial structures does not evoke a consistent response from the adrenal (sympathetic) nerve whereas cardiovascular parameters (BP and HR) and splanchnic nerve activity (including the renal nerve) are influenced. Thus, the available evidence points to a preliminary conclusion that large diameter afferents (including group I and II fibres) may evoke a response from selective parts of the sympathetic nervous system.

1.4.2. Somato-autonomic reflexes in humans

1.4.2.1. Clinical reports regarding manipulative therapies for cardiovascular dysfunctions

McGee (1992) reported a case of a significant reduction in BP observed in a hypertensive patient following a series of chiropractic adjustments (manipulation of the spine) over a period of 4 and a half weeks. In addition, medication usage by the patient was reduced to half and no BP rebound was observed at 3 weeks follow up. Similarly, Plaugher & Bachman (1993) reported that a patient undergoing antihypertensive treatment (for essential hypertension) was able to stop medication use under a physician's instruction following 7 chiropractic treatments. This return to a normotensive condition was confirmed at an 18-month follow up. A large case series study documented the effects of cervical manipulation on patients suffering hypertension following a course of treatment (average 25.1 days). The study also reported progress in a long-term follow-up (average 3.6 years). The study reported that the hypertension was eased after a course of treatment in a significant proportion of patients who were observed. Furthermore the majority of patients (75.9%) who initially responded with a reduction in BP were still free from hypertension at follow up (Wei et al. 1989).

In addition to studies in the literature reporting the effects of manipulation on hypertension, there are two case studies reporting on the effect of manipulation on arrhythmia. One case study was reported that the arrhythmia was resolved immediately after a spinal manipulation treatment (Igarashii & Budgell 2000; Budgell & Igarashi 2001).

These clinical reports suggest that somatic dysfunctions may result in visceral dysfunction and the visceral dysfunction appears be eased by manipulative therapies. Therefore, a question arises whether it is plausible to suggest that manipulative therapies might impact on cardiovascular dysfunctions such as hypertension. Case reports and case series report on observations in clinical practice and these may form the basis for further research (Portney & Watkins 2000, p. 14). The case study is a practical method of research, however, the strength of evidence provided by this type of study is limited. This is because there may be a large number of factors that influence the outcome of the study and generalizations cannot be made from a small number of single case study reports. It was indeed found in an international survey that chiropractor's positive attitude towards treatment for non-musculoskeletal conditions tended to influence a patient's perception of non-musculoskeletal symptom relief (Leboeuf-Yde et al. 2005). On the other hand, experimental studies are used to clarify a "cause and effect", that is, the relationship between the clinical observation and the intervention used in clinic. Therefore, studies carried out under more controlled conditions are definitely required to justify whether or not manipulative therapies normalise cardiovascular function and studies and these studies are reviewed below.

1.4.2.2. Randomised Clinical Trials

The randomised clinical trial provides the most powerful evidence (gold standard) for the effectiveness of an intervention. So far, five randomised clinical trials have been published that investigate the effectiveness of spinal manipulative therapies for hypertension.

Morgan et al. (1985) conducted a study using 29 patients allocated into two groups. The first group received spinal manipulation for the first 6 weeks and massage (sham) for the subsequent 6 weeks and the second group received the sham intervention first, followed by spinal manipulation. Blood pressure was recorded for both groups over the 12 weeks, and at a 6 weeks follow-up period. The study failed to demonstrate a change in BP over the intervention and follow up periods. Later, Yates et al. (1988) assigned 21 patients into 3 groups (treatment, placebo, and no intervention control) within a single trial. The treatment group received actual manipulation to the upper back using a mechanical therapeutic instrument, the placebo group received a sham intervention by use of the same instrument without the addition of any mechanical force, and the control group stayed in the treatment room for the same period of time without receiving any intervention at all. It was found that both systolic (SBP) and diastolic BP (DBP) were significantly lowered (by approximately 15 and 13 mmHg, respectively) in the treatment group during a single treatment session. Patients in the "treatment" group also experienced a reduction in the level of their anxiety, which was not reported by the other groups (Yates et al. 1988).

A longitudinal (pilot) study (8-week treatment and 8-week follow up) was carried out in the United States using hypertensive patients (either stage I or II). Study participants were allocated into three groups; spinal manipulation (n = 9), brief massage (n = 8), and no treatment control (n = 6) (Plaugher et al. 2002). In the study, no consistent BP change was observed in the spinal manipulation or massage group over the treatment or follow up periods while DBP in the control group appeared to consistently decrease over the course of the study. According to the authors' sample size estimation, 255 patients were required in order to achieve 80 % statistical power for their study design (Plaugher et al. 2002). More recently, Bakris et al. (2007) carried out a blind randomised control trial (upper cervical manipulation *vs.* placebo) and recruited 50 patients with stage I hypertension. Patients were divided into

two groups, either manipulation or placebo (2-week wash out from anti-hypertensive medication). The intervention was administered during the first week only and observations were then made every week up to the eighth week. The cervical manipulation group experienced significant reductions in both SBP and DBP (by 17 and 10 mmHg, respectively) (Bakris et al. 2007).

The above randomised trials examined the effectiveness of spinal manipulation for hypertension comparing spinal manipulation with massage therapy as a placebo and/or with a no intervention control. In addition to these studies, one randomised clinical trial employed a non-physical therapeutic type of intervention in individuals with high-normal BP or stage I hypertension (Goertz et al. 2002). Comparisons were made between spinal manipulation with diet management (n = 71) and diet management itself (n = 69). Instruction in diet management (including low fat and low salt recipes) in both groups was provided by a registered dietician. Following a 4-week trial, both groups exhibited significant reductions in SBP and DBP but no difference between these groups was observed, suggesting that no additional effect was obtaining by combining spinal manipulation with diet management to manage stage I hypertension (Goertz et al. 2002).

1.4.2.3. Experimental studies

Several studies have examined whether the procedures of physical therapies (somatic stimuli) themselves influence autonomic drives and/or cardiovascular function. A single case study investigated whether spinal manipulation had an impact on autonomic drives in a normotensive patient with neck and shoulder pain (Driscoll & Hall 2000). The study documented the effect of a course of chiropractic treatment over a period of 5 weeks (10 visits). The study reported that cardiac sympathovagal balance tended to shift towards sympathetic dominance and BP decreased (Driscoll & Hall 2000). Knutson (2001) examined

the effect of spinal manipulation delivered to the upper cervical spine on BP in patients who ranged between being normotensive and hypertensive. The study found that spinal manipulation decreased SBP by 10 mmHg and the reduction was greater in the more elderly group (greater than 55 years old).

Fujimoto et al. (1999) demonstrated that decreases in HR and BP were induced by various forms of innocuous mechanical stimuli applied to the neck; including spinal manipulation, 90° passive neck rotation, and rhythmic neck rotation in human subject. Of note in this study was that different levels of consciousness during the manipulative interventions resulted in different results; that is, if the subject fell asleep during the interventions, parameters of their cardiovascular function were often the reverse of the those of the non-sleeping subjects (Fujimoto et al. 1999). Budgell and colleagues examined the effect of spinal manipulation on cardiac autonomic nervous function in normotensive young adults using HRV (Budgell & Hirano 2001; Budgell & Polus 2006). These studies used a pretest-posttest design and compared different types of manipulative procedures including a "high-velocity and low-amplitude thrust" that produced an audible sound (authentic) and a similar intervention without any audible sound (sham). A significant shift in cardiac sympathovagal balance towards sympathetic dominance was found following the high-velocity and low-amplitude thrust delivered to the upper cervical (Budgell & Hirano 2001) and upper thoracic (Budgell & Polus 2006) spines. While the sham intervention delivered to either upper cervical or thoracic spine did not induce significant change in HRV parameters, a reduction in HR was found across the interventions (Budgell & Hirano 2001; Budgell & Polus 2006).

In addition, a large clinic-based study, which involved 539 patients and 96 chiropractors, was carried out in the U.S. (Zhang et al. 2006). The study demonstrated that a single chiropractic treatment significantly decreased HR and altered HRV parameters (indicating a shift towards

vagal dominance) compared with parameters obtained at a pre-treatment visit. The treatment also lowered patients' pain levels. Further, some patients (n = 111) were followed up for 4 weeks and these patients continued to demonstrate slight but progressive decrease in HR over this follow-up period. HRV parameters tended to alter over the 4-week follow-up measurement period, however the study did not report pre-treatment HRV values so no conclusions can be drawn as to the effect of treatment on these parameters. In contrast, it was clear that pain levels significantly decreased immediately after treatment but the decreased pain level returned to the previous level at the subsequent visit (Zhang et al. 2006).

A research group in Queensland, Australia have conducted a series of studies on the influence of cervical mobilization on the autonomic nervous, cardiovascular, and respiratory systems (McGuiness et al. 1997; Vicenzino et al. 1998; Sterling et al. 2001). It was found that all dependent variables (HR, BP, and respiratory rate) increased in response to cervical mobilization delivered both posterior-to-anterior (McGuiness et al. 1997) and lateral-to-medial (Vicenzino et al. 1998) directions in asymptomatic (no neck pain) young adults, indicating sympathetic activation. Similarly, cervical mobilization (applied posterior-to-anterior) resulted in an increase in skin conductance and a reduction in skin temperature in chronic (more than 3 month) neck pain patients, suggesting that the sympathetic nervous system in skin was activated by the intervention (Sterling et al. 2001).

Interestingly, BP and HR responses to cervical mobilization were opposite to those reported for spinal manipulation (McGuiness et al. 1997; Vicenzino et al. 1998; Fujimoto et al. 1999; Driscoll & Hall 2000; Knutson 2001). This difference in study result was recognised by these authors. The cervical mobilization was performed over a period of 30 to 60 seconds while the spinal manipulation is a fast manoeuvre (McGuiness et al. 1997). It has been hypothesised that different forms of manipulative interventions may influence different sensory beds in

axial structures; that is spinal manipulation may influence the deeper sensory beds while mobilization or massage-like stimulation is likely to be absorbed by the superficial structures (Bolton & Budgell 2006).

The effects of different forms of manipulative interventions on autonomic drives and cardiovascular function have also been examined. Delaney et al. (2002) demonstrated that a trigger point massage procedure (20 minutes) directed to the neck and back region induced reductions in HR and BP and a cardiac sympathovagal balance shift towards vagal dominance. Similarly, a significant reduction in HR was observed 20 minutes following back massage (for approximately 24 minutes) (Ouchi et al. 2006). This study also demonstrated that the HR change was accompanied by a reduction in chromogranin-A concentration in the saliva and activation of brain regions including the amygdala, basal forebrain, and cerebellar vermis revealed by a positron emission tomography. Thus, it may be suggested that back massage is associated with mental relaxation as well as reductions in HR (Ouchi et al. 2006). In addition to these psychological effects in relatively healthy volunteers, it was also found that 20-minute massage to the back were psychologically and physiologically beneficial for patients prior to cardiac catheterization (McNamara et al. 2003). However, even though these therapeutic interventions appear to influence autonomic nervous and cardiovascular functions, it is not clear whether the manipulative interventions *per se* have an impact on these functions, or whether the responses to the interventions were due to psychological influences, or whether both physiological and psychological factors contribute to the outcomes observed.

1.4.2.4. Alterations in somatosensory inputs from the neck by non-manipulative procedures

As summarised above (*section 1.3.3*), HDNF has been employed to investigate the effect of vestibular activation on autonomic nervous and cardiovascular functions. This procedure also activates sensory receptors of the neck, so the involvement of neck proprioceptive inputs in

autonomic nervous and cardiovascular function needed to be clarified. In order to address this issue, neck flexion was performed in the side-lying posture instead of the prone posture for HDNF (Normand et al. 1997). It was assumed that neck flexion in the lateral decubitus posture minimised any change in otolith organ activity while allowing the alteration of neck proprioceptive inputs (although the vestibular apparatus could be activated during head position change). Therefore, the neck flexion manoeuvre in the side-lying posture is capable of examining the influence of neck sensory inputs on autonomic and cardiovascular functions. There have been a few studies that have examined the involvement of neck sensory inputs in autonomic nervous and cardiovascular function using the neck flexion manoeuvre (Normand et al. 1997; Ray & Hume 1998; Lee et al. 2001; Watenpaugh et al. 2002). Some of these studies found that neck flexion in the side-lying posture (in turn, changes in neck proprioceptive inputs) induced sympathetically-induced responses including increases in muscle sympathetic nerve activity, BP, and HR (or shortened R-R intervals) (Normand et al. 1997; Lee et al. 2001), whereas others found no significant effect of neck sensory activation (Ray & Hume 1998; Watenpaugh et al. 2002). More recently, Evans and Polus (2005) employed passive neck rotation on the horizontal plane (concomitant with no head motion) and observed that neck rotation was associated with a shift in cardiac sympathovagal balance towards vagal dominance.

Collectively, the literature reporting the influence of neck afferents on cardiovascular function is conflicting. Additionally, the interventions used in these studies may stimulate various kinds of sensory receptors even including baroreceptors of the carotid arteries transmurally (Bent et al. 2006). Therefore, the contribution of neck receptors to autonomic and cardiovascular functions is yet to be clarified (Bolton & Ray 2000).

1.5. Conclusion

This literature review has focused on the contributions of somatic sensory inputs (including the vestibular apparatus) to autonomic regulation of cardiovascular function in both animals and humans. It is evident that activation of the vestibular apparatus (particularly, the otolith organs) is associated with both changes in autonomic drives and cardiovascular responses in animals and humans, known as the vestibulo-autonomic reflex. Of particular note, the vestibulo-autonomic reflex is thought to contribute to autonomic regulation of cardiovascular function in a "feedforward" manner, whereas the baroreflex predominantly participates in short-term cardiovascular regulation as a "feedback" mechanism.

In addition to vestibular-related changes in autonomic drives and cardiovascular responses, the contribution of somatosensory inputs (other than the vestibular system) to these functions has been investigated. In general, the findings of these studies suggest that noxious stimuli induce consistent and powerful responses of the autonomic and cardiovascular systems, while innocuous stimuli evoke inconsistent or negligible responses. There is general agreement that innocuous stimuli from muscles (i.e., activation of muscle spindles and Golgi tendon organs) are not involved in somato-autonomic (or -vesceral) reflexes. It is worth noting however, that this conclusion was drawn by data obtained from experiments which mechanically or chemically stimulated the "limb" muscles. Few studies have examined the effects of innocuous mechanical stimulation of the "axial" structures including muscle proprioceptors on autonomic drives and cardiovascular function. In humans, somatically-induced cardiovascular responses seem to be documented in a more clinically-driven way, however, there is some evidence that mechanical stimulation of the neck may influence cardiac autonomic and cardiovascular functions using therapeutic procedures such as spinal manipulation. Those studies tend to report that mechanical stimulation similar to spinal manipulation types of mechanical stimuli induce significant changes in autonomic drives and

cardiovascular responses. In contrast, several studies, which used natural head-neck motion rather than externally applied therapeutic types of interventions to the body, tend to produce results that are contradictory and inconclusive.

In spite of some contradictory results, the contribution of neck sensory inputs to autonomic regulation of cardiovascular function in humans requires further investigation. This is particularly important where spinal manipulation-like stimuli are used to evoke neck sensory inputs, as this is a treatment technique commonly utilised by the manual therapy professions. Accordingly, improved understanding of the autonomic and resultant cardiovascular effects of this type of stimulation could affect the health care of many people. Also of importance, spinal manipulation-type mechanical stimuli are likely to cause slight head displacement during its application, possibly leading to vestibular activation (i.e., the vestibulo-autonomic reflex). Therefore, the primary purpose of this thesis is to investigate the contribution of innocuous somatosensory afferent inputs from the neck to autonomic regulation of cardiovascular function in awake humans using techniques that are capable of isolating neck sensory afferent inputs from vestibular inputs. Firstly, the effects of a mechanical stimulus to the neck, with the purpose of simulating cervical manipulation, will be examined using a therapeutic instrument that significantly minimises head displacement during stimulus application. The subsequent studies in this thesis will focus on the contribution of the neck muscle proprioceptors using different techniques – namely the muscle conditioning manoeuvre and a vibratory stimulus.

The specific research questions addressed by this thesis are as follows:

1. Are there any similarities and differences in autonomic and cardiovascular parameters between different static postures particularly between two horizontal postures (prone and supine)?

2. Is it possible to observe changes in autonomic drives and cardiovascular function to a mechanical stimulus to the neck simulating cervical manipulation in humans while head displacement is significantly minimised? If so, is it an arousal effect or genuine response?
3. Do neck muscle proprioceptive afferent inputs contribute to autonomic regulation of cardiovascular function in seated humans?
4. Do neck muscle proprioceptive afferent inputs contribute to autonomic regulation of cardiovascular function in lying humans?
5. Do neck muscle proprioceptive afferent inputs contribute to autonomic regulation of cardiovascular function during mild orthostatic stress in humans with a constant vestibular stimulus?

Chapter 2

Experimental Techniques

2.1. Introduction

This chapter will describe all experimental techniques used in subsequent chapters of this thesis. These descriptions may help readers understand the basic mechanisms of operation of equipment and analytical methods to prepare raw data for statistical analysis as well as the advantages and disadvantages associated with measurement technique used in this thesis.

2.2. Electrocardiography

The electrocardiograph is a non-invasive means of recording the electrical activity of the heart and a commonly used technique in experimental studies and clinical practice. Unlike skeletal muscles, the muscle of the heart has spontaneously generates action potentials and the action potentials spread from one muscle cell of the heart to another. This widespeard of action potentials precedes the muscle contraction of the heart (Mohrman & Heller 2006).

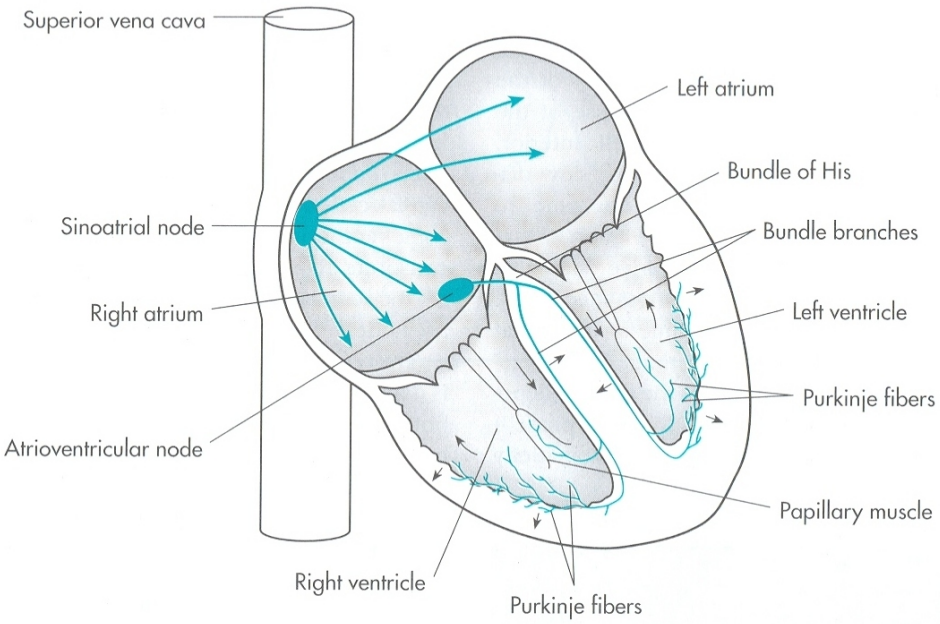

Figure 2.1. Electrical transmission through the heart.
Action potentials occurred at the sinoatrial node spreads the atria and subsequently the ventricles through the atrioventricular node and His bundles. Adapted from Berne & Levy (2001).

As illustrated in Figure 2.1, action potentials are initiated at the sinoatrial node and spread to the atria (Berne & Levy 2001). The spread of action potentials reaches the atrioventricular node and transmits to the ventricles via the His bundle and its branches (Berne & Levy 2001).

This series of action potential transitions in the heart constitutes one cycle of the heart beat and can be viewed on the electrocardiogram (ECG) as the summation of voltage changes between two points on the surface of the body (Mohrman & Heller 2006). Figure 2.2 shows typical ECG signal recorded from an asymptomatic individual using the 3-lead method. The waveform demonstrates three major peaks (P, QRS, and T waves). The P wave reflects the depolarization of the atria, the QRS complex indicates depolarization of the ventricles, and the T wave shows the repolarization of the ventricles (Berne & Levy 2001; Mohrman & Heller 2006).

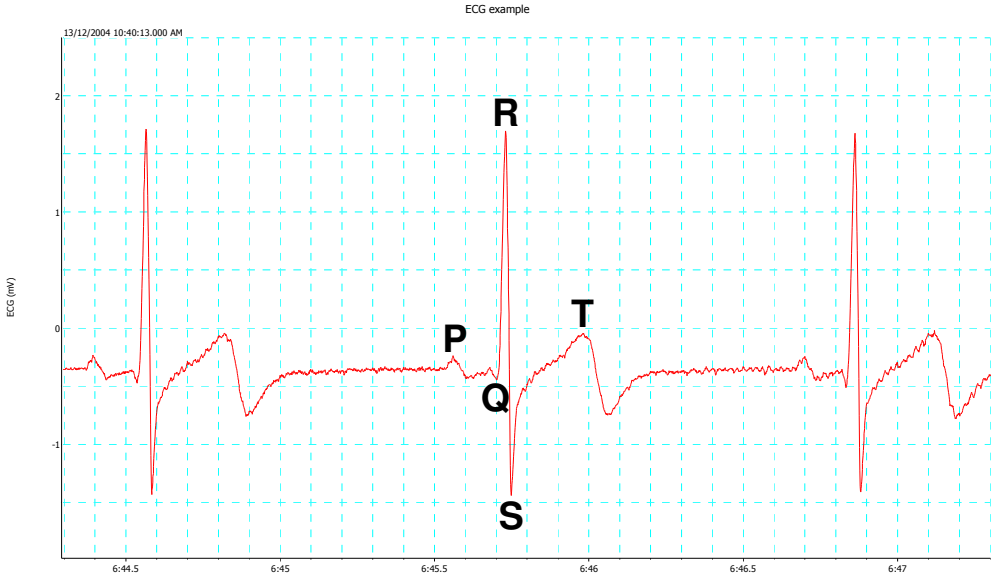

Figure 2.2. The electrocardiographic signals recorded using the 3-lead method.
A significant peak of signal appears periodically on the display (i.e., R wave).

In this research project, the ECG was recorded using a 3-lead method. Three disposable electrodes (Blue Sensor, Medicostest, Denmark) were positioned, with the negative electrode over the manubrium and the positive and earth electrodes at the left and right axillary lines (over the 5th intercostal space). Prior to electrode placement, the skin around the electrode attachment area was cleaned using a 70% isopropyl alcohol soaked swab (BRIEMARPAK®, VIC, Australia). Electrodes were attached after the skin dried. The same method has been used in previous studies (Budgell & Hirano 2001; Budgell & Polus 2006).

Detected signals were amplified (BIO Amp ML 132, ADInstruments, Bella Vista, NSW, Australia) and stored on a personal computer.

2.3. Heart rate variability

In healthy individuals, the heart beats appear to be constant in resting condition. However, the intervals of one heart beat to another are not completely homogeneous but slightly different. This variability of heart beat interval has been known to be strongly influenced by respiration; that is heart rate (HR) is accelerated during the inspiration and retarded during the expiration (Malik & Camm 1995). However, other mechanisms also contribute to the occurance of the spontaneous fluctuations of HR.

2.3.1. Heart rate variability as an indicator of the modulation of the cardiac autonomic nervous system

To advance the knowledge about the causes of cardiovascular oscillation (including HR), Sayers and his colleagues introduced the power spectrum analysis in 1973 (Malliani 2000). The power spectrum analysis is capable of quantifying the variability of heart beat intervals and the authors identified that there were three peaks in the spectrum around 0.03, 0.1, and 0.25 Hz (Malliani 2000). Later, Akselrod et al. (1981) linked each peak of the power spectrum analysis of HR with the autonomic nervous and renin-angiotensin systems in conscious trained dogs. The influence of each system was characterised using blockade of each system, and it was found that a parasympathetic blocker diminished both the mid- (0.12 Hz) and high-frequency (around 0.4 Hz) power spectral peaks and decreased low-frequency power peak (around 0.04 Hz). In contrast, infusion of a β-sympathetic blocker resulted in an inconsistent reduction in the low-frequency peak of the power spectral band whereas the infusion of both β-sympathetic and parasympathetic blockers diminished most of the peaks associated with the power spectrum. In addition to sympathetic and parasympathetic involvement in the power

spectrum, the low-frequency peak area was significantly increased following renin-angiotensin blockade. Therefore, Akselrod et al.'s study (1981) indicated that the fluctuations of HR over time are influenced by the autonomic nervous and renin-angiotensin systems and it is possible to quantify these influences using power spectral analysis. Similarly, in humans, Pomeranz et al. (1985) examined the distribution of sympathetic and parasympathetic drives influences in the power spectrum using sympathetic (propranolol) and parasympathetic (atropine) blockades in the supine and standing postures. The high frequency (HF) band (2.224-0.28Hz) of power spectrum was reduced by atropine infusion during both supine and standing postures, but not propranolol. The low frequency (LF) band (0.04-0.12Hz) was decreased by only atropine infusion during the supine posture, but the effect of propranolol infusion was additive to atropine during standing. Thus, Pomeranz et al.'s study (1985) showed that fluctuations in HR was influenced by the autonomic nervous system in humans as well, and that the parasympathetic drive influenced both the low- and high-frequency bands of the power spectrum while the sympathetic drive contributed to the low-frequency band particularly in the standing position.

In addition to these pharmacological studies, the influence of physiological manoeuvres on the power spectral analysis of HR oscillations (i.e., heart rate variability; HRV) may reflect autonomic regulation of the heart. One of the examples may be autonomic regulation of cardiovascular function during orthostasis, because the autonomic nervous system largely contributes to the short-term regulation of cardiovascular function as summarised above (see *section 1.2*). Montano et al. (1994) investigated the effect of a graded head-up tilt (0°-90°, by 15° intervals except 75°) on the power spectral analysis of HRV. It was found that changes in power of the spectral band tended to be linearly correlated with the angles of tilt; that is low-frequency power increases and high-frequency power decreases as tilt angle increases. Although the absolute value of the LF power did not exhibit tilt angle-dependency, the

correlation between LF power and tilt angle was improved ($r \geq 0.70$) by normalising the power of the spectral band. Similar trends were reported by others (Mukai & Hayano 1995; Akatsu et al. 1999; Ishibashi et al. 1999; Bahjaoui-Bouhaddi et al. 2000). Collectively, HRV analysis seems to be influenced by the degree of orthostatic stress and in turn may indicate the influence of the autonomic nervous system on the heart. Especially, changes in sympathetic and parasympathetic nervous functions tend to be reciprocal in these studies.

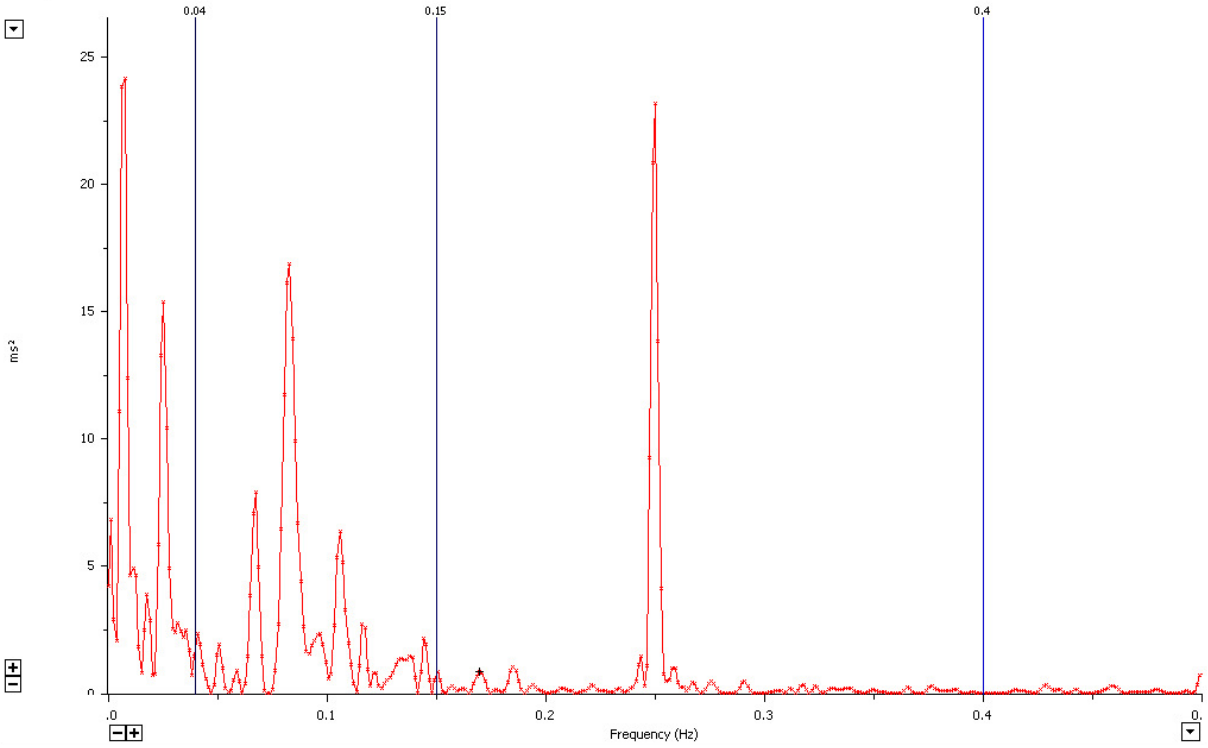

Figure 2.3. An example of spectral analysis of heart rate variability.

In order to standardise use of HRV, a united team of European and North American experts released the task force report (Task Force 1996). One of the important requirements is the length of the recording period. Using the fast Fourier transform analysis, three spectral bands of HRV can be obtained, very low frequency (VLF) ($\leq$ 0.04Hz), LF (0.04-0.15 Hz), and HF (0.15-0.4 Hz) power bands. The optimal duration for a short-term recording is 5 minutes. However, it was suggested to avoid including the VLF power in interpretation because this component of HRV obtained from a short-term recording ($\leq$ 5 minutes) is not physiologically well-defined (Task Force 1996). To determine each band of the power spectrum, a time

interval at least 10 times longer than the longest wavelength of the band range is required, so at least approximately 1 and 2 minutes are necessary for the HF and LF power, respectively (Task Force 1996).

As discussed above, the HF power of the spectral band of HRV reflects HR modulation by the parasympathetic drive. In contrast, the contribution of the cardiac autonomic nervous system to the LF power band of HRV is controversial. Some studies suggest that the LF power band of HRV reflects HR modulation by the sympathetic drive whereas others suggest that this component reflect both sympathetic and parasympathetic drives to the heart (Task Force 1996). Particularly, when overall power of HRV changes, the normalised units of the LF and HF are more meaningful than the absolute values since the normalised units indicate the proportion of each division of the spectral power to the entire power (Malliani et al. 1994). The normalization of both LF and HF power bands provides physiological significance or interpretation (Malliani et al. 1994). The normalization of each component (LF and HF) of HRV analysis is calculated by dividing the absolute power of the LF or HF power by the total power (TP) minus the VLF power component (Task Force 1996). Additionally, the ratio of the LF component to the HF component (LF/HF) is calculated as an expression of HR modulation of the sympathetic drive or the sympathovagal balance (Task Force 1996).

In addition to the power spectral analysis of HRV mentioned above, called the frequency-domain analysis, the oscillation of HR over time can be revealed using either a statistical or geometric manipulation (the time-domain analysis). Because the frequency-domain analysis provides physiologically-interpretable variables, this analysis is thought to be more useful than the time-domain analysis in this aspect (Task Force 1996). In contrast, the parameters obtained from the time-domain analysis can approximately be translated to the parameters of the frequency-domain analysis (Task Force 1996). For example, the standard deviation of all

R-R intervals (SDNN) is equivalent to the TP of the frequency domain analysis and the square root of the mean of the sum of the squares of differences between adjacent R-R intervals (RMSSD) is approximate to the HF power component of the frequency spectrum (Task Force 1996). The translation of HRV parameters obtained by the time-domain analysis to frequency-domain analysis parameters may be possible for data recorded over 24 hours (Task Force 1996; Stein et al. 2005). Also, it was reported that a few parameters obtained from a 5-minute recording including RMSSD strongly indicated parasympathetic (vagal) drive (Polanczyk et al. 1998).

Therefore, the extent of cardiac autonomic nervous modulations can be estimated by analysing fluctuations of heart beat intervals using either the frequency- or time-domain analysis. It is important to keep in mind that HRV analysis does not indicate the tone of the cardiac autonomic nervous system (Task Force 1996). In other words, HRV parameters are qualitative rather than quantitative (Cohen & Tan 2006), whereas this analysis does provide plausible physiological meanings to observations and has a potential for clinical use (Cerutti 2006).

2.3.2. Factors that influence heart rate variability parameters

It has been demonstrated that body position influences HRV parameters as summarised above. There are also a number of other factors that influence HRV parameters. In order to standardise an individual's condition prior to recording ECG, those influential factors need to be considered and controlled. For preparation during routine clinical autonomic testing, some restrictions are applied. For example, consumption of food, caffeine, and nicotine should be stopped three hours and alcohol intake has to be abstained for 12-14 hours before testing (Low 1997). Also, it has been suggested that medications such as anticholinergics and parasympathomimetic agents might be withdrawn under the guidance of physicians (Low

1997). In addition to these routine restrictions for autonomic testing, it has been reported that other factors that may influence HRV include the circadian rhythm (Bonnemeier et al. 2003), water ingestion (Routledge et al. 2002), exercise (for review see Carter et al. 2003), clothes tightness (Miyatsuji et al. 2002), and psychological stress (Ishibashi et al. 1999). Therefore, these factors should also be considered prior to data collection.

2.4. Non-invasive blood pressure measurement

Blood pressure (BP) measurements were obtained using the Portapres® device (Medel-2, Finapres Medical Systems BV, Amsterdam, The Netherlands) in the studies of this thesis. The Portapres® device provides non-invasive and continuous beat-to-beat BP and HR measurements from the finger. The measurement is based on the "volume-clamp" method advocated by Penaz (Finapres Medical Systems BV 2002). The "volume-clamp" technique seeks to maintain a constant volume of the finger artery by changing the pressure of an inflatable finger cuff wrapped around the recorded finger while the finger artery volume changes over time. Changes in finger artery volume are immediately detected by the photoplethysmograph installed in the finger cuff and forwarded to the servo-controller system of the device. Thus, pressure of the inflated finger cuff is regulated as the artery volume of the finger changes. During inflation of the finger cuff, the transmural pressure of the finger is maintained at zero and the pressure applied by the finger cuff is equivalent to intra-arterial pressure (Low 1997). Thus, by measuring finger cuff pressure, the Portapres® device is able to indirectly obtain the intra-arterial pressure of the finger.

There have been a large number of studies that compared BP measurement between intra-arterial pressure recordings (gold standard) and the Finapres™ device that employs the "volume-clamp" method (Imholz et al. 1998; Silke & McAuley 1998). It has been documented that the accuracy of the Finapres™ device measurement meets with the

requirement of the American Association for the Advancement of Medical Instruments (i.e. < ± 5 mmHg of BP) (Imholz et al. 1998). There are reports that the precision of systolic BP (SBP) measures are not as great as diastolic (DBP) measures (Imholz et al. 1998; Silke & McAuley 1998). However, a further report suggests better accuracy and less variability BP measurements when a similar device with hydrostatic correction system is used (i.e., the Portapres® device) (for review see Hirschl et al. 1999). Therefore, BP measurements using the "volume-clamp" method such as the Portapres® device appears to provide an acceptable level of accuracy for BP measures for experimental studies, but not for clinical diagnostic purposes (Finapres Medical Systems BV 2002).

In order to obtain optimal BP measurement using the Portapres® device, there are a few points to be noted. First of all, it is important to use the appropriate size of finger cuffs. There are three sizes of finger cuffs of the Portapres® device (45-55, 55-65, and 65-75 mm). As the Portapres® device estimates the pressure of the finger cuff, which is required to clamp the volume of finger artery, a loose finger cuff fitting would tend to lead to an overestimation of BP values. Secondly, it is also critical to maintain sufficient finger blood flow (FiBF) during data recording. Vasoconstriction occurs due to cold ambient temperature. In order to maintain sufficient FiBF, it is suggested to warm the hand (Finapres Medical Systems BV 2002) and cover the hand with a light-weight cloth (Imholz et al. 1998). In addition, conducting examinations in a warm environment (> 22°C) is recommended because vasoconstriction may occur by an individual's perception of coldness is worse than a cold hand itself (Imholz et al. 1998). Furthermore, Low (1997) further suggests that the hand and shoulder need to be relaxed and excessive abduction or extension of the arm should be avoided.

2.5. Finger Blood Flow

It is accepted that regulatory mechanisms governing blood flow in the finger differ from those controlling muscle blood flow. Circulation in the hand has more arteriovenous shunts which are aimed at thermal regulation. The blood vessels in the finger are innervated by sympathetic nerves. Unlike the sympathetic nerve outflow to the muscle vasculature, the sympathetic nerves only constrict the blood vessels in the skin. The synapses of the skin sympathetic nerves secrete acetylcholine and vasoconstriction occurs (Levick 1995, p. 242). Hence, often the term of "skin blood flow" indicates FiBF and in this thesis, the term of "skin blood flow" is used equally as the term of "FiBF".

The primary purpose of FiBF measurement in this study was to detect the influence of an arousal effect on BP measurements by means of the Portapres® device, since an accurate measurement of the Portapres® device requires sufficient blood flow in the finger and a vasoconstrictor response from skin blood vessels is thought to be associated with an arousal reaction (Macefield et al. 1998). In order to measure blood flow in the finger, a photoplethysmograph (MLT1020FC, ADInstruments, Bella Vista, NSW, Australia) was used in this study. Data were fed into the PowerLab (PowerLab/8SP, ADInstruments, Bella Vista, NSW, Australia) after amplification (ML221, Bridge Amp, ADInstruments, Bella Vista, NSW, Australia) and filtering (bandwidth of 0.1-100 Hz) before viewing the signal on-line on the computer display.

The amplitude of the photoplethysmograph waveform depends on the amount of blood flow in the finger. Despite filtering the obtained data, the lowest points of each pulse cycle on the photoplethysmogram (PPG) were variable. On occasion, the lowest points were unable to be determined due to baseline fluctuations. In order to sort this issue out, it was necessary to consult the distributor of the equipment (ADInstrument, NSW, Australia). It was suggested to

take three steps to reduce noise and quantify the obtained photoplethysmographic data. Figure 2.4 demonstrates the sequence of steps used to prepare PPG data for analysis. These computations were carried out using Chart software (ADInstrument).

1. Obtain PPG data (magenta)

2. Filter PPG data (orange): A 40 Hz low-pass filter was used to reduce noise.

3. Positively integrate PPG data (navy): Filtered PPG data were further calculated using a positive integral.

4. The height of the integrated PPG waveform was detected to provide an indication of FiBF volume.

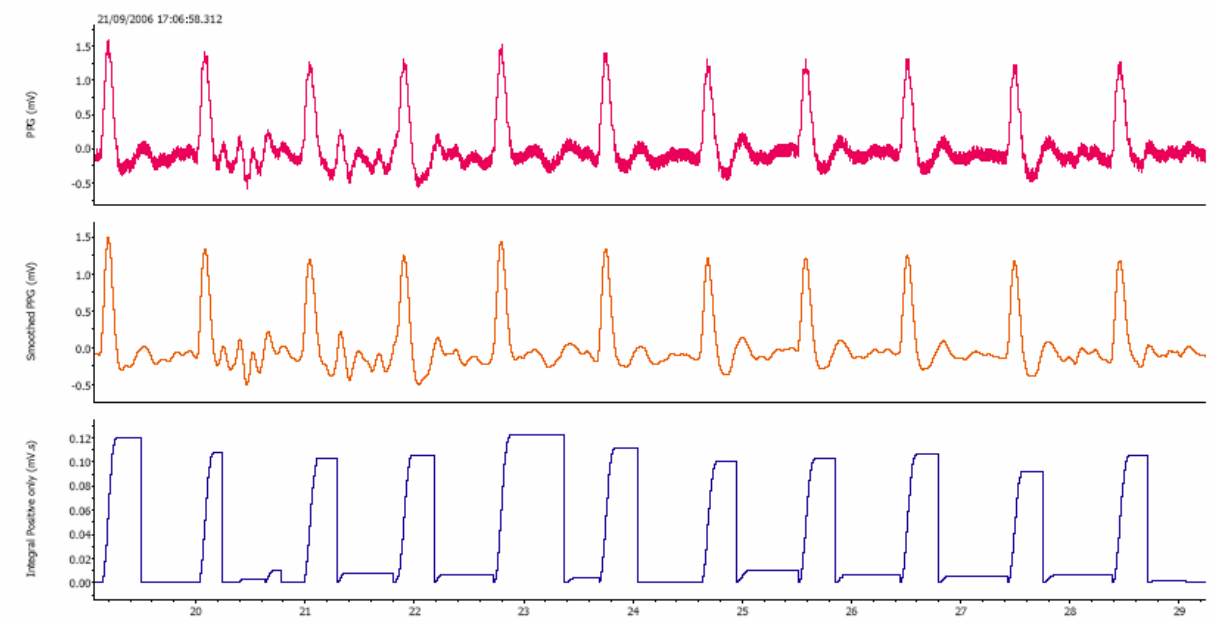

Figure 2.4. Quantification of photoplethysmogram data.
The data window shows raw photoplethysmogram (PPG) signals (*Top*), Filtered PPG signals (*Middle*), and positively integrated PPG signals (*Bottom*). The height of the integrated PPG waveform was obtained as an indication of finger blood flow volume.

2.6. Skin potential change

The measurement of skin potential changes provides information on sudomotor activity, which is controlled by the sympathetic nervous system. When the sympathetic nerve in the skin is stimulated and sweat is released from the sweat gland, the extent of hydration in the skin changes (Boucsein 1992). This leads to a change in the resistance of the surface of the

skin (Boucsein 1992). Therefore, the resistance change of the skin can be measured as a potential change on the skin and in turn sudomotor activity.

In the studies of this thesis, three Ag/AgCl electrodes (Red Dot™, 3M™, MN, USA) were used. Two electrodes were attached over the eminence and the dorsum of the thenar. The earth electrode was attached on the middle of the ventral forearm. Unlike bipolar electrode use for ECG or electromyography, it is recommended that skin cleaning is not necessary unless the skin is extremely oily (Boucsein 1992). The detected signals with differential recording were amplified and fed into the PowerLab® (PowerLab/8SP, ADInstruments, Bella Vista, NSW, Australia). The signals were filtered (bandwidth 0.3-1k Hz) and visualised on computer display during the experiment. The obtained signals of skin potential change were stored on computer and analysed off-line using Chart software (Chart for Windows V5.4.1., ADInstruments, Bella Vista, NSW, Australia).

After Donadio and colleagues (2002a; 2002b), a significant change in skin potential was defined when the amplitude of changes in the skin potential had exceeded 5% of the baseline, which is the maximum amplitude of spontaneous skin potential change before interventions (e.g., mechanical stimulation). The time window of the analysis was limited to 7 seconds after the stimulus. The primary purpose of skin potential change measurement was to detect an arousal response rather than to investigate the effect of the intervention on skin sympathetic nerve activity. Therefore, assessment of the measurement was based on whether skin potential was changed or not, but not the degree of skin potential change.

2.7. Limb blood flow

Blood flow through vessels is dependent upon peripheral resistance as well as cardiac output (Berne and Levy, 1997, p.142). Peripheral resistance, which indicates how stiff the blood

vessels are, is modulated intrinsically and extrinsically; involving mechanisms such as myogenic response, local chemical substances, hormones, and sympathetic innervation (Boron & Boulpaep 2003, pp. 478, 566). In general, blood vessels are mainly innervated by sympathetic nerves (Mohrman & Heller 2006). Although parasympathetic nerves innervate blood vessels in the brain and heart, the influence of this innervation on blood vessel control seems to be minimal (Mohrman & Heller 2006). The sympathetic nervous system acts to constrict and dilate blood vessels. Therefore, blood flow measurements indirectly provide an indicator of the tension of the vasculature and this, in turn, is an indicator of sympathetic nervous outflow to the blood vessels.

Several techniques to measure muscle blood flow in the limbs are available. One of these is the "strain-gauge plethysmograph (SGP)", which was used in the studies of this thesis. This technique provides an indirect and non-invasive measure of blood outflow to limb muscles (Appenzeller & Oribe 1997, p. 680). Since the relationship between changes in volume and circumference of the limbs is linear, skeletal muscle blood inflow can be estimated by measuring the change in volume of the limb during venous occlusion (Appenzeller & Oribe 1997).

2.7.1. Equipment

In order to measure skeletal muscle blood flow using the strain-gauge plethysmograph (EC6 Strain Gauge Plethysmograph, Hokanson, WA, USA), a mercury-in-silastic band was wrapped around the largest circumference of the limb (the forearm or calf). Any change in limb volume was detected as a change in length of the strain gauge system. The The mercury-in-Silastic band is attached with strain gauges, so electrical impedance change attributing to a change in length of the elastic band is detected by stain gauges. It is recommended to use a

strain gauge, whose length is 2 cm shorter than the actual circumference of the recorded limb (D.E. Hokanson Inc. 1998).

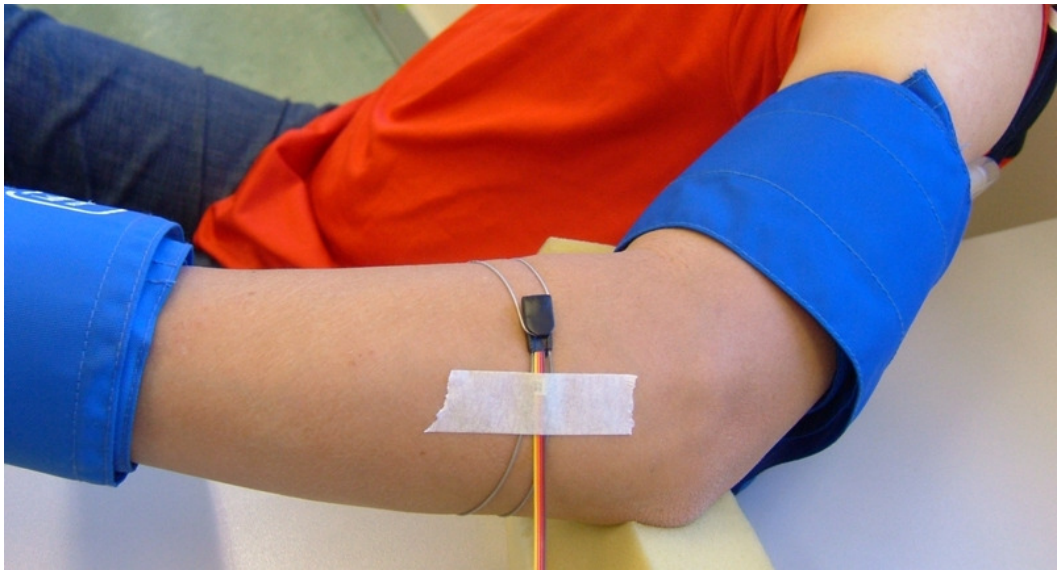

Figure 2.5. A strain gauge and two inflatable cuffs attached around the left arm.
The left arm in the figure is placed on the dest for photo taken purpose. The limb to be recorded was positioned above heart level during data recording.

Two cuffs are required for SGP use. For forearm blood flow (FoBF) measurement, for example, cuffs are wrapped around the upper arm and wrist. For calf blood flow measurement, cuffs are wrapped around the thigh and ankle.

The purpose of the proximal cuff (around the upper arm or thigh) is to occlude venous return to the heart. In this study, an upper arm or thigh cuff was inflated to 50 mmHg during venous occlusion. This level of pressure arrests venous return but not arterial outflow to the muscles, although it has been suggested that the cuff pressure reauired for venous occlusion may slightly interfere with arterial outflow (Pallares et al. 1994). During upper arm or thigh cuff inflation, the distal part of the limb from the cuff begins to swell. The rate of this swelling indicates blood outflow rate to the limb.

The other cuff (around the wrist or ankle) is used to exclude circulation in the hand or foot. This is because circulation in the hand is physiologically controlled differently from the

forearm (Benjamin et al. 1995; Wilkinson & Webb 2001). It was also demonstrated that the exclusion of hand circulation reduces intra-individual variability of strain-gauge plethysmography (SGP) measurements (Burggraaf et al. 2000). To exclude hand or foot circulation, a cuff was wrapped around the wrist or ankle was inflated to 180-200 mmHg. This cuff pressure was maintained for at least 1 minute before data collection commenced in order to stabilise the volume of the forearm or calf and during data collection.

In order to inflate the proximal cuff (around the upper arm or thigh), a cuff inflator (E20 Benchtop Cuff inflator, Hokanson, WA, USA) and air-compressor (AG101 Air source, Hokanson, WA, USA) were used. The proximal cuff can reliably be inflated and deflated using a cycle timer. Cuff inflation takes less than 0.3 seconds.

Importantly, the limb attached with the strain gauge was elevated above heart level during data recording using high density foam blocks in order to enhance venous drainage.

2.7.2. Analysis of recorded strain-gauge plethysmogram

An example of the strain-gauge plethysmogram (SGP) recorded from the left forearm is presented in Figure 2.6. The arrow indicates when the upper arm cuff was inflated. A rapid elevation in volume of the upper arm can be seen immediately after the cuff was inflated. This is because as the proximal cuff inflates, blood distal to the cuff is forced into the forearm or calf, resulting in a sudden increase in volume of the forearm or calf. This initial increase was excluded from data analysis.

To quantify the data of the SGP, a linear portion of the volume trace was selected and a line drawn tangential to the volume peaks. The gradient of the slope of the line was calculated as ΔmV divided by Δt (mV/second). Just before each trial started, the amplitude of the electrical

signal change was calibrated and a 1 % change was determined. Thus, the actual obtained unit was % per second. The unit of blood outflow to the muscle vasculature was expressed as millilitres per 100 millilitre tissue per minute (ml/100ml tissue/min) which is equivalent to the rate of the electrical signal change of the SGP for a minute (% per minute). Consequently, the obtained value (% per second) was multiplied by 60 and muscle blood flow values were determined.

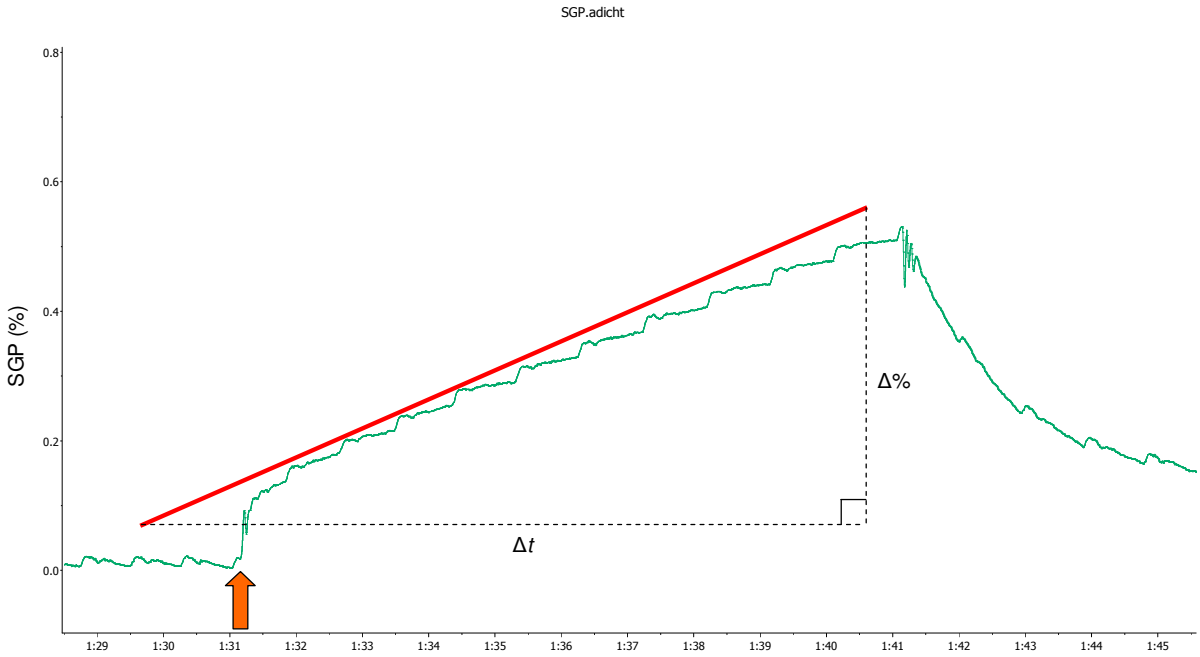

Figure 2.6. An example of strain-gauge plethysmogram recorded from the left forearm.
Arrow indicates the beginning of cuff inflation wrapped around the left upper arm. A red line indicates the linear increase in forearm volume which was chosen for quantification.

2.7.3. Advantages and disadvantages of strain-gauge plethysmography use

There are advantages when using this measurement while there are also a few limitations. First of all, the SGP provides a non-invasive measurement of limb blood flow. Psychological stress and noxious stimuli are known to influence cardiovascular function. Consequently, these factors may distort study results. For this reason, non-invasive tests are strongly recommended to evaluate autonomic nervous function (Low 1997). Secondly, the SGP has high reproducibility (Roberts et al. 1986; Pallares et al. 1994). Importantly, since there is no gold standard for limb blood flow measurement, the accuracy of SGP measurement may not

be definite (Joyner et al. 2001). However, it has been found that the comparability of SGP with the Doppler ultrasound was high (Joyner et al. 2001) to moderate (Pallares et al. 1994). In addition, the SGP has better reproducibility than Doppler ultrasound (Pallares et al. 1994). Therefore, despite the lack of absolute accuracy, the moderate to high comparability with another measurement tool of may indicate the reasonability of SGP use for limb blood flow measurement.

On the other hand, there were also limitations associated with use of this equipment. Firstly, since SGP measures a change in volume of the limb during venous occlusion, measurements can be taken every 15-20 seconds rather than continuous measurement. Therefore, using this equipment, it is not possible to capture a transient change in limb blood flow so cardiovascular changes can be detected only if they are sustained changes. Secondly, during data collection, hand or foot circulation needs to be excluded in order to isolate the influence of skin (hand or foot) blood flow. A cuff wrapped around the wrist or ankle was inflated slightly above SBP (that is, 180-200 mmHg in the studies of this thesis). Prolonged arrest of arterial flow may lead to ischaemic symptoms such as numbness and a cold sensation in the hand or foot. For safety, it was recommended that arterial arrest should be limited to 13 minutes (Wilkinson & Webb 2001). Therefore, SGP may not be suitable for a long-term experimental design or the measurement protocol may need to be amended to take these issues into consideration.

Despite these issues, it is considered that SGP is a useful technique to measure muscle blood outflow to the forearm or calf (Joyner et al. 2001). In this thesis, forearm (muscle) blood flow was used in the studies in Chpater 5 and calf (muscle) blood flow was used in the studies in Chapter 6 and 7.

2.8. Activator® instrument

In order to simulate spinal manipulation, an Activator® instrument was used in the studies of this thesis (AAI III, Activator Methods International LTD., AZ, USA) (Figure 2.7). The Activator® instrument is a hand-held device and capable of delivering reproducible force to a specific region of the body (Fuhr & Smith 1986). This instrument is widely employed by chiropractors in their daily clinical practice and is an alternative to manually-delivered spinal manipulation (Haldeman 2005). Unlike a manually-delivered spinal manipulation, the Activator® instrument does not require displacement of any parts of the body when themechanical stimulus is applied. Thus, this instrument is capable of simulating cervical manipulation without concomitant head movement and the activation of the vestibular apparatus.

Figure 2.7. An Activator® instrument.

There have been sporadic neurophysiological studies related to use of this instrument. For example, Symons et al. (2000) investigated the effect of the Activator® instrument on motor responses using surface electromyography (sEMG) and showed that a single mechanical impulse induced a phasic electromyographic (EMG) signal in approximately 68 % of their deliveries. The phasic EMG response was presumed to be an involuntary reflexive response originating from activation of the muscle spindle (Symons et al. 2000). Similar reflexive EMG responses have been reported in other studies, which used the (manually delivered) high-velocity low-amplitude spinal manipulation (Herzog et al. 1995; Herzog et al. 1999). In

addition to the non-invasive recording, Colloca and colleagues directly recorded the action potential of the mixed nerve root in patients undergoing surgery, in response to a mechanical stimulus delivered using an Activator® instrument (Colloca et al. 2000; Colloca et al. 2003; Colloca et al. 2004). Thus, these studies suggest that although afferent types to be activated by the mechanical stimulus cannot be specified from the data, the mechanical stimulus of the instrument is sufficient to activate somatosensory receptors including cutaneous, muscle, and articular receptors and to induce somatic-motor response.

The mechanical stimulus was applied to the upper neck using the minimum force setting of the AAI III. Its characteristics were estimated as approximately 130 N of peak force and 0.37 N per second (Ns^{-1}) of force impulse (Colloca et al. 2005) and similarly 190 N and 0.5 Ns^{-1} (AW Fuhr 2008, personal communication, 28 February). The duration of the mechanical impulse generated by the Activator® instrument is 0.1 to 5 milliseconds (Fuhr & Menke 2005). It has been suggested that a threshold level of mechanical force is required to activate different types of somatosensory receptors (Gillette 1987 as cited by Fuhr & Menke 2005), whereas the duration of the mechanical impulse applied to the body influences the quantity of somatosensory inputs (Pickar & Kang 2006; Pickar et al. 2007). An important consideration is that the investigator or clinician may unknowingly apply variable degrees of pressure through the Activator instrument and this could result in a variable degree of delivered force. However, the AAI III is equipped with a "preload control frame", which restricts the degree of inconsistent compression of the instrument as it makes contact with the body prior to delivery of the mechanical impulse (Fuhr & Menke 2005). As a result, this AAI III is able to generate a reproducible force (< 8% variance) (Keller et al. 1999).

2.9. Muscle conditioning

Muscle consists of a number of muscle fascicles and is bundled with fascia. The ends of the muscle merge with the tendon which then attaches to bone. Each muscle fascicle consists of muscle cells which contain myofibrils, consisting of thin (actin) and thick (myosin) myofilaments. The myosin and actin are arranged in a hexagonal array with the myosin filament positioned in the centre. Each actin and myosin set is separated by a Z line and this unit is called the sarcomere.

Since muscles cross joint(s), muscle contributes not only to generating force but also to sensing position (angle) and movement of the joint and tension in the muscle. Muscle is therefore equipped with sensory receptors including muscle spindles which lie in parallel with extrafusal muscle fibres and Golgi tendon organs that are located at the musculo-tendonous junction. Each organ is responsible for detecting a different sensory mechanical stimulus.

2.9.1. Receptors in skeletal muscle

There are two types of sensory receptors in skeletal muscles as follows;

Muscle spindle: The role of muscle spindles is to detect length changes of muscle so muscle spindles are activated when they are stretched. A more detailed understanding of muscle stretch is achieved by a combination of signals from the different types of sensory endings within the muscle spindle. Muscle spindles consist of 7-12 specialised muscle fibres called intrafusal fibres and there are up to three types of intrafusal fibres in a muscle spindle – they are bag_1, bag_2, and chain fibres. Each intrafusal fibre receives innervation from Ia afferent fibre which repeatedly branches and terminates as a primary ending at the central portion of the intrafusal fibre. Bag_2 and chain fibres also receive also receive innervation from group II nerve fibres which terminate as secondary endings in the juxtaequatorial zone of these

intrafusal fibres. In addition, each intrafusal fibre receives a motor innervation; bag_1 fibres are innervated by γ dynamic fibres while bag_2 and chain fibres are innervated by γ static fibres.

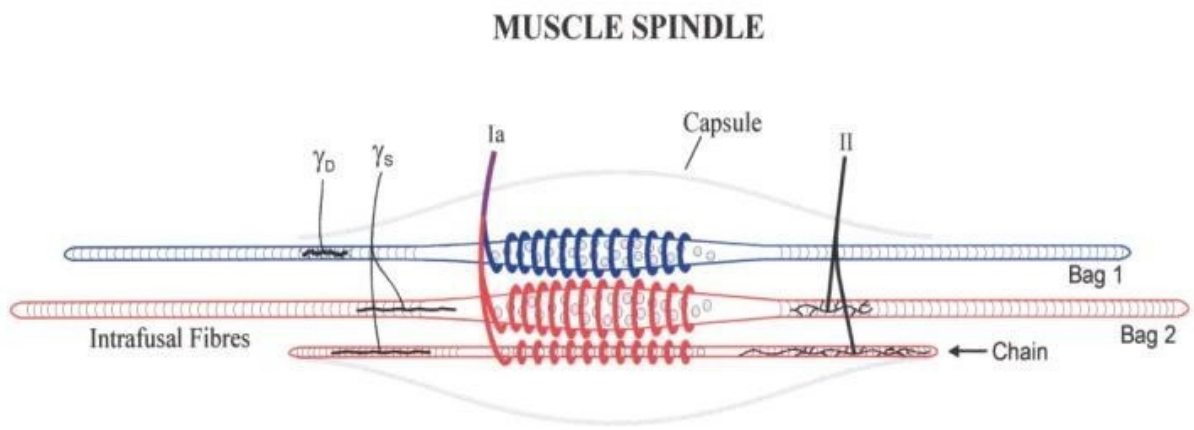

Figure 2.8. A summary of mammalian muscle spindle structure.
There are three types of intrafusal fibres (bag_1, bag_2, and chain). Ia afferents innervate all types of intrafusal fibres but II afferents innervate bag_2 and chain fibres (not bag_1 fibres). Bag_1 fibres are innervated by γ dynamic (γ_D) fibres and both bag_2 and chain fibres are innervated by γ static (γ_S) fibres. Adapted from Proske (1997).

Primary endings respond to dynamic muscle stretch (such as tapping) while secondary endings respond to static (sustained) stretch. Because intrafusal muscle fibres lie in parallel with extrafusal fibres, muscle spindles fall slack and their afferent signals can be undetectable if muscle is contracted and the length of the entire muscle is shortened. However, because the muscle spindle also receives a γ motor innervation, the tension of muscle spindles can be maintained (by shortening of the two ends of the intrafusal fibre) even though the whole muscle shortens so the muscle spindle can still respond to length changes. Particularly, when voluntary muscle contraction occurs, both α and γ motor neurons are activated. Therefore, muscle spindle sensitivity can be maintained during muscle contraction.

Golgi tendon organ: Golgi tendon organs are responsible for muscle tension detection. This type of receptor is activated when the muscle is stretched as well as when the muscle is contracted. The sensory inputs of Golgi tendon organs are transmitted to the CNS via Type Ib afferents.

2.9.2. Mechanism of muscle contraction

One of the roles of skeletal muscle is to generate force. Any physical tasks are achieved by coordinated muscle contractions in each part of the body. The mechanism of muscle contraction is well described in the literature (Boron & Boulpaep 2003, pp. 234-42; Berne 2004, pp. 227-33; Guyton & Hall 2006, pp. 75-82). Muscle contraction is triggered by motor neuron activation. The action potential is transmitted along the α motor efferent fibre. Once the action potential reaches the neuromuscular junction, acetylcholine is released. The released acetylcholine is received by acetylcholine receptors on the muscle fibre membrane. The action potential spreads along the sarcolemma to the T tubule system. The action potential in the T tubule signals the sarcoplasmic reticulum and calcium ions are released. As a result, calcium ion concentration within the sarcolemma increases and the process of muscle contraction commences. Calcium ion concentration in the muscle is crucial for the process of muscle contraction.

2.9.3. Thixotropic property of muscle

Muscle contraction occurs in response to an increase in calcium ion concentration in the sarcolemma. In other words, the connection of actin and myosin filaments (associated with muscle contraction) is a calcium ion dependent reaction. However, even in the presence of low levels of Ca^{2+}, a resting muscle has some tension. This was first demonstrated by Hill (1968) who concluded that muscle exhibited thixotropic behaviour. Thixotropy refers to the behaviour of certain types of fluid; at rest the fluid becomes viscous, however with movement (e.g., stirring) the viscosity decreases. Hill (1968) proposed that the tension of a resting muscle could be attributed to a small number of actin-myosin connections (cross-bridges), which are quite stable and have a “long life”.

Although a number of studies have pointed to the presence of stable cross-bridges in a resting muscle and this concept seems to be widely accepted, there has been no direct evidence to demonstrate their presence. Campbell and Laike (1998) showed that a resting muscle exhibited "stiffness" and that this stiffness developed gradually (later than 1 second) following a stretch. The authors attributed this to the formation of stable cross-bridges between actin and myosin and explained that stable cross-bridge formation in a resting muscle was an intracellar calcium ion concentration dependent phenomenon (Campbell & Lakie 1998). On the other hand, Proske and Gregory (1985) demonstrated that replacement of calcium ions with potassium ions in Ringer solution (therefore, the osmolarity of the solution was the same) did not alter the muscle's thixotropic behaviour. Further, since elimination of adenosine triphosphate (ATP) (by metabolic exhaustion and ATP poisoning) induced an indication of an increase in the number of stable cross-bridges, it was concluded that ATP was more important for stable cross-bridge formation in a resting muscle (Proske & Stuart 1985).

As the extrafusal fibres contain actin and myosin filaments, the intrafusal fibres also contain actin and myosin filaments. Although the number of those filaments in the intrafusal fibres is quite small compared with the extrafusal fibres (Guyton & Hall 2006, p. 675), the intrafusal fibres still can form stable cross-bridges.

2.9.4. Muscle conditioning

In order to alter the mechanical sensitivity of muscle spindles, the studies in this thesis employed the muscle conditioning manoeuvre. Proske and colleagues have developed a well-characterised technique for limb muscle conditioning (Morgan et al. 1984; Gregory et al. 1987, 1988; Jahnke et al. 1989). Review of the muscle conditioning manoeuvre has been made recently (Proske et al. 1993; Proske & Morgan 1999).

By exploiting the thixotropic property of muscles, Morgan et al. (1984) showed that the muscle spindle afferent response to tetanic fusimotor stimulation was altered by muscle conditioning in a systematic way; for example, the muscle spindle afferent response was larger if the muscle had been held at a shorter-than test length following repetitive stretch to break existing stable cross-bridges and to eliminate the previous history of muscle spindles, while the response was smaller if the muscle had been held at a longer-than test length. Further, Edin and Vallbo (1988) confirmed muscle history- (muscle conditioning-) dependent changes in muscle spindle afferent discharge rate using microneurographic recording in humans. It was also shown that the previous muscle history could be removed by muscle contraction at a strength sufficient to involve both α- and γ-motor neurons (Proske & Stuart 1985; Jahnke et al. 1989). Recently, it was estimated that 30% of maximum voluntary contraction might be sufficient to activate all muscle spindles by γ motoneuron activation (Winter et al. 2005). Following this modification of the muscle conditioning protocol, the contribution of proprioceptive inputs from muscle spindles to reflex and kinaesthetic control was extensively investigated using isometric contraction at a longer- or shorter- than test length of a muscle to condition the muscle (Gregory et al. 1987; Gregory et al. 1990; Polus et al. 1991; Allen et al. 2007).

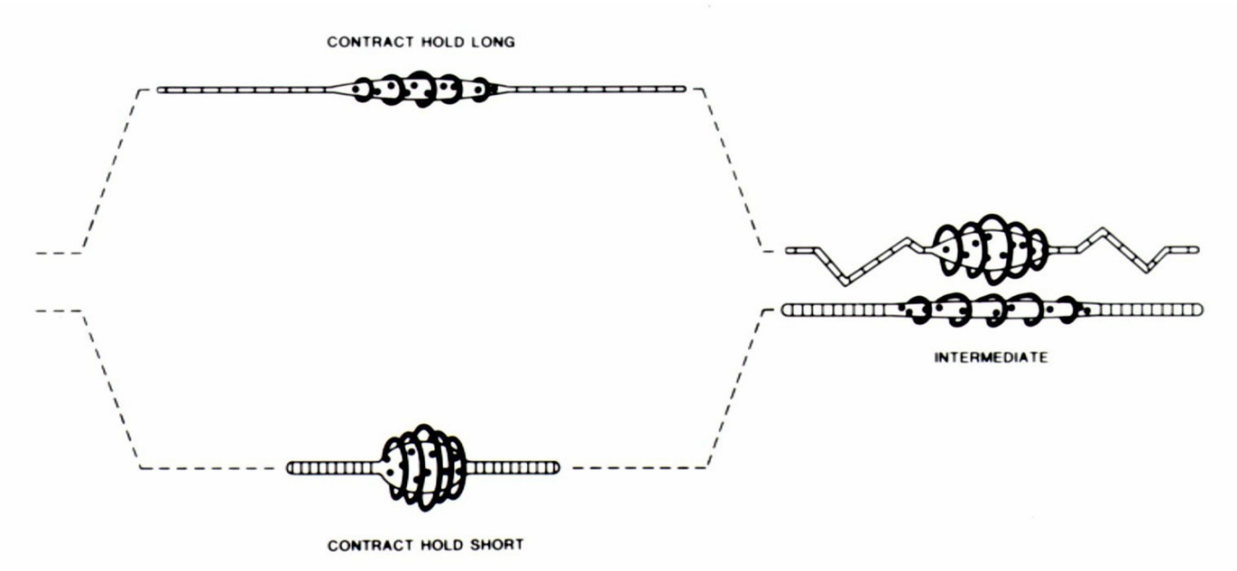

Figure 2.9. Two different forms of the muscle conditioning manoeuvre.
(Personal communication Proske).

To summarise the muscle conditioning manoeuvre, at a longer-than test length (upper trace of Figure 2.9), a muscle is contracted isometrically for a few seconds at a sufficient contraction strength to engage both α and γ motoneurons. The reason for the isometric contraction is to remove any pre-existing stable cross-bridges. The muscle is then relaxed and held at the long length for further 5 seconds. During this time, stable cross-bridges of the muscle begin to re-form at the prevailing length as Morgan et al. (1984) demonstrated that stable cross-bridge formation gradually plateaus over 3 seconds following its removal. Consequently, the muscle is *passively* returned to the intermediate (test) length. This form of the muscle conditioning has been termed "hold-long conditioning". The end result of hold-long conditioning is a reduction in the mechanical sensitivity of muscle spindle, because the muscle conditioning manoeuvre at the longer length induces the formation of stable cross-bridges of the intrafusal fibres at the long length and the stable cross-bridges which have formed at the longer length are maintained even at the intermediate (test) length. As a result, the intrafusal muscle fibres (and extrafusal muscle fibres) fall slack and the mechanical sensitivity of the muscle spindle decreased at the intermediate length.

The symmetrically opposite form of the muscle conditioning manoeuvre has been termed "hold-short conditioning" (lower trace of Figure 2.9). At a shorter-than test length, a muscle is contracted isometrically for a few seconds and subsequently held for further 5 seconds. During this time, stable cross-bridges begin to form at the short length. The muscle is then passively brought back to the intermediate (test) length. Once again, because there has been no active contraction, the stable cross-bridges that had formed at the shorter-than test length do not break. This results in a rise in tension of the intrafusal fibres and an increase in the mechanical sensitivity of the muscle spindle.

Notably, the resulting reduction or increase in mechanical sensitivity of the muscle spindle is manifested as a relative reduction or increase in the resting discharge in spindle afferent fibres between different forms of the muscle conditioning manoeuvre (i.e., hold-long vs. -short conditioning) rather than absolute changes in mechanical sensitivity of the muscle spindle.

The muscle conditioning manoeuvre creates a known muscle history, leaving the muscle spindles in a defined mechanical state. If a muscle is not conditioned, each fibre in the muscle may have a different history of contraction and length change so that different muscle spindles in the muscle are left in a different and unknown mechanical state. As demonstrated previously, the mechanical state of the muscle spindle depends on the muscle history and this can alter its biological functions (Polus et al. 1991). Therefore, by standardising when muscle contraction occurred and how muscle length has changed prior to examination, it is first possible to create a known mechanical state of muscle spindles and investigate its contribution to biological mechanisms.

2.9.5. Morphological difference in intramuscular receptors between the neck and limb

In order to investigate the contribution of muscle spindles in the neck to autonomic regulation of cardiovascular function in awake humans, the muscle conditioning manoeuvre was employed to alter the mechanical state of muscle spindles in the neck. The concept of muscle conditioning use and the protocol in this thesis was based on that developed by Proske and colleagues in the limb (Proske et al. 1993). However, morphological difference in intramuscular receptors (particularly muscle spindles and tendon organs) between the neck and limb may need to be taken into account.

It has been suggested that the basic function of muscle spindles appears similar between the limbs and axis (Richmond & Abrahams 1979). However, significant differences in muscle

spindles between the neck and limb have been found by morphological studies in cats (Richmond & Abrahams 1975) and humans (Boyd-Clark et al. 2002; Liu et al. 2003). Common findings of these studies include a higher density of muscle spindles, fewer intrafusal fibre contents, and more complex spindle arrays in the neck in contrast to the limbs. Furthermore, spindle-tendon organ complexes are often found in the neck (Richmond & Abrahams 1975).

Muscle conditioning alters not only the mechanical sensitivity of muscle spindles, but also the resting tension of the muscle because the muscle conditioning procedure also affects the extrafusal fibres (Whitehead et al. 2001). It is not known how the morphological differences of the intramuscular receptors of the neck, compared to the limb, influence muscle conditioning and its outcome. Accordingly, this thesis assumed that the muscle conditioning manoeuvre was capable of altering the "quantity of muscle proprioceptive afferent inputs" (such as muscle spindles and tendon organs) in the neck in a systematic way.

There have been a few indicative studies which have applied muscle conditioning to the axial muscles. In cats, it was shown that muscle conditioning influenced the resting discharge of muscle spindles in the lumbar paraspinal muscles (Pickar & Kang 2001; Ge et al. 2005). Also, human studies showed that head position sense was influenced by neck muscle conditioning (Owens et al. 2006; Repka & Polus 2008).

2.10. Surface electromyography

It is critical to ensure that muscles are contracted and relaxed as required when employing the muscle conditioning manoeuvre. Therefore, in order to monitor neck muscle activity during data collection, sEMG was used in the studies of this thesis.

The sEMG is a means of recording the electrical activity of muscles and provides safe, uncomplicated, and non-invasive measurement (Cram et al. 1998). The electrical activity recorded by the electromyograph is the sume of the action potentials, which originate from individual motor units and spread along the membranes of the muscle fibres. Thus, on the surface of the body, it is possible to record the electrical activity of the muscles under the recording electrodes as "spatial and temporal summation of individual action potentials" (Sornmo & Laguna 2005).

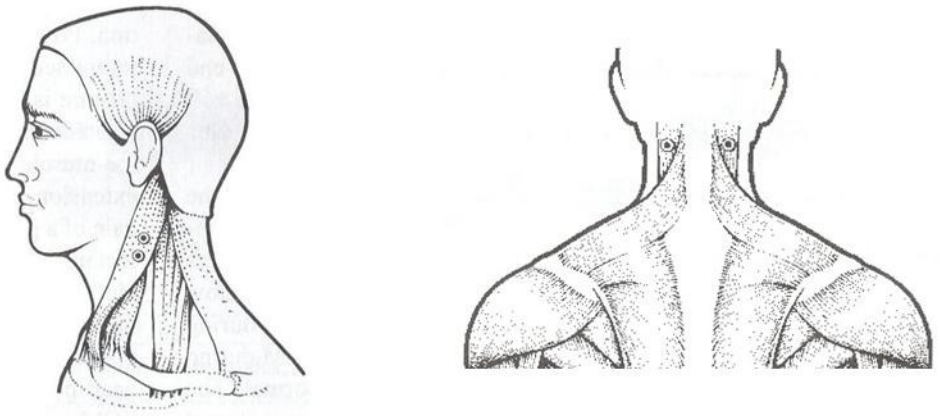

Figure 2.10. Electrode placements for the sternocleidomastoid (left) and dorsal neck muscles (right).
Bipolar electrodes were placed after skin cleaning and drying the cleaned area. Modified from Cram et al. (1998).

After skin cleaning using 70 % ethyl alcohol, electrodes (Blue Sensor, Medicostest, Denmark) were attached over either side of the dorsal neck muscles lateral to the C_4 spinal process with an interelectrode distance of 5 cm. The earth electrode was positioned over the T_1 spinous process. For each sternocleidomastoid muscle recording, the electrodes were attached over the muscle belly in parallel with muscle fibres (3.5 cm interelectrode distance) and the earth electrode was positioned at the level of the C_6 spinous process after Cram et al. (1998, pp. 262, 9). The EMG signals were filtered using a bandwidth of 10-500 Hz and a 50 Hz notch filter.

Whereas there are great advantages of sEMG use, there are also limitations. Firstly, the sEMG detects electrical activity from only the superficial muscles (Sommerich et al. 2000b). The

dorsal neck muscles consists of several layers (Cramer & Darby 2005). Therefore, it is unlikely to obtain muscle activity data from muscles of the deeper layers such as the rectus capitis posterior minor muscle using sEMG. Secondly, associated with recordings of the neck muscles, signals of the sEMG include artefacts such as breathing, swallowing as well as the ECG (Cram et al. 1998; Sommerich et al. 2000b). These artefacts may hide signals of actual muscle activity. However, respiratory-related artefact is particularly recognisable as the occurrence of this artefact synchronises with the inspiratory phase of participant's respiration. The respiratory-related artefact might be reduced or diminished by avoidance of exertive respiration. Also, when muscle activity is low, artefacts of the ECG signals can be recognised as a large spike appears periodically in EMG signals. Because the studies of this thesis employed the ECG concurrently, ECG artefacts can be distinguished by comparing with the actual ECG data.

2.11. Vibratory stimulus

As summarised in *section 2.9*, the muscle spindle is activated by muscle stretch (Brodal 1998). In addition to muscle stretch, a vibratory stimulus also activates muscle spindles (Macefield 2005; Fallon & Macefield 2007). Although there are two types of muscle spindle endings, that is the primary and secondary endings, the primary ending of the muscle spindle is exceptionally sensitive to a vibratory stimulus while the secondary ending is not as sensitive as the primary ending (Boudreau & Tsuchitani 1973). However, this behaviour of spindle endings seems to be not applicable under all circumstances. For example, Burke et al. (1976) demonstrated that 62 % of secondary endings discharged at a vibration frequency of 50Hz while Roll et al. (1989) found that only 27 % of secondary endings exhibited 1:1 driving at vibration frequencies up to 60 Hz. Also, the majority of primary endings (32 out of 33) synchronised their discharge rate at vibration frequencies up to 220Hz, which was the upper limit of frequencies used in the Burke et al.'s study (1976). On the other hand, Roll et al.

(1989) showed that a significant number of primary endings ceased to synchronise their discharge at vibration frequencies as low as 80 Hz and few primary endings exhibited synchronization at frequencies higher than 140 Hz. More recently, Fallon and Macefield (2007) found that only 14 % of recorded muscle spindle afferents (both Ia and II) were responsive to the vibratory stimulus. It is important to note that the above studies all tested spindle afferent responses to vibration while the muscle was at rest.

These differences in muscle spindle response appeared to have resulted from characteristics of the vibratory stimuli used in their studies. Firstly, Burke et al. (1976) employed a vibratory stimulus with an amplitude of 1.5 mm while the other two studies used smaller amplitudes of vibration (0.2 to 0.5 mm in Roll et al. 1989; up to 1 mm in Fallon & Macefield 2007). Indeed, Fallon and Macefield (2007) investigated the influence of frequency and amplitude of the vibratory stimulus on muscle spindle discharge rate, and showed that both primary and secondary endings of the muscle spindle tended to exhibit discharge rates that synchronised with vibration frequency as vibration amplitude increased. Secondly, although both studies employed relatively small amplitudes of vibration, differences in study results between Roll et al. (1989) and Fallon and Macefield (2007) were attributed to mechanical differences of the vibratory stimuli used in their studies. While Roll et al. (1989) used an "eccentrically-weighted DC motor", Fallon and Macefield (2007) used a vibrator that produced a repetitive tapping-like stimulus (i.e., "small sinusoidal displacement") (Fallon & Macefield 2007). The eccentrically-weighted DC motor was presumed to produce a mechanical stimulus that induced greater acceleration and velocity than the tapping stimulus used by Fallon and Macefield (2007).

Unlike the muscle spindle, it is rare for the Golgi tendon organ to be activated by a vibratory stimulus in a resting muscle. However, it was demonstrated that Golgi tendon organs become

responsive to a vibratory stimulus during a voluntary muscle contraction (Roll et al. 1989; Fallon & Macefield 2007). In particular, it was shown that tendon organs exhibited synchronization with vibration frequency between 10 and 40 Hz (Roll et al. 1989).

The application of a vibratory stimulus may also be accompanied with an illusion of movement. This vibration-related movement illusion was presumed to be attributed to the primary ending (Proske et al. 2000; Fallon & Macefield 2007) and appeared to be related to the number of muscle spindle afferents recruited as well as the characteristics of the vibration stimulus (e.g. amplitude and frequency) (Proske et al. 2000).

In the studies in this project, a custom-built vibratory apparatus was used (Scientific Concepts, Glen Waverley, VIC, Australia) (Figure 2.11). The vibrator consisted of a DC motor eccentrically positioned on a shaft which was embedded in a plastic tube that measured 35 mm in diameter and 65 mm in length. The DC motor had an adjustable frequency of vibration ranging between 40 and 120 Hz. The amplitude was specified as approximately 1.5 mm at 70 Hz.

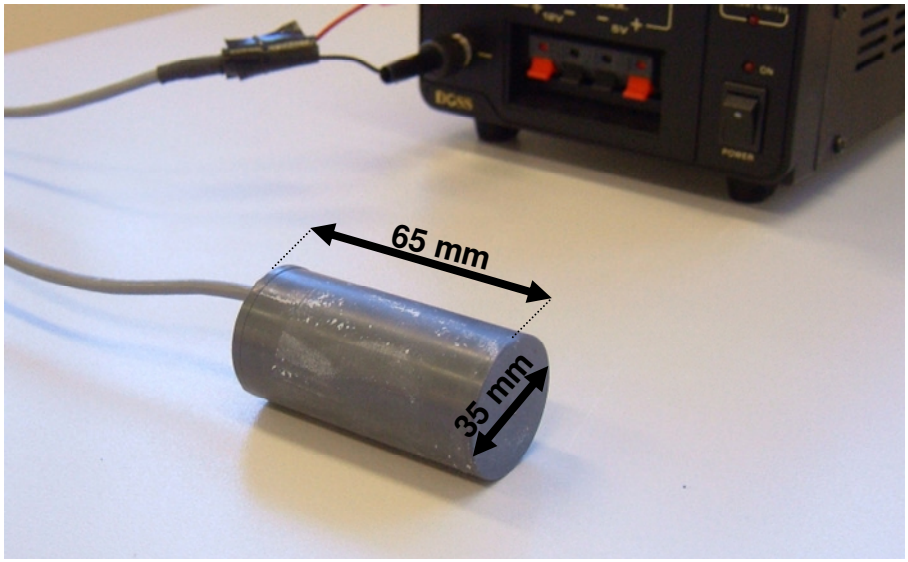

Figure 2.11. A custom-built vibratory apparatus.

2.12. Head-up tilt

Head-up tilt (HUT) is a common test used to evaluate autonomic nervous function (Low 1997). Compared with active standing, HUT provides a more controlled orthostatic stress (e.g. velocity, amplitude, and direction) and it also minimises factors that act to compensate cardiovascular regulation such as lower limb muscle contractions. Cardiovascular responses to HUT are characterised by an immediate initial increase in HR and an accompanying reduction in BP (Appenzeller & Oribe 1997; Low 1997). Blood pressure and HR values stabilise within the following 1-2 minutes (Appenzeller & Oribe 1997; Low 1997). In contrast, in patients with neurogenic orthostatic hypotension, autonomic regulation of cardiovascular function fails, so the initial HR increase is attenuated and the decreased BP is exaggerated (Appenzeller & Oribe 1997).

2.12.1. The determination of tilt angle

Head-up tilt has been used not only for clinical examination but also for experimental purposes. Sixty-degrees HUT is widely used (Appenzeller & Oribe 1997), but in this research project head up tilt was used in combination with the neck muscle conditioning manoeuvre. As summarised in *section 2.9*, it is critical that the neck muscles remain relaxed after the completion of the manoeuvre so that the muscle history that is created by the muscle conditioning manoeuvre is maintained. There was concern that a higher angle of HUT might have caused neck muscle contraction to support the head and this, in turn, would result in a loss of the created muscle history. Therefore, the angle of HUT used in this thesis was chosen as the lowest angle capable of inducing a cardiovascular response.

Iwase et al. (1987) demonstrated that HR and muscle sympathetic nerve activity recorded from the tibial nerve progressively increased as tilt angle increased (0°-90°) and the increases were significantly correlated to the angle of HUT (the burst rate of muscle sympathetic nerve

activity; r = 0.988, HR; r = 0.946). Saito et al. (1997) showed more detailed responses of autonomic drives and cardiovascular function to graded HUT by 5°. In their study, muscle sympathetic nerve activity recorded from the tibial nerve began to increase at 5° HUT. A significant increase in HR was observed at tilt angles greater than 15°. Muscle blood flow decreased at tilt angles higher than 20°. In contrast, BP did not change even in the 30° HUT position (Saito et al. 1997). Similarly, Bahjaoui-Bouhaddi et al. (2000) examined the effects of graded HUT (-10°, 10°, 30°, 45°, and 60°) on changes in autonomic drives and cardiovascular function including change in plasma catecholamine levels. There were no significant changes in any parameters at -10° or 10°, but HR and blood concentrations of adrenaline and noradrenaline increased progressively in response to greater HUT angles (>30°) while dopamine concentration did not change across the different angles of HUT (Bahjaoui-Bouhaddi et al. 2000). More recently, cardiovascular responses to graded HUT (20°, 40°, and 60°) were examined and compared between males and females (Shoemaker et al. 2001). Overall cardiovascular responses to HUT were similar in both genders, but HR significantly increased at 20° compared with the supine posture in females only. Otherwise, cardiovascular parameters tended to change linearly with respect to an increase in tilt angle; for example, muscle sympathetic nerve activity recorded from the common peroneal nerve significantly increased and stroke volume decreased compared with the supine position. Also, increases in BP and total peripheral resistance were observed at 40° and 60° (Shoemaker et al. 2001). Furthermore, Shamsuzzaman et al. (1998) examined differences in cardiovascular responses to active and suspended (passive) HUT (20°, 40°, and 60°). It was demonstrated that active HUT, where subjects supported their own weight in the tilted position, induced greater increases in HR and muscle sympathetic nerve activity at tilt angles greater than 40°, while there were no significant differences in any parameters at 20° between active and passive HUT (Shamsuzzaman et al. 1998).

In addition to measures of cardiovascular function and muscle sympathetic nerve activity, the literature also reports on the effect of graded HUT on HRV (an indicator of cardiac autonomic nervous drives) (Montano et al. 1994; Mukai & Hayano 1995; Akatsu et al. 1999; Ishibashi et al. 1999; Bahjaoui-Bouhaddi et al. 2000). In one study, graded HUT was performed from 0° to 60° and then to 90° and it was shown that the LF component of HRV progressively increased, while the HF component linearly decreased with the incremental rises in HUT that were used in this study (Montano et al. 1994). Both changes in HRV were accompanied by a progressive increase in HR. This suggests that there is a relationship between the various parameters of HRV and tilt angle, which were best observed when the HF and LF components of HRV were normalised (Montano et al. 1994). A further study demonstrated that a threshold angle of HUT had to be exceeded before changes in HRV parameters became significantly different from values recorded in the horizontal posture (0° HUT) (Mukai & Hayano 1995). It was found that LF and LF/HF began to increase at 10° compared with the supine position while HF was significantly decreased at tilt angles greater than 30° (Mukai & Hayano 1995). These data suggest that a shift to sympathetic dominance occurs at 10° HUT while parasympathetic withdrawal becomes evident later, at 30° HUT. Similarly, Ishibashi et al. (1999) demonstrated that increases in the LF component and LF/HF were significant at HUT angles greater than 20°.

Therefore, these studies indicated that an angle of 20° HUT provided a minimal but sufficient angle to induce both cardiac autonomic drive and cardiovascular changes. Further, it was suggested that at this angle the influence of lower extremity muscle contractions during HUT made negligible impact on cardiovascular responses (i.e., Shamsuzzaman et al. 1998).

2.12.2. The operation of the tilt table

The examination table used in this study was manufactured for clinical use. Although the table has a tilt function, it does not have a function to stop tilting at a particular angle. The table is electrically operated; the tilt function is operated by a peddle switch. Closing the switch by stepping on the pedal initiates to tilt the table. Releasing the peddle stops motion – at whatever angle the table happens to be at when the switch is released. In order to stop the table at the particular angle used during the experiment, a cord of a set length was attached to the table. At the end of the cord a lead weight was secured. To commence tilt, the weight was placed on the pedal. The weight was heavy enough to close the switch and this action initiated table tilt. The length of the cord was adjusted so that when the angle of the tilt was equivalent to 20°, the weight lifted off the pedal which stopped the motion of the table. The table then remained at the 20° HUT position for as long as required. In this way, the angle of table tilt was able to be reproduced for each experiment. It was found in a preliminary experiment that a small degree of slippage of the tilt table occurred after the tilt angle was attained. To prevent slippage, a stopper was inserted under the edge of the tilt table so as to maintain the same angle of HUT throughout data collection.

Chapter 3

Effects of body position on autonomic regulation of cardiovascular function

3.1. Introduction

As body requirements change, autonomic nervous output regulates cardiac function (e.g., heart rate; HR), to maintain a stable internal environment (Boron & Boulpaep 2003). At first glance, the heart appears to beat regularly, however, the interval between one heartbeat and the next is not the same. Further, embedded within these beat-to-beat variations in length of interval between successive heartbeats are inherent rhythms, at specific frequencies. These changing frequencies constitute what are referred to, collectively, as heart rate variability (HRV) and can be revealed through power spectral analysis. The power spectrum characterises the strength (or power) of these frequencies and reflects sympathetic and parasympathetic (vagal) contributions to their generation. That is, a power spectral analysis of HRV can be used, non-invasively, to quantify sympathetic and parasympathetic output to the heart.

Body position significantly influences cardiac autonomic drive in humans. In healthy adults, HRV has been compared across supine and right- and left-side lying postures (Ryan et al. 2003); supine and right-side lying postures (Kalisnik et al. 2001); supine and sitting postures (Sipinkova et al. 1997); supine and standing postures (Chen et al. 1999; Radhakrishna et al. 2000; Siebert et al. 2004); and supine, standing, and head-up and -down tilt postures (Iida et al. 1999). In healthy adults, cardiac autonomic modulation does not change significantly with different recumbent postures (Kalisnik et al. 2001; Ryan et al. 2003), but is clearly different between supine and vertical postures (standing or sitting). Sympathetic nervous function predominates in vertical postures, while the vagal drive predominates in recumbent postures.

Autonomic function also has been examined in patients who were prone and under general or spinal anaesthesia (Tetzlaff et al. 1998), and in chronic heart failure patients in right and left side-lying and supine postures (Kuo & Chen 1998; Fujita et al. 2000). In patients with heart

disease, the right recumbent posture is associated with enhanced vagus activity (when compared with supine and left recumbent postures) (Kuo & Chen 1998; Fujita et al. 2000).

To date, there have been no direct comparisons of the modulation of cardiac autonomic drives in supine and prone postures, or in prone and sitting postures. Although commonly assumed by patients undergoing manual therapy, effects of these postures on autonomic and cardiovascular function may differ. Therefore, the purpose of Chpater 3 is to establish the impact of recumbent and sitting postures (particularly, the difference between supine and prone) on autonomic nervous regulation of cardiovascular function.

Because healthy, young adults were focused in this study, other factors, which can modify cardiovascular adjustments to changes in posture, were presumed absent. For example, elderly people with systolic hypertension show poor cardiovascular adjustments to changes in posture from horizontal to vertical when compared with normotensive elderly people (James & Potter 1999).

3.2. Methods

This study was approved by the RMIT Human Research Ethics Committee. Written, informed consent was obtained from participants before commencement of experiments (see Appendix 1 for a Plain Language Statement and Appendix 4 for a consent form), and all study protocols were conducted in accordance with the Declaration of Helsinki.

3.2.1. Participants

Eligible participants were between 18 and 35 years old and in good general health. Nineteen young adults responded to advertisements placed around the RMIT University campus, but four were excluded: due to high blood pressure (two), medication use (one), and benign

arrhythmia (one). Participants (nine males and six females) were 24 ± 3 years old and had a body mass index (BMI) of 22.2 ± 3.5 kg/m^2 (expressed as mean ± standard deviation [SD]). None were smokers, used medication, or had a history of cardiovascular disease, diabetes mellitus, or cancer. Prior to the experiment, to assess general health status and account for factors that might influence autonomic drives and cardiovascular function, participants completed general health, cardiovascular, and pre-experimental questionnaires (see Appendix 2, 3, and 5, respectively). These focused on medical history, current health status, tobacco and medication use, and food and caffeine intake. Participants also completed questionnaires after each experimental session, regarding unpleasant sensations or discomforts during the experiment (see Appendix 6). Discomfort was assessed using a 10 cm, visual analogue scale (VAS), where 0 indicated "complete comfort" and 10 "worst pain imaginable."

3.2.2. Measurement of autonomic function

Heart rate, HRV, and systolic (SBP) and diastolic (DBP) blood pressure were measured. A 3-lead electrocardiogram (ECG) allowed measurement of quick changes in HR (Appenzeller & Oribe 1997), and visualization of the QRS waveform. Disposable electrodes (Blue Sensor, Medicostest, Denmark) were positioned, with the negative electrode over the manubrium and the positive and earth electrodes at the left and right axillary lines (over the 5th intercostal space). Using the obtained ECG signals, R-R intervals were calculated (Chart for Windows V5.1.1 with HRV extension V1.0.1, ADInstruments, Bella Vista, NSW, Australia) and the power spectrum of HRV was derived for the period of each intervention. The high frequency (HF) (0.15-0.4 Hz) component of the HRV power spectrum reflects parasympathetic activity (Task Force 1996) and the low frequency (LF) (0.04-0.15 Hz) reflects a combination of sympathetic and parasympathetic activity (Task Force 1996). The ratio of LF to HF (LF/HF) was adopted to determine the predominance of cardiac sympathetic drive. Because it is

unreliable over short recording periods (Task Force 1996), the power of the very low frequency (VLF) component (0-0.04Hz) was not calculated.

3.2.3. Measurement of cardiovascular function

A Portapres® (Model-2, Finapres Medical Systems, The Netherlands) continuously measured blood pressure (BP) and HR, using a finger cuff around the middle finger of the right hand. The Portapres® uses a hydrostatic height correction to transform measured BP values to those expected at the level of the heart (cf., Netea et al. 2003). Results were transferred to the data acquisition system (Chart for Windows) and displayed on a computer monitor, in real time.

3.2.4. Posture definition

Autonomic drives and cardiovascular function were measured during prone, supine, and sitting postures. Participants were encouraged to position themselves comfortably, but once settled, were asked to remain still for recording.

Prone: Participants laid horizontally on a treatment table with hands on hand rests. The headrest was designed to facilitate participants' breathing and was adjusted to minimise neck flexion, extension, and rotation.

Supine: Participants lay on the table with a contoured pillow supporting their natural cervical lordosis.

Sitting: A custom-designed chair supported participants' upright posture while minimising body and head movement. Footrests permitted comfortable knee flexion and both seat cushion and back support were provided. Immediately prior to recording, a helmet frame fixed the participant's head in a neutral position.

3.2.5. Experimental procedures

Recordings of HR and BP in the three postures were made in an air-conditioned laboratory, with white noise minimising disturbing sounds. Participants were asked to abstain from food and caffeine-containing beverages for at least 4 hours prior to data collection, and from alcoholic beverages and exercise for at least 12 hours. Two experiments (prone versus supine and prone versus sitting) were conducted on different days. To help minimise diurnal variation, participants were encouraged to schedule each experiment for the same time of day. The vestibular system is responsible for balance (Berne 2004), and is thought to influence autonomic and cardiovascular activities (Bolton & Ray 2000; Cui et al. 2001; Bolton et al. 2004). Therefore, to minimise vestibular organ activation, participants were instructed to avoid head motion during recording; they were also encouraged to stay awake.

Adjustment of autonomic function to a particular posture is thought to occur within 5 minutes (Appenzeller & Oribe 1997). Therefore, to stabilise autonomic outflow to cardiovascular organs before definitive recordings for each posture, participants were asked to make themselves comfortable, and then remain still for 5 minutes. Additionally, through respiratory sinus arrhythmia, a participant's respiratory rhythm can influence HRV components (Yasuma & Hayano 2004). To standardise this impact, participants were asked (following the rest period) to synchronise their breathing to a metronome set at 0.25 Hz (15 times a minute) for 5 minutes.

Day 1: prone–supine

In the prone posture, HR and BP were measured continuously during both resting and breath-synchronised phases. Participants then moved to a supine posture, and recordings were repeated.

Day 2: prone–sitting

Identical to Day 1, except that prone posture was followed by sitting posture.

Prior to re-commencing data collection after a posture change, a 5-minute relaxation period was included to re-stabilise cardiovascular function.

To confirm normal autonomic nervous function (Shibahara et al. 1996), participants were asked to place a hand in a bucket of icy water (the cold pressor test), for as long as they could tolerate, but not longer than 1 minute. There was concern that the effect of the cold pressor test might remain during the data collection period if the test was performed prior to data collection. The test is known to have a significant impact on cardiovascular function; however it is not known how long the effect of the test lasts. Therefore, the cold pressor test was performed at the end of Day 2. Blood pressure and HR were monitored during the test; had a significant sudden drop in BP and/or HR been observed, the cold pressor test would have been terminated and excluded the participant from the study. Participants were also excluded if they did not respond to this test.

3.2.6. Data analysis

Heart rate, mean arterial pressure (MAP), and SBP and DBP were recorded during rest and synchronised breathing periods in each posture. Mean values of each parameter were computed using Chart for Windows and analysed with the statistical software package SPSS (V12.0.1 for Windows, SPSS Inc., U.S.A).

Electrocardiographic data recorded during synchronised breathing periods in each posture were analysed off-line (Chart V5.1.1 with HRV extension for Windows V1.0.1) for frequency spectrum characteristics, including LF and HF (absolute and normalised), and LF/HF. Paired

samples *t*-tests were used to compare postures. When measurements for a variable deviated markedly from the normal distribution, the Wilcoxon signed-rank test was used (and reported as z scores). The statistical significance level for each comparison was set at $p < 0.05$.

Reproducibility of cardiovascular and autonomic parameters on different days was examined via the intraclass correlation coefficient (ICC). Values above 0.75 were considered to indicate good reliability, lower values poor to moderate (Portney & Watkins 2000). Paired samples *t*-tests were conducted to check for consistent differences in these parameters across recording days.

To evaluate cold pressor test response, minimum values of BP and HR were compared against mean values of BP and HR recorded during synchronised breathing periods in the sitting posture. A normal response to the cold pressor test was defined as a change in BP and/or HR to at least 2 standard deviations of reference values.

In order to confirm that a five-minute relaxation before data collection is sufficient to stabilise cardiovascular function, stability of BP and HR was assessed. Each parameter recorded during a controlled breathing period (following a five-minute relaxation) was averaged every one minute. Stability of BP and HR was assessed using a one-way repeated measures analysis of variance (ANOVA), Friedman test, paired t-test, or Wilcoxon signed-rank test based on normality assessment (using a Kolmogorov-Smirnov test). In order to avoid type I error, alpha level was adjusted using Bonferroni correction for pairwise comparisons. Statistical significance was set at $p < 0.05$ per comparison for the results of a one-way repeated measures ANOVA because probability (*p* values) were automatically calculated by the statistics software (SPSS). However, for paired t-test and Wilcoxon signed-rank test, 0.05 was

divided by the number of comparison (which is five) so the *p* values of these test needed to be less than 0.01 in order to satisfy with statistical significance level.

3.3. Results

A few participants reported a small degree of experiment-related discomfort (VAS = 0.64 ± 1.69 on Day 1, 0.12 ± 0.52 on Day 2). All responded normally to the cold pressor test.

3.3.1. Day 1: prone–supine

Differences in HRV components between prone and supine postures are presented in Table 3.1, and MAP, systolic and diastolic BP, and HR are shown in Figure 3.1. Between prone and supine postures, MAP [t (14) = 6.28, $p < 0.001$, d = 1.26], systolic BP [t (14) = 4.56, $p < 0.001$, d = 1.01], diastolic BP [t (14) = 7.26, $p < 0.001$, d = 1.38], and HR [t (14) = 5.04, $p < 0.001$, d = 0.48] were all significantly higher in the prone posture than in the supine. In contrast, components of HRV did not differ between postures: total power (TP) [z (15) = -0.74, p = 0.46], LF [z (15) = -1.53, p = 0.13], normalised LF [t (14) = -0.042, p = 0.97, d = 0.01] HF [z (15) = -1.53, p = 0.26], normalised HF [t (14) = -0.13, p = 0.90, d = 0.03], and LF/HF [t (14) = 0.24, p = 0.81, d = 0.07].

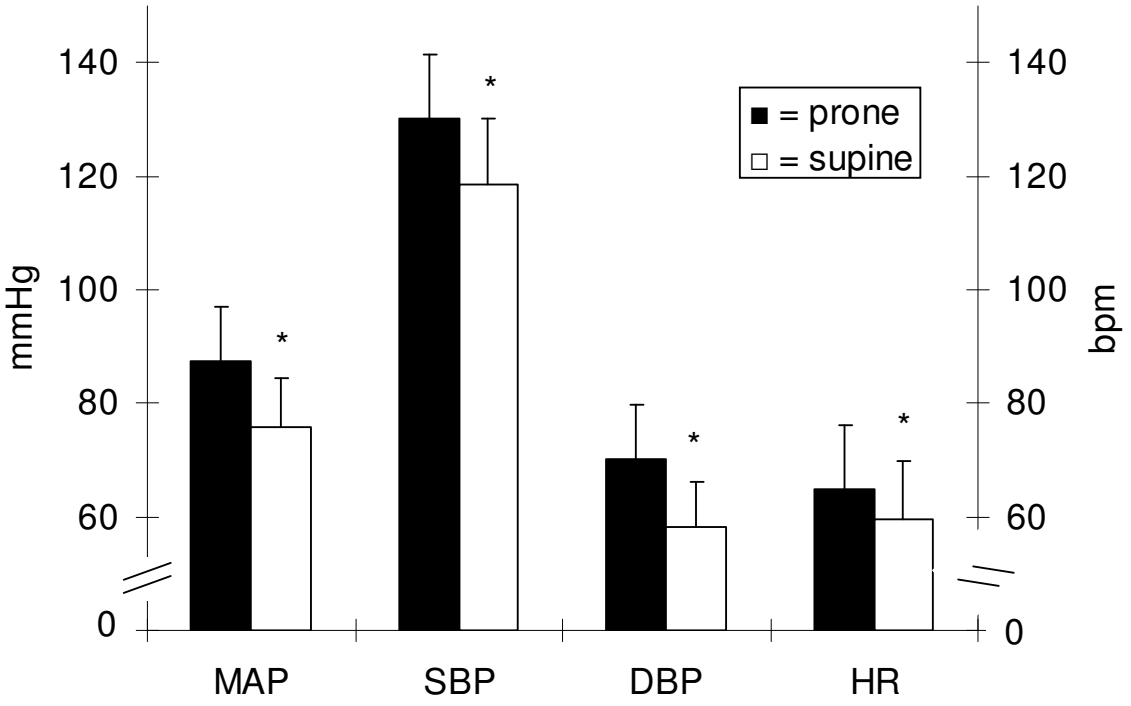

Figure 3.1. Comparison of blood pressure and heart rate between two horizontal postures (prone ■ and supine □)
Data are expressed as mean ± SD and compared using paired *t*-tests. Left scale applies to MAP, SBP, and DBP, right scale only to HR; DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure, mmHg = millimetre of mercury, bpm = beats per minute, and * = statistically significant difference from value in prone posture.

3.3.2. Day 2: prone–sitting

Differences in HRV components between prone and sitting postures are presented in Table 3.1, and BP and HR in Figure 3.2. Both autonomic and cardiovascular parameters differed between postures. In the prone posture, MAP [t (14) = 5.32, $p < 0.001$, $d = 0.96$], systolic BP [t (14) = 5.84, $p < 0.001$, $d = 1.36$], and diastolic BP [t (14) = 5.73, $p < 0.001$, $d = 1.01$] were significantly higher, and HR [t (14) = -3.61, $p = 0.003$, $d = 0.55$] was significantly lower. For HRV during sitting, normalised LF values were significantly higher [t (14) = 4.38, $p = 0.001$, $d = 1.13$] and both normalised and absolute HF values were significantly lower [t (14) = 4.76, $p < 0.001$, $d = 1.16$] and [z (15) = -3.18, $p = 0.001$]. These differences were reflected in LF/HF, which was also significantly higher in the sitting posture [z (15) = -3.35, $p = 0.001$].

Between postures, TP [z (15) = 1.14, p = 0.26] and absolute LF [z (15) = -0.51, p = 0.61] were not significantly different.

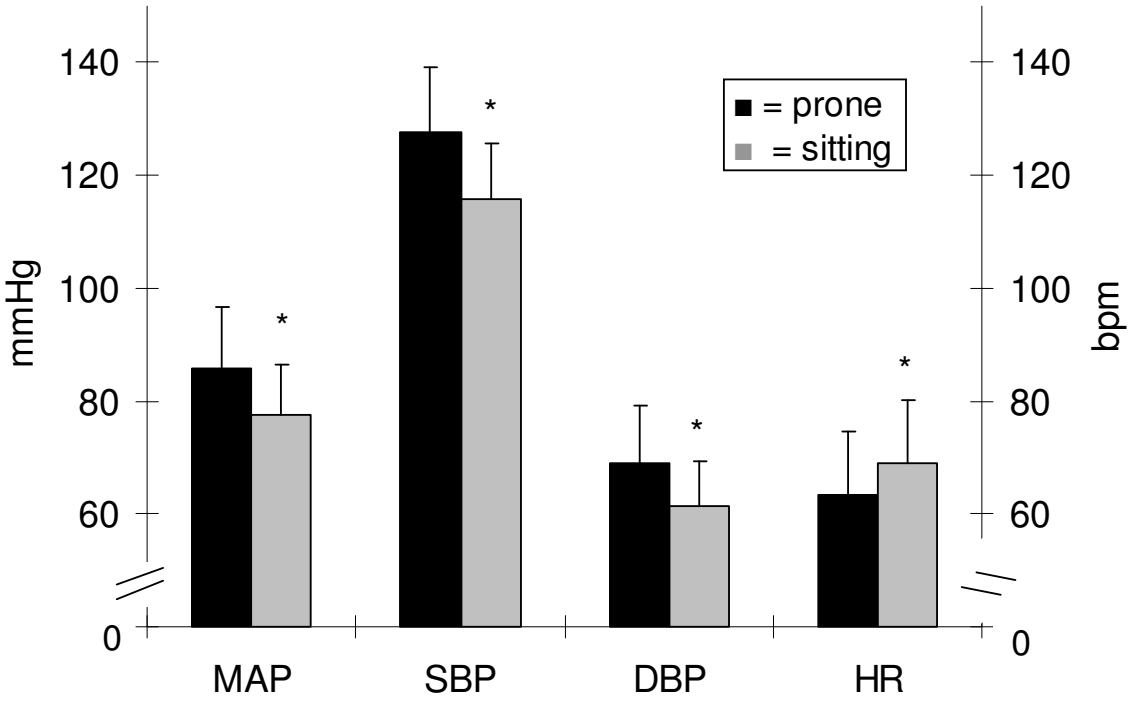

Figure 3.2. Comparison of blood pressure and heart rate between the horizontal (prone ■) and vertical (sitting ■) postures
Data are expressed as mean ± SD and compared using the paired *t*-tests. Left scale applies to MAP, SBP, and DBP, right scale only to HR; DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure, mmHg = millimetre of mercury, bpm = beats per minute, and * = statistically significant difference from a value in the prone posture.

Table 3.1. Comparison of heart rate variability parameters on Day 1 and Day 2; N = 15.

	Day 1			Day 2		
	Prone	**Supine**	(*p*)	**Prone**	**Sitting**	(*p*)
TP (ms^2)	4896.39 ± 5579.34	4076.27 ± 4215.74	0.46 (w)	5004.72 ± 5797.60	2746.36 ± 2643.12	0.26 (w)
LF (ms^2)	1023.57 ± 1505.68	963.55 ± 1095.13	0.13 (w)	800.09 ± 791.30	667.95 ± 731.74	0.61 (w)
LF (nu)	40.70 ± 21.12	40.93 ± 19.85	0.97 (t)	37.61 ± 18.46	59.87 ± 20.88	0.001 (t)
HF (ms^2)	2069.20 ± 3292.70	1975.40 ± 3304.52	0.26 (w)	2145.69 ± 3320.78	577.78 ± 650.45	0.001 (w)
HF (nu)	55.91 ± 21.22	56.61 ± 19.67	0.90 (t)	58.42 ± 18.12	35.84 ± 20.87	< 0.001 (t)
LF/HF	0.98 ± 0.76	0.93 ± 0.69	0.81 (t)	0.82 ± 0.65	3.00 ± 3.07	0.001 (w)

Data are expressed as mean ± SD; (*t*) = paired *t*-test, (w) = Wilcoxon signed-rank test, HF = high frequency of HRV power spectrum, LF = low frequency, LF/HF = ratio of low frequency to high, TP = total power, ms^2 = milliseconds squared, nu = normalised unit.

3.3.3. Reproducibility of autonomic nervous and cardiovascular parameters

Parameters measured in the prone posture on days 1 and 2 were used for reproducibility analysis. Table 3.2 presents descriptive data and ICC values for all HRV components. Table 3.3 shows reproducibility of BP and HR values. Several components of HRV (TP, normalised LF, and absolute and normalised HF) and HR demonstrated good reliability. Cardiovascular parameters, including MAP and BP (systolic and diastolic), showed poor reproducibility.

Table 3.2. Reproducibility of heart rate variability parameters recorded in the prone posture on two different days; N = 15.

parameters	ICC
TP (ms^2)	0.95*
LF (ms^2)	0.74
LF (nu)	0.78*
HF (ms^2)	0.95*
HF (nu)	0.78*
LF/HF	0.69

ICC = Intraclass correlation coefficient, HF = high frequency of HRV power spectrum, LF = low frequency, LF/HF = ratio of low frequency to high, TP = total power, ms^2 = milliseconds squared, nu = normalised unit, * = good reproducibility (ICC > 0.75).

Table 3.3. Reproducibility of blood pressure and heart rate recorded in prone posture on two different days; N = 15.

	Prone Day 1	Prone Day 2	ICC
MAP (mmHg)	87 ± 9.7	86 ± 9.8	0.13
SBP (mmHg)	130 ± 11.2	129 ± 9.8	0.25
DBP (mmHg)	70 ± 9.4	70 ± 8.7	0.062
HR (bpm)	65 ± 11.5	63 ± 11.4	0.86*

Data are expressed as mean ± SD; ICC = Intraclass correlation coefficient, DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure, mmHg = millimetre of mercury, bpm = beats per minute, * = good reproducibility (ICC > 0.75).

3.3.4. Stability of cardiovascular parameters during the controlled breathing period

In order to confirm that a five-minute relaxation was sufficient to stabilise cardiovascular function in each posture, the stability of cardiovascular parameters (BP and HR) was examined using data recorded during controlled breathing following five-minute relaxation.

3.3.4.1. Stability of cardiovascular parameters during prone posture

The results of a one-way repeated measures ANOVA showed that there was a significant time effect on HR during the prone posture on Day 2 [Wilks' $\Lambda = 0.41$, $F\ (4,11) = 3.90$, $p = 0.033$, $\eta^2 = 0.59$] but not on Day 1 [Wilks' $\Lambda = 0.66$, $F\ (4,11) = 1.44$, $p = 0.29$, $\eta^2 = 0.34$].

Pairwise comparisons showed that there was a significant difference in HR during the prone posture on Day 2 between at the second and third minutes [$t\ (14) = 3.68$, $p = 0.025$, $d = 0.95$] (Table 3.4).

Table 3.4. Stability of heart rate during the prone posture on Day 1 and Day 2

	1 min	2 min	3 min	4 min	5 min
Day 1 prone	65±12.9	65±11.4	65±11.0	64±11.8	65±10.8
Day 2 prone	63±12.3	64±11.7*	63±11.6	62±11.1	63±11.2

Data was expressed as mean ± SD min = minute. Asterisk (*) indicates a significant difference between the second and third minute.

The results of a one-way repeated measures ANOVA showed that there was a significant time effect on MAP during the prone posture on Day2 [Wilks' $\Lambda = 0.44$, $F\ (4,11) = 3.57$, $p = 0.042$, $\eta^2 = 0.57$] but not on Day 1 [Wilks' $\Lambda = 0.51$, $F\ (4,11) = 2.63$, $p = 0.092$, $\eta^2 = 0.49$].

Pairwise comparisons showed that there was a significant difference in MAP during the prone posture on Day 2 between at the first and second minutes [$t\ (14) = -3.92$, $p = 0.015$, $d = 1.01$] (Table 3.5).

Table 3.5. Stability of mean arterial pressure during the prone posture on Day 1 and Day 2

	1 min	2 min	3 min	4 min	5 min
Day 1 prone	87±9.3	88±9.6	87±9.9	88±10.3	88±9.6
Day 2 prone	85±8.3*	86±9.4	86±9.9	87±10.6	87±11.1

Data was expressed as mean ± SD min = minute. Asterisk (*) indicates a significant difference between the second and third minute.

During the prone position, there was a significant time effect on DBP on Day 1 [$\chi^2(4, N = 15) = 11.31$, $p = 0.023$] and Day 2 [Wilks' $\Lambda = 0.36$, $F\ (4,11) = 4.90$, $p = 0.016$, $\eta^2 = 0.64$]. On Day 1, there was a significant difference in DBP between the first and second minutes [$t\ (14) = -3.22$, $p = 0.006$, $d = 0.83$]. On Day 2, DBP at the first minute was significantly different from the second [$t\ (14) = -4.64$, $p = 0.004$, $d = 1.20$], the fourth [$t\ (14) = -3.61$, $p = 0.028$, $d = 0.93$], and the fifth [$t\ (14) = 3.64$, $p = 0.027$, $d = 0.94$] minutes (Table 3.6).

Table 3.6. Stability of diastolic blood pressure during the prone posture on Day 1 and Day 2

	1 min	2 min	3 min	4 min	5 min
Day 1 prone	70 ± 9.2	70 ± 9.3*	70 ± 9.9	71 ± 9.8	71 ± 9.2
Day 2 prone	68 ± 7.6	70 ± 8.5*	70± 9.0	70 ± 9.4*	71 ± 9.7*

Data was expressed as mean ± SD min = minute. Asterisk (*) indicates a significant difference from the first minute.

During the prone position there was a significant time effect on SBP on Day 1 [Wilks' $\Lambda = 0.31$, $F\ (4,11) = 6.26$, $p = 0.007$, $\eta^2 = 0.70$] but not on Day 2 [$\chi^2(4, N = 15) = 4.05$, $p = 0.40$]. Pairwise comparisons showed a significant difference in SBP between the first and second minutes on both Day 1 and 2 [$t\ (14) = -3.60$, $p = 0.029$, $d = 0.93$ and $t\ (14) = -3.89$, $p = 0.002$, $d = 1.00$, respectively] (Table 3.7).

Table 3.7. Stability of systolic blood pressure during the prone posture on Day 1 and Day 2

	1 min	2 min	3 min	4 min	5 min
Day 1 prone	129 ± 10.7	132 ± 11.3*	129 ± 12.1	131 ± 12.8	130 ± 12.1
Day 2 prone	126 ± 7.9	130 ± 9.2*	128 ± 9.6	129 ± 12.4	130 ± 12.5

Data was expressed as mean ± SD min = minute. Asterisk (*) indicates a significant difference from the first minute.

3.3.4.2. Stability of cardiovascular parameters during supine posture

During the supine posture, the results of a one-way repeated measures ANOVA showed that there was no significant time effect on HR [Wilks' $\Lambda = 0.86$, $F\ (4,11) = 0.43$, $p = 0.78$, $\eta^2 = 0.14$], on MAP [Wilks' $\Lambda = 0.67$, $F\ (4,11) = 1.38$, $p = 0.30$, $\eta^2 = 0.33$], on DBP [Wilks' $\Lambda = 0.69$, $F\ (4,11) = 1.24$, $p = 0.35$, $\eta^2 = 0.31$], or on SBP [Wilks' $\Lambda = 0.692$ $F\ (4,11) = 1.68$, $p = 0.22$, $\eta^2 = 0.38$].

3.3.4.3. Stability of cardiovascular parameters during sitting posture

During the sitting position, the result of a Friedman test showed a non-significant time effect on HR [$\chi^2(4, N = 15) = 3.63$, $p = 0.46$] and the results of a one-way repeated measures ANOVA showed a non-significant time effect on MAP [Wilks' $\Lambda = 0.68$, $F\ (4,11) = 1.28$, $p = 0.34$, $\eta^2 = 0.32$], on DBP [Wilks' $\Lambda = 0.63$, $F\ (4,11) = 1.62$, $p = 0.24$, $\eta^2 = 0.37$], or on SBP [Wilks' $\Lambda = 0.77$, $F\ (4,11) = 0.83$, $p = 0.53$, $\eta^2 = 0.23$].

3.4. Discussion

The current study recorded HR, HRV, and beat-to-beat BP, as measures of autonomic and cardiovascular function, comparing prone and supine postures (Day 1) and prone and sitting postures (Day 2). The reproducibility of these parameters which was measured in the prone posture on both days was also examined.

Parameters of autonomic efferent drives (HRV) and cardiovascular function (BP and HR) were affected less by changes between the two horizontal postures (prone and supine) than changes between horizontal (prone) and vertical (sitting). Between prone and supine, there was no significant difference in HRV parameters indicative of a change in autonomic balance to the heart, but HR and BP were significantly higher in the prone posture. In contrast, between prone and sitting postures, there were significant differences in HRV parameters, with a shift towards sympathetic dominance during sitting.

Others have examined cardiovascular regulation during different horizontal postures in adults. For example, Pump et al. (2002) observed cardiovascular parameters (including BP and HR) over a period of 9 hours: supine posture for 3 hours, then either supine or prone for 6 hours. In the prone posture, HR, total peripheral resistance, and sympathetic nerve activity increased, and stroke volume decreased. However, there was no difference in BP between these two postures. Tabara et al.'s (2005) study design was similar to the current study. Cardiovascular variables (including BP and HR) were measured over a short time frame, with participants in the supine and then prone postures. Unlike the current study, these authors found that BP in the prone posture was significantly lower than in the supine. In the present study, changes in HR were consistent with those reported by Tabara et al. (2005) and Pump et al. (2002).

In contrast to our current results, Pump et al. (2002) observed no change in BP between supine and prone postures. Tabara et al. (2005) did observe a change, but BP was lower in prone than that in supine. These discrepancies probably resulted from differences in methodology: Tabara et al. measured BP and HR only 1 minute after posture changed from supine to prone and Pump et al. (2002) recorded BP and HR at the start and every 90 minutes thereafter, for 9 hours. After a shift from supine to standing, 1 to 2 minutes may be required to stabilise consequent cardiovascular adjustments and up to 5 minutes to complete most

autonomic adjustments (Appenzeller & Oribe 1997). Cardiovascular function is controlled by regulatory mechanisms involving the neural, renal, and endocrine systems, each operating within a different time frame. For example, baro- and chemoreceptors are involved in cardiovascular adjustments as soon as arterial pressure is altered, whereas blood volume control by the kidneys plays a role in BP regulation several hours later (Guyton & Hall 2006). To minimise the impact that the very act of changing postures might have on the results, participants in the current study maintain each posture for 5 minutes prior to data collection. Therefore, the different changes observed between Pump et al.'s (2002) or Tabara et al.'s study (2005) and the current study may be because each study recorded cardiovascular activity at a different phase of the cardiovascular adjustment cycle. The mean age of volunteers for Tabara et al. (2005) was 50 ± 11 years, for the present study it was 24 ± 3 years. Finally, approximately one-third of Tabara et al.'s participants were considered hypertensive (although they had no history of cardiovascular disease and were not being treated for hypertension). The current study employed only healthy volunteers, with no signs or symptoms of hypertension. It is known that both age and hypertensive condition have an impact on cardiovascular regulation associated with posture changes (James & Potter 1999). Therefore, these differences in participant population and timing of data collection from the previous studies (Pump et al. 2002; Tabara et al. 2005) might have led to some contradictory results.

The current study showed significantly higher HR and BP in the prone posture than these in the supine. Toyota and Amaki (1998) measured haemodynamic changes associated with prone posture during general anaesthesia in surgical patients and observed decreases in end-systolic and end-diastolic left ventricular area and left ventricular volume. They ascribed these changes to reduction of venous flow (caused by compression of the inferior vena cava) and augmentation of left ventricular filling resistance (caused by compression of the thorax)

during the prone posture (Toyota & Amaki 1998). This has been supported by Pump et al. (2002), who postulated that compression of the thorax might have been responsible for their observed decreased stroke volume. In turn, this was thought to attenuate arterial pulse waves, which inhibited baroreflexes and subsequently increased sympathetic nervous activity. Thus, the condition of the cardiovascular system is thought to be quite different during prone and supine postures. A decrease in central venous flow may cause pooling and increased blood volume in peripheral vessels. Consequently, vessel constriction is induced in fingers and BP increased (the Bayliss myogenic response) (Levick 1995). The prone posture is likely to cause facial tissue compression that does not occur during the supine posture, and trigeminal afferents from there modulate cardiac function (Schaller 2004). Therefore, in movements between prone and supine postures, cardiovascular parameters (HR and BP) might be regulated by different reflexive neural and non-neural factors. An example of a non-neural factor could be vessel myogenic activity, which is not associated with the cardiac autonomic nervous system.

In the current study, HRV parameters indicative of autonomic nervous activity to the heart did not differ between the two horizontal postures and were associated with a large standard deviation (reflecting large individual differences within the sample in the current study). Further, calculation of Cohen's *d* revealed a very small effect size. Based on these results, post-hoc analysis revealed that approximately 50 participants would have been required to reliably detect (power = 0.8) a difference in HRV parameters between prone and supine postures. With the sample of the current study and the effect of posture change on cardiac autonomic activity so small, HRV analysis could not reveal related differences in autonomic regulation of the heart.

With a horizontal to vertical posture change, a hydrostatic gradient is introduced and cardiovascular adjustments may occur to maintain adequate perfusion to the brain. It was found that HR increased and HRV parameters indicated a shift to sympathetic dominance during the sitting posture in the current study. In contrast, BP was higher in the prone than in the sitting posture. Arterial BP is a product of cardiac output (HR × stroke volume) and total peripheral resistance (Berne & Levy 1997). Shamsuzzaman et al. (1998) has shown that antigravity muscle activity influences sympathetic outflow to the muscle vasculature and cardiovascular parameters including HR and BP during postural change. It is likely that the sitting posture minimised antigravity muscle involvement. This is because participants were asked to keep still during the data collection period unless their posture became uncomfortable. Additionally, participants were observed throughout the data collection period to ensure that they were not drifting off to sleep or for any signs of movement. Therefore, even though leg muscle contraction was not monitored using a rigorous methods (e.g., surface electromyogram), the contribution of leg muscle contraction to the cardiovascular system in the study is believed to be minimal. Consequently, one possible explanation for the finding of the current study that BP was higher in the prone posture than in the sitting, may be that there was little change in total peripheral resistance secondary to vascular compression induced by skeletal muscle contraction, resulting in a minimal change in BP during the sitting posture.

The current study demonstrated that components of HRV are highly reproducible, across days, which is consistent with former studies (Pitzalis et al. 1996; Kowalewski & Urban 2004). Kowalewski and Urban (2004) used a 12-month follow-up and found that components of HRV were consistent. Others, however, have asserted that HRV parameters are not a consistent tool for measuring autonomic nervous function (Toyry et al. 1995; Lord et al. 2001). There are several possible explanations for this contradiction. First, a minimal 2 to 5 minute recording period is required for accurate HRV analysis (Task Force 1996). One

minute recording period (Toyry et al. 1995) may have made it hard to assess whether measures of HRV are reproducible. For HRV analysis, respiration rate is usually fixed, and because this influences the location of the central frequency within the HF band of the power spectrum (Eckberg 2000). Lord et al. (2001) set the respiration rate at 0.167 Hz, which may have resulted in inadequate separation of LF and HF components of the power spectrum, leading to decreased reproducibility. It is critical that measures of HRV be conducted under well-controlled circumstances.

In addition, it was demonstrated consistency in HR and HRV components across different recording days in the current study. Measurements of BP, however, varied from day to day within individuals. Because their comfort was a priority, participants determined their final body position and daily BP measurements may have been influenced by changes in central blood flow, due to different pressures on the vena cava (Toyota & Amaki 1998). Another explanation might be diurnal variations in participants' hydration levels. Under normal conditions, plasma osmolality regulates vasopressin secretion (Rhoades & Tanner 2003), which in turn constricts blood vessels (Boron & Boulpaep 2003). The participants in the current study were asked to forgo food and caffeinated beverages for 4 hours prior to data collection, and alcoholic beverages for 12 hours. Otherwise, food and fluid intake was not governed. A participant's plasma osmolality may have varied daily, and this may have influenced recorded BP.

Finally, the stability of cardiovascular parameters (BP and HR) was assessed using data recorded during the controlled breathing period following a 5-minute relaxation period in each posture. A 5-minute relaxation prior to data collection was sufficient to stabilise the cardiovascular parameters in the supine and sitting postures. However, significant fluctuations in BP and HR were observed in the prone posture so five-minute was not a enough duration

for the posture. It is not possible to identify a cause of the cardiovascular fluctuation during the prone posture from the data of the current study. However, as discussed above, blood pooling in the peripheral vasculatures may be likely to occur during the prone posture due to inferior venocava compression and a reduction in venous return to the heart (Toyota & Amaki 1998). Thus, it may take longer to stabilise cardiovascular function during the prone posture. It was thought that the prone posture included uncertain factors with respect to cardiovascular function so was not reliable posture for the subsequent studies in this thesis due to unstable cardiovascular function although the prone posture has great accessibility to the paraspinal structures. Therefore, the supine and sitting would be used in the next chapter in order to investigate the effect of mechanical stimulation simulated spinal manipulation on autonomic regulation of cardiovascular function in awake humans.

Chapter 4

The effects of a single mechanical impulse to the neck on autonomic regulation of cardiovascular function

4.1. Introduction

Circulation problems are a serious issue world-wide. In Australia, high blood pressure (BP) was listed as the most common condition of the cardiovascular disease. Ten percent of the Australian population have a reported hypertensive condition (Australian Bureau of Statistics 2002). Furthermore, surprisingly there are nearly two million medication users for circulatory problems in Australia, and over three-quarters of those are taking medications because of hypertension (Australian Bureau of Statistics 1999).

Effective treatment for hypertension may not only involve the taking of medications but may also include other interventions including diet and lifestyle management as an adjunct to medical care (Goertz et al. 2002). The role of manipulative therapies such as chiropractic in the disorder is not clear (Goertz et al. 2002). In professional practice, manual therapists seem to experientially treat several visceral complaints (Budgell 1999). Some case reports have reported on the effectiveness of manipulative therapy for arrhythmia (Igarashii & Budgell 2000; Budgell & Igarashi 2001) and hypertension (Wei et al. 1989; Plaugher & Bachman 1993).

In order to elucidate the mechanism of these clinical observations, a few experimental studies have been carried out and demonstrated that spinal manipulation to the neck might be capable of evoking changes in autonomic (Budgell & Hirano 2001; Zhang et al. 2006) and cardiovascular (Vicenzino et al. 1998; Fujimoto et al. 1999; Knutson 2001; Dimmick et al. 2006) functions. Although it has not been rigorously investigated, spinal manipulation appears to involve a head position alteration when practitioners or investigators deliver manipulative procedures to the spine. As well-documented, the vestibular apparatus influences autonomic regulation of cardiovascular function in animals (Yates & Miller 1994) and humans (Essandoh et al. 1988; Cui et al. 1997a; Ray & Hume 1998; Cui et al. 2001).

Thus, due to the nature of the manipulative procedure, it is difficult to elucidate the physiological mechanism responsible for autonomic and cardiovascular effects in humans, while some animal studies have demonstrated that manipulating paraspinal structures might evoke autonomic drive and cardiovascular changes (Sato & Swenson 1984; Bolton et al. 1998; Kang et al. 2003).

Therefore, the aim of the study in Chapter 4 was to investigate whether cervical manipulation in the absence of head displacement influences autonomic and cardiovascular function in awake asymptomatic humans. To minimise vestibular effects associated with head displacements as a result of cervical manipulation, the Activator® Instrument (AAI III, Activator Methods International LTD., AZ) was employed to produce a brief mechanical impulse directed to the upper cervical region, simulating cervical manipulation.

4.2. Methods

This study was approved by the RMIT Human Research Ethics Committee, and written informed consent was obtained from participants before the commencement of the first experimental session (see Appendix 7 for a Plan Langeuage Statement and Appendix 4 for a concent form).

4.2.1 Participants

Potential participants were recruited through advertisements placed around the university. For inclusion into this study, participants needed to be aged between 18 and 35 years old and in generally good health. Twelve young healthy adults volunteered and 11 of them (9 males and 2 females, 27 ± 4.56 years old, BMI = 22.31 ± 3.58 kg/m^2; expressed as mean ± SD) completed this study. One person withdrew their participation as they were unable to attend all experimental sessions. They were required to be non-smokers and non-medication users,

report no history of cardiovascular diseases (e.g., known arrhythmia, stroke and myocardial infarction), and report no history of diabetes mellitus or cancer. Participants were asked to complete general health, cardiovascular and pre-experimental questionnaires before any experimental procedures commenced (see Appendix 2, 3, and 5, respectively). Also, once participants were included in the study, they were also asked to complete a post-experimental questionnaire after each experimental session (see Appendix 6). This was used to assess participant's perceptions about the mechanical stimulus and to document any unpleasant sensations as well as to determine their level of discomfort during the experimental sessions. In order to assess discomfort, a visual analogue scale (VAS) was used where participants marked a 10cm line where 0 indicated "complete comfort" and 10 indicated "the worst pain imaginable".

4.2.2. Instrumentation

Heart rate variability (HRV), heart rate (HR) and BP were employed as parameters to assess autonomic regulation of cardiovascular activities in this study. Mechanical stimulus was delivered using the AAI III, which is widely employed by chiropractors in their daily clinical practice. The instrument was set at the minimal force setting.

4.2.2.1. Autonomic Nervous function measurement

A 3-lead electrocardiogram (ECG) was employed in this study. Using the recorded ECG signals, R-R intervals were calculated and the power spectrum of HRV was obtained (Chart for Windows V5.1.1 with HRV extension V1.0.1, ADInstruments, Bella Vista, NSW, Australia).

In this study, the frequency range 0-0.04 Hz was considered as very low frequency (VLF), low frequency (LF) ranged between 0.04-0.15 Hz and 0.15-0.4Hz was indicative of the hight

frequency (HF) range. However, the VLF component of the HRV power spectrum is unreliable when short recording times are used (Task Force 1996). Therefore, the VLF was not taken into account in this study.

4.2.2.2. Cardiovascular function measurement

Blood pressure and HR were measured with a Portapres® (Portapres Model-2, Finapres Medical Systems, The Netherlands) device which can record beat-to-beat BP automatically, non-invasively and continuously. On-line visualization of the pressure waveform was achieved through the connection of the output of the Portapres® device to a data acquisition system, "Chart for Windows V5.1.1" (ADInstruments, Bella Vista, NSW, Australia).

4.2.3. Posture

In this study, autonomic and cardiovascular responses to the mechanical stimulus were recorded in two different postures — supine and sitting postures. Participants were encouraged to position themselves comfortably but once settled they were asked to remain as still as possible during the recording period.

For the supine posture, participants were asked to lie face-up on a chiropractic technique table. A contoured pillow was provided to support their natural neck lordosis. The right hand, instrumented with the Portapres® was placed on a stool which was positioned to the side of the technique table at the same height as the table.

For the sitting posture participants were asked to sit on a custom-designed experimental chair that was used to support an upright sitting posture while minimising body and head movement. A head-frame was attached to the frame of the chair and could be lowered and fastened over the participant's head. This head-frame was used to discourage head movement

during the recording period. For participants' comfort, foot position on the chair apparatus was adjusted allowing for a comfortable degree of knee flexion. A cushion and a back support were also provided. Once participants were satisfied with their posture, the head frame was gently lowered over the participant's head and their head position was gently fixed in a neutral position.

4.2.4. Experiment procedures

This study was conducted in an air-conditioned laboratory (mean ± SD; 22.01 ± 1.15 C°) with a consistently produced noise environment in order to minimise any sudden disturbing sounds. Data recording was continued over the entire experimental period. This study consisted of four sessions (sham and authentic mechanical impulse × two postures). The order of the postures and stimuli were semi-randomised; that is, the order of the two postures was randomised and the order of delivery of each stimulus within each posture was also randomised.

Participants were asked to fast for at least 4 hours, and to abstain from any caffeine-containing beverages for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the experiment. Participants were asked to refrain from any exercise for 12 hours before the experiment, and to participate in the second session of this study as near to the same hour of the first session as possible.

Once the participants had made themselves comfortable in any given posture, they were advised to keep still and quiet unless their posture became uncomfortable. They were also encouraged to stay awake. Participants were instructed to avoid any head motion during the entire experimental session in order to minimise the influence of vestibular organ activation

on the autonomic and cardiovascular systems (Essandoh et al. 1988; Ray & Hume 1998; Bolton & Ray 2000; Cui et al. 2001).

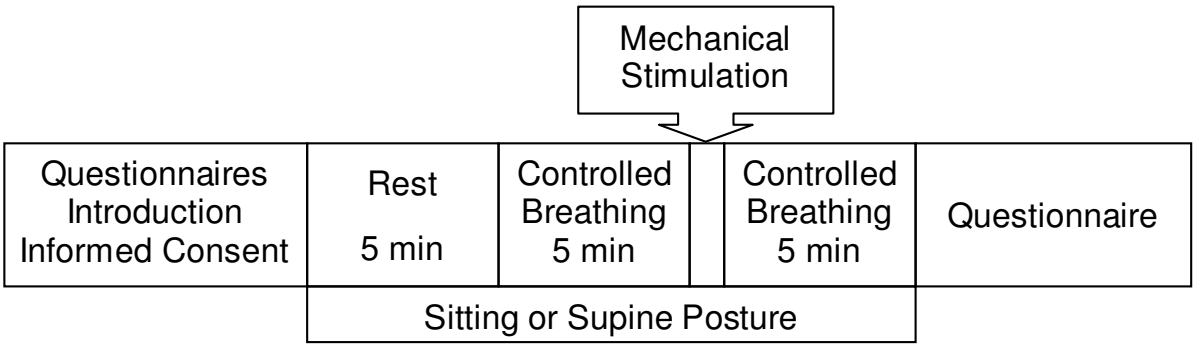

Figure 4.1. Time course of experimental sessions.
The intervention was conducted at meddle of 10-minute controlled breathing following 5-minute rest.

As shown in Figure 4.1, participants were asked to synchronise their breathing with a metronome rhythm (0.25Hz, which is equivalent to 15 times per minute) for 10 minutes following the 5-minute rest period. This is due to respiratory cycle events, that are related to a rhythmic change in HR, known as the *respiratory sinus arrhythmia* (Yasuma & Hayano 2004). This impacts on HRV analysis and needs to be normalised throughout the experimental sessions. At the mid-point of the controlled breathing period, a mechanical stimulus was delivered to the right lateral aspect of the transverse process of the atlas. This location was found between the tip of the mastoid process and the angle of the mandible (Field 2001, p. 158). To determine stimulus location, the area was gently palpated and marked by the same investigator for each participant. For the "authentic" stimulus, the mechanical impulse was delivered through the investigator's finger tip. For the "sham" stimulus the investigator's finger tip was placed on the stimulating site of the neck, but the mechanical impulse was applied over the dorsal aspect of the investigator's hand.

At the end of one of the experimental sessions (just after the 5-minute controlled breathing post "mechanical stimulation" period) in the sitting posture, participants were asked to put their hand into a bucket filled with icy water (the cold pressor test). This procedure aimed to

examine the status of autonomic nervous function (Shibahara et al. 1996). The response to the cold stimulus is normally to increase BP and cardiac output (Appenzeller & Oribe 1997, p. 692) but a decrease of BP and HR is also clinically observed (Shibahara et al. 1996). During the cold pressor test, BP and HR were continuously monitored. In the current study the cold pressor test was continued for as long as the participant could tolerate their hand in the icy water, but no longer than 1 minute. The evaluation of BP and HR responses to the cold pressor test has been described in *section 3.2.6*.

4.2.5. Data Analysis

This study used a pre- and post-study design. All variables, which were recorded during the 10-minute controlled breathing periods, were analysed. Mean values of each parameter (HR, mean arterial pressure; MAP, diastolic BP; DBP, and systolic BP; SBP) were computed using Chart for Windows V5.1.1. These data were analysed with the statistical software package SPSS (V13 for Windows, SPSS Inc., U.S.A).

Electrocardiography data recorded during the controlled breathing period pre- and post- the mechanical stimulus were analysed off-line using Chart V5.1.1 with HRV extension for Windows V1.0.1. Five-minute blocks of data were analysed for frequency spectrum characteristics, including total power (TP), absolute and normalised LF, absolute and normalised HF, and the ratio of LF to HF (LF/HF). Data were analysed using paired *t*-tests, or where the data deviated markedly from the normal distribution, the Wilcoxon signed rank test was employed. The significance level per comparison was $p < 0.05$. Data were expressed as mean ± SEM

Collected cardiovascular data including HR and BP were divided into 10-second bins and the mean of the data were compared before and after the intervention. To follow up the effects of

each intervention on cardiovascular function, a one-way repeated measures analysis of variance (ANOVA) with post-hoc testing of pairwise comparisons using Bonferroni adjusted α levels was used. The factor for this analysis was time (mean value of 60-second pre-intervention and 1st to 6th 10-second bins post-intervention). Additionally differences in cardiovascular response to the different kinds of mechanical stimuli were also tested by a two-way repeated measures ANOVA with Bonferroni correction (factors were time and kinds of mechanical stimuli). Significance was accepted at $p < 0.05$.

4.3. Results

According to the pre-experimental questionnaire, two participants reported neck stiffness before the experiment started; one reported neck stiffness in all four sessions (VAS = ranged from 1.5 to 2.4) and the other reported neck stiffness on three session (VAS = 3.6). One participant reported a mild level of neck pain and stiffness in one session (VAS = 1.9) and another participant reported slight neck stiffness in one session (VAS = 1). No one reported that any symptoms became worse or any new symptom arose on completing the post-experiment questionnaire. All 11 participants responded to the cold pressor test and none reported that any mechanical stimuli were painful.

4.3.1. Effects on heart rate variability parameters

Data recorded during the controlled breathing periods were divided into two 5-minute blocks (pre- and post-intervention) and compared. The results of all HRV parameters are presented in Table 4.1 and 4.2.

In this study, few differences in HRV parameters between pre- and post-intervention were observed. There was a significant shift in the ratio of the power of the LF and HF bands of the

frequency spectrum toward sympathetic dominance observed after the sham manipulation in the sitting posture [z (11) = -2.13, p = 0.033].

Table 4.1. HRV parameters pre- and post-mechanical stimulus with the Activator® Instrument in the sitting posture

stimulus	Parameters	Pre	Post	*p*
SIAU	TP (ms^2)	2259.72 ± 375.92	2532.88 ± 689.92	0.72 (w)
	LF (ms^2)	524.27 ± 93.75	553.35 ± 138.60	0.79 (t)
	LF (nu)	58.64 ± 6.31	56.01 ± 6.09	0.86 (w)
	HF (ms^2)	472.56 ± 6195.82	667.56 ± 368.55	0.53 (w)
	HF (nu)	36.78 ± 65.89	39.98 ± 6.15	0.53 (w)
	LF/HF	2.70 ± 0.88	1.97 ± 0.40	0.42 (w)
SISH	TP (ms^2)	3047.74 ± 1007.53	2566.58 ± 602.69	0.86 (w)
	LF (ms^2)	897.19 ± 446.02	809.71 ± 259.62	0.79 (w)
	LF (nu)	55.28 ± 4.99	62.34 ± 5.51	0.066 (t)
	HF (ms^2)	575.01 ± 211.36	658.88 ± 286.23	1.00 (w)
	HF (nu)	38.54 ± 4.72	34.06 ± 5.42	0.15 (t)
	LF/HF	1.74 ± 0.27	3.34 ± 1.32	0.033 (w)

Data are expressed as mean ± SEM SIAU; authentic manipulation in sitting posture, SISH; sham manipulation in sitting posture, TP; total power, LF; low frequency power, HF; high frequency power, LF/HF; the ratio of low frequency to high frequency, ms^2; millisecond square, un; normalised unit, t; paired t-test, w; Wilcoxon singed-rank test

Table 4.2. HRV parameters pre- and post-mechanical stimulus with the Activator® Instrument in the supine posture

interventions	parameters	Pre	Post	*p*
SUAU	TP (ms^2)	4419.19 ± 1351.50	5750.83 ± 1673.22	0.13 (w)
	LF (ms^2)	696.73 ± 145.39	672.86 ± 139.53	0.83 (t)
	LF (nu)	39.61 ± 6.15	39.54 ± 5.67	0.98 (t)
	HF (ms^2)	1787.86 ± 814.02	2158.78 ± 1141.52	0.86 (w)
	HF (nu)	53.81 ± 5.52	54.43 ± 4.99	0.82 (t)
	LF/HF	0.94 ± 0.21	0.93 ± 0.23	0.94 (t)
SUSH	TP (ms^2)	5389.17 ± 1635.23	5226.55 ± 1712.59	0.37 (w)
	LF (ms^2)	874.13 ± 183.14	1067.58 ± 325.29	0.93 (w)
	LF (nu)	45.38 ± 5.58	44.33 ± 7.28	0.82 (t)
	HF (ms^2)	1662.93 ± 712.01	1784.21 ± 817.41	0.79 (w)
	HF (nu)	48.14 ± 5.28	50.75 ± 6.89	0.58 (t)
	LF/HF	1.24 ± 0.30	1.27 ± 0.31	0.72 (w)

Data are expressed as mean ± SEM SUAU; authentic manipulation in supine posture, SUSH; sham manipulation in supine posture, TP; total power, LF; low frequency power, HF; high frequency power, LF/HF; the ratio of low frequency to high frequency, ms^2; millisecond square, un; normalised unit, t; paired t-test, w; Wilcoxon singed-rank test

4.3.2. Effects on cardiovascular parameters

Heart rate and BP were employed as indicators of cardiovascular function in this study. Collected data after the intervention were divided into 10-second bins, and 60-second mean values of HR and BP prior to the intervention were used as the baseline.

In the supine posture, there were significant changes in MAP with the "authentic" and "sham" procedures [Wilks' $\Lambda = 0.82$, $F\ (6, 4) = 7.49$, $p = .036$, $\eta^2 = 0.92$ and Wilks' $\Lambda = 0.71$, $F\ (6, 4) = 8.73$, $p = .027$, $\eta^2 = 0.93$, respectively]. Post-hoc testing of pairwise comparisons using Bonferroni adjusted α levels indicated that the authentic manipulation decreased MAP from

its pre-stimulus value by approximately 7 mmHg at 20 and 30 seconds [t (9) = 7.49, p = 0.001, d = 2.37 and t (9) = 4.56, p = 0.029, d = 1.44], and the sham manipulation was associated with a reduction of 10 mmHg at 20 seconds [t (9) = 5.42, p = 0.009, d = 1.71] (Figure 4.2). Immediately after the maximum decrease, BP steadily increased and tended to stabilise to pre-intervention levels. This occurred within the 60 second measurement period. However, changes were not observed in BP in the sitting posture or in HR recorded in both postures (p > 0.05). In addition, cardiovascular responses to "authentic" and "sham" interventions were similar in both the sitting posture [MAP; Wilks' Λ = 0.98, F (1, 9) = 0.15, p = 0.71, η^2 = 0.016, HR; Wilks' Λ = 0.98, F (1, 9) = 0.17, p = 0.69, η^2 = 0.018] and in the supine posture [MAP; Wilks' Λ = 0.99, F (1, 9) = 0.63, p = .81, η^2 = 0.007, HR; Wilks' Λ = 0.98, F (1, 9) = 0.17, p = 0.69, η^2 = 0.019].

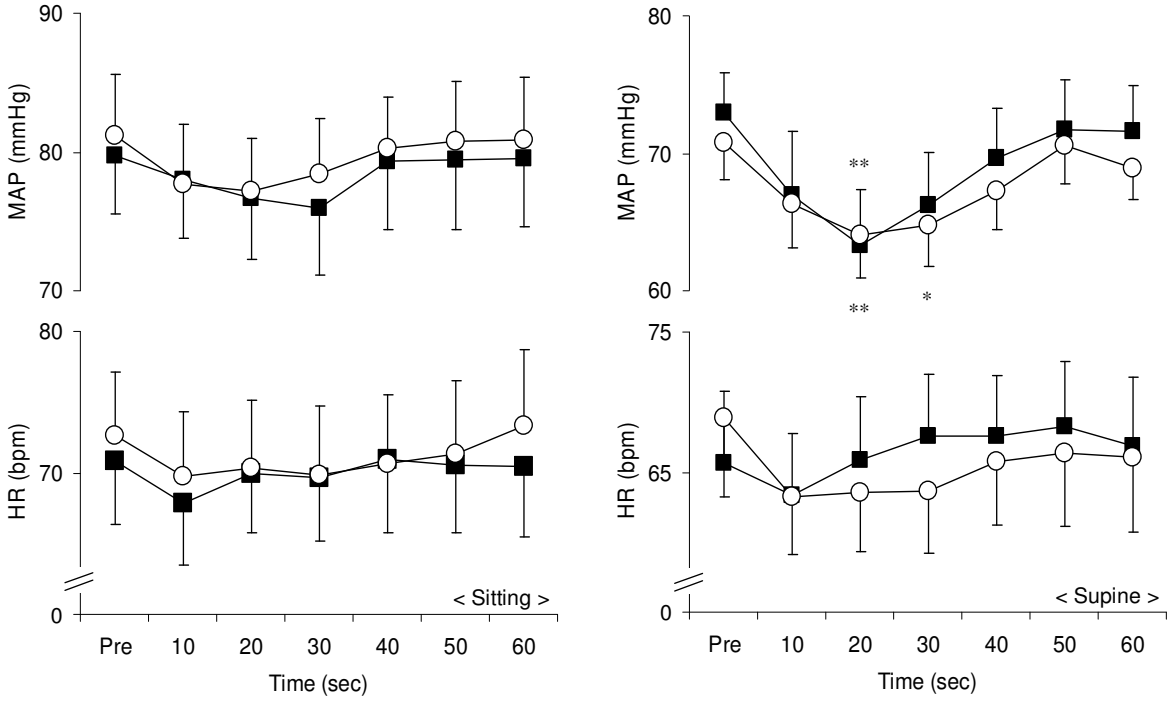

Figure 4.2. Changes in mean arterial pressure (MAP) and heart rate (HR) during sitting (left) and supine (right) after both interventions (authentic ○, sham ■).
Data are expressed as mean ± SEM Collected data after intervention were averaged into 10-second bins; these were compared with the mean value of the final 60 seconds prior to intervention using a one-way repeated measures ANOVA with post-hoc testing of pairwise comparisons using Bonferroni adjusted α levels, * p < 0.05; ** p < 0.01

Similarly, during sitting, there was a significant time effect on DBP in response to the authentic intervention ["authentic"; Wilks' $\Lambda = 0.065$, F (6, 4) = 9.67, $p = 0.023$, $\eta^2 = 0.94$], but no significant changes in DBP for the sham intervention [Wilks' $\Lambda = 0.22$, F (6, 4) = 2.31, $p = 0.22$, $\eta^2 = 0.78$] or SBP ["authentic"; Wilks' $\Lambda = 0.29$, F (6, 4) = 1.66, $p = 0.33$, $\eta^2 = 0.71$, "sham"; Wilks' $\Lambda = 0.12$, F (6, 4) = 4.75, $p = 0.077$, $\eta^2 = 0.88$]. Despite a significant time effect revealed by the multivariate test, the authentic intervention did not induce a significant change in DBP.

In the supine position, there was no significant time effect on SBP for either intervention ["authentic"; Wilks' $\Lambda = 0.28$, F (6, 4) = 1.75, $p = 0.31$, $\eta^2 = 0.72$, "sham"; Wilks' $\Lambda = 0.37$ F (6, 4) = 1.13, $p = 0.48$, $\eta^2 = 0.63$]. However, the multivariate test showed a significant time effect for DBP in response to the sham intervention [Wilks' $\Lambda = 0.055$, F (6, 4) = 11.48, $p = 0.017$, $\eta^2 = 0.95$], but not the authentic intervention [Wilks' $\Lambda = 0.10$, F (6, 4) = 5.72, $p = 0.057$, $\eta^2 = 0.90$]. Regardless of the insignificant time effect on DBP for the authentic intervention, DBP significantly decreased at 20 and 30 seconds following the authentic intervention [t (9) = 5.35, $p = 0.001$, $d = 1.69$ and t (9) = 4.65, $p = 0.025$, $d = 1.47$, respectively]. Similarly, DBP significantly decreased at 20 seconds following the sham intervention [t (9) = 5.38, $p = 0.01$, $d = 1.70$].

Table 4.3. Diastolic and systolic blood pressure responses to the mechanical stimulus applied to the neck during sitting

parameters	interventions	pre	post					
			10 sec	20 sec	30 sec	40 sec	50 sec	60 sec
DBP (mmHg)	Authentic	64 ± 5	61 ± 4	60 ± 4	62 ± 4	63 ± 4	64 ± 5	65 ± 4
	Sham	64 ± 4	62 ± 4	60 ± 4	60 ± 4	63 ± 5	63 ± 5	63 ± 5
SBP (mmHg)	Authentic	121 ± 6	122 ± 5	120 ± 5	119 ± 6	120 ± 5	121 ± 5	122 ± 6
	Sham	124 ± 6	126 ± 6	123 ± 6	119 ± 7	122 ± 7	123 ± 7	123 ± 7

Data are presented as mean ± SEM Asterisk (*) indicates a significant difference from the pre-intervention value, DBP = diastolic blood pressure, SBP = systolic blood pressure, sec = second.

Table 4.4. Diastolic and systolic blood pressure responses to the mechanical stimulus applied to the neck in the supine position

parameters	interventions	pre	post					
			10 sec	20 sec	30 sec	40 sec	50 sec	60 sec
DBP (mmHg)	Authentic	55 ± 2	51 ± 2	49 ± 2*	50 ± 2*	52 ± 2	54 ± 2	53 ± 2
	Sham	59 ± 3	54 ± 5	54 ± 4*	54 ± 4	54 ± 4	54 ± 4	54 ± 4
SBP (mmHg)	Authentic	113 ± 5	109 ± 5	105 ± 6	105 ± 5	107 ± 5	112 ± 5	110 ± 5
	Sham	113 ± 3	106 ± 4	100 ± 4	102 ± 4	107 ± 4	109 ± 3	111 ± 4

Data are presented as mean ± SEM Asterisk (*) indicates a significant difference from the pre-intervention value, DBP = diastolic blood pressure, SBP = systolic blood pressure, sec = second.

4.4. Discussion

This study examined whether a simulated cervical manipulative procedure itself influences autonomic and cardiovascular function in young healthy adults. The results showed that a single mechanical stimulus applied to the neck evoked a significant change in cardiovascular function. The mechanical stimulus was a simulated spinal manipulative procedure delivered using an AAI III. Previous studies have indicated that a mechanical stimulus delivered using an instrument of this type is capable both of evoking a compound action potential (directly recorded from a mixed nerve root in patients undergoing lumbar spine surgery) (Colloca et al. 2000; Colloca et al. 2003; Colloca et al. 2004) and reflexive motor responses (Symons et al. 2000) in humans. These studies provide evidence that a mechanical stimulus with the type of instrument in the current study is presumably capable of activating somatosensory receptors as well as motor responses. Further, the stimulus was delivered in such a way that minimised vestibular involvement. It is arguable that previous investigations which examined the effect of spinal manipulation to the neck on autonomic and cardiovascular function in humans did not exclude the potential involvement of the vestibular system in the recorded changes in autonomic drives and cardiovascular function.

The current study result provides some support for the notion that a simulated manipulative procedure to the neck may be capable of influencing cardiovascular function in humans, and is consistent with clinical reports (Yates et al. 1988; Plaugher & Bachman 1993) and previous studies (Fujimoto et al. 1999; Knutson 2001). In other words, this study suggests that neck afferents may participate in modifying cardiovascular function in the absence of vestibular inputs.

It has been shown that afferent inputs from the neck terminate in the vestibular nuclei as well as many other anatomically related regions including nucleus X in the CNS (Bolton 1998). Signals to these areas are integrated for the purpose of regulating body movement and posture in response to head/neck movements. Typical reflex responses include the "cervico-spinal reflex", which stabilises body position, the "cervico-ocular reflex", which maintains eye position, and the "cervico-collic reflex" which keeps a steady head position with respect to the trunk (Bolton 1998). Following posture changes, supplying blood to the vital organs (especially the brain) also needs to be adequately regulated. In the 1970s, it was observed that bilateral vestibular nerve section in the cat impaired orthostatic reflexes (Doba & Reis 1974). It is thought that vestibular system reflexes may act in a "feedforward" manner to reduce overabundant BP perturbations that accompany orthostasis (Bolton & Ray 2000). It has been well-documented that the vestibular system can modulate sympathetic activity in animals (Yates & Miller 1994) and humans (Essandoh et al. 1988; Cui et al. 1997a; Ray & Hume 1998; Cui et al. 2001). There is evidence to suggest a contribution from neck afferents to these vestibulo-sympathetic reflexes, however, support for this proposal is still equivocal in humans (Normand et al. 1997; Ray & Hume 1998; Tobal et al. 2002; Watenpaugh et al. 2002). In an animal study conducted by Bolton et al. (1998), neck afferent participation in sympathetic regulation during nodding head movements was examined. Autonomic responses were recorded from the splanchnic nerve in anaesthetised cats and compared pre- and post-

dorsal root resection in the upper cervical region (C_{1-3}). Only after section of C_{1-3} dorsal roots was modulation of splanchnic nerve activity evident, and this modulation was synchronised to the induced head movements. Because this modulation was not evident prior to C_{1-3} dorsal root section, it suggested that inputs from the neck interact with vestibular inputs to modulate vestibulo-sympathetic reflexes.

From the current study, it is still not possible to deny that there are some other factors which may be responsible for the change in cardiovascular and autonomic functions. This question has arisen because there were similar trends of cardiovascular response that occurred for both "authentic" and "sham" procedures. Further, only the "sham" intervention was associated with a change in balance of cardiac autonomic nervous activity towards sympathetic dominance during the sitting posture. One explanation for this result is that the "sham" procedure used in this study may still have had an impact on autonomic drives and cardiovascular function. To deliver the "sham" manipulation, the investigator gently placed his finger tip on the neck and applied the mechanical impulse with the Activator® Instrument over the dorsum of the hand. Use of the instrument is associated with an audible (and sudden) 'click' sound. Previous studies have shown that sudden noises are associated with an "arousal" reaction which increases (or decreases) HR and/or BP, releases sweat and constricts skin blood vessels (Macefield et al. 1998; Donadio et al. 2005). Beat-to-beat BP was recorded from the finger in this study, and it is important to sustain enough blood flow in the finger for an accurate measurement. However, as a result of the arousal effect, the device might have lost a pulse signal of sufficient amplitude and this may have led to a decrease in BP. Another possible explanation was that the mechanical impulse was transmitted through the dorsum of the hand and the finger tip to the neck although this was not investigated in this study. Therefore, the "sham" intervention in this study may have been contaminated and may still have had an interventional effect.

4.5. Transition to a follow-up study

One of the difficulties in conducting autonomic and cardiovascular research in awake humans is that it is not possible to completely eliminate psychological factors. The mechanical impulse employed in the previous study included not only a mechanical stimulus but also a sudden clicking sound from the mechanical impulse device (AAI III). Previous studies have examined responses of autonomic drives and cardiovascular function that are considered to be a psychological reaction to various stimuli; including sudden noise (Macefield et al. 1998), electrical stimulus (Donadio et al. 2002a; Donadio et al. 2002b) and a visual flash (Donadio et al. 2002a). The reactions to those sudden surprising stimuli were changes in sudomotor and skin vasoconstrictor activity, and an accompanying decrease in BP and muscle sympathetic nerve activity. However, no change in HR was observed. Notably, those studies employed similar BP recording methods to the study reported above (i.e., use of the Finapres™ (that provides non-invasive and continuous BP measurement from the finger), and the outcomes observed in those studies were quite similar to those reported in this study (no change in HR and a transient reduction in BP). Therefore, it was suspected that the interventions used in the earlier part of this chapter (Watanabe & Polus 2007) might induce an arousal reaction and the cardiovascular responses might have resulted from a psychological startle resulting in a sudden change in finger blood flow (FiBF).

The purpose of this part of the Chapter 4 (follow-up study) was to examine whether the cardiovascular responses observed previously were due to an arousal reaction.

4.6. Methods

4.6.1. General procedures

Generally healthy young adults aged between 18 and 35 years old were sought for this study. Prior to commencement of the first experimental session, written informed consent was

obtained from all volunteers and general health, cardiovascular risk, pre-experimental questionnaires were completed. This study was approved by the RMIT Human Research Ethics Committee (see Appendix 9 and 10 for a Plain Language Statement and Appendix 4 for a consent form).

An attempt was made to recruit those participants, who were involved in the previous study. However, it was not possible to recruit all of the same individuals. Therefore, the present study was treated as two independent experimental sessions; the first session employed the same interventions as the previous study (Watanabe & Polus 2007) and the second session used two additional interventions (see below for further descriptions). For comparison purposes, data in the previous study is presented below with newly obtained data.

For preparation, participants were asked to fast and refrain from any caffeine beverages for at least 4 hours before, and to avoid any alcoholic beverages and rigorous exercise for at least 12 hours before the session. These restrictions were applied to each experimental session. Participants lay on an examination table on their back and their head-neck position was comfortably supported with a small neck pillow. Once participants were comfortable with their position, following a 5-minute relaxation period, they were instructed to synchronise their respiratory rhythm with a metronome for ten minutes (paced at 0.25 Hz). Five minutes after the controlled breathing period started, one form of the mechanical stimulus was delivered to the neck and data collection was continued for a further 5 minutes during which time the respiratory rate was still controlled.

4.6.2. Equipment

In addition to the outcome measures recorded in the previous study (see *section 4.2.2*), FiBF and change in skin potential were recorded in this study. Finger blood flow was measured

from the right index finger using a finger clip photoplethysmograph (MLT1020FC, ADInstruments, Sydney) (bandwidth 0.1-100 Hz). Skin potential change was recorded using three disposable electrodes (Red Dot™, 3M™, MN, USA) attached over the thenar eminence, the dorsum of the hand and the ventral aspect of the right forearm (bandwidth 0.3-1k Hz). Signals from the photoplethysmograph and skin potential change were fed into the data acquisition system (PowerLab/8SP, ADInstruments, Bella Vista, NSW, Australia) after amplification.

4.6.3. Mechanical stimulus

The mechanical stimulus was delivered with the same device used in the previous study (AAI III). For the first session of this study, the same interventions were employed as the previous study (Watanabe & Polus 2007), which included the "authentic" procedure, executed by delivering a single mechanical impulse to the neck over the examiner's finger placed on the stimulus point (MIF) and the "sham" procedure, carried out by delivering the same mechanical impulse to the examiner's dorsal hand (MIH) with the finger positioned over the lateral edge of the transverse process of the first cervical vertebra.

For the second session of this study, two additional forms of mechanical impulse were employed. The first delivered the mechanical impulse directly to the transverse process of the first vertebrae (MID). The MID intervention was the same as the MIF intervention but without finger placement on the neck. The second stimulus was carried out by placing the finger only on the neck without any mechanical impulse delivered (FIN).

4.6.4. Data analysis

This study used a pretest-posttest design. To evaluate the effects of the mechanical stimuli, variables (HR, BP, and FiBF) were averaged into ten-second bins. The data for BP included

MAP, SBP, and DBP. Since BP responses observed in the previous study lasted up to 30 seconds (Watanabe & Polus 2007), pre-mechanical stimulus values (average of the fifth minutes) were compared with four 10-second data blocks of the post-stimulus period. The method used to assess skin potential change in this study was after Donadio and colleagues (2002a; 2002b). The evaluation of skin potential change has been described in *section 2.6*. Data analysis was carried out using commercialised software, Chart for Windows V5.1.1. (ADInstruments, Bella Vista, NSW, Australia).

4.6.5. Statistical analysis

Statistical analysis for this study was conducted with the statistical software package (SPSS V13 for Windows, SPSS Inc., U.S.A). The main analyses of interest were designed around a 2×2 fully within-subjects ANOVA for HRV parameters and a 2×5 fully within-subjects ANOVA for cardiovascular parameters in this study. The two within-subjects factors were intervention (the MIF vs. MIH interventions in the first session and the MID vs. FIN interventions in the second session) and time (pre- and post-intervention for HRV parameters, and pre-intervention and four subsequent ten-second bins for cardiovascular parameters). Further, in order to examine whether the changes in BP were affected by FiBF, FiBF values were added as a covariate to the separate analyses of the second session (i.e., a repeated measures analysis of covariance, ANCOVA). The linear mixed models approach to repeated measures ANCOVA was chosen over the more conventional general linear modelling approach for a few reasons. Firstly, the linear mixed models approach deals more conveniently with covariates of the analysis. This is because the covariates for the repeated measures ANCOVA are variable across the different levels of the factors (time and intervention). Secondly, there were some missing data and the missing values are not easily taken into account of by the general linear model approach without either losing cases or conducting missing value estimation. In contrast, because the linear mixed models approach

accommodates missing data, it is possible to include participants who have incomplete data in the analysis.

In addition to the overall analyses looking for main effects, interactions, and relationships with covariates, more focussed single degree of freedom analyses using post-hoc pairwise comparisons with Bonferroni correction were performed on both repeated measures ANOVA and ANCOVA in order to examine more closely the specific effects on the interest. The per-comparison significance level was set at $p < 0.05$. However, because of the exploratory nature of these comparisons, all p levels were Bonferroni-corrected automatically by SPSS software.

4.7. Results

Thirteen young adults completed the first session and 11 completed the second session. Seven of them completed both sessions. Physical characteristics of participants are summarised in Table 4.5.

Table 4.5. Physical characteristics of participants in the first and second sessions

	First session	Second session
Age (years old)	27.1 ± 4	29.5 ± 3
Genders (Male/Female)	9 / 4	6 / 5
Body Mass Index (kg/m^2)	21.99 ± 3.37	21.20 ± 3.13

Data are expressed as mean ± SD

According to the pre-experimental questionnaire, mild to moderate levels of current neck stiffness were reported by two participants in the first session (VAS = 2.5 ± 1.8) and three participants in the second session (VAS = 2.6 ± 2.1). However, according to post-experimental survey, there was no significant development of neck stiffness, neck pain or

discomfort procedures during the trial. Therefore, it could be said that all interventions used in this study were innocuous mechanical stimuli.

4.7.1. First session

4.7.1.1. Acute effects of the mechanical stimulus on cardiovascular function

Cardiovascular parameters (MAP, DBP, SBP, and HR) were followed-up for 40 seconds after the application of each form of mechanical stimulus.

For MAP, the results of the two-way repeated measures ANOVA showed that there was significant time effect [Wilks' $\Lambda = 0.026$, $F\ (4,8) = 73.79$, $p < 0.001$, $\eta^2 = 0.97$], but no intervention effect [Wilks' $\Lambda = 0.99$, $F\ (1,11) = 0.12$, $p = 0.73$, $\eta^2 = 0.011$] or time-by-intervention interaction [Wilks' $\Lambda = 0.87$, $F\ (4,8) = 0.29$, $p = 0.87$, $\eta^2 = 0.13$]. Post-hoc pairwise comparisons showed MAP significantly decreased 10 and 20 seconds after the MIF intervention [$t\ (11) = 4.27$, $p = 0.013$, $d = 1.23$ and $t\ (11) = 8.30$, $p < 0.001$, $d = 2.40$, respectively] and 20 and 30 seconds after the MIH intervention [$t\ (11) = 6.43$, $p = 0.001$, $d = 1.86$ and $t\ (11) = 4.16$, $p = 0.016$, $d = 1.20$, respectively] (Figure 4.3). Those responses were not significantly different between those interventions.

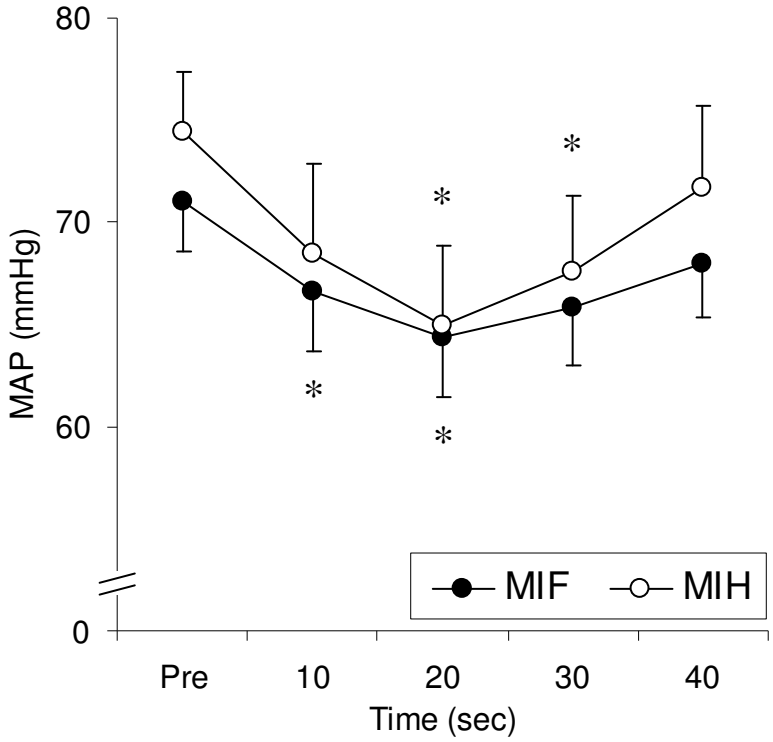

Figure 4.3. Comparison of mean arterial pressure response to each intervention.
Data were expressed as mean ± SEM MAP = mean arterial pressure, MIF = Mechanical impulse to the neck over the finger. MIH = Mechanical impulse over the dorsal hand. Asterisk (*) indicates a significant difference from pre-intervention value ($p < 0.05$).

For DBP, the results of the two-ways repeated measures ANOVA showed a significant time effect [Wilks' $\Lambda = 0.018$, F (4,8) = 107.90, $p < 0.001$, $\eta^2 = 0.98$], but no intervention effect [Wilks' $\Lambda = 0.94$, F (1,11) = 0.70, $p = 0.42$, $\eta^2 = 0.06$] or time-by-intervention interaction [Wilks' $\Lambda = 0.84$, F (4,8) = 0.40, $p = 0.81$, $\eta^2 = 0.17$]. Post-hoc pairwise comparisons showed DBP significantly decreased 10 and 20 seconds after the MIF intervention [t (11) = 4.27, p = 0.013, d = 1.23 and t (11) = 8.89, $p < 0.001$, d = 2.56, respectively] and 20 and 30 seconds after the MIH intervention [t (11) = 4.43, p = 0.001, d = 1.28 and t (11) = 3.86, p = 0.027, d = 1.11, respectively]. However, there was no difference in DBP between the MIF and MIH interventions.

For SBP, the results of the two-ways repeated measures ANOVA showed a significant time effect [Wilks' $\Lambda = 0.14$, F (4,8) = 12.86, p = 0.001, $\eta^2 = 0.87$], but no intervention effect

[Wilks' $\Lambda = 0.99$, $F\ (1,11) = 0.081$, $p = 0.78$, $\eta^2 = 0.007$] or time-by-intervention interaction [Wilks' $\Lambda = 0.82$, $F\ (4,8) = 0.44$, $p = 0.78$, $\eta^2 = 0.18$]. Post-hoc pairwise comparisons showed SBP significantly decreased not only 20 seconds after the MIF intervention [$t\ (11) = 4.34$, $p = 0.012$, $d = 1.25$], but also 20 and 30 seconds after the MIH intervention [$t\ (11) = 3.97$, $p = 0.022$, $d = 1.15$ and $t\ (11) = 3.78$, $p = 0.030$, $d = 1.09$, respectively]. However, there was no difference in SBP between the MIF and MIH interventions.

For HR, the results of the two-way repeated measures ANOVA showed that there were significant time effect [Wilks' $\Lambda = 0.33$, $F\ (4,8) = 4.03$, $p = 0.045$, $\eta^2 = 0.67$] and a time-by-intervention interaction [Wilks' $\Lambda = 0.34$, $F\ (4,8) = 3.89$, $p = 0.048$, $\eta^2 = 0.66$], but no intervention effect [Wilks' $\Lambda = 0.97$, $F\ (1,11) = 0.36$, $p = 0.56$, $\eta^2 = 0.032$]. However, post-hoc pairwise comparisons showed there were no significant changes in HR following either intervention or any difference between the interventions.

Table 4.6. Cardiovascular parameters before and after each intervention

Parameters	Interventions	Pre	Post			
			10 sec	20 sec	30 sec	40 sec
MAP (mmHg)	MIF	71 ± 2	67 ± 3*	64 ± 3*	66 ± 3	68 ± 3
	MIH	74 ± 3	68 ± 4	65 ± 4*	68 ± 4*	72 ± 4
DBP (mmHg)	MIF	54 ± 2	51 ± 2*	49 ± 2*	50 ± 2	52 ± 2
	MIH	59 ± 3	54 ± 4	51 ± 4*	55 ± 3*	57 ± 4
SBP (mmHg)	MIF	114 ± 4	110 ± 4	106 ± 5*	107 ± 5	108 ± 5
	MIH	116 ± 4	108 ± 4	102 ± 4*	104 ± 4*	110 ± 4
HR (bpm)	MIF	68 ± 5	63 ± 4	63 ± 4	63 ± 4	65 ± 4
	MIH	66 ± 4	64 ± 4	66 ± 4	67 ± 4	67 ± 4

Data were expressed as mean ± SEM DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure, MIF = Mechanical impulse to the neck over the finger, MIH = Mechanical impulse on the dorsum hand, sec = seconds, Asterisk (*) indicates a significant difference from pre-intervention value ($p < 0.05$).

4.7.1.2. The effects of the mechanical stimulus on heart rate variability parameters

There were no significant changes in HRV parameters or differences between the interventions according to the results of the two-way repeated measures ANOVA nor were there any significant post-hoc pairwise comparisons.

Table 4.7. The effects of the mechanical stimulus on heart rate variability parameters in the first session.

Parameter	Interventions	Pre	Post	*p*
TP (ms^2)	MIF	4093.19±11.5614	5236.01±1447.64	0.083
	MIH	4933.35±1407.41	4780.61±1469.50	0.40
LF (ms^2)	MIF	637.87 ± 128.63	662.99 ± 123.78	0.81
	MIH	788.00 ± 164.58	962.66 ± 283.30	0.45
LF (nu)	MIF	36.87 ± 5.49	39.85 ± 5.32	0.46
	MIH	42.15 ± 5.33	41.40 ± 6.58	0.84
HF (ms^2)	MIF	1689.04 ± 687.96	1943.50 ± 969.89	0.50
	MIH	1572.53 ± 602.32	1672.07 ± 690.64	0.41
HF (nu)	MIF	57.38 ± 5.23	54.72 ± 4.83	0.53
	MIH	52.18 ± 5.37	54.21 ± 6.39	0.63
LF/HF	MIF	0.84 ± 0.19	0.94 ± 0.21	0.52
	MIH	1.11 ± 0.27	1.13 ± 0.28	0.89

Data were expressed as mean ± SEM TP = total power, LF = low frequency power, HF = high frequency power, LF/HF = the ratio of low frequency to high frequency, ms^2 = millisecond square, un = normalised unit, MIF = Mechanical impulse to the neck over the finger, MIH = Mechanical impulse delivered over the dorsal hand.

4.7.2. Second session

4.7.2.1. Acute effects of the mechanical stimulus on cardiovascular function

For MAP, the results of the two-way repeated measures ANOVA showed that there were significant time [F (4,90) = 4.19, p = 0.004] and intervention [F (1,90) = 4.46, p = 0.037] main effects. Post-hoc pairwise comparisons showed that MAP significantly decreased at 20 seconds after the MID intervention [t (10) = 3.56, p = 0.006, d = 1.07], but there were no significant changes after the FIN intervention. There was a significant difference between the MID and FIN interventions at baseline [t (10) = 2.02, p = 0.046, d = 0.61], but not after the interventions.

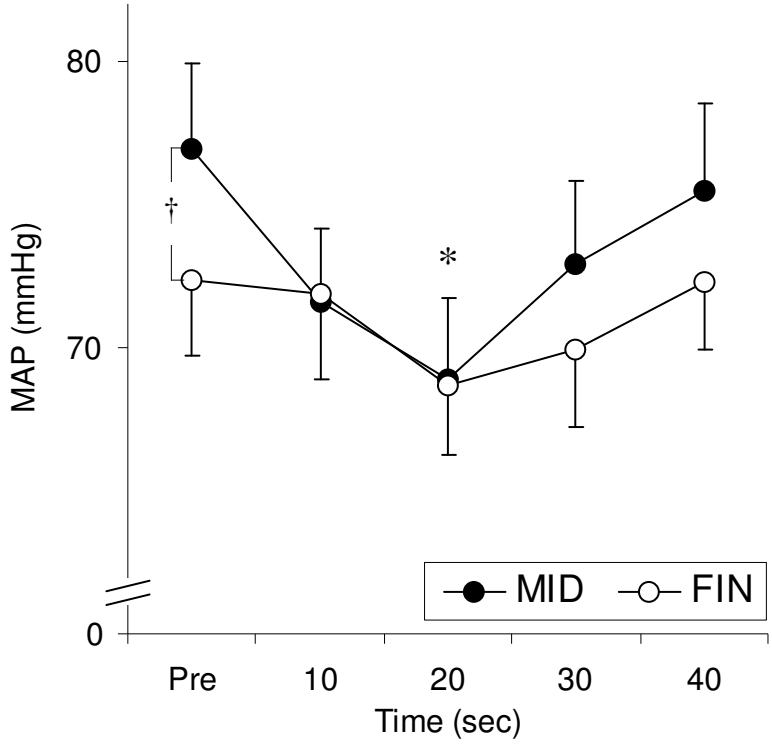

Figure 4.4. Comparison of mean arterial pressure response to each intervention.
Data were expressed as mean ± SEM MAP = mean arterial pressure, MID = Mechanical impulse directly to the neck, FIN = Finger contact on the neck only, sec = seconds, Asterisk (*) indicates a significant difference from pre-intervention value ($p < 0.05$), Cross (†) indicates a significant difference in MAP between the MID and FIN interventions ($p < 0.05$).

Due to a significant difference in MAP at baseline, it might not be meaningful to compare MAP responses to each intervention (the MID and FIN), so the data regarding the MAP responses were further explored. In order to examine the magnitude of differences in MAP change between the two interventions over the time, effect size (d) and its 95% confidence interval were obtained at each time point, based on data of post-hoc pairwise comparisons. The 95% confidence intervals of the effect size shown in Figure 4.5 indicated that there was no notable change in MAP difference over the recording period. However, there was a trend that the effect size decreased at 10 and 20 seconds and returned towards pre-intervention level from 30 seconds after the application of the intervention. In other words, the magnitude of the difference in MAP between the two interventions decreased at 10 and 20 seconds after the stimulation and then rebounded towards the pre-intervention level. Therefore, this trend of

MAP difference change might suggest that the two interventions (the MID and FIN) induced different MAP responses, particularly at 10 and 20 seconds after the stimulation.

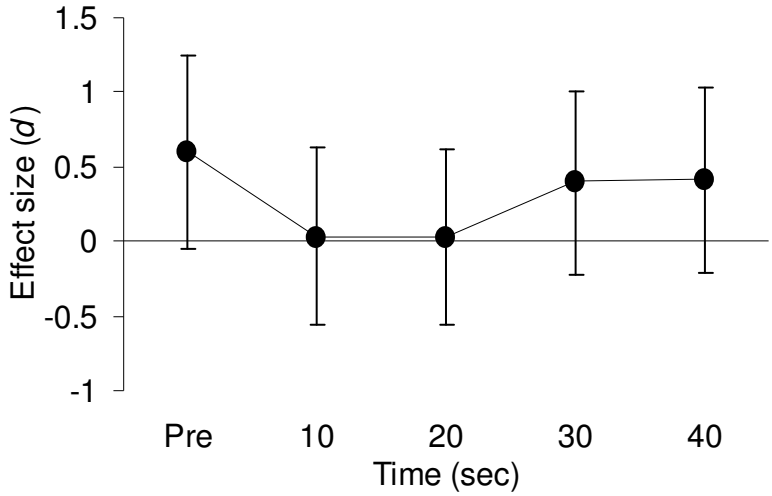

Figure 4.5. Changes in effect size on mean arterial pressure over a recording period.
Data are expressed as mean ± 95% confidence interval. *d* = effect size, MAP = mean arterial pressure, sec = second.

For DBP, the results of the two-way repeated measures ANOVA showed that there was a significant time main effect [F (4,90) = 3.86, p = 0.006], but no intervention main effect [F (1,90) = 3.80, p = 0.054] or time-by-intervention interaction [F (4,90) = 0.49, p = 0.74]. Post-hoc pairwise comparisons showed that DBP significantly decreased at 20 seconds after the MID intervention [t (11) = 3.17, p = 0.021, d = 0.95], but there were no changes after the FIN intervention. There was no significant difference in DBP between the MID and FIN interventions.

For SBP, the results of the two-way repeated measures ANOVA showed that there were significant time [F (4,90) = 3.39, p = 0.013] and intervention [F (1,90) = 7.32, p = 0.008] main effects but no time-by-intervention interaction [F (4,90) = 1.17, p = 0.33]. Post-hoc pairwise comparisons showed that SBP significantly decreased at 20 seconds after the MID intervention [t (11) = 3.45, p = 0.009, d = 1.04], but there were no changes after the FIN intervention. There was a significant difference in SBP between the MID and FIN interventions during the pre-intervention period [t (11) = 2.27, p = 0.026, d = 0.68].

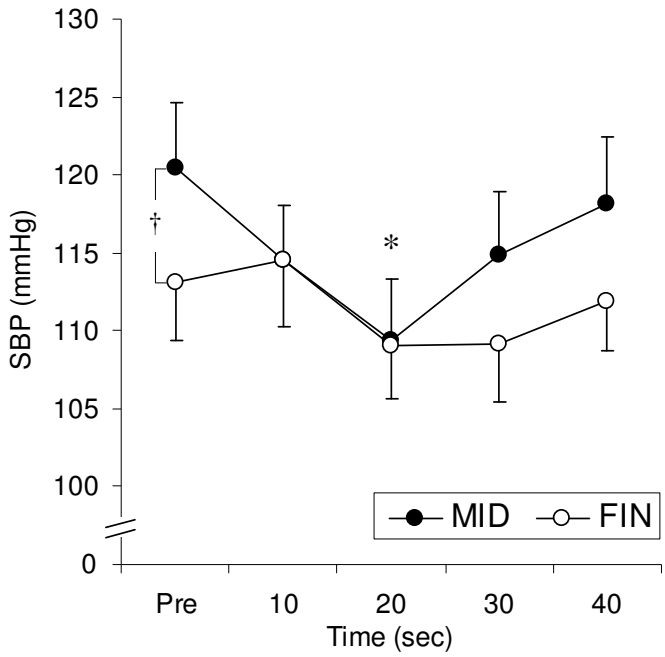

Figure 4.6. Comparison of mean arterial pressure response to each intervention.
Data were expressed as mean ± SEM SBP = systolic blood pressure, MID = Mechanical impulse directly to the neck, FIN = Finger contact on the neck only, sec = seconds, Asterisk (*) indicates a significant difference from pre-intervention value ($p < 0.05$), Cross (†) indicates a significant difference in SBP between the MID and FIN interventions ($p < 0.05$).

Due to a significant difference in SBP at baseline, it might not be meaningful to compare SBP responses to each intervention (the MID and FIN), so the data regarding the SBP responses were further explored. In order to examine the magnitude of differences in SBP change between the two interventions over time, effect size (*d*) and its 95% confidence interval were obtained at each time point, based on data from the post-hoc pairwise comparisons. The 95% confidence intervals of the effect size shown in Figure 4.7 indicated that there was no notable change in SBP difference over the recording period. However, there was a trend showing that the effect size decreased at 10 and 20 seconds and returned towards pre-intervention level from 30 seconds after the application of the interventions. In other words, the magnitude of the difference in SBP between the two interventions decreased at 10 and 20 seconds after the stimulation and then rebounded towards the pre-intervention level. Therefore, this trend of SBP difference change might suggest that the two interventions (the MID and FIN) induced different SBP responses, particularly at 10 and 20 seconds after the stimulation.

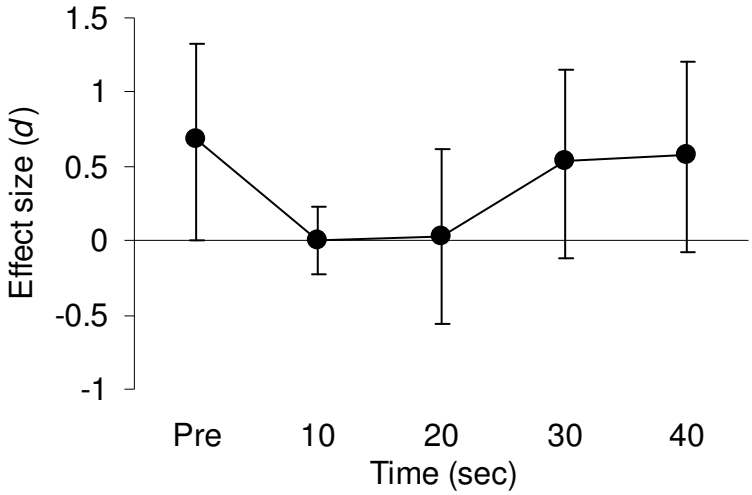

Figure 4.7. Changes in effect size on mean arterial pressure over a recording period.
Data are expressed as mean ± 95% confidence interval. *d* = effect size, SBP = systolic blood pressure, sec = second.

For HR, the results of the two-way repeated measures ANOVA showed that there were no significant time and intervention effects.

Table 4.8. Blood pressure and heart rate responses to each intervention

Parameters	Interventions	Pre	Post			
			10 sec	20 sec	30 sec	40 sec
MAP (mmHg)	MID	77 ± 3†	72 ± 3	69 ± 3*	73 ± 3	75 ± 3
	FIN	72 ± 3	72 ± 3	69 ± 3	70 ± 3	72 ± 3
DBP (mmHg)	MID	56 ± 2	51 ± 2	49 ± 2*	53 ± 2	55 ± 2
	FIN	52 ± 2	51 ± 2	49 ± 2	51 ± 2	53 ± 2
SBP (mmHg)	MID	120 ± 4†	114 ± 4	109 ± 4*	115 ± 4	118 ± 4
	FIN	113 ± 4	115 ± 4	109 ± 4	109 ± 4	112 ± 4
HR (bpm)	MID	60 ± 3	58 ± 3	59 ± 3	59 ± 3	60 ± 3
	FIN	59 ± 3	59 ± 3	58 ± 3	58 ± 3	59 ± 3

Data were expressed as mean ± SEM DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure, MID = Mechanical impulse directly to the neck, FIN = Finger contact on the neck only, sec = seconds, Asterisk (*) indicates a significant difference from pre-intervention value ($p < 0.05$), Cross (†) indicates a significant difference between the MID and FIN interventions ($p < 0.05$).

4.7.2.2. The influence of finger blood flow on blood pressure measure

In addition to BP responses to each intervention *per se* (analysed using repeated measures ANOVA), BP changes following each intervention were examined while the influence of FiBF was taken into account (ANCOVA). This analysis was aimed to elucidate whether the BP change observed was associated with a change in FiBF itself.

For MAP, the results of the two-way repeated measures ANCOVA showed that there was a significant time effect [F (4,71.46) = 3.17, p = 0.019], but there was no overall effect of FiBF [F (1,71.29) = 0.61, p = 0.44]. Post-hoc pairwise comparisons showed that MAP significantly decreased at 20 seconds after the MID intervention [t (11) = 3.04, p = 0.033, d = 0.92], but there were no significant changes after the FIN intervention. In contrast, there was no significant difference between the MID and FIN interventions.

For DBP, the results of the two-way repeated measures ANCOVA showed that there was a significant time effect [F (4,71.23) = 3.15, p = 0.019], but no influence of FiBF on the whole same comment as above [F (1,71.57) = 0.38, p = 0.54]. Post-hoc pairwise comparisons showed that there were no significant changes in DBP following both MID and FIN interventions or difference between these interventions.

For SBP, the results of the two-way repeated measures ANCOVA showed that there was a significant time effect [F (4,70.77) = 2.65, p = 0.040] and overall influence of FiBF [F (1,78.55) = 8.04, p = 0.006]. Post-hoc pairwise comparisons showed that MAP significantly decreased at 20 seconds after the MID intervention [t (11) = 3.17, p = 0.022, d = 0.96], but there were no significant changes after the FIN intervention. Further, there was no significant difference between the MID and FIN interventions.

Table 4.9. Blood pressure responses to each intervention when the influence of finger blood flow is taken into account.

Parameters	Interventions	Pre	Post			
			10 sec	20 sec	30 sec	40 sec
MAP (mmHg)	MID	78±2.8	72±2.9	70±2.9*	74±2.9	77±2.9
	FIN	75±2.9	75±2.9	72±2.9	73±2.9	75±2.9
DBP (mmHg)	MID	57±2.6	52±2.7	51±2.7	54±2.6	56±2.6
	FIN	54±2.6	53±2.6	51±2.6	53±2.7	55±2.7
SBP (mmHg)	MID	121±4.7	113±4.7	110±4.7*	115±4.7	117±4.7
	FIN	117±4.7	119±4.7	113±4.7	115±4.7	117±4.7

Data were expressed as mean ± SEM DBP = diastolic blood pressure, MAP = mean arterial pressure, SBP = systolic blood pressure, MID = Mechanical impulse directly to the neck, FIN = Finger contact on the neck only, sec = seconds, Asterisk (*) indicates a significant difference from pre-intervention value ($p < 0.05$).

4.7.2.3. Changes in skin potential

A significant change in skin potential was seen in 7 out of 9 volunteers after the MID intervention and 6 out of 9 volunteers after the FIN intervention.

4.7.2.4. Effects of the mechanical stimulus on heart rate variability parameters

The results of the two-way repeated measures ANOVA showed that there was a significant time effect on absolute value of LF component [Wilks' $\Lambda = 0.53$, $F\ (1,10) = 9.04$, $p = 0.013$, $\eta^2 = 0.48$], but no intervention effect [Wilks' $\Lambda = 0.98$, $F\ (1,10) = 0.20$, $p = 0.67$, $\eta^2 = 0.019$] or time-by-intervention interaction [Wilks' $\Lambda = 0.94$, $F\ (1,10) = 0.59$, $p = 0.46$, $\eta^2 = 0.056$]. Pairwise comparisons demonstrated that the absolute value of LF component significantly increased following the MID intervention but not the FIN.

The results of the two-way repeated measures ANOVA showed that there was a significant time effect on normalised LF component [Wilks' $\Lambda = 0.42$, $F\ (1,10) = 13.85$, $p = 0.004$, $\eta^2 = 0.58$], but no intervention effect [Wilks' $\Lambda = 0.95$, $F\ (1,10) = 0.49$, $p = 0.50$, $\eta^2 = 0.047$] or

time-by-intervention interaction [Wilks' $\Lambda = 0.99$, $F\ (1,10) = 0.15$, $p = 0.71$, $\eta^2 = 0.014$]. Pairwise comparisons demonstrated that the normalised LF component of HRV significantly increased following the MID intervention but not the FIN.

The results of the two-way repeated measures ANOVA showed that there was a significant time effect on normalised HF component [Wilks' $\Lambda = 0.46$, $F\ (1,10) = 11.54$, $p = 0.007$, $\eta^2 = 0.54$], but no intervention effect [Wilks' $\Lambda = 0.96$, $F\ (1,10) = 0.38$, $p = 0.55$, $\eta^2 = 0.036$] or time-by-intervention interaction [Wilks' $\Lambda = 1.00$, $F\ (1,10) = 0.035$, $p = 0.86$, $\eta^2 = 0.003$]. Pairwise comparisons demonstrated that the normalised HF component of HRV significantly decreased following the MID intervention but not the FIN.

The results of the two-way repeated measures ANOVA showed that there was a significant time effect on LF/HF [Wilks' $\Lambda = 0.53$, $F\ (1,10) = 8.96$, $p = 0.014$, $\eta^2 = 0.47$], but no intervention effect [Wilks' $\Lambda = 1.00$, $F\ (1,10) = 0.12$, $p = 0.74$, $\eta^2 = 0.012$] or time-by-intervention interaction [Wilks' $\Lambda = 1.00$, $F\ (1,10) = 0.005$, $p = 0.94$, $\eta^2 = 0.001$]. Pairwise comparisons demonstrated that LF/HF significantly increased following the MID intervention but not the FIN. There were no significant differences in any HRV parameters between these interventions.

Table 4.10. The effects of the different mechanical stimuli on heart rate variability parameters in the second session.

Parameter	Intervention	Pre	Post	*p*
TP (ms^2)	MID	3208.88±715.31	4079.19±759.90	0.081
	FIN	3361.30±496.50	3468.16±561.93	0.84
LF (ms^2)	MID	555.93±166.78	920.46±285.96	0.039*
	FIN	699.64±133.45	927.11±196.75	0.062
LF (nu)	MID	35.81±6.22	45.28±6.89	0.005*
	FIN	41.19±4.86	48.15±5.58	0.19
HF (ms^2)	MID	902.69±162.11	1024.02±212.92	0.29
	FIN	1053.68±216.88	936.87±143.89	0.42
HF (nu)	MID	61.57±6.32	53.08±6.76	0.008*
	FIN	57.28±4.79	50.01±5.64	0.18
LF/HF	MID	0.81±0.24	1.25±0.35	0.006*
	FIN	0.87±0.20	1.33±0.37	0.11

Data were expressed as mean ± SEM TP = total power, LF = low frequency component, HF = high frequency component, LF/HF = the ratio of low frequency to high frequency, ms^2 = millisecond square, un = normalised unit, MID = Mechanical impulse directly to the neck, FIN = Finger contact on the neck only, Asterisk (*) indicates a significant difference between pre- and post-intervention ($p < 0.05$).

4.8. Overall discussion

This part of Chapter 4 examined whether cardiovascular responses to a mechanical impulse delivered to the neck in the earlier part of this chapter resulted from a psychological arousal reaction to the stimulus. To this end, additional measurements (fBF and changes in skin potential) were employed. Additionally, two further forms of mechanical stimulus were included in the second session of the follow-up study.

In the first session of the follow-up study, data obtained from two newly recruited participants were added. Similar cardiovascular responses to both MIF and MIH interventions were

observed as the earlier of this chapter (i.e., Watanabe & Polus 2007); that is a significant reduction in MAP at 20 seconds (although the duration of the effects were slightly different) and no change in HR or any HRV parameters. In addition, in the second session of the follow-up study, the results of the acute effects of the mechanical impulse (the MID intervention) on cardiovascular function (analysed using repeated measure ANOVA) in this part of Chapter 4 also showed quite similar outcomes to those observed in the initial study presented earlier in this chapter (i.e., Watanabe & Polus 2007) whereas the FIN intervention did not induce any significant changes in HRV or cardiovascular parameters.

The BP responses observed in both sessions (the results of "ANOVA") were consistent with a previous study that delivered a sudden electrical stimulus to induce arousal (Donadio et al. 2002b). Their study showed that mean BP was reduced by approximately 2-3 % of pre-stimulus values while R-R intervals did not change significantly. Despite the greater amplitude of BP changes in the study of this chapter, the trends of the BP responses to any type of mechanical stimulus to the neck were similar to Donadio et al's study (2002b) except the FIN intervention (finger contact on the neck only). Thus, it was suspected that BP responses observed in this chapter might be due to an arousal effect. This is particularly because BP was recorded from the finger using the Portapres® device and a sudden change in FiBF (due to vasoconstriction), which may occur as a consequence of an arousal response, was presumed to influence the BP recording. Therefore, in order to address this issue, FiBF values were included in statistical analyses (i.e., ANCOVA).

The results of repeated measures ANCOVA showed that cardiovascular responses to the MID intervention (direct mechanical impulse to the neck) were not significantly influenced by the inclusion of the skin vasoconstrictor influence in the statistical analysis except for DBP. Thus, the results indicate that cardiovascular responses to the MID intervention were not dependent

on FiBF change (arousal response) but presumably due to mechanical stimulus. In contrast to the genuine responses, a change in skin potential was observed in response to both the MID and the FIN interventions in many participants in this study (78 % and 67%, respectively). This observation may also indicate that the MID intervention induced an arousal response as it did for the FIN intervention. Further evidence that the MID intervention induced genuine responses is given by the results of the statistical analyses of HRV parameters. In response to the MID intervention, HRV parameters indicated a shift of cardiac sympathovagal balance towards sympathetic dominance (see Table 4.10), while the other interventions did not induce any changes in HRV parameters (see also Table 4.7). Furthermore, the changes in HRV parameters observed in the current study were consistent with previous studies which examined the effect of spinal manipulation (Budgell & Hirano 2001; Budgell & Polus 2006). Thus, the observed changes in BP and HRV parameters may have been induced by the mechanical stimulus (the MID intervention). However, it is not possible to justify from the data of the present study that the observed HRV parameter changes were the primary or secondary effect of the MID intervention. This is because the HRV analysis used in the present study required a set of 5-minute ECG data and indicates accumulative chages in autonomic drives to the heart. Hence, the 5-minute HRV data may not reflect transient or short-term changes in cardiac autonomic drives. Since there were significant reductions in BP in response to the MID intervention, it may be possible to initiate the baroreflex to return the decreased BP as a feedback mechanism of cardiovascular function and in turn, sympathetic drive to the heart was increased. Therefore, further investigation is required to clarify a shift in cardiac sympathovagal balance occurred due to the mechanical stimulus (primary) or the consequence of baroreflex (secondary). In the present study, therefore, the results suggest that the BP responses constitute genuine effects of the mechanical stimulus, however neither an arousal effect can be excluded nor the effects of the mechanical stimulus on autonomic drive to the heart are conclusive.

What is significant is that this study suggests that an innocuous mechanical stimulus applied to the neck is capable of influencing cardiovascular function. An arousal effect may also be superimposed on this genuine response. Since there was no report on the post-experimental questionnaire that any of the mechanical stimuli were painful or caused any symptoms, it is reasonable to conclude that the mechanical stimuli employed in the present study were innocuous. It may then be asked whether an innocuous mechanical stimulus *per se* can evoke cardiovascular responses. Sato et al. (1997a) extensively reviewed autonomic or visceral responses to somatic stimulus (somato-autonomic/-visceral reflexes). It has been generally found that noxious stimuli such as pinching the skin or the injection of inflammatory substances into various somatic tissues (including paraspinal tissues) induces powerful and consistent cardiovascular responses (e.g. increases in HR and BP) while innocuous stimuli such as brushing and mobilising joints within their physiological range evoke inconsistent or small responses (for review, see Sato et al. 1997a).

Of note is that many studies related to somato-autonomic/-visceral reflexes appeared to employ noxious stimuli and to examine the extremities rather than the axial structures. In contrast, there have been few studies examined the effects of an innocuous stimulus applied to spinal and paraspinal structures. Sato and Swenson (1984) examined the effect of a mechanical stimulus to the spinal column on autonomic drives and cardiovascular function in anaesthetised rats. In their study, a mechanical load (0.5-3.0 kg) was applied laterally to the spinal column of the thoracic or lumbar region after the muscles of the regions were transected. In response to the mechanical stimulus ($\geq$ 2.0 kg), there were significant reductions in BP and renal sympathetic nerve activity and an increase in adrenal nerve activity following a transient decrease. Whereas significant autonomic drive and cardiovascular responses to a mechanical load applied to the spinal column were observed in their study, the type of the stimulus remained to be characterised, innocuous or noxious (see discussion in

Sato & Swenson 1984). Later, Lund et al. (1999) investigated its effect on cardiovascular function in conscious rats using a different type of mechanical stimulus (that is a massage-like stroking stimulus). The stroking stimulus, which was thought likely to stimulate various types of mechanoreceptors including Pacinian corpuscles, Merkel disc endings, and free nerve endings was delivered to the abdomen or back (Lund et al. 1999). The experiments were carried out after habituating the rats to the study protocol over several days. The control procedure (only holding rats for 5 minutes) increased HR and BP compared with pre-holding value. When five minutes of the stroking stimulus was applied to the abdomen or back of rats held in the same way as the controls, suppression effects on increases in HR and BP were found. Thus, Lund et al. (1999) suggest that a mechanical stimulus (i.e., stroking) is capable of evoking significant cardiovascular responses in conscious animals as well.

Bolton and colleagues investigated the influence of neck sensory stimulus on autonomic nervous function. Bolton et al. (1998) examined the responses of the splanchnic (sympathetic) and respiratory nerves to neck sensory stimulus by means of electrical stimulus, which was assumed to likely activate muscle spindles and tendon organs, and head movement in the sagittal plane in anaesthetised cats. In response to the neck electrical stimulus, a spinal nerve (innervating abdominal muscles) and the hypoglossal and splanchnic nerves were all influenced. In addition to electrical stimulus, it was demonstrated that these nerve responses to the head motion were significantly influenced by the transection of the $C_{1\text{-}3}$ dorsal roots. Therefore, their study indicates not only that neck afferents including the muscles independently influenced splanchnic (sympathetic) and respiratory nerve activity, but also that the role of the neck sensory inputs was either synergistic or antagonistic for efferent outflows in these nerves with the vestibular inputs (Bolton et al. 1998). More recently, the effect of neck structure stimulus, which was formed as the rotational displacement of the cervical spine but not whole head-neck movement, was investigated on adrenal efferent activity (Bolton et

al. 2006). Various degrees of cervical rotation (ranged from 2° to 30°) were tested, however, unlike the study on the splanchnic nerve (Bolton et al. 1998), the influence of neck afferent stimulus on adrenal efferent activity was inconsistent, suggesting that the neck afferents may not influence adrenal nerve activity, but some other branch of the splanchnic nerve (Bolton et al. 2006).

In conclusion, this study results may indicate that mechanical stimulus to the neck is capable of initiating a response that impacts on autonomic regulation of cardiovascular function in awake humans. However, the arousal effect also influences the autonomic regulation of cardiovascular function in a similar manner. Hence, it seems that the genuine effect of mechanical stimulus delivered to the neck superimposes on the arousal effect. However, the origin of the cardiovascular responses seen in this chapter is assumed non-nociceptors but not specified. Therefore, the following chapters will focus on whether a specific group of sensory receptors in the neck (i.e., muscle proprioceptors) influence autonomic regulation of cardiovascular function in awake humans.

Chapter 5

The contribution of neck proprioceptive inputs to autonomic regulation of cardiovascular function in the sitting posture (muscle conditioning & vibration study)

5.1. Introduction

After cervical structure damage such as whiplash injury, patients may complain of dizziness, loss of balance and disturbances in walking (Madeleine et al. 2004). These phenomena indicate that sensory afferent inputs from neck structures contribute to posture control and inadequate posture control may occur once neck sensory afferent inputs are disturbed by neck injuries such as whiplash injury. It has also been reported that head repositioning ability in whiplash injury patients is impaired compared with non-injured people (Treleaven et al. 2003; Madeleine et al. 2004). Likewise, experimental studies have demonstrated that standing balance of healthy volunteers may deteriorate after induced neck muscle fatigue (Schieppati et al. 2003; Gosselin et al. 2004; Vuillerme et al. 2005). It has been suggested that muscle fatigue influences muscle spindles directly and indirectly via a reflex mediated through small diameter fibres (Bjorklund et al. 2000). Furthermore, artificial increases in proprioceptive inputs from the neck induced by a vibratory stimulus results in a postural illusion, depending on where a stimulus is applied (see Kasai et al. 2002) and altered motion patterns during stepping (Bove et al. 2002; Kasai et al. 2002). Therefore, clinical reports and experimental studies clearly indicate the importance of neck proprioceptive afferent inputs in body schema perception and motor control.

Another component of motor control is the regulation of visceral functions. For example, it is essential that appropriate cardiovascular adjustments are made with a change in posture. When moving from lying to standing, body fluid movement occurs and approximately 300 to 800 ml of blood pools in the legs (Berne & Levy 2001, p. 220). Involvement of the vestibular system in autonomic regulation of cardiovascular function during posture change is well-known (Balaban & Yates 2004). On the other hand, the involvement of neck proprioceptors is less clear in cardiovascular regulation. According to Bolton et al. (1998), stimulation of large diameter neck afferents can influence sympathetic outflow in anaesthetised animals. A few

human studies have also examined the influence of neck afferents on cardiovascular regulation, but results from these studies are conflicting (Normand et al. 1997; Ray & Hume 1998; Lee et al. 2001; Watenpaugh et al. 2002).

Therefore, the purpose of the studies in Chapter 5 is to investigate the contribution of neck proprioceptive afferent inputs to autonomic regulation of cardiovascular function in humans. The techniques used in this chapter were muscle conditioning and a vibratory stimulus, which preferentially targets the muscle proprioceptors. Chapter 5 consists of two sections – 1) muscle conditioning and vibration section and 2) vibration follow up section.

5.2. Methods

5.2.1. General procedures

This section of this chapter consists of two studies – 1) muscle conditioning study and 2) vibration study. For both studies, healthy young adults aged between 18 and 35 years old were sought. Prior to commencement of the first experimental session, written informed consent was obtained from all of volunteers and general health, cardiovascular risk, and pre-experimental questionnaires were completed. This study was approved by the RMIT Human Research Ethics Committee (see Appendix 11 and 16 for Plain Language Statements and Appendix 13 and 17 for consent forms).

5.2.2. Equipment

In both studies, as described earlier, a three-lead electrocardiograpm (ECG) (in *section 3.2.2*), beat-to-beat blood pressure (BP) using the Portapres® device (in *section 3.2.3*), finger blood flow (FiBF) using a photoplethysmograph, and changes in skin potential (in *section 4.6.2*) were recorded.

In addition to these measurements, in both studies, a strain-gauge plethysmograph (SGP) was used to indirectly measure muscle blood flow in the forearm. For SGP recording, a mercury-in-silastic tube was fastened around the largest circumference of the left forearm and a cuff was wrapped around the left upper arm and left wrist.

An upper arm cuff was positioned around the upper arm. This cuff was periodically inflated to 50 mmHg during the experiment in order to stop venous return from the forearm to the heart without interfering with arterial inflow to the muscles. Thus, the forearm progressively swells during upper arm cuff inflation and the rate of change in forearm volume is used as a measure of arterial outflow to the muscles. In order to enhance venous return during deflation of the upper arm cuff, the forearm was elevated by resting it on a high-density foam block and the point of the forearm where the strain-gauge was attached was positioned above heart level. Additionally, the left hand was placed slightly (approximately 10 cm) higher than the strain gauge level. A 10-second inflation and 5-second deflation cycle is commonly used in the literature (Benjamin et al. 1995; Wilkinson & Webb 2001). However, in preliminary experiments using this inflation-deflation cycle period, it was found that venous drainage after cuff deflation was sometimes incomplete and that the volume of the forearm did not return to baseline before the next cycle of inflation started. Consequently, the measurement may become inaccurate. Thus, 10-second inflation and deflation cycle was chosen for this study. This cycle provided an adequate amount of plethysmogram data as well as sufficient time for emptying vessels during upper arm cuff deflation.

In addition to the upper arm cuff, a wrist cuff was continuously inflated at 180 to 200 mmHg during data collection in order to exclude the influence of hand circulation. This is important because of the physiological difference in circulatory regulation between the forearm muscles and the hand (Wilkinson & Webb 2001).

5.2.3. Muscle conditioning

The technique used to alter the quantity of muscle proprioceptive afferent inputs from the neck in this study was muscle conditioning. Participants sat comfortably in a custom-built chair with rotating seat pan. The chair had a straight back support, and a harness was fitted across the shoulders of the participant to prevent movement of the torso during the experiment. A head-frame was also positioned over the head to discourage any head movement. The muscle conditioning manoeuvre was applied to the right rotator muscles of the neck including the left sternocleidomastoid muscle, left scalene muscles and the right dorsal neck muscles (Cramer & Darby 2005). When the head and neck are rotated left (as if looking behind over the left shoulder), the right neck rotator muscles are stretched. Thus, when the seat pan of the chair was rotated towards the right while the head position remained still, passive left rotation of the neck was induced and the length of the left rotator muscles is increased as shown in Figure 6.1. Therefore, the right neck rotator muscles were assumed to be more stretched at 30° than at 10°.

In this study, muscle conditioning was carried out in the neck left rotated position at 10° and 30° and data collection was made at the 20° neck rotation position. In order to carry out the muscle conditioning protocol, participants first sat in the chair with the harness and head-frame attached and the head-neck in the straight position. The seat pan of the chair was then rotated to 10° or 30° while the head remained in the straight-ahead position. Participants were then instructed to contract their neck muscles for a few seconds against the resistive force of the investigator's hand which was positioned over the cheek and mandible of the participant, "as if looking over the right shoulder". After the isometric voluntary contraction of the right rotator muscles of the neck, the rotated position was maintained for a further five seconds. The neck was then passively rotated back to 20° and data collection commenced. Since hold-30° conditioning was carried out at an angle where the right rotator muscles of the neck are

lengthened, it might best translate to hold-long conditioning and result in a decreased quantity of muscle proprioceptive afferent inputs from the neck right rotators. Conversely, hold-10° conditioning may be equivalent to hold-short conditioning and result in an increased quantity of muscle proprioceptive afferent inputs from the neck right rotator muscles. It was assumed that other somatosensory afferent inputs including those from cutaneous and articular receptors were consistent during data collection following each form of muscle conditioning while afferent inputs from the muscle proprioceptors were quantitatively different. The order of hold-10° or -30° conditioning was randomised before the experiment commenced.

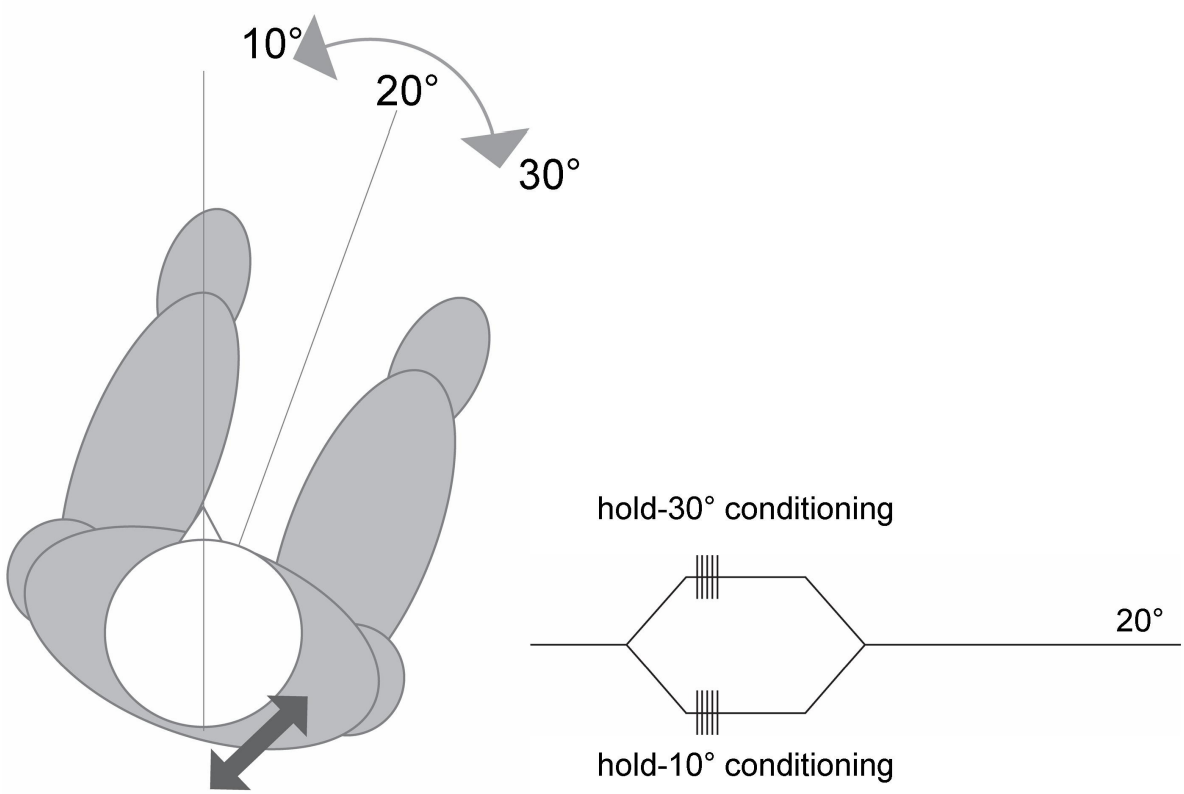

Figure 5.1. The left panel of the diagram shows passive neck rotation with stable head position and muscle conditioning manoeuvre to the head-neck right rotator muscles in the sitting position.
Data were collected at the 20° neck rotation position after muscle conditioning manoeuvre was carried out at 10° or 30° neck rotation. The right panel represents a change in the length of the head-neck right rotator muscles during the muscle conditioning manoeuvre. The muscles were lengthened during hold-30° conditioning and shortened during hold-10° conditioning. Vertical lines indicate voluntary muscle contraction.

Initially, the study was carried out with vision excluded in order to minimise clues relating to head and body position. However, it was quite difficult for participants to relax their neck muscles especially during passive neck rotation. Relaxing the neck muscles was critical for a successful muscle conditioning protocol. Therefore, neck muscle activity recorded as the surface electromyogram (sEMG) was displayed on an oscilloscope (HAMEG Instruments GmbH, Mainhausen, Germany) placed in front of the participant so that they could monitor their own neck muscle activity and relax the neck muscles as required (biofeedback).

5.2.4. Vibratory stimulus

Another technique to alter the quantity of muscle proprioceptive afferent inputs from the neck used in this section was a vibratory stimulus using a custom-built vibratory apparatus (35 mm in diameter and 65 mm in length size) (Scientific Concepts, Glen Waverley, VIC, Australia). The vibratory apparatus was vertically attached on the right side of the dorsal neck using adhesive tape. The cephalad side of the vibrator was placed at the level of the C_2 spinous process. The attachment site of the vibrator was chosen where the strongest illusion of head movement was induced when the vibratory stimulus was delivered. The head motion illusion was, in particular, intended to be "as if the head is turning over the left shoulder" (i.e. the head is turning towards "9 o'clock" from "12 o'clock"). In order to avoid excessive psychological stress but obtain a sufficient vibratory stimulus, a frequency of 70 Hz (1.5 mm amplitude) was chosen for this study.

5.2.5. Preparation for experiments

For preparation, participants were asked to fast and refrain from any caffeine beverages for at least 4 hours before, and to avoid any alcoholic beverages and rigorous exercise for at least 12 hours before their participation.

Both studies in this section were carried out in the sitting position with participants sitting in the same custom-built chair described earlier (*section 5.2.3*). The chair has adjustable foot rests and the position of the foot rests were adjusted to ensure a comfortable posture at the hip and knee. After participants were fully instrumented, the head-frame was attached over the head (and a blindfold was applied for the vibration study only).

5.2.6. Experiment protocol – muscle conditioning study

The muscle conditioning study consisted of two sessions – including the heart rate variability (HRV) and SGP recording sessions. To familiarise subjects with the research protocol, each participant was required to attend at least one two-hour training session in the laboratory. On the actual data collection day, the order of the muscle conditioning manoeuvre (hold-10° and 30° conditioning) was randomised by tossing a coin. However, the HRV recording session was always carried out first. Between HRV and SGP recording sessions, a relaxation period was provided for approximately 20 minutes but this period was extended if necessary. During the relaxation period, participants relaxed, usually reading a book or newspaper. After the relaxation period, the SGP session commenced. The order of rotation angles for the muscle conditioning was opposite to the order of the HRV session (e.g. conducted at 30° rotation in SGP session if first muscle conditioning was carried out at 10° in HRV session).

5.2.6.1. Experiment protocol – muscle conditioning study (heart rate variability recording session)

Once participants were seated comfortably in the chair (following instrumentation), a 5-minute relaxation period commenced. During the final minute of the relaxation period, the participant was instructed to synchronise their respiratory rate with a metronome rhythm (0.25Hz, which is equivalent to 15 counts per minute). Familiarization with paced breathing occurred during the training session(s), however, the one-minute controlled breathing period

was aimed to re-familiarise participants with paced breathing and to allow participants to seek a comfortable depth of breathing. During this time participants remained seated in the head-body straight position. At the beginning of the trial, the seat pan of the chair was moved to induce passive left neck rotation (See Figure 5.1). The chair was moved to the angle as decided prior to commencement of the trial, for example, 10°. Participants were instructed to contract their neck right-rotator muscles for three seconds by resisting the examiner's hand which was positioned over the participants' cheek and mandible. This head-neck angle was then held for five seconds, after which time the chair was gently rotated back to 20°, and data was collected for five minutes at this neck rotation position. After data collection, the chair was rotated to the other angle for the muscle conditioning manouevre (in this case, 30°) and the same procedures (i.e., isometric voluntary contraction, five-second relaxation, and gentle rotation of the chair) were repeated. Data were collected at 20°-neck rotated position.

5.2.6.2. Experiment protocol – muscle conditioning study (strain-gauge plethysmogram recording session)

Once participants were seated comfortably in the chair, a 5-minute relaxation period commenced as carried out in the HRV recording session. At the final minute of the relaxation period, the wrist cuff was inflated to 180-200 mmHg in order to exclude hand circulation. This inflation pressure was maintained for at least one minute in order to stabilise blood volume of the forearm before the first muscle conditioning procedure of the SGP recording session commenced. Up to this point, participants were in the head-body straight position. The seat pan of the chair was then turned to an angle for the muscle conditioning manoeuvre; for example, 30°. The order of neck rotation angle for the muscle conditioning manoeuvre (10° or 30°) was reversed from the HRV recording session. Following the three-second isometric voluntary muscle contraction and five-second relaxation period, the chair was rotated to the test position (20°). As soon as the chair reached the 20° neck rotation position,

the upper arm cuff was inflated to 50 mmHg. The 10-second inflation-deflation cycle was automatically continued for 2 minutes (six cycles in total). Immediately after this recording time, the chair was rotated to another muscle conditioning angle (10°, in this case), the same procedures were repeated, and data collection was made for two minutes.

5.2.7. Experiment protocol – vibration study

The second study of this chapter applied a vibratory stimulus to the right dorsal neck. To familiarise participants with the vibratory stimulus and upper cuff inflation and deflation cycle, the vibratory stimulus was delivered for 1 minute with 1-minute intervals and the cuff around the upper arm was periodically inflated and deflated prior to actual data collection. This rehearsal sequence was repeated three times. After this familiarization period, the postural illusion created by the vibratory stimulus was formally rated by participants (1 = no, 2 = slight, 3 = moderate, 4 = strong, and 5 = very strong) (see Appendix 18 for a head motion illusion scale).

Once participants were seated comfortably in the chair (following instrumentation), a 5-minute relaxation period commenced. At the final minute of the relaxation period, the wrist cuff was inflated to 180-200 mmHg. Once the volume of the left forearm stabilised, the pre-vibration measurement period commenced followed by a vibration period and post-vibration period. Each phase of the experiment lasted for one minute (Figure 5.2). As soon as data collection of the post-vibration period finished, the wrist cuff was released and a relaxation period was provided for five minutes or until post-ischemia symptoms including warmness and pins-and-needles sensation in the left hand were completely eased. The same procedures (data collection) were repeated five times.

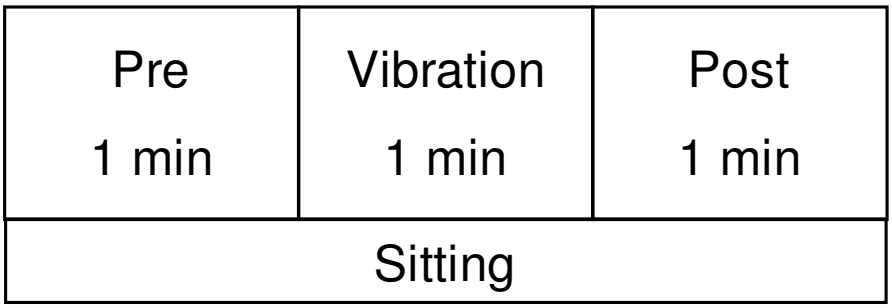

Figure 5.2. The experimental protocol of the vibration study.
A vibratory stimulus was applied to the right dorsal neck following and followed by one-minute recording periods.

5.2.8. Data analysis

The analyses of SGP and skin potential change were carried out in the same way in both muscle conditioning and vibration studies using commercialised software (Chart for Windows V5.1.1, ADInstruments, Castle Hill, NSW, Australia). Strain-gauge plethysmogram data was quantified after D.E. Hokanson Inc. (1998) (for more details see in section 2.7.2.). Quantified data were expressed as volume flow in millilitres per 100 millilitre tissue per minute (ml/100ml tissue/min) and values over a one-minute period were averaged in each phase of the experiment (pre-, during, and post-vibration). The method used to assess change in skin potential in this study was the same as that used by Donadio and colleagues (2002a; 2002b). A significant change in skin potential was accepted when the amplitude of change in skin potential exceeded 5% of the pre-vibration value. For the vibration study, averaged data from the 60 seconds prior to the onset of vibratory stimulus was used as the reference value and compared with skin potential data up to 7 seconds after the onset and cessation of the vibratory stimulus.

5.2.8.1. Data analysis of heart rate, blood pressure, and finger blood flow for muscle conditioning study

All variables (heart rate; HR, BP, and FiBF) following each form of muscle conditioning were averaged into both 10-second and one-minute bins. Ten-second average data were used for

capturing transient changes in cardiovascular parameters in response to different forms of the muscle conditioning manoeuvre whereas 1-minute average data were purposed to confirm whether the observed muscle conditioning-dependent changes in cardiovascular function was prolonged-action. Signals of the ECG were recorded over a 5-minute period and these data were used for HRV analysis (Chart for Windows V5.1.1 with HRV extension V1.0.1, ADInstruments, Bella Vista, NSW, Australia).

5.2.8.2. Data analysis of heart rate, blood pressure, and finger blood flow for vibration study

For the pre-vibration period, HR, BP and FiBF were averaged over a period of 1 minute. For vibration and post-vibration periods, these parameters were averaged into 20-second periods.

5.2.8.3. Data analysis of forearm blood flow for both (muscle conditioning and vibration) studies

Forearm blood flow (FoBF) was averaged into 1-minute bins. Therefore, for muscle conditioning study, two 1-minute bins were obtained for each form of the manoeuvre (i.e., the first and second minutes). For vibration study, three 1-minute bins were obtained for each vibration trial (i.e., pre-, during, and post-vibration periods).

5.2.9. Statistical analysis

Statistical analysis for this study was conducted with the statistical software package (SPSS V14 for Windows, SPSS Inc., U.S.A). The normal distribution of the data was assessed using a Kolmogorov-Smirnov test. This normality assessment, determined whether the parametric or nonparametric equivalent statistical test was used (see below regarding specific statistical tests). Statistical significance was set at $p < 0.05$.

5.2.9.1. Statistical analysis for muscle conditioning study

A paired *t*-test or Wilcoxon signed-rank test was used to compare averaged values for each time period for each variable between hold-10° and -30° conditioning.

5.2.9.2. Statistical analysis for vibration study

In order to examine whether there was a main effect of time for each variable, a one-way repeated measures analysis of variance (ANOVA) or a Friedman test was used. Following this initial analysis, post-hoc pairwise comparisons were made and the α level was adjusted by Bonferroni correction to avoid a type I error. Following the Friedman test, a paired *t*-test or Wilcoxon signed-rank test was used for pairwise comparisons.

5.3. Results

5.3.1. Muscle conditioning study

Eleven young adults (7 males and 4 females) participated in the muscle conditioning study. The average age of the group was 24.5 ± 4 years old and their body mass index (BMI) was 23.44 ± 2.13 kg/m^2 (expressed as mean ± SD). Two participants reported neck stiffness (visual analogue scale; VAS = 1.4 and 5.2) prior to the commencement of the experiment. No participant reported development of neck stiffness or pain after the experiment which did not present before the experiment. Neither was there any report that procedures during the experiment produced discomfort. Three participants reported difficulty in staying awake during the experiment.

5.3.1.1. Cardiovascular responses to muscle conditioning during paced breathing (heart rate variability recording session)

There were no differences in the 1-minute averaged cardiovascular parameters (HR, BP, and FiBF) between the two forms of conditioning.

In contrast, more detailed data, which were averaged every 10 seconds, showed some significant differences in cardiovascular parameters between hold-10° and 30° conditioning, however, these differences were random (see Figure 5.3). For example, mean arterial pressure (MAP) was significantly lower by 3 mmHg at 150 seconds following hold-10° conditioning when compared with hold-30° conditioning at the same time period [t (10) = -2.72, p = 0.022, d = 0.82]. Following hold-30° conditioning, diastolic BP (DBP) was significantly lower at 20 seconds [z (11) = -2.31, p = 0.021] and higher at 150 seconds [t (10) = -2.26, p = 0.048, d = 0.68] compared with hold-10° conditioning. Systolic blood pressure (SBP) was significantly lower by 5 mmHg at 150 seconds following hold-10° conditioning in comparison with hold-30° conditioning [t (10) = -2.34, p = 0.041, d = 0.71]. Finger blood flow was greater by 14 % at 30 seconds after hold-30° conditioning compared with hold-10° conditioning [t (10) = -2.73, p = 0.021, d = 0.82]. In contrast, there was no significant difference in 10-second averaged HR between these forms of muscle conditioning over the 5-minute recording period.

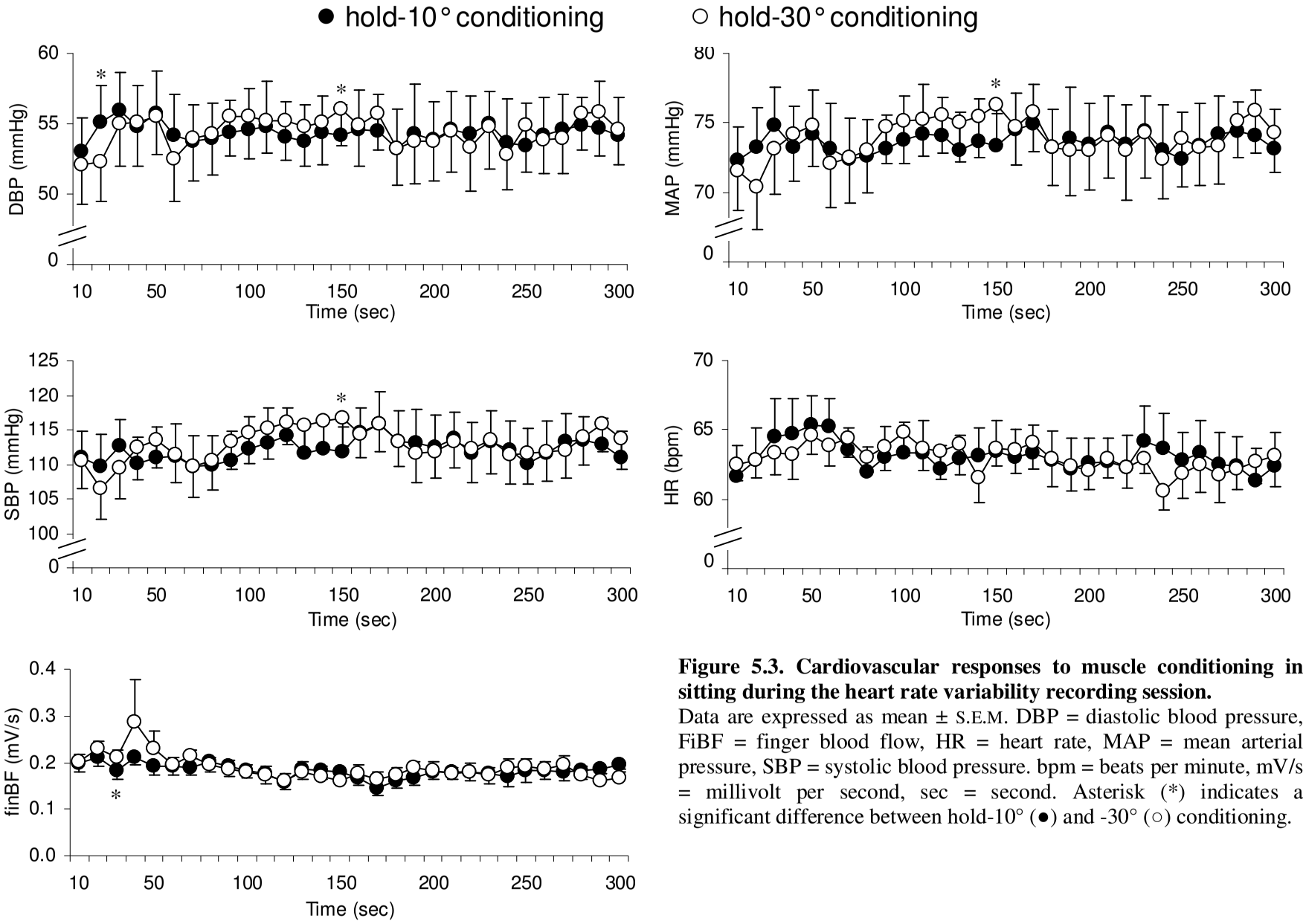

Figure 5.3. Cardiovascular responses to muscle conditioning in sitting during the heart rate variability recording session.
Data are expressed as mean ± S.E.M. DBP = diastolic blood pressure, FiBF = finger blood flow, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure. bpm = beats per minute, mV/s = millivolt per second, sec = second. Asterisk (*) indicates a significant difference between hold-10° (●) and -30° (○) conditioning.

5.3.1.2. Cardiac autonomic nervous response to muscle conditioning

Heart rate variability analysis was used to examine the effect of muscle conditioning in the head-neck right rotators on the modulation of autonomic nervous drive to the heart. The results of the paired *t*-test and Wilcoxon signed-rank test revealed insignificant differences in all parameters between hold-10° and -30° conditioning. However, individual data did show differences , but these were inconsistent (Figure 5.4-5.6).

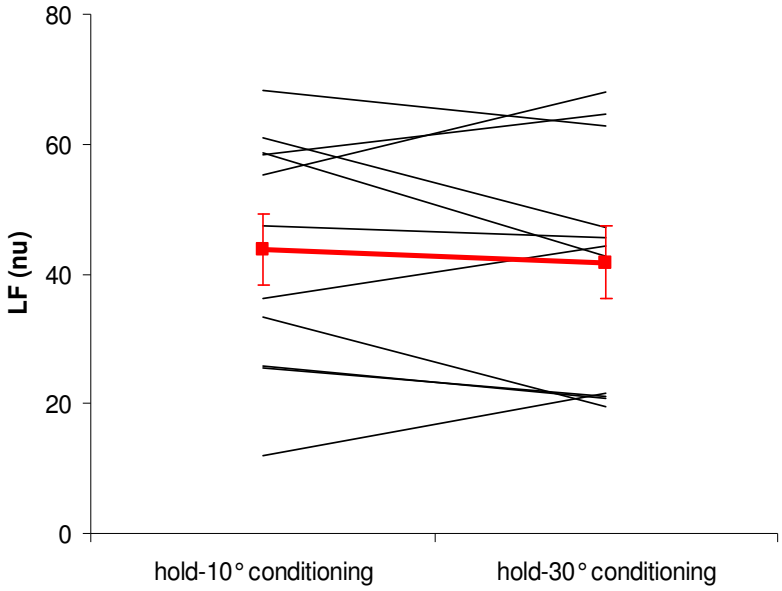

Figure 5.4. Group average and individual responses of normalised low frequency power component of heart rate variability to muscle conditioning.
Thick line (═) indicates the group average and is expressed as mean ± SEM Thin line (—) indicates the individual response. LF = low frequency component of heart rate variability, nu = normalised unit. Please note that the second bottom individual response appears as a thick line because two participants exhibited similar responses.

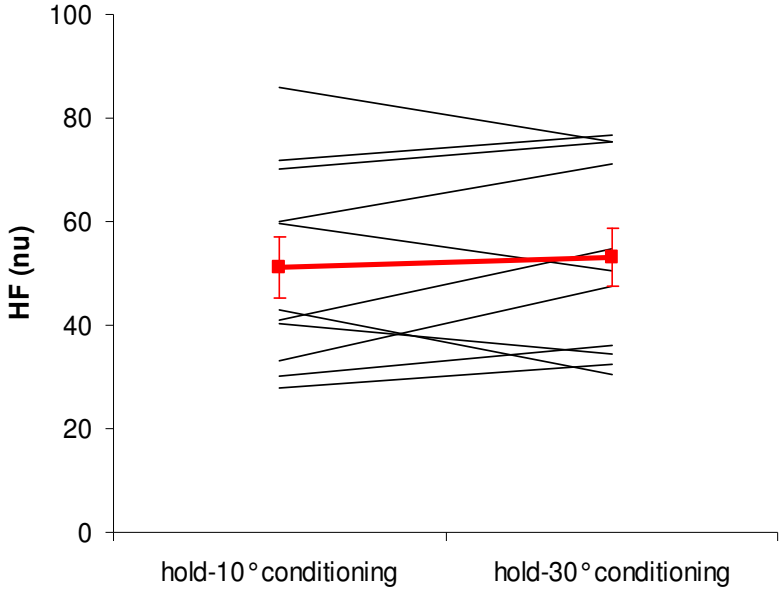

Figure 5.5. Group average and individual responses of normalised high frequency power component of heart rate variability to muscle conditioning.
Thick line (═) indicates the group average and is expressed as mean ± SEM Thin line (—) indicates the individual response. HF = high frequency component of heart rate variability, nu = normalised unit.

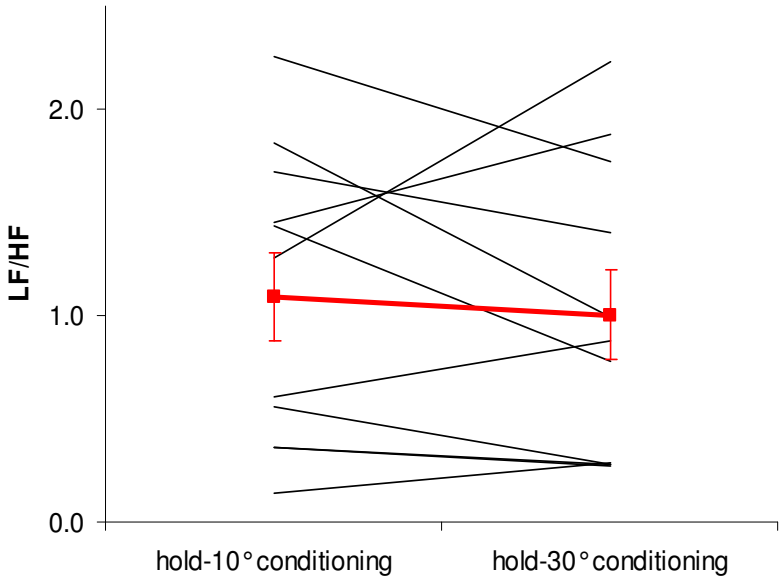

Figure 5.6. Group average and individual responses of the ratio of low frequency component to high frequency component of heart rate variability to muscle conditioning.
Thick line (═) indicates the group average and is expressed as mean ± SEM Thin line (—) indicates the individual response. LF/HF = the ratio of low frequency power component to high frequency power component of heart rate variability. Please note that the second bottom individual response looks a thick line due to two participants exhibited quite similar values.

Table 5.1. Comparisons of heart rate variability parameters between the two different forms of muscle conditioning of the head-neck right rotators.

parameters	hold-10° conditioning	hold-30° conditioning	*p*	*t* or *z*	*d*
TP (ms^2)	6126.83 ± 1620.47	5690.07 ± 896.22	0.79	-0.27	N/A
LF (ms^2)	1975.06 ± 816.14	1614.39 ± 430.37	0.86	-0.18	N/A
LF (nu)	43.78 ± 5.49	41.70 ± 5.61	0.51	0.68	0.20
HF (ms^2)	2124.17 ± 597.89	1933.72 ± 453.41	0.53	-0.62	N/A
HF (nu)	51.22 ± 5.83	53.21 ± 5.64	0.52	-0.67	0.20
LF/HF	1.09 ± 0.21	1.00 ± 0.22	0.59	0.56	0.17

Data are expressed as mean ± SEM Effect size (*d*) was reported when a paired *t*-test was used only. TP = total power of heart rate variability, LF = low frequency power component, HF = high frequency power component, LF/HF = the ratio of low frequency power component to high frequency power component, ms^2 = milliseconds squared, nu = normalised unit.

5.3.1.3. Cardiovascular responses to muscle conditioning during strain-gauge plethysmogram recording session

During the SGP recording session, FoBF responses after each form of muscle conditioning was measured. During this session, BP, HR, and FiBF were also measured. The results of the Wilcoxon signed-rank test showed that there was no significant difference in FoBF at the first and second minutes following the two forms of muscle conditioning [z (11) = -0.13, p = 0.89 and z (11) = -1.16, p = 0.25]. In terms of the other cardiovascular parameters, there were no significant differences in 1-minute average data but transient changes were found in 10-second data of a few parameters. For example, DBP and MAP were lower at 100 seconds following hold-30° conditioning than hold-10° conditioning [t (10) = 2.61, p = 0.026, d = 0.79 and t (10) = 2.49, p = 0.032, d = 0.75, respectively]. Additionally, FiBF was greater by approximately 25 % at 110 seconds following hold-30° conditioning than hold-10° conditioning [t (10) = 2.27, p = 0.046, d = 0.69].

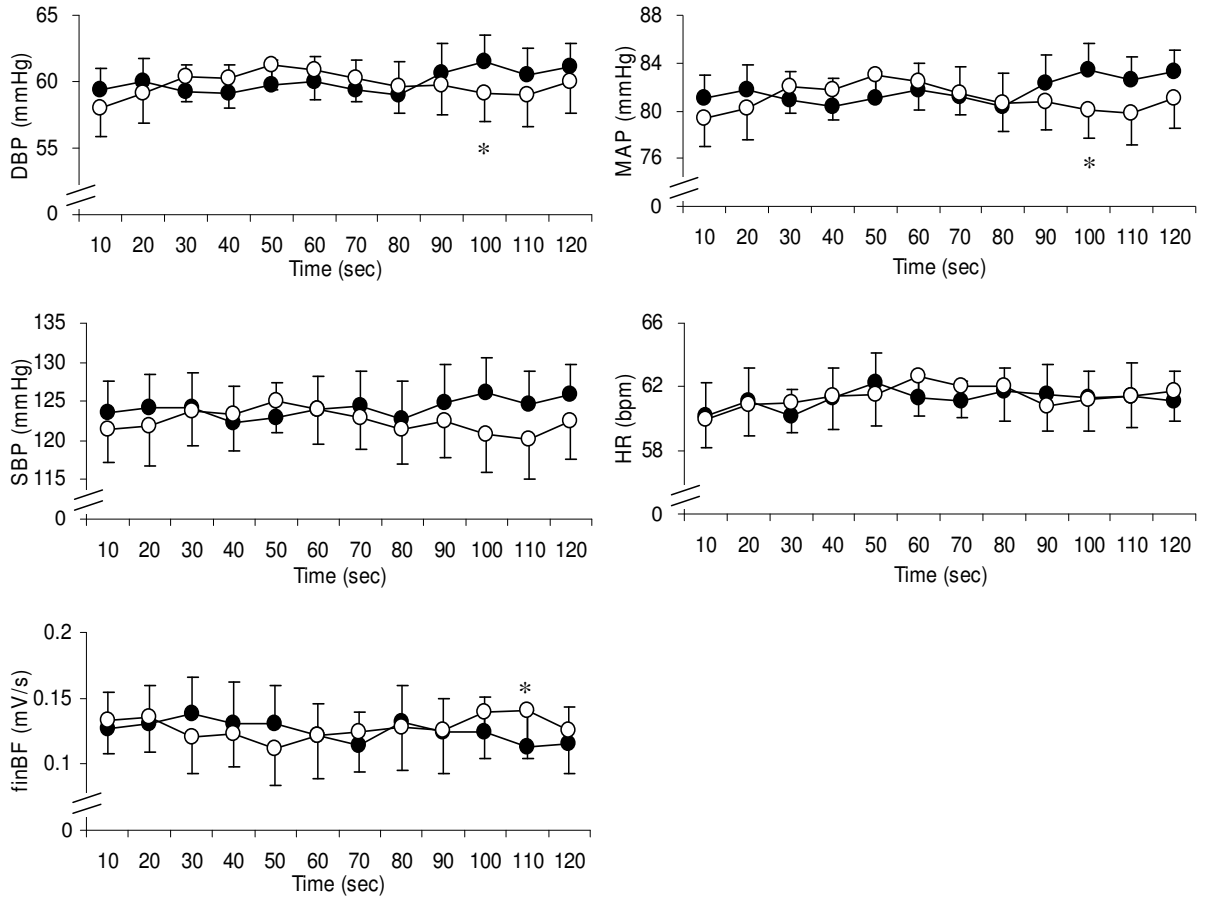

Figure 5.7. Cardiovascular responses to muscle conditioning in sitting during the strain-gauge plethysmograph recording session.
Data are expressed as mean ± SEM DBP = diastolic blood pressure, FiBF = finger blood flow, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure. bpm = beats per minute, mV/s = millivolt per second, sec = second. Asterisk (*) indicates a significant difference between hold-10° (●) and -30° (○) conditioning.

5.3.2. Vibration study

Nine young adults (5 males and 4 females) participated in the vibration study. The average age of participants was 26.0 ± 4 years old and they had an average BMI of 24.41 ± 5.55 kg/m^2 (expressed as mean ± SD). One participant reported neck stiffness (VAS = 2.3) before the experiment started. There were no reports of post experiment neck stiffness or any new symptoms which did not present before the experiment. Neither were there any reports that the procedures of the experiment produced any discomfort. Three participants reported difficulty in staying awake during the experiment.

The vibratory stimulus used in this study tended to evoke an illusion of head motion in 7 out of the 8 participants (the questionnaire used to record an illusion of head motion could not be found for one participant). The illusion of head motion was characterised as the head turning towards the left side. A summary of the intensity of the illusion recorded for each participant is summarised in Table 5.2. The participant who did not perceive any illusion of head motion reported that they felt that their eyes were moving towards the left during vibration.

Table 5.2. Participants' perceptions on head motion illusion during the right dorsal neck vibration

The intensities of illusion	***N***
No	1
No to Mild	1
Mild	1
Moderate	4
Moderate to Strong	1
Total	8

5.3.2.1. Blood pressure responses to unilateral neck vibration

The result of the repeated measures ANOVA demonstrated that there was no significant time effect for MAP [Wilks' $\Lambda = 0.082$, $F\ (6,3) = 5.57$, $p = 0.094$, $\eta^2 = 0.34$], DBP [Wilks' $\Lambda = 0.182$, $F\ (6,3) = 2.25$, $p = 0.27$, $\eta^2 = 0.82$], or SBP [Wilks' $\Lambda = 0.056$, $F\ (6,3) = 8.44$, $p = 0.054$, $\eta^2 = 0.94$]. Pairwise comparison showed significant reductions in SBP 20 and 40 seconds after cessation of the vibratory stimulus to the neck [$t\ (8) = 5.54$, $p = 0.011$, $d = 1.85$ and $t\ (8) = 4.45$, $p = 0.045$, $d = 1.48$, respectively] (Figure 5.8), but not in MAP and DBP.

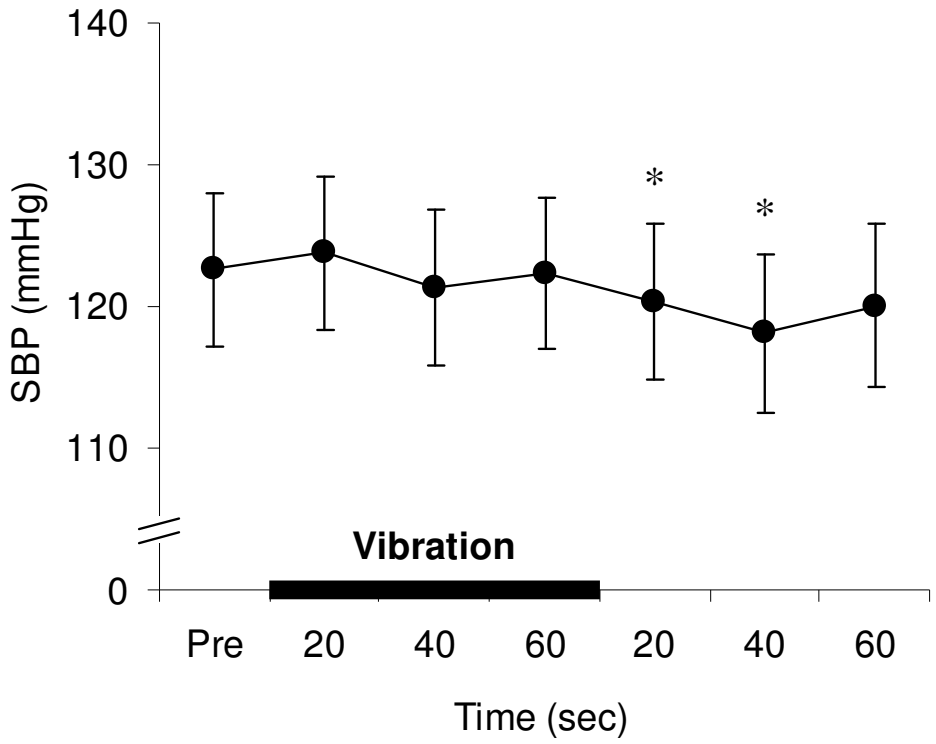

Figure 5.8. Systolic blood pressure response to neck vibration.
Data are expressed as mean ± SEM SBP = systolic blood pressure, sec = second, Asterisk (*) indicates a significant difference from pre-vibration value ($p < 0.05$).

5.3.2.2. Finger blood flow response to unilateral neck vibration

The result of the repeated measures ANOVA demonstrated that there was no significant time effect for FiBF [Wilks' $\Lambda = 0.072$, F (6,3) = 6.48, $p = 0.077$, $\eta^2 = 0.93$]. However, pairwise comparisons showed FiBF significantly increased at 20 and 40 seconds following the cessation of neck vibration [t (8) = -6.40, $p = 0.006$, $d = 2.13$ and t (8) = -4.89, $p = 0.018$, $d =$ 1.63, respectively] (Figure 5.9).

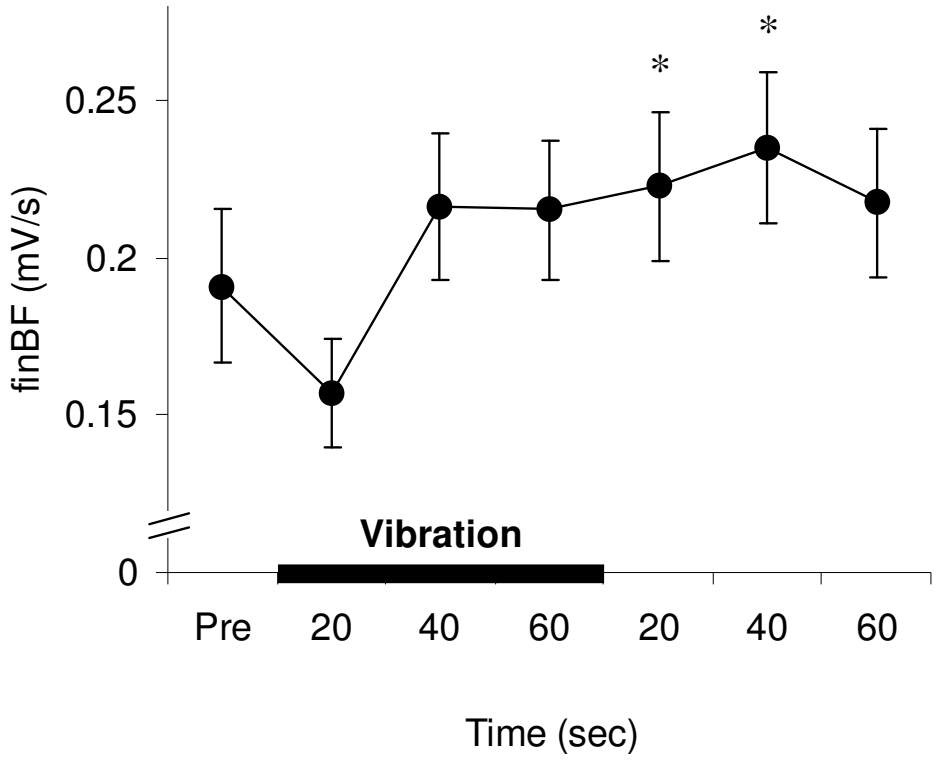

Figure 5.9. Finger blood flow response to neck vibration.
Data are expressed as mean ± standard error of mean. FiBF = finger blood flow, sec = second, Asterisk (*) indicates a significant difference from pre-vibration value ($p < 0.05$).

5.3.2.3. Forearm blood flow response to unilateral neck vibration

The result of the repeated measures ANOVA demonstrated that there was a significant time effect for FoBF [Wilks' $\Lambda = 0.24$, F (2,7) = 10.97, $p = 0.007$, $\eta^2 = 0.76$]. Pairwise comparisons showed that FoBF significantly decreased by approximately 11 % both during and after vibration [t (8) = 5.03, $p = 0.003$, $d = 1.68$ and t (8) = 3.34, $p = 0.03$, $d = 1.11$, respectively] (Figure 5.10).

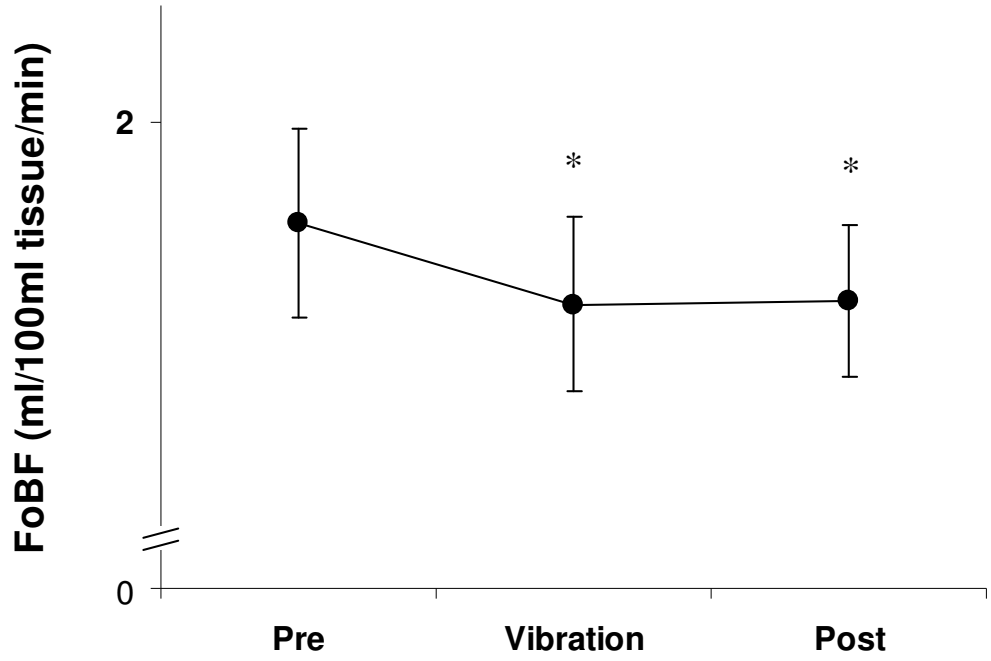

Figure 5.10. Forearm blood flow response to neck vibration.
Data are expressed as mean ± standard error of mean. FoBF = forearm blood flow, ml/100ml tissue/min = millilitres per 100 millilitre tissue per minute, Asterisk (*) indicates a significant difference from pre-vibration value ($p < 0.05$).

5.3.2.4. Heart rate response to unilateral neck vibration

The result of the Friedman test showed that there was no significant time effect for HR [$\chi^2(6, N = 9) = 10.33$, $p = 0.11$]. Pairwise comparisons showed insignificant changes in HR over the recording period.

5.3.2.5. Change in skin potential in response to the onset and cessation of unilateral neck vibration

A significant change in skin potential was observed following the onset of vibration in 14 trials of 45 trials, and following the cessation of vibration in 11 of 44 trials (one data value following the cessation of vibration was missing) trials. There was no clear trend in sudomotor activation in response to the vibratory stimulus, although a significant change in skin potential was observed more often in latter trials (Table 5.3).

Table 5.3. Individual skin potential response to the right dorsal neck vibration

ID	trials									
	1st		2nd		3rd		4th		5th	
	on	off	on	off	on	off	on	off	on	off
1								+		
2						+				+
3					+	+	+	+	+	
4						+		+	NA	
5	+	+								
6									+	
7	+				+	MS	+		+	+
8	+				+		+	+		
9							+		+	+
Total	3	1	0	0	3	3	4	4	4	3

Cross (+) indicates a significant change in skin potential, on = the onset of vibration, off = the offset of vibration, NA = no data collection, MS = missing data. Please note that ID is used for just convenience and does not indicate certain participant in later table, Table 5.8.

5.4. Discussion

In this study, the contribution of proprioceptive afferent inputs from the neck muscles to autonomic regulation of cardiovascular function was investigated in sitting humans and consisted of two experiments. In each experiment, either muscle conditioning or a vibratory stimulus was employed to alter the quantity of neck muscle proprioceptive afferent inputs. In an experiment with the muscle conditioning manoeuvre, it was found that there were transient and random but statistically significant differences in BP and FiBF between hold-10° and -30° conditioning. More specifically, at early phase of data collection (≤ 30 seconds) BP tended to be lower and FiBF to be greater following hold-30° conditioning, which was presumed to induce greater quantity of muscle proprioceptive inputs from the head-neck right rotator muscles than hold-10° conditioning. Conversely, at later of data collection (at 150 seconds), BP became greater following hold-30° conditioning. In the second experiment in this chapter, a vibratory stimulus was employed to preferentially activate muscle spindles of the right dorsal neck and it was found that there was a significant reduction in FoBF during application of the vibratory stimulus. Moreover, the vibratory effects persisted after the vibratory stimulus was terminated. Additionally, SBP and FiBF significantly decreased only following the

cessation of vibration. Collectively, the results in these experiments indicate that neck muscle proprioceptive inputs may contribute to cardiovascular function in awake humans.

5.4.1. The contribution of large afferent inputs from muscles to autonomic regulation of cardiovascular function

Interventions used in this study included muscle conditioning and a vibratory stimulus which both presumably altered the quantity of large afferent inputs from muscles (see *section 2.9* and *2.10*). These techniques enabled the study to investigate the participation of group I and II afferent inputs in autonomic regulation of cardiovascular function, although a vibratory stimulus may also stimulate cutaneous receptors and presumably Golgi tendon organs. This is because the neck muscles in the sitting position were likely active (even though the head position was held using a head-frame) and the application of a vibratory stimulus is capable of activating Golgi tendon organs during (even weak) muscle contraction (Roll et al. 1989; Fallon & Macefield 2007).

Autonomic drive and visceral responses induced by somatic stimulation are known as somato-autonomic and -visceral reflexes and these reflexes have been extensively examined especially in animals (Sato et al. 1997a). In order to evoke autonomic drive and/or visceral responses, various kinds of stimulation were employed such as pinching, brushing, and inflammatory chemicals. Noxious stimuli in general induce more consistent and profound autonomic and visceral responses; for example, sympathetic activation and increases in HR and BP (Sato et al. 1997a). These powerful responses are known to originate from free endings via small diameter afferents (group III and IV). On the other hand, innocuous stimuli tend to induce non-significant or inconsistent responses and particularly larger diameter afferents such as group I and II fibres originating from muscle spindles and Golgi tendon organs are thought not to participate in somato-autonomic or -visceral reflexes (Coote 1975;

Sato et al. 1997a). A few studies have investigated the involvement of large diameter afferents (group I and II) in somato-autonomic and -visceral reflexes using vibration (McCloskey et al. 1972), electrical stimulation (Sato et al. 1981), and succinylcholine (Sato et al. 1982). These studies found no significant effect of muscle large afferent activation on cardiovascular function. However, noteworthily, these studies stimulated only the limb muscles to reach these conclusions. It seemed that differences between limb and axial muscles were dismissed. So far, there have been only a few studies that have examined the effects of neck afferent stimulation on the autonomic nervous system in anaesthetised animals (Bolton et al. 1998; Bolton et al. 2006). It was found that stimulation of neck muscle large afferents induced responses from the splanchnic nerve and that the splanchnic nerve response to head motion around the sagittal plane became evident only following neck afferent dissection, suggesting that neck afferent inputs influence splanchnic nerve activity in an antagonistic manner to vestibular input (Bolton et al. 1998). In contrast, innocuous stimulation of neck afferents did not significantly influence adrenal efferent nerve activity (Bolton et al. 2006). These studies may indicate that innocuous neck afferent stimulation has an impact on autonomic regulation in particular target organs but such stimulation is thought not to produce a global response. Therefore, the contribution of large diameter afferents from the neck muscles to autonomic regulation presumably needs to be considered separately from those from the limb muscles.

5.4.2. Inconsistent effects of neck muscle conditioning on autonomic regulation of cardiovascular function in sitting humans

The muscle conditioning study found only transient and inconsistent changes in cardiovascular function. The following paragraphs consider possible explanations for the results obtained.

The muscle conditioning study might have failed to change the quantity of dorsal neck muscle proprioceptive inputs to the central nervous system (CNS). Visual feedback was used in order to improve the success of neck muscle conditioning by improving neck muscle relaxation. However, by providing a visual clue, participants were aware of their body schema and even if there were an alteration of neck muscle proprioceptive inputs, the impact of this change might have been negated. In support of this notion a previous study demonstrated that the presence of visual input reduced cardiovascular instability associated with posture change in vestibulectomised conscious cats (Jian et al. 1999). Additionally, the monitoring of neck muscle activity was carried out using sEMG in this study. Although the study protocol attempted to minimise neck muscle activity, the activity of deep neck muscles was unknown from the data obtained in this study. Therefore, muscle conditioning might have been unsuccessful in the deep neck muscles. It is critical to ensure that the conditioned muscle is passive (not contracting) during data collection in order to maintain the created muscle history (Morgan et al. 1984; Proske et al. 1993). As a result, it was thought that the protocol of neck muscle conditioning used in this study might not have been successful to alter muscle proprioceptive inputs in a systematic way and therefore, the method was not capable of revealing any consistent effect on autonomic regulation of cardiovascular function.

Alternatively, it was not possible to confirm from this study that muscle conditioning to the head-neck right rotator muscles influenced muscle proprioceptors in the muscle as intended. Muscle conditioning protocol was developed by the limb muscles (Proske et al. 1993). However, the architecture of neck muscles is more complex than limb muscles. Ge et al. (2005) applied muscle conditioning to the lumber multifidus muscles in cats and the determination of forms of muscle conditioning was made by muscle spindle afferent response to spine motion, but not based on spine motion itself. For example, if ventral movement of the spinous process increases muscle spindle afferent discharges, the spinous process was held at

the position for hold-long conditioning following muscle history elimination. It is not possible to apply the determination method (hold-long or -short conditioning) to the neck muscles in awake humans. Owens et al. (2006) applied the muscle conditioning manoeuvre to the neck muscles and found that neck muscle conditioning in the head-lateral flexion position revealed more distortion towards flexion. This may suggest that the outcome of the muscle conditioning manoeuvre in the limb muscles does not best translate to that in the paraspinal muscles. As a result of individual difference in response of neck muscle proprioceptor to each form of muscle conditioning, HRV responses varied across individuals were seen and group average appeared insignificant (Figure 5.4-5.6 and Table 5.1).

5.4.3. Post-vibratory effect

In the vibration study, cardiovascular responses to neck vibration were observed not only during the stimulation period but also following cessation of the stimulus. There is a possible explanation to the result that the vibratory effects may have lasted for some time after termination of the vibratory stimulus. There are a number of reports in the literature that document post vibration effects. Such effects include illusions of limb motion (Roll et al. 1980, quoted in Gilhodes et al. 1992), increased postural sway (Wierzbicka et al. 1998) and on-going, involuntary muscle contraction (Gilhodes et al. 1992). Such effects could last for as long as 15 minutes following neck vibration (Wierzbicka et al. 1998).

Microneurography has also been used to investigate post-vibratory effects on muscle spindle discharge. This recording method may contribute to elucidating the origin of the post-vibration effect and whether the posteffect creates changes in muscle spindle discharge. Ribot-Ciscar et al. (1998) demonstrated that the resting discharge of muscle spindles decreased after termination of the vibratory stimulus and this depression lasted for up to 40 seconds in their study. The authors also found that a minor proportion of muscle spindles

(13.5% of sampled muscle spindles) did increase their discharge for approximately 30 seconds post stimulus (Ribot-Ciscar et al. 1998). It was thought that the primary endings of muscle spindles displayed reductions in discharge rates (quoted in Ribot-Ciscar et al. 1995) and it is these endings that are most sensitive to the effects of vibration.

Collectively, the literature demonstrates that there are strong posteffects of vibration on various body functions. While vibration produces short-term changes in muscle spindle discharge (in the periphery) it is thought that a supraspinal component may be involved in more long-lasting effects that, in turn, influences sensorimotor integration over longer periods.

5.4.4. Transition to the follow-up study

This section of this chapter demonstrated that a unilateral neck vibration was capable of influencing autonomic regulation of cardiovascular function in sitting young healthy adults. However, some questions arose associated with the use of the vibratory stimulus. Therefore, the purpose of the next study was to address these issues; 1) to determine whether the previous results were peculiar to the neck or a non-specific response and 2) to clarify whether there was a laterality in the cardiovascular responses observed in response to the vibration stimulus. To achieve these purposes, similar experiments were repeated with the same outcome measures and the same frequency of a vibratory stimulus used earlier in this chapter was applied to the right tibialis anterior muscle (non-specific effect) and the left dorsal neck (laterality).

5.5. Methods for vibration follow-up study

5.5.1. General procedures

For this follow-up study, the participants, who participated in the previous neck vibration study were approached. However, it was not possible to contact all participants. Thus, this follow-up study sought other generally healthy young adults as well (aged between 18 and 35 years old) and therefore this study was treated as a separate study from the previous vibration study.

5.5.2. Estimation of sample size for vibration follow-up study

The sample size for the vibration follow-up study was estimated based on effect sizes obtained in the earlier study of this chapter. Table 5.4 summarises estimates sample sizes for the outcome measures BP, FiBF, and FoBF in order to achieve a statistical power of 80% when statistical significance was set at $p < 0.05$. Sample size estimation for HR was not made because an effect size could not be obtained.

Table 5.4. Sample size estimation in accordance with the vibration study in Chapter 5

Parameters	Obtained effect size	*df*	Estimated sample size
Diastolic BP	0.82	6	4
Systolic BP	0.94	6	4
Mean arterial pressure	0.34	6	17 to 22
Finger blood flow	0.93	6	4
Forearm blood flow	0.76	2	6 to 8

Sample size was estimated after Portney and Watkins (2000).

5.5.3. Equipment

The same equipment was used to deliver the vibratory stimulus and to measure cardiovascular responses to vibration as the neck vibration study earlier in this chapter (*section 5.2.2* and *5.2.4*).

5.5.4. Vibratory apparatus attachment

In order to address the issues arisen in the earlier part of Chapter 5, a vibratory apparatus used in the previous study was applied to the left dorsal neck and the right tibialis anterior. The order of vibration location was randomised before the first experiment commenced and each experiment was carried out on separate days.

Neck Vibration; The vibratory apparatus was vertically attached on the left side of the dorsal neck using adhesive tape. The cephalad side of the vibrator was placed at the level of the C_2 spinous process. The attachment site of the vibrator was chosen where the strongest illusion of head movement was induced when the vibratory stimulus was switched on. The head motion illusion was, in particular, intended to be "as if the head is turning over the right shoulder".

Leg Vibration; The vibratory apparatus was attached over the muscle belly of the right tibialis anterior and parallel with the muscle fibres using adhesive tape. Pre-wrap tape was first placed on the leg to before attaching the vibrator. The right foot was secured to a footplate to prevent motion of the foot during and after vibration using Velcro straps.

5.5.5. Experiment protocol

The same experiment protocol as the earlier study of Chapter 5 was used (see *section 5.2.7*).

5.5.6. Data analysis

Heart rate, BP and FiBF were averaged into 1 minute periods for the pre-vibration period and into 20-second bins for the vibration and post-vibration periods. The quantification of the SGP measures and the evaluation of skin potential change were carried out as described earlier (see *section 5.2.8*).

5.5.7. Statistical analysis

Statistical analysis for this study was conducted with the statistical software package (SPSS V15.0 for Windows, SPSS Inc., U.S.A). The normal distribution of data was assessed using a Kolmogorov-Smirnov test. Based on the normality assessment, the appropriate parametric or nonparametric equivalent statistical test was chosen, so a two-way repeated measures ANOVA was used for DBP, MAP, FiBF, and FoBF. For the two-way repeated measures ANOVA, the two factors were "vibration location" (leg or the left dorsal neck) and "time" - 7 levels for DBP, SBP, and FiBF (pre-vibration, during vibration at 20, 40, and 60 seconds, and post-vibration at 20, 40, and 60 seconds) and 3 levels for FoBF (pre-, during, and post-vibration). For the other parameters (HR and SBP), parametric (a one-way repeated measures ANOVA and paired *t*-test) and nonparametric (a Friedman test and Wilcoxon signed-rank test) tests were used as required. Initially, a Bonferroni correction was used for *post hoc* comparisons. However, no significant results for HR, BP, and FiBF were found. Therefore, the statistical results were reported without α level adjustment for these parameters although it was recognised that there was a risk of introducing a familywise error. The significance level was set at $p < 0.05$.

5.6. Results for follow-up study

Ten young adults (seven males and three females) participated in this study. The characteristics of the participant group were 24.7 ± 5 years old and 23.55 ± 2.67 kg/m^2 of

BMI (expressed as mean ± SD). Neck stiffness was reported on the day of neck vibration by three participants (VAS = 2.0 ± 0.2; *M* ± *SD*) and on the day of leg vibration by one participant (VAS = 1.7). One participant reported neck stiffness which they did not have before the leg vibration session (VAS = 1), but otherwise there was no report that development of neck stiffness or symptoms not seen before occurred as a result of the experimental intervention or that the procedures used in the experiment were uncomfortable.

A vibratory stimulus to the left dorsal neck in this study evoked an illusion of head motion in nine out of ten participants. The most common head motion illusion was turning of the head towards the right side, "as if looking behind over the right shoulder" (reported by seven of nice participants). The other two participants described the illusion as "the head moving forward" and "the head extending". The intensity of the perception of head motion illusion are summarised in Table 5.5. Interestingly, one participant reported during the familiarization session that the illusion of head motion was much less when the eyes were open (and looking down through the gap in the blindfold) (rated 1.5/5) than when the eyes were closed (rated 3/5) although this comparison of movement illusion between present and absent vision was not intended. In response to the leg vibration, no participant reported the perception of head motion, but several different perceptions relating to the vibrated leg were reported (Table 5.6.). The vibratory stimulus to the right tibialis anterior in this study evoked various illusions of leg motion or sensation in the right leg, but not head motion illusion.

Table 5.5. The intensities of head motion illusion associated with unilateral (left) neck vibration

The intensities of illusion	*N*
No	1
Mild	4
Mild to Moderate	1
Moderate	3
Strong	1
Total	10

Table 5.6. The characteristics of participants' perceptions associated with the vibratory stimulus over the right tibialis anterior muscle

Characteristics	*N*
Hamstrings tightness	2
Knee extension	2
Tibialis anterior tightness	1
Dorsiflexion of foot	1
Plantarflexion of foot	1
Total	7

Note; dorsiflexion and knee extension were reported by the same participant.

5.6.1. Blood pressure response to vibration on the neck and leg

For MAP, the results of the two-way repeated measures ANOVA showed that there was no main effect of time [Wilks' $\Lambda = 0.26$, $F\ (6,4) = 1.90$, $p = 0.28$, $\eta^2 = 0.74$] or vibration location [Wilks' $\Lambda = 0.97$, $F\ (1,9) = 0.28$, $p = 0.61$, $\eta^2 = 0.031$] or time-by-location interaction [Wilks' $\Lambda = 0.43$, $F\ (6,4) = 0.88$, $p = 0.58$, $\eta^2 = 0.57$]. However, pairwise comparisons demonstrated that MAP significantly decreased immediately after cessation of neck vibration [$t\ (9) = 3.04$, $p = 0.014$, $d = 0.96$]. There was no difference in MAP between vibration of the neck and leg at any of the time periods used in this study.

For DBP, the results of the two-ways repeated measures ANOVA showed that there was no main effect of time [Wilks' Λ = 0.098, F (6,4) = 6.13, p = 0.050, η^2 = 0.90] or vibration location [Wilks' Λ = 1.00, F (1,9) = 0.006, p = 0.94, η^2 = 0.001] or time-by-location interaction [Wilks' Λ = 0.27, F (6,4) = 1.83, p = 0.29, η^2 = 0.73]. However, pairwise comparisons demonstrated that DBP decreased at 20 seconds after the commencement of leg vibration [t (9) = 2.98, p = 0.017, d = 0.93] and decreased by approximately 1 mmHg (from 67 mmHg to 66 mmHg) at 20 seconds after cessation of neck vibration [t (9) = 2.78, p = 0.022, d = 0.88]. There was no difference in DBP between vibration of the neck and leg at any of the time periods used in this study.

For SBP, the results of the one-way repeated measures ANOVA and Friedman test showed that there was no significant main effect of time of neck vibration [χ^2(6, N = 10) = 4.50, p = 0.61] or of leg vibration [Wilks' Λ = 0.25, F (6,4) = 2.00, p = 0.26, η^2 = 0.75]. However, pairwise comparisons demonstrated that SBP significantly decreased at 20 seconds following cessation of both neck and leg vibration [z (10) = -2.12, p = 0.028 and t (9) = -2.42, p = 0.039, d = 0.81, respectively]. There was no significant difference in SBP between vibration of the neck and leg during any of the time periods.

5.6.2. Heart rate response to vibration on the neck and leg

For HR, the results of the Friedman test showed that there was no main effect of time for either neck or leg vibration [χ^2(6, N = 10) = 11.61, p = 0.071 and χ^2(6, N = 10) = 9.26, p = 0.16, respectively]. However, pairwise comparisons demonstrated that HR significantly decreased at 40 and 60 seconds of the leg vibration period [z (10) = -2.50, p = 0.013 and z (10) = -2.29, p = 0.022, respectively] and at 20 seconds following cessation of neck vibration [z (10) = -2.80, p = 0.005]. There was no difference in HR between vibration of the neck and leg at any of the time periods used in this study.

5.6.3. Finger blood flow response to vibration of the neck and leg

The results of the two-way repeated measures ANOVA showed that there was no main effect of time [Wilks' $\Lambda = 0.11$, $F\ (6,4) = 5.45$, $p = 0.061$, $\eta^2 = 0.89$] or vibration location [Wilks' $\Lambda = 1.00$, $F\ (1,9) = 0.003$, $p = 0.96$, $\eta^2 < 0.001$] or any time-by-location interaction [Wilks' $\Lambda = 0.13$, $F\ (6,4) = 4.48$, $p = 0.084$, $\eta^2 = 0.87$] for FiBF. However, pairwise comparisons demonstrated that FiBF increased at 40 seconds after the onset of leg vibration [$t\ (9) = -2.50$, $p = 0.026$, $d = 0.79$] and at 20, 40 and 60 seconds after cessation of neck vibration [at 20 seconds; $t\ (9) = -2.43$, $p = 0.031$, $d = 0.77$, at 40 seconds; $t\ (9) = -2.50$, $p = 0.029$, $d = 0.79$, at 60 seconds; $t\ (9) = -2.50$, $p = 0.043$, $d = 0.79$]. There was no difference in HR between vibration of the neck and leg at any of the time periods used in the study.

Table 5.7. The summary of blood pressure, heart rate, and finger blood flow responses to neck and leg vibration.

	Location	Pre	Vibration			Post		
			20 sec	40 sec	60 sec	20 sec	40 sec	60 sec
MAP	**Neck**	87 ± 3	86 ± 4	86 ± 3	87 ± 3	86 ± 3*	86 ± 4	86 ± 3
(mmHg)	**Leg**	88 ± 2	88 ± 2	88 ± 2	88 ± 2	88 ± 2	88 ± 2	88 ± 2
DBP	**Neck**	67 ± 3	67 ± 4	67 ± 3	67 ± 3	67 ± 3*^	67 ± 4	67 ± 3
(mmHg)	**Leg**	68 ± 2	67 ± 2*	63 ± 3	68 ± 2	68 ± 2	68 ± 2	68 ± 2
SBP	**Neck**	126 ± 4	126 ± 5	125 ± 4	126 ± 4	125 ± 4*	125 ± 4	126 ± 4
(mmHg)	**Leg**	128 ± 2	129 ± 2	128 ± 2	128 ± 2	130 ± 2*	129 ± 2	129 ± 2
HR	**Neck**	65 ± 2	65 ± 2	64 ± 2	64 ± 2	63 ± 2*	65 ± 2	64 ± 2
(bpm)	**Leg**	67 ± 2	66 ± 2	66 ± 2*	66 ± 2*	66 ± 2	66 ± 3	66 ± 3
FiBF	**Neck**	1.2 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2*	1.3 ± 0.2*	1.3 ± 0.2*
(mV/s)×10^{-1}	**Leg**	1.2 ± 0.2	1.1 ± 0.2	1.3 ± 0.2*	1.3 ± 0.2*	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.2

Data was expressed as mean ± SEM MAP = mean arterial pressure, DBP = diastolic blood pressure, SBP = systolic blood pressure, HR = heart rate, FiBF = finger blood flow, mmHg = millimetres of mercury, bpm = beats per minute, mV/s = millivolt per second, sec = second. Asterisk (*) indicates a significant difference from pre-vibration value ($p < 0.05$). Please note that the change in diastolic blood pressure at 20 seconds following neck vibration (^) was 0.71 mmHg (from 67.28 mmHg to 66.57 mmHg). Therefore, although statistical significance is presented, the significance does not reflect the value because the value has been rounded to the first decimal place.

5.6.4. Forearm blood flow response to vibration on the neck and leg

The result of the two-way repeated measures ANOVA showed that there was a significant main effect of time for FoBF [Wilks' $\Lambda = 0.37$, $F\ (2,8) = 6.72$, $p = 0.019$, $\eta^2 = 0.63$], but no main location effect [Wilks' $\Lambda = 0.90$, $F\ (1,9) = 0.96$, $p = 0.35$, $\eta^2 = 0.097$] or time-by-location interaction [Wilks' $\Lambda = 0.70$, $F\ (2,8) = 1.74$, $p = 0.24$, $\eta^2 = 0.30$]. Pairwise comparisons with Bonferroni correction demonstrated that FoBF significantly decreased by approximately 8 % during neck vibration only [$t\ (9) = 3.23$, $p = 0.032$, $d = 1.02$] (Figure 5.11). However, FoBF did not differ between leg and neck vibration for the time periods used in this study.

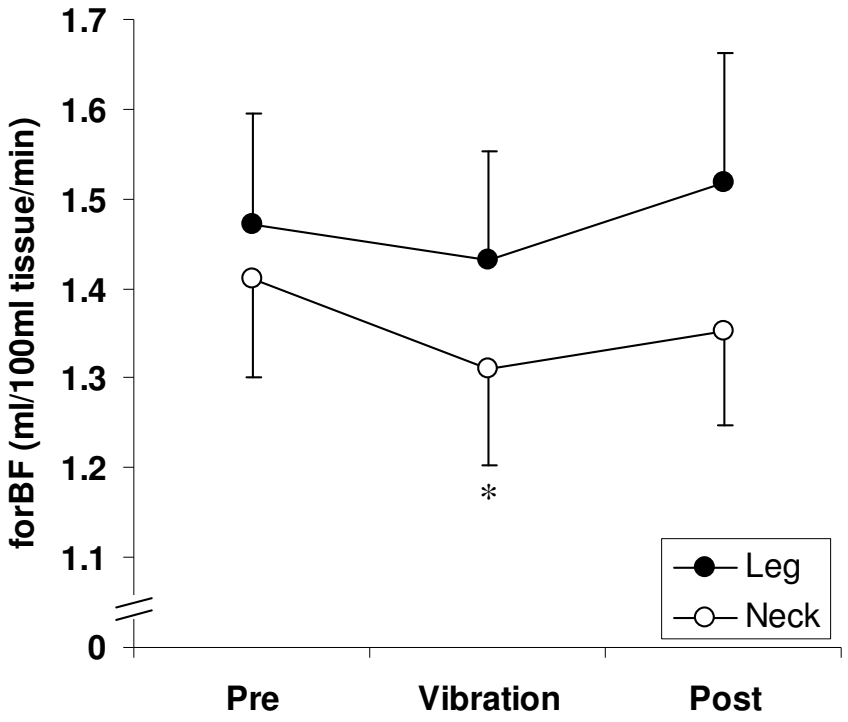

Figure 5.11. Comparison of forearm blood flow response between neck and leg vibration.
Data are expressed as mean ± SEM FoBF = forearm blood flow, ml/100ml tissue/min = millilitre per 100 millilitre tissue per minute, Leg = vibratory stimulus to the right tibialis anterior, Neck = vibratory stimulus to the left dorsal neck, Asterisk (*) indicates significant difference from pre-vibration value ($p < 0.05$).

5.6.5. Change in skin potential in response to the onset and cessation of unilateral neck vibration

Of 45 trials of leg vibration, a significant change in skin potential was observed following the onset of vibration in 8 trials and following the cessation of vibration in 7 trials. Of 47 trials of neck vibration, a significant change in skin potential was observed following the onset of vibration in 15 trials and following the cessation of vibration in 12 trials. Significant sudomotor activation was seen in three trials following the onset of vibration in one participant and in four trials following the cessation of vibration in another participant.

Table 5.8. Individual skin potential response to neck and leg vibration.

ID	Neck vibration										Leg vibration									
	1st		2nd		3rd		4th		5th		1st		2nd		3rd		4th		5th	
	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
1							NA		NA								NA		NA	
2	+			+		+		+	+	+									NA	
3							+	MS					+							
4			+				+	+			+		+						NA	
5		+							+	+										+
6								+	+						+					+
7			+	+			+		+					+	+	+				
8	+			+		+			NA			+					+	+		
9			+						+		+						+		NA	
10					+				+	+								+		
Total	2	1	3	3	1	2	3	3	6	3	2	1	2	1	2	1	2	2	0	2

Cross (+) indicates significant change in skin potential, on = the onset of vibration, off = the offset of vibration, NA = no data collection, MS = missing data. ID = participant number. Please note that ID is used for convenience and does not relate to a specific participant in any earlier tables (e.g., Table 5.3).

5.7. Overall discussion

The purpose of this section of this chapter (vibration follow-up study) was to clarify whether cardiovascular responses observed earlier in this chapter were due to a non-specific response and, therefore due to arousal or a genuine response. Additionally, this study aimed to investigate whether the side of the neck to which the vibratory stimulus was applied (i.e., laterality of the stimulus) determined cardiovascular responses to the stimulus because an increase in ipsilateral FiBF and a decrease in contralateral FoBF were observed in the earlier study of Chapter 5. Unilateral neck vibration revealed similar cardiovascular responses to the previous study, that is, a reduction in FoBF during vibration as well as an increase in FiBF and a reduction in BP following the cessation of the vibrator stimulus. On the other hand, leg vibration revealed different cardiovascular responses from neck vibration including increases in FiBF and SBP and decreases in HR and DBP during the vibratory stimulus period. Therefore, these results suggest that (1) there was no significant effect of laterality for cardiovascular responses to unilateral neck vibration and (2) the cardiovascular responses observed in the previous study were not likely to have occurred in response to arousal. However, a Bonferroni correction was not used in the statistical analyses in the latter part of Chapter 5 (except FoBF). Thus, the possibility exists that a type I error occurred – that is, falsely rejecting the null hypothesis that vibration of the neck had no effect on cardiovascular function. In addition to a possibility of the statistical error, the responses of BP and HR to a vibratory stimulus in this study were quite small; for example, a reduction in HP by 1 bpm. However, HR is predominantly controlled by the autonomic nervous system (Levy et al. 2007). Blood pressure is determined by physiological factors such as cardiac output and reripheral resistance, and these factors are also influenced by multiple factors (Levy et al. 2007). Hence, it can be said that both BP and HR are end-products of cardiovascular regulatory mechanism. Therefore, the small change in BP and HR does not necessarily mean that the activation of neck muscle proprioceptors using a vibratory stimulus does not have an

impact on the cardiovascular system. The small changes in cardiovascular parameters (BP and HR) may rather indicate that there is possibly a contribution of neck muscle proprioceptive inputs to the regulatory mechanism of the cardiovascular system.

5.7.1. Genuine response or non-specific response

In order to clarify whether the observation in the earlier part of this chapter was due to neck vibration or a non-specific response (arousal), this part of the chapter employed vibratory stimuli to the leg and the neck. The common findings of the neck vibration in this chapter were 1) a decrease in FoBF during application of the vibratory stimulus and 2) an increase in FiBF and a reduction in SBP following cessation of neck vibration. In contrast, reports in the literature concerning the characteristics of an arousal response include an inhibition of muscle sympathetic nerve activity (Donadio et al. 2002a; Donadio et al. 2002b), sudomotor and skin vasoconstrictor responses (Macefield et al. 1998), and an increase or reduction in BP (Holand et al. 1999; Donadio et al. 2002b), but no HR change (Macefield et al. 1998; Donadio et al. 2002b). Therefore, the cardiovascular responses to neck vibration in this chapter exhibit different characteristics from that of the arousal reaction.

In this study, two different indicators of vasomotor activity were obtained (i.e., skin and muscle blood flow). Because the results show an increase in FiBF (i.e., an inhibition of skin sympathetic nerve activity) and a reduction in FoBF (i.e., an increase in muscle sympathetic nerve activity), these reactions of skin and muscle vasculature to neck vibration appear contradictory. These phenomena were observed regardless of the side of the neck to which the vibratory stimulus was applied in the present study. Similar observations have also been reported by Cui and colleagues (Cui et al. 1997a; Cui et al. 1999). It was demonstrated that horizontal semicircular canal activation by caloric stimulation resulted in an activation of muscle sympathetic nerve activity (Cui et al. 1997a) and an inhibition of skin sympathetic

nerve activity (Cui et al. 1999). Taken together with the results of these studies (Cui et al. 1997a; Cui et al. 1999) and the current study, the responses of the skin and muscle sympathetic nerve to either caloric vestibular stimulation or neck vibration are presumably opposite. In contrast to evidence on a change in neural control of vascular beds, it is known that acute change in BP alters blood flow to any tissue (subsequently, organs) (Guyton & Hall 2006). Although the current study recorded a change in FoBF as an indirect measure of neural control of muscle vasculature in the forearm, a change in blood perfusion resulted from BP change may lead to misinterpretation of data. The present study showed complex cardiovascular responses to neck vibration; a reduction in BP accompanying with a decrease in FoBF and an increase in FiBF. If a reduction in FoBF was induced just by vasoconstriction in the forearm muscles, it might have resulted in an increase in BP due to an increase in total peripheral resistance. Actually, the function of vasculatures is regulated by not only neral factors but also others including humoral and locally-induced chemical factors (Klabunde 2005). Hence, FoBF might have been altered by either decreased BP or muscle sympathetic nerve, or both while the involvement of other factors in cutaneous (finger) vasculature control needs to be considered besides the different mechanisms of neural control of the vasculature in skin and muscle. Its local factor has been further discussed below.

The current study has not completely refuted the involvement of the arousal effect. This is because this study found that a vibratory stimulus to both the neck and leg was associated with a significant change in skin potential (sweat release). This physiological change is also commonly observed as a consequence of the arousal response (Macefield et al. 1998). In addition, studies have shown that the amplitude of the skin potential change tends to undergo habituation resulting in a reduction in amplitude of the response. However, this habituation phenomenon was found to occur later than 10 times of psychological stimulation (Cariga et al. 2001; Donadio et al. 2005). In deed, it was found that there was no tendency for a decrease

in the occurrence frequency of a significant skin potential change for the totality of vibration trials (Table 5.8). Further, the vibratory stimulus was also delivered three times during the familiarization session before the actual experiment commenced. This means that a total of eight vibration trials were conducted on each participant and there was no evidence of any real habituation of the sweat response observed.

Accompanying sweating, it is believed that sweat gland activity results in the local release of "kallikrein", which is an enzyme to enhance the formation of the vasodilator polypeptide, "kallidin" (bradykinin) (Webster and Pierce 1963, quoted in Kelman 1977, p. 153). This hypothesis is supported by some evidence, however, the relationship between the local release of bradykinin and vasodilation has become controversial because a conflicting observation has been reported more recently (for review see Kellogg 2006). In the latter section of this chapter, a significant change in skin potential was observed more often in the neck vibration trials and an increase in FiBF was coincidentally found. Therefore, besides the effect of the neck vibration itself on cardiovascular function, sudomotor activation occurred as a part of an arousal response and subsequently vasodilation of the finger artery might have occurred due to sweat release-related substances. As a result of local vasodilation, peripheral resistance decreased and BP measured from the finger was decreased. Collectively, the observed cardiovascular responses in the present study (i.e., skin and muscle blood flow) are unlikely artifacts.

5.7.2. Changes in respiration

The possible mechanisms of the cardiovascular responses observed in this study were summarised above. In addition to these, the contribution of respiration to cardiovascular function cannot be disregarded as respiration is known to have a large impact on autonomic regulation of cardiovascular function. Jammes et al. (1981) demonstrated that the application

of a vibratory stimulus to the calf muscles is associated with increases in tidal volume and mean inspiration flow rate in awake humans regardless of the presence or absence of tonic muscle contraction associated with the vibration. Also, stimulation of large afferents from the neck muscles (i.e., muscle spindles and Golgi tendon organs) results in changes in motor outflows to lingual and abdominal musculature, which are related to respiratory control (Bolton et al. 1998). Respiration is well-known to influence autonomic regulation of cardiovascular function. In this latter study, participants breathed spontaneously (there was no paced breathing as was performed during the HRV recording session of the earlier study in this chapter). Therefore, changes in respiratory pattern, volume and/or rate change in response to the onset and cessation of the vibration might have occurred and, in turn, the respiratory-related change might secondarily influence cardiovascular parameters recorded in this study.

5.7.3. Laterality of cardiovascular response in awake humans

The other purpose of this section in this chapter was to confirm whether the cardiovascular responses to unilateral neck vibration were dependent on the side of vibration application. The latter part of this chapter indicated that the side of unilateral neck vibration (right or left) did not affect cardiovascular responses. Kimura et al. (1995) employed a noxious mechanical stimulus (pinching) and showed that cardiac sympathetic nerve responses to the stimulus did not exhibit laterality in CNS intact rats. However, laterality of the response became evident in spinalised rats (Kimura et al. 1995), indicating that sensory integration occurs in the CNS and right-and-left autonomic outflows may already be similar by the time when the sympathetic outflows reach the preganglionic neuron. In addition, Bolton et al. (1998) demonstrated that similar sympathetic nerve activity was recorded in response to both ipsi- and contralateral neck muscle afferent stimulation (presumably originating from muscle spindles and tendon organs) in CNS intact anaestheised cats. Neuroanatomically, it has also been documented that neck sensory afferents project to the ipsi- and controlateral sides of the central cervical nuclei

(Bolton 1998) and of the medial vestibular nuclei (Xiong & Matsushita 2001). Since studies in this chapter employed all healthy young, adults, it may be presumed that all participants were CNS intact and that the neck sensory afferents activated by the vibratory stimulus were bilaterally projected at both spinal and supraspinal levels. Therefore, even though the vibratory stimulus was unilateral (on the right and left), sympathetic outflows to the vasculatures were presumably not different. Consequently, similar cardiovascular responses were induced by application of the vibratory stimulus on the right and left sides of the neck in this chapter.

Chapter 6

The contribution of proprioceptive inputs from the dorsal neck muscles to autonomic regulation of cardiovascular function in the supine posture

6.1. Introduction

Chapters 4 and 5 examined whether somatosensory inputs from the neck have an impact on autonomic regulation of cardiovascular function in awake humans. Particularly, Chapter 5 attempted to dissect the origin of the cardiovascular responses observed in Chapter 4 and therefore focused on the contribution of dorsal neck muscle spindles to autonomic regulation of cardiovascular function. To do so, the muscle conditioning manoeuvre and a vibratory stimulus were employed to preferentially alter the quantity of neck muscle proprioceptive inputs including those from muscle spindles. The vibratory stimulus induced significant cardiovascular responses during and after cessation of the stimulus. On the other hand, the muscle conditioning manoeuvre failed to reveal observable cardiovascular responses in seated humans. It was suspected that the muscle conditioning manoeuvre was unsuccessful because the neck muscles were not completely relaxed during the manoeuvre and data collection periods. Thus, the question of the contribution of neck proprioceptive afferents to cardiovascular function was not resolved.

Several studies have examined the possible contribution of neck afferents to cardiovascular function. A common method used is neck flexion in the side-lying posture in humans (Normand et al. 1997; Ray & Hume 1998; Lee et al. 2001; Watenpaugh et al. 2002). However, reported study results are conflicting. The neck flexion manoeuvre applied in the side-lying position might be expected to alter the quantity of neck sensory inputs while maintaining a constant vestibular signal, although the vestibular apparatus may be activated during head displacement from the midline to neck-flexion positions. It is also important to note that the neck flexion manoeuvre in the side-lying posture stimulates neck sensory receptors including cutaneous, articular, and intramuscular receptors. Concurrently, the head-neck flexion position may stimulate baroreceptors of the carotid arteries transmurally (Bent et al. 2006) even though there is no direct evidence that neck flexion alters intramural pressure

of the carotid arteries and in turn its afferent inputs. Therefore, it is not possible to determine which receptors/structures might be involved in any observed cardiovascular responses to neck flexion in the side-lying posture. Also, activation of multiple sensory receptors potentially counteracts the influence of each afferent input. Hence, further investigation is warranted using different manoeuvres.

Therefore, Chapter 6 aimed to clarify whether neck sensory inputs contribute to autonomic regulation of cardiovascular function during horizontal postures, particularly focusing on the contribution of neck muscle proprioceptive inputs. To achieve this, this chapter examined the application of the muscle conditioning manoeuvre to the dorsal neck muscles while participants lay in the supine position. This manoeuvre, at least when carried out in limb muscles, is capable of systematically altering the quantity of muscle proprioceptive inputs while those of other somatosensory afferents such as cutaneous and articular receptors were presumed to remain the same. Therefore, it was hypothesised that if the muscle conditioning manoeuvre to the neck behaves in the same way as observed in limb muscles (Gregory et al. 1987; Gregory et al. 1990; Polus et al. 1991; Allen et al. 2007) and muscle proprioceptive inputs from the dorsal neck do influence cardiac autonomic drives and cardiovascular function, measured parameters should exhibit conditioning dependent changes.

6.2. Methods

This study was approved by the RMIT Human Research Ethics Committee. All study protocols were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the commencement of the experiment (see Appendix 19 for a Plain Language Statement and Appendix 13 for a consent form). This study consisted of two sessions – heart rate variability (HRV) recording and strain-gauge plethysmograph (SGP) recording sessions.

6.2.1. Participants

Sixteen young adults volunteered and 15 of them completed HRV recording session of this study (one participant failed to complete the study due to difficulty in scheduling experiments after the first day of their participation). The participants (8 males and 7 females) were aged 25.4 ± 4.6 and had a BMI of 22.7 ± 2.5 kg/m^2 (expressed as mean ± SD). Of the 15 participants, 13 participants (7 males and 6 females) completed SGP recording session. They were all in good general health, non-smokers, and not on medication. On the first day of their participation, general health, cardiovascular, and pre-experimental questionnaires were completed (see Appendix 12, 3, and 14, respectively). At the end of each experiment, participants completed a post-experiment questionnaire (see Appendix 15) to reveal any unpleasant or uncomfortable procedures, which might affect results. The level of discomfort was assessed using a visual analogue scale (VAS), where 0 represented "complete comfort" and 10 represented "the worst imaginable pain".

6.2.2. Equipment

As described earlier, a three-lead electrocardiogram (ECG) (in *section 3.2.2*), beat-to-beat blood pressure (BP) using the Portapres® device (in *section 3.2.3*), finger blood flow (FiBF) using a photoplethysmograph, and changes in skin potential (in *section 4.6.2*) were recorded.

Surface electromyogram (sEMG) was employed to monitor neck muscle activity (the paraspinal and sternocleidmostoid muscles). This monitoring was aimed at determining that neck muscles were active and at rest as required. Particularly, relaxing the neck muscles was critical for successful muscle conditioning (see below for the details of muscle conditioning). The details of the sEMG have been described in *section 2.10*.

In addition to these measurements, for the SGP recording session only, a strain-gauge plethysmograph was used to measure muscle blood inflow to the calf (EC6 Plethysmograph, Hokanson Inc, Bellvue, WA, USA). The strain-gauge was attached around the largest circumference of the left calf.

A thigh cuff was positioned proximal to the knee. This cuff was periodically inflated to 50 mmHg during the experiment in order to stop venous return from the calf to the heart without interfering with arterial inflow to the muscles. Thus, the calf progressively swells during thigh cuff inflation and the rate of change in calf volume is used as a measure of arterial outflow to the muscles. In order to enhance venous return during deflation of the thigh cuff, the left heel rested on a stand covered with high density foam. The height of the foot was determined as the point of the calf where the strain-gauge was attached was positioned above heart level. A ten-second inflation-deflation cycle of the thigh cuff was used in the experiment.

All signals were sampled at 1k Hz. Signals of ECG, FiBF, skin potential changes and sEMG were recorded using a data acquisition system, Chart for Windows V 5.4.1 (ADInstruments, Bella Vista, NSW, Australia) after amplification. The signals of the Portapres® and SGP were directly transferred to the PowerLab® (PowerLab/8SP, ADInstruments, Bella Vista, NSW, Australia).

6.2.3. Muscle conditioning

Muscle conditioning was used to manipulate the quantity of muscle proprioceptive afferent inputs from the dorsal neck. The muscles were conditioned in the neck-flexion, -extension, and -intermediate positions. The protocol of muscle conditioning adopted was similar to that of previous studies (Gregory et al. 1987; Gregory et al. 1990; Polus et al. 1991; Allen et al. 2007).

The dorsal neck muscles are predominantly extensors of the head and neck when they contract bilaterally (Cramer & Darby 2005). Thus, it was assumed that hold-flexion conditioning in the present study might best translate to hold-long conditioning and result in a decrease in the quantity of muscle proprioceptive afferent inputs from the dorsal neck. Conversely, the hold-extension conditioning in the present study was thought to be equivalent to hold-short conditioning and result in an increase in the quantity of muscle proprioceptive afferent inputs. Muscle conditioning was performed on an examination table, which had an adjustable head piece allowing neck flexion and extension. In order to induce sufficient neck extension, the cervicothoracic junction was positioned at the fulcrum between the head piece and the rest of the table.

For hold-flexion conditioning, head position was first moved from the head-body straight (the intermediate) position to the neck-flexion position by rotating the head piece in the sagittal plane. Participants were then instructed to contract their dorsal neck muscles for a few seconds (isometric voluntary contraction of the dorsal neck muscles) by extending their head "as if they were looking at the wall behind them". This head position was held for a further five seconds following cessation of the muscle contraction before the head piece and head-neck position were gently and passively returned to the intermediate position. Since table used in this study has only limited range of head piece movement, a foam wedge was attached on the head piece of the table in order to increase the degree of neck flexion.

For hold-extension conditioning, the head piece and head position were lowered to the neck-extension position. An isometric voluntary contraction of the dorsal neck muscles and subsequent relaxation was completed as for hold-flexion conditioning before the head piece and head position were gently returned to the intermediate position.

Hold-intermediate conditioning was carried out while the head-neck remained in the horizontal position. The participant was first asked to perform an isometric voluntary contraction for a period of at least three seconds as described above. The participant was then asked to relax their neck muscles for the remainder of the trial. For hold-intermediate muscle conditioning data collection commenced 15-20 seconds after the end of the voluntary dorsal neck muscle contraction. The variation in the time to commence data collection was because of differences in the time taken to return a participant's head to midline (intermediate) position after hold-flexion or -extension conditioning in each individual. The time delay used for hold-intermediate conditioning for a particular participant reflected the time delay which occurred during the other two forms of the manoeuvre.

6.2.4. Study protocol

In order to familiarise participants with parts of the study protocol including the muscle conditioning manoeuvre and metronome-paced breathing, training sessions were held before data were actually collected. On the day of data collection, participants were asked to fast and abstain from any caffeine-containing beverages for at least 4 hours and to refrain from alcohol consumption and rigorous exercise for at least 12 hours before data collection commenced.

Participants lay supine on the examination table and were secured with a Velcro belt positioned around their upper abdomen for safety during the experiment. The tightness of the belt was adjusted so that it did not impede breathing or the comfort of the participant.

6.2.4.1. Study protocol – heart rate variability recording session

Once participants were comfortable with their posture, they were asked to remain still for 5 minutes. They were then instructed to synchronise their respiratory rate with a metronome (0.25Hz). After 1-minute of controlled breathing, the muscle conditioning manoeuvre was

conducted. As soon as muscle conditioning was completed (generally 20 to 25 seconds) paced breathing and data collection commenced for a period of 5 minutes. At the end of the data collection period, participants were requested to stop paced breathing and relax in the supine position for 5 minutes. The same sequence was repeated for each form of muscle conditioning. The order of muscle conditioning (hold-flexion, -intermediate, or -extension) was randomised prior to the experiment.

6.2.4.2. Study protocol – strain-gauge plethysmogram recording session

Participants were positioned supine on the examination table as described above. However, for these trials, the left foot was place on a support that raised the leg (calf) above heart level. Once participants were comfortable with their posture, they were asked to remain still for 5 minutes. At the final minute of the 5-minute relaxation period, a cuff around the left ankle was inflated at 200 mmHg in order to exclude foot circulation. This pressure was maintained for at least 1 minute until the volume of the calf stabilised (the change in calf volume was viewed on-line on a computer display). Then muscle conditioning of the dorsal neck muscles was performed. As soon as head position was returned to the intermediate position, the thigh cuff was inflated to 50 mmHg and data collection commenced. For hold-intermediate conditioning, the thigh cuff was inflated 15-20 seconds after cessation of isometric contraction of the dorsal neck muscles. Ten seconds after thigh cuff inflation, the cuff was automatically deflated for 10 seconds and then inflated again. This 10-second inflation-and-10-second deflation cycle was repeated 6 times over a 2-minute period. After this time, the ankle cuff was deflated, and the participant rested for a period of 5 minutes before the same sequence was repeated for each form of muscle conditioning.

6.2.5. Data analysis

All cardiovascular parameters (heart rate; HR, BP, FiBF, and calf blood flow), obtained following the onset of each form of muscle conditioning (hold-flexion, -intermediate, and -extension), were averaged into 1-minute periods. Heart rate variability analysis was based on the full 5-minute data.

Since the primary purpose of this study was to investigate the contribution of muscle proprioceptive inputs from the dorsal neck to autonomic regulation of cardiovascular function and the muscle conditioning manoeuvre was employed to alter the quantity of these inputs in a systematic way, cardiovascular parameters measured in this chapter were compared between hold-flexion and -extension conditioning. The consequence of hold-intermediate conditioning was assumed to eliminate "slack" in muscle spindles and to create a muscle history at the prevailing length of the muscle (Wise et al. 1998). Therefore, values of parameters following hold-intermediate conditioning were used as a reference. If the neck muscle proprioceptive inputs participate in autonomic regulation of cardiovascular function, the parameters should exhibit dependency to the forms of the muscle conditioning manoeuvre relative to the values obtained following hold-intermediate conditioning. To examine this, values measured following hold-intermediate conditioning were subtracted from those following hold-flexion (Δ_{flex}) or -extension (Δ_{ext}) conditioning. This analytical procedure has been used in a previous study, which examined the effect of muscle conditioning on muscle spindle resting discharge of the lumbar muscles in anaesthetised cats (Ge et al. 2005). Statistical analysis for comparison of cardiovascular parameters (HR, BP, FiBF, and calf blood flow) between Δ_{flex} and Δ_{ext} was carried out using a paired t-test or Wilcoxon signed-rank test based on normality assessment using a Kolmogorov-Smirnov test.

Due to the primary purpose of this study, it is not appropriate to compare cardiovascular parameters obtained prior to establishing known muscle history. However, cardiovascular function is controlled by multiple regulatory systems. Further, even though the order of conditioning manoeuvres was randomised, it is possible that cardiovascular changes occurred over the data collection period. It was important to establish that cardiovascular function did not alter for the duration of the experiment. Therefore, cardiovascular parameters before the application of either form of muscle conditioning were compared. For this analysis, a one-way repeated measures analysis of variance (ANOVA) with Bonferroni's correction was employed after normal distribution was confirmed. For all statistical analyses, significance level was set at $p < 0.05$.

In addition to cardiovascular parameters, skin potential change was recorded since sudomotor activation can be seen as a consequence of an arousal response (Macefield et al. 1998). The evaluation of skin potential change has been described in *section 2.6*.

6.3. Results

According to the pre-experiment questionnaire, 3 participants reported a mild to moderate level of neck stiffness (VAS = 2.1-3.2). One participant reported a mild level of symptoms as recorded by the VAS (a score of 2), but this participant did not specify whether this score reflected neck stiffness or pain. Two participants reported neck stiffness (VAS = 1 and 2) and another participant experience discomfort on their shoulders during experiment (VAS = 5) in the post-experimental questionnaire. The results of the one-way repeated measures ANOVA with *post hoc* Bonferroni's correction showed that there were no differences in any cardiovascular parameters among the three forms of muscle conditioning before the muscle conditioning manoeuvre.

6.3.1. Comparisons of cardiovascular parameters between hold-flexion and -extension conditioning obtained during the heart rate variability recording session

Cardiovascular parameters (HR, BP, and FiBF) were compared between hold-flexion (Δflex) and -extension (Δext) conditioning.

Systolic blood pressure following hold-extension conditioning was significantly higher than hold-flexion conditioning [t (14) = -2.49, p = 0.026, d = 0.64]. Otherwise, there was no difference in the cardiovascular parameters between the two forms of muscle conditioning.

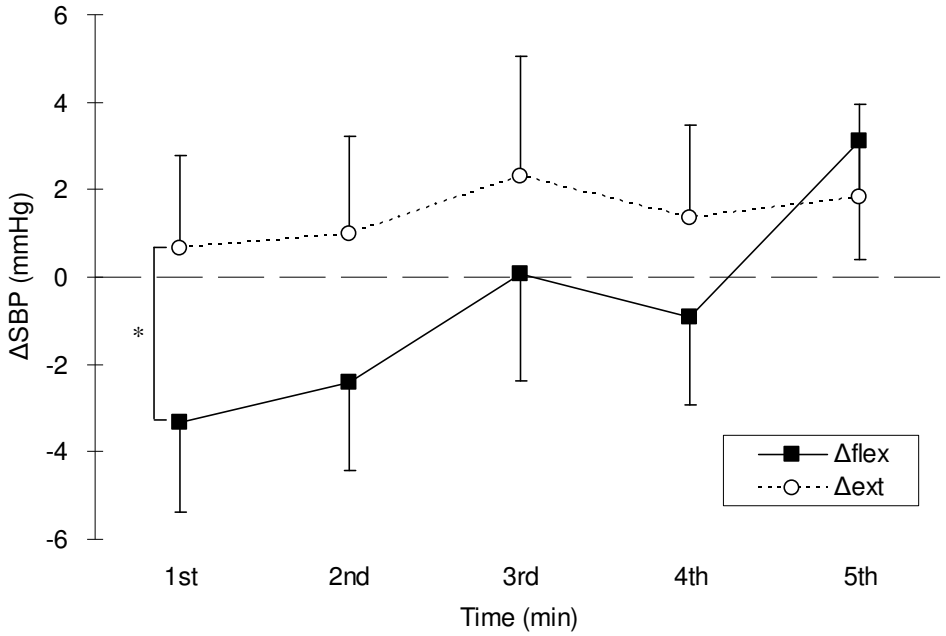

Figure 6.1. Muscle conditioning-dependent change in systolic blood pressure during the controlled breathing period in the supine posture.
Data are presented as mean ± SEM SBP = systolic blood pressure. Δflex = values of SBP following hold-intermediate conditioning were subtracted from values recorded after hold-flexion conditioning. Δext = values of SBP following hold-intermediate conditioning were subtracted from values recorded after hold-extension conditioning. Asterisk (*) indicates a significant difference between Δflex and Δext ($p < 0.05$).

Table 6.1. Comparisons of cardiovascular parameters between hold-flexion and -extension conditioning during heart rate variability recording session

parameters	conditioning	1 minute	2 minute	3 minute	4 minute	5 minute
DBP	**Δflex**	-1 ± 1	0 ± 1	1 ± 1	0 ± 1	1 ± 1
(mmHg)	**Δext**	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1
SBP	**Δflex**	-3 ± 2*	-2 ± 2	0 ± 2	-1 ± 2	3 ± 3
(mmHg)	**Δext**	1 ± 2	1 ± 2	2 ± 3	1 ± 2	2 ± 2
MAP	**Δflex**	-2 ± 1	-1 ± 1	0 ± 2	0 ± 1	2 ± 2
(mmHg)	**Δext**	1 ± 2	1 ± 1	2 ± 2	1 ± 1	1 ± 2
HR (bpm)	**Δflex**	1 ± 1	1 ± 1	0 ± 1	0 ± 1	-1 ± 1
	Δext	1 ± 1	1 ± 1	1 ± 1	0 ± 1	0 ± 1
FiBF	**Δflex**	0.03 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.01
(mV/s)	**Δext**	0.01 ± 0.02	0.02 ± 0.02	0.01 ± 0.02	0.00 ± 0.02	-0.01 ± 0.02

Data are presented as mean ± SEM DBP = diastolic blood pressure, SBP = systolic blood pressure, MAP = mean arterial pressure, HR = heart rate, FiBF = finger blood flow. Δflex = values of cardiovascular parameters following hold-intermediate conditioning were subtracted from values recorded after hold-flexion conditioning. Δext = values of cardiovascular parameters following hold-intermediate conditioning were subtracted from values recorded after hold-extension conditioning. Asterisk (*) indicates a significant difference between Δflex and Δext ($p < 0.05$).

6.3.2. Comparisons of cardiovascular parameters between hold-flexion and -extension conditioning obtained during strain-gauge plethysmogram recording session

Cardiovascular parameters were also recorded during the strain-gauge plethysmograph recording session (n = 13). There were no significant differences in all parameters between the two forms of muscle conditioning.

Table 6.2. Comparisons of cardiovascular parameters between hold-flexion and -extension conditioning during strain-gauge plethysmogram recording session

parameters	conditioning	1 minute	2 minute
DBP	**Δflex**	0 ± 2	2 ± 2
(mmHg)	**Δext**	-1 ± 1	0 ± 1
SBP	**Δflex**	-3 ± 3	1 ± 3
(mmHg)	**Δext**	-2 ± 3	0 ± 2
MAP	**Δflex**	-1 ± 3	1 ± 3
(mmHg)	**Δext**	-2 ± 2	0 ± 2
HR (bpm)	**Δflex**	-1 ± 1	0 ± 1
	Δext	-1 ± 1	0 ± 1
FiBF	**Δflex**	0.03 ± 0.01	0.01 ± 0.01
(mV/s)	**Δext**	0.02 ± 0.01	0.002 ± 0.01

Data are presented as mean ± SEM DBP = diastolic blood pressure, SBP = systolic blood pressure, MAP = mean arterial pressure, HR = heart rate, FiBF = finger blood flow. Δflex = values of cardiovascular parameters following hold-intermediate conditioning were subtracted from values recorded after hold-flexion conditioning. Δext = values of cardiovascular parameters following hold-intermediate conditioning were subtracted from values recorded after hold-extension conditioning.

6.3.3. Comparisons of heart rate variability parameters between hold-flexion and -extension conditioning

The indicators of cardiac autonomic nervous drives, HRV parameters, were compared between the two forms of muscle conditioning. These parameters were obtained from ECG signals recorded over a 5-minute period. Paired t-tests showed that there were no significant differences in HRV parameters between the two forms of muscle conditioning.

Table 6.3. Comparisons of heart rate variability parameters between hold-flexion and -extension conditioning conditioning

parameters	Δflex	Δext	*T*	*p*	*d*
TP (ms^2)	-497.64 ± 699.31	572.85 ± 464.50	-1.57	0.14	0.41
LF (ms^2)	-150.20 ± 190.64	106.31 ± 133.55	-1.54	0.15	0.40
LF (nu)	-1.54 ± 2.75	3.05 ± 2.56	-1.57	0.14	0.41
HF (ms^2)	-114.77 ± 102.31	-193.62 ± 141.29	0.56	0.59	0.14
HF (nu)	1.31 ± 2.72	-3.46 ± 2.55	1.58	0.14	0.41
LF/HF	0.02 ± 0.13	0.10 ± 0.11	-0.53	0.61	0.14

Data are presented as mean ± SEM *d* = Cohen's *d*, TP = total power, LF = low frequency power, HF = high frequency power, LF/HF = the ratio of low frequency power to high frequency, ms^2 = millisecond square, nu = normalised unit. Δflex = values of heart rate variability parameters following hold-intermediate conditioning were subtracted from values recorded after hold-flexion conditioning. Δext = values of heart rate variability parameters following hold-intermediate conditioning were subtracted from values recorded after hold-extension conditioning.

6.3.4. Comparisons of calf blood flow between hold-flexion and -extension conditioning

Data of calf blood flow was obtained from 13 participants. Calf blood flow was not significantly different between the two forms of muscle conditioning at both the first and second minutes [t (12) = -0.99, p = 0.34, d = 0.27 and t (12) = -1.30, p = 0.22, d = 0.36, respectively].

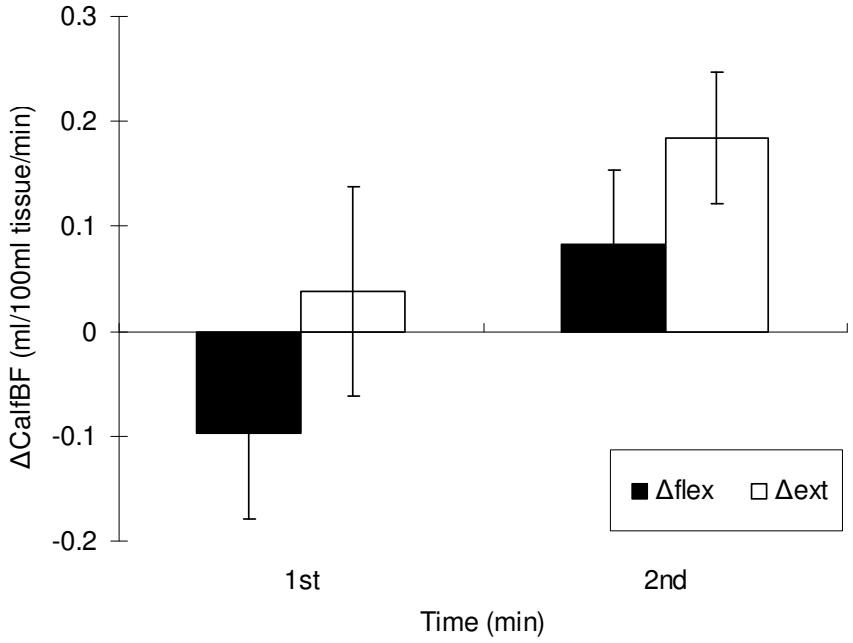

Figure 6.2. Comparisons of calf blood flow between hold-flexion and -extension conditioning at the first and second minutes following each form of muscle conditioning.
Data are presented as mean ± SEM. CalfBF = calf blood flow, Δflex = values of calf blood flow following hold-intermediate conditioning were subtracted from values recorded after hold-flexion conditioning. Δext = values of calf blood flow following hold-intermediate conditioning were subtracted from values recorded after hold-extension conditioning.

6.3.5. Skin potential change and muscle conditioning

A significant change in skin potential was found in three out of 15 participants during the HRV recording session and in seven out of 13 participants during the SGP recording session. During the HRV recording session, significant skin potential change was found in three participants following hold-extension conditioning, in two of these participants following hold-flexion conditioning, and in one of these participant following hold-intermediate conditioning. In these three participants, a change in skin potential was observed during the third trial, during the first trial in two participants, and during the second trial in one participant only. Therefore, skin potential change session was observed on rare occasion and randomly during the HRV recording.

During the SGP recording session, skin potential change occurred more frequently following hold-extension conditioning (in six participants), compared with hold-intermediate and -flexion conditioning, (one and two participants, respectively). Because the order of the form

of the muscle conditioning manoeuvre was randomised, the sequence of the manoeuvre may not influence skin potential change. It was confirmed that in these seven participants, a change in skin potential was observed during the first trial in four participants, during the second trial in two participants, and during the third trial in three participants.

6.4. Discussion

The purpose of Chapter 6 was to re-examine whether neck muscle proprioceptive inputs influence autonomic regulation of cardiovascular function during lying postures by means of the muscle conditioning manoeuvre. One of the possibilities for the conflicting results in the previous studies (Normand et al. 1997; Ray & Hume 1998; Lee et al. 2001; Watenpaugh et al. 2002) was due to undetermined sensory inputs, which may counteract the influence of each afferent input involved during the neck flexion manoeuvre. Therefore, this chapter employed the muscle conditioning manoeuvre which aimed to alter the quantity of neck muscle proprioceptive inputs rather than undefined sensory inputs from the neck. However, although SBP appeared to show a muscle conditioning-dependent change during the HRV recording session, most parameters did not change significantly between the two forms of muscle conditioning; that is, hold-flexion and -extension conditioning.

6.4.1. Muscle conditioning

The application of the muscle conditioning manoeuvre to the dorsal neck was aimed to examine the contribution of muscle proprioceptive afferent inputs to cardiovascular regulation while other sensory inputs did not alter such as cutaneous and articular receptors. Thus, it was assumed that measured parameters might have exhibited the dependency of the form of the muscle conditioning manoeuvre if the manoeuvre to the neck behaved in the same way as observed in limb muscles (Gregory et al. 1987; Gregory et al. 1990; Polus et al. 1991; Allen et

al. 2007). However, muscle conditioning-dependent changes in most of parameters were not seen in this study. There are two possible explanations on this result related to the manoeuvre.

First of all, it is not possible to determine whether neck muscle conditioning was functional from data of the present study. Although it has been shown that head position sense may be influenced by neck muscle conditioning in humans (Owens et al. 2006; Repka & Polus 2008), there is no direct evidence that the manoeuvre to the neck muscles alter the quantity of neck proprioceptive inputs in a systematic way. Compared with the limb muscles such as the biceps brachii and triceps surae muscles used in previous studies (Wise et al. 1996; Allen et al. 2007), the stream of individual dorsal neck muscle is unique. For example, the semispinalis capitis and semispinalis cervicis muscles lie longitudinally from the head or cervical spine while there are a large number of muscles attached obliquely such as obliquus capitis inferior and superior and splenius capitis muscles (Cramer & Darby 2005). Thus, it is unknown how the complex architecture of the dorsal neck muscles affects the success of the muscle conditioning manoeuvre. Therefore, it was unknown whether the muscle conditioning manoeuvre in this study was successful.

Secondly, sEMG was used to monitor neck muscle activity in this study. Surface EMG is a convenient technique to measure muscle activity because it is non-invasive and facile. On the other hand, the neck muscles, are layered and only superficial layers of the muscles can be measured by the sEMG (Sommerich et al. 2000a). Therefore, it was not possible to know whether the deep neck muscles were active or passive as required for the muscle conditioning manoeuvre.

From the results in Chapter 5 and 6, it is still not possible to conclude that muscle conditioning is not functional in the neck because the outcome parameters used in this study

were not a direct measure of the quantity of afferent inputs to the CNS. Therefore, further investigation is essential for this matter.

6.4.2. Involvement of muscle small diameter afferents

The neck flexion manoeuvre in the side-lying posture used in previous studies (Normand et al. 1997; Ray & Hume 1998; Lee et al. 2001; Watenpaugh et al. 2002) may involve mechanosensitive receptor input via group III fibres from the muscles. This is because it is well-known that muscle afferents in these groups have a significant impact on cardiovascular function, whereas larger diameter muscle afferents (i.e., group I and II) are not involved in cardiovascular regulation (for review see Sato et al. 1997a). The mechanosensitive receptors are activated by muscle stretch and pressure as demonstrated in studies where calf muscle stretch was associated with an increase in HR in humans (Gladwell & Coote 2002; Gladwell et al. 2005). In addition, Cui et al. (2006) demonstrated that calf muscle stretch increased HR as well as BP and muscle sympathetic nerve activity. These experiments were carried out in the absence of pain accompanying the muscle stretch, so that these cardiovascular responses were presumably due to activation of muscle mechanoreceptors connecting with group III fibres.

The above discussion was based on studies that used limb muscles but to my knowledge there is no study that has examined the participation of group III afferent inputs from the neck muscles (i.e., mechanosensitive receptors) in cardiovascular regulation. However, it was demonstrated that activation of small diameter fibres of the neck (by isometric muscle contraction) resulted in increased HR, BP, and muscle sympathetic nerve activity at both 10 and 30 % maximum voluntary contraction (Steele & Ray 2000). Although cardiovascular response to muscle contraction likely involves both mechanoreceptor activation and the influence of central commands (see discussion in Cui et al. 2006), Steele and Ray's study

(2000) may indicate that stimulation of mechanosensitive receptors (via group III fibres) in the neck also induces cardiovascular responses.

In addition, muscle conditioning alters not only the quantity of muscle proprioceptive inputs but also the resting tension of the muscle (Whitehead et al. 2001). There might be a possibility that the muscle conditioning manoeuvre induced sufficient tension in the neck muscles to activate smaller diameter muscle afferent nerves (group III fibre). However, since significant cardiovascular responses were not observed, it must be concluded that muscle conditioning did not activate group III afferent fibres in this study. Further investigation is required before any conclusions may be drawn about the involvement of group III afferents associated with the muscle conditioning manoeuvre.

6.4.3. The significance of neck muscle proprioceptive inputs

Physiologically speaking, it may be necessary to consider how important neck muscle proprioceptive inputs are at rest. In this chapter, the muscle conditioning manoeuvre was performed in the supine posture and the posture was maintained during data collection following the head being returned to the intermediate (original) position. Therefore, if the muscle conditioning manoeuvre in this study was effective as demonstrated previously in the limb muscles (for review see Proske et al. 1993, see also section 2.9.4.), the central nervous system may interpret the positional relationship between the head and the rest of the body as head-neck flexion following hold-extension conditioning and head-neck extension following hold-flexion conditioning. However, in this circumstance, the cardiovascular system does not need to re-distribute body fluid because the head-neck displacement (but not body movement) may not cause significant body fluid movement. On the other hand, when the vestibular apparatus, especially the otolith organ, is activated, cardiovascular function more likely requires to be regulated. This is because the vestibular activation is induced by a posture

change and consequently body fluid shift such as occurs when moving from lying to standing. Postural control-related reflexes occur as a result of integration of sensory information from the neck and vestibular apparatus. Similarly, it has been proposed that the contribution of neck muscle proprioceptive inputs to autonomic regulation of cardiovascular function may be evident when the vestibulo-sympathetic reflex occurs (Bolton et al. 2006). Therefore, the influence of muscle proprioceptive inputs from the neck to the cardiovascular control may only become evident associated with orthostasis (i.e., vestibular activation).

In the next chapter, the contribution of muscle proprioceptive inputs from the neck to autonomic regulation of cardiovascular function will be examined during mild orthostatic stress to engage the vestibular and cardiovascular regulatory systems.

Chapter 7

The contribution of proprioceptive inputs from the dorsal neck muscles to autonomic regulation of cardiovascular function during a mild orthostatic stress

7.1. Introduction

The studies in Chapter 5 and 6 were carried out with participants in a resting condition (sitting or supine). Neither cardiac autonomic drive nor cardiovascular parameters altered remarkably in response to the muscle conditioning manoeuvre while only small changes were observed in response to neck vibration, which induces a large and possibly unphysiological change in neck proprioceptive inputs to the central nervous system (CNS). Thus, it was proposed that the signals from neck afferent might be inhibited under resting conditions and therefore not influence autonomic and cardiovascular function. Since significant cardiovascular changes are not required when body position remains still, the results observed in Chapters 5 and 6 may be biologically sound.

On the other hand, the significance of neck sensory inputs to the CNS is evident when posture control-related responses occur. Associated with posture control, sensory inputs from the vestibular apparatus are integrated centrally with proprioceptive inputs from the neck (Rubin et al. 1975). Because posture change may induce the gravitational re-distribution of blood towards feet, cardiovascular function needs to be re-adjusted. Now, it is known that the vestibular system detects head position and motion in space as well as influences autonomic and cardiovascular functions (see *section 2.1*). Accordingly, it may be hypothesised that neck afferent inputs also participate in autonomic regulation of cardiovascular function associated with posture change as the sensory integration of signals from the vestibular apparatus and neck is known to occur in posture control.

Therefore, the purpose of the study in Chapter 7 was to investigate whether proprioceptive inputs from the neck muscles contribute to autonomic regulation of cardiovascular function associated with orthostasis in awake humans. Since the baroreflex is an important feedback mechanism for cardiovascular adjustments during posture change, there is a latency before

cardiovascular responses to baroreceptor input are revealed, estimated to be as long as 10 seconds (Denise et al. 2007). Hence, it is thought that the vestibular system is the primary contributor to the initial phase of cardiovascular adjustment following orthostasis (Olufsen et al. 2006, p. 1363). Therefore, it was hypothesised that, by focusing on the early (initial 15 seconds) period of cardiovascular adjustment after orthostasis, the interaction and contribution of neck proprioceptive inputs to cardiovascular control to vestibular-induced cardiovascular regulation could be examined.

7.2. Methods

This study was approved by the RMIT Human Research Ethics Committee. All study protocols were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the commencement of the experiment (see Appendix 20 for a Plain Language Statement and Appendix 13 for a consent forms). This study consisted of two sessions – heart rate variability (HRV) recording and strain-gauge plethysmograph (SGP) recording sessions.

7.2.1. Participants

Sixteen young adults volunteered and 14 of them involved in this study while two were excluded; one due to difficulty in scheduling experiments after the first day of their participation and the other due to falling asleep during data collection. Participants (seven males and seven females) were aged 25.7 ± 4.6 and had a BMI of 22.7 ± 2.6 kg/m^2 (expressed as mean ± SD). Fourteen participants participated in HRV recording session and 10 of them completed SGP recording session also. When 2 of the 4 participants, who did not involve in the SGP recording session, withdrew their participation due to leg muscle tightness during left leg rise and the SGP was not available when the others were being involved in this study. They were all in good general health, non-smokers, and not on medication. On the first day of

their participation, general health, cardiovascular, and pre-experimental questionnaires were completed (see Appendix 12, 3, and 14, respectively). At the end of each experiment, participants completed a post-experiment questionnaire (see Appendix 15) to reveal any unpleasant or uncomfortable procedures, which might affect results. The level of discomfort was assessed using a visual analogue scale (VAS), where 0 represented "complete comfort" and 10 represented "the worst imaginable pain".

7.2.2. Equipment

As described earlier, a three-lead electrocardiogram (ECG) (in *section 3.2.2*), beat-to-beat blood pressure (BP) using the Portapres® device (in *section 3.2.3*), finger blood flow (FiBF) using a photoplethysmograph, and changes in skin potential (in *section 4.6.2*) were recorded. Also, the details of surface electromyogram (sEMG) and SGP for calf blood flow measurement appeared in *section 6.2.2*.

7.2.3. Muscle conditioning

The details of muscle conditioning appeared earlier (see *section 6.2.3*).

7.2.4. Study protocol

In order to familiarise participants with parts of the study protocol including the muscle conditioning manoeuvre, training sessions were held before data were actually collected. On the day of data collection, participants were asked to fast and abstain from any caffeine-containing beverages for at least 4 hours and to refrain from alcohol consumption and rigorous exercise for at least 12 hours before data collection commenced. The order of forms of muscle conditioning (hold-flexion, -intermediate, and -extension conditioning) was randomised before the experiment commenced, but HRV recording session was always

carried out first. Then, SGP recording session was run with the opposite order of forms of muscle conditioning.

During the experiment, participants lay supine on the tilt table and were secured with a Velcro belt positioned around their upper abdomen for safety during the head-up tilt (HUT). To minimise clues of their body schema, participants were blindfolded and a thick high density foam block (Clark Rubber, Bundoora, VIC, Australia) was placed under their feet.

7.2.4.1. Study protocol – heart rate variability recording session

For HRV recording session, once participants were comfortable with their posture, they were asked to remain still for 5 minutes. They were then instructed to synchronise their respiratory rate with a metronome (0.25Hz). After 1-minute of controlled breathing, the muscle conditioning manoeuvre was conducted. Each participant was tilted on three occasions following a different form of muscle conditioning on each occasion. As soon as muscle conditioning was completed (generally 20 to 25 seconds), participants were tilted up (to 20 degrees) and recommenced paced breathing for a further 5 minutes. After this period, they were tilted back to the supine position and rested for 5 minutes. The same sequence was repeated for each form of muscle conditioning.

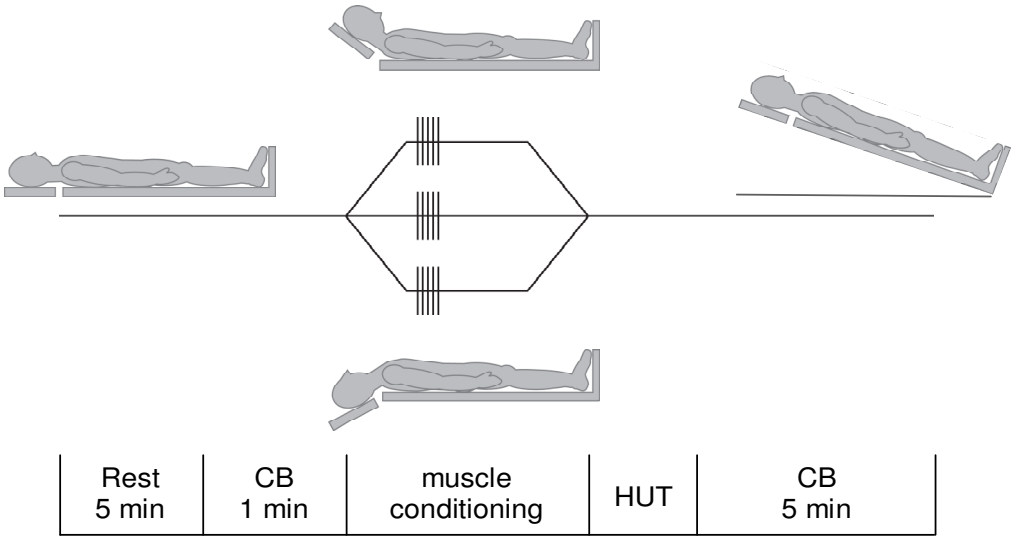

Figure 7.1. Experiment protocol.
This sequence was applied to each occasion of each form of muscle conditioning. Trace in the middle of the figure represents the length of dorsal neck muscles and spikes in the trace indicate muscle contraction. Note that the window width of experiment protocol description on the bottom of the figure does not accord with the time window. CB = controlled breathing, HUT = head-up tilt.

7.2.4.2. Study protocol – strain-gauge plethysmograph recording session

For SGP recording session, participants lay in supine on the tilt table and the left leg was raise. The calf was positioned above the heart level when participants were tilted. However, if participants felt excessive stretch of their leg muscles, the calf position was lowered as the calf position was higher than the pelvic in the tilted position. Once participants were comfortable with their posture, they were asked to remain still for 5 minutes. At the final minute of the 5-minute relaxation period, a cuff around the left ankle was inflated at 200 mmHg in order to exclude foot circulation. This pressure was maintained for at least 1 minute until the volume of the calf stabilised (the change in calf volume was viewed on computer display). Then muscle conditioning was performed to the dorsal neck muscles. As soon as the head position was returned to the intermediate position, participants were tilted up as conducted in HRV recording session. Immediately after tilt table had reached to 20° and stopped, another cuff wrapped around the thigh was inflated at 50 mmHg. Ten seconds after the cuff inflation, automatically the cuff was deflated for 10 seconds and inflated again. This 10-second inflation-and-10-second deflation cycle was repeated 6 times over 2 minutes. After

the measurement, participants were tilted back to supine posture, the ankle cuff was deflated, and a 5-minute relaxation period was provided. The same sequence was repeated for each form of muscle conditioning.

7.2.5. Data analysis

All obtained data were stored on a computer and analysed using Chart software (Chart for Windows V5.1.1, ADInstruments, Bella Vista, NSW, Australia).

7.2.5.1. Cardiovascular function

In order to evaluate whether changes in the quantity of proprioceptive afferent inputs from dorsal neck muscles induced different cardiovascular responses to a mild orthostatic stress, the recorded cardiovascular variables of BP, heart rate (HR), and FiBF were averaged into 5-second bins and expressed as % change with respect to the averaged 30-second value of the pre-conditioning period (100%). An average of the final 5 seconds of the pre-conditioning period, which was also expressed as % change with respect to the averaged 30-second pre-conditioning value, was compared with each of three periods (5, 10, and 15 seconds after HUT). Heart rate responses were followed-up to 5 minutes. Heart rate was averaged into 1-minute bins and expressed as % change (of baseline as defined above).

7.2.5.2. Heart rate variability analysis (1 minute)

Heart rate variability is generally accepted as an indicator of the balance of autonomic nervous drive to the heart (Task Force 1996). In order to investigate the effect of an alteration in neck proprioceptive afferent inputs from the dorsal neck muscles on cardiac autonomic nervous function, HRV analysis was performed on 1-minute epochs of the ECG signal recorded 1 minute before the muscle conditioning manoeuvre and periods of 2 to 5 minutes after HUT (Chart for Windows V5.1.1 with HRV extension V1.0.1, ADInstruments, Bella

Vista, NSW, Australia). A one-minute recording is the minimal requirement to determine the HF component of the power spectrum of HRV using conventional frequency domain analysis (Task Force 1996). Thus, this study reported total power (TP) and both absolute and normalised values of high frequency (HF) power (bandwidth; 0.15-0.4Hz) which indicates vagal drive to the heart (Task Force 1996). In addition to the frequency domain analysis, results of the time domain analysis were also reported; that is SDNN (standard deviation of all NN intervals) and RMSSD (the square root of the mean of the sum of the squares of differences between adjacent NN intervals). The SDNN and RMSSD may be interpreted in the same way as TP and HF, respectively (Task Force 1996). Each value was expressed as % change during HUT with respect to pre-conditioning values which were expressed as 100%.

7.2.5.3. Heart rate variability analysis (5 minutes)

In addition to 1-minute HRV analysis, 5-minute HRV parameters were also obtained such as both absolute and normalised values of low frequency (LF) power and the ratio of LF to HF (LF/HF). In order to normalise HRV parameters, these parameters following hold-flexion and -extension conditioning were subtracted by values of hold-intermediate conditioning. The subtracted values were then compared between the two forms of muscle conditioning.

7.2.5.4. Strain-gauge plethysmogram analysis

In order to examine whether blood inflow to the calf muscles is dependent on the quantity of muscle proprioceptive inputs from the dorsal neck, SGP was used. Data was obtained every 20 seconds over 2 minutes and three 20-second data were averaged. Therefore, two 1-minute data were obtained in each form of muscle conditioning. In order to normalise individual variability of this data, SGP data following hold-flexion and -extension conditioning were subtracted by hold-intermediate conditioning. Then, comparisons were made between the subtracted values at the first and second minutes in the HUT position.

7.2.5.5. Skin potential change

In addition to the cardiac autonomic drive and cardiovascular parameters, skin potential change was recorded since sudomotor activation can be seen as a consequence of arousal response (Macefield et al. 1998). Skin potential change was compared between immediately before the muscle conditioning manoeuvre was commenced and after HUT was completed following the manoeuvre. The evaluation of skin potential change has been described in *section 2.6*.

7.2.6. Statistical analysis

All statistical analysis was carried out using the statistical software package SPSS (V15.0 for Windows, SPSS Inc., U.S.A). Statistical significance was set at $p < 0.05$ for all analyses.

7.2.6.1. Blood pressure, heart rate, and finger blood flow

Prior to the statistical analysis, normality of the data was assessed using a Kolmogorov-Smirnov test. Based on the results of the normality assessment, statistical analyses for BP, HR, and FiBF were carried out using a one-way repeated measures analysis of variance (ANOVA), Friedman test, paired t-test, or Wilcoxon signed-rank test. In order to avoid a type I error, α level was adjusted with Bonferroni correction (Munro 2001). In addition, for 1-minute HR data, the *F* statistic was used to assess differences in the extent of the variation of HR in response to HUT among different forms of muscle conditioning, since group averages of HR response following each muscle conditioning seemed quite similar but standard deviations appeared to be different (see Appendix for *F* value determination).

7.2.6.2. Heart rate variability (1 minute)

To examine changes in HRV parameters between the supine and HUT positions (2 to 5 minutes) in each conditioning trial, a one-sample t-test was used (to examine whether the

value significantly differed from 100 %) and α level was adjusted by Bonferroni correction. Further, to examine differences in HRV parameters among the three different forms of muscle conditioning, HRV parameters were compared using a one-way repeated measures ANOVA, Friedman test, paired t-test or Wilcoxon signed-rank test (depending on the result of the normality assessment) and α level was adjusted by Bonferroni correction.

7.2.6.3. Heart rate variability (5 minutes) and strain-gauge plethysmograph

In order to examine the muscle conditioning dependency of cardiac autonomic nervous activity and calf muscle blood flow, a paired t-test or Wilcoxon signed-rank test was used as normality assessment indicated.

7.3. Results

No participant reported neck pain before or after the experiment or any discomfort associated with the muscle conditioning manoeuvre, measurement, or other procedures (e.g. paced breathing and HUT).

7.3.1. Heart rate response to head-up tilt (< 15 seconds)

Responses of HR to 20-degree HUT following the three muscle conditioning are presented in Figure 7.2.

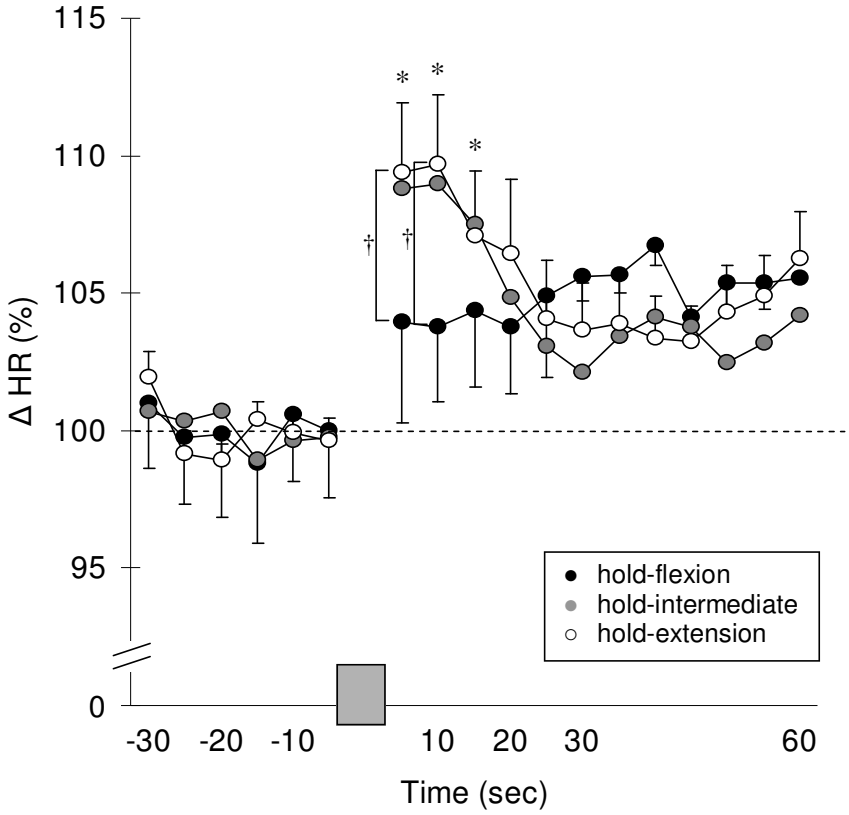

Figure 7.2. Different heart rate responses to head-up tilt following different forms of neck muscle conditioning
The muscle conditioning manoeuvre and 20° head-up tilt were executed during the period of the shaded box. Data were averaged over 5 seconds and expressed as mean ± SEM 100% refers to the average of pre-tilt value (for 30 seconds) in each condition. HR = heart rate, sec = second. Asterisk (*) indicates significant difference from pre-tilt value. Cross (†) indicates significant difference between hold-flexion and -extension conditioning. Following hold-extension conditioning, HR significantly increased and its increase was sustained for 15 seconds. Change in HR for hold-intermediate conditioning was significant only at 15 seconds after 20° head-up tilt.

Significant time effects for HR were found following hold-intermediate and -extension conditioning [Wilks'Λ = 0.49, $F(3,11) = 3.82$, $p = 0.043$, $\eta^2 = 0.51$ and $\chi^2(3, N = 14) = 22.54$, $p < 0.001$, respectively], but not following hold-flexion conditioning [Wilks'Λ = 0.83, $F(3,11) = 0.77$, $p = 0.54$, $\eta^2 = 0.17$]. Pairwise comparisons showed that HR significantly increased at 15 seconds after HUT following hold-intermediate [$t(13) = -3.46$, $p = 0.025$, $d = 0.92$] and -extension [at 5 seconds; $t\ (13) = -4.19$, $p = 0.001$, $d = 1.12$, at 10 seconds; $z\ (14) = -3.11$, $p = 0.002$, at 15 seconds; $t\ (13) = -3.54$, $p = 0.004$, $d = 0.95$] conditioning.

The result of the repeated measures ANOVA showed that there was no difference in HR among the three different forms of muscle conditioning at baseline [Wilks'Λ = 0.99, $F(2,12)$ = 0.055, p = 0.95, η^2 = 0.009]. There were significant differences between hold-flexion and -extension conditioning at 5 and 10 seconds after HUT [t (13) = -3.54, p = 0.044, d = 0.75 and z (14) = -2.54, p = 0.011, respectively], but not at 15 seconds.

7.3.2. Mean arterial pressure response to head-up tilt (< 15 seconds)

Responses of mean arterial pressure (MAP) to the moderate HUT following each muscle conditioning are presented in Figure 7.3 below.

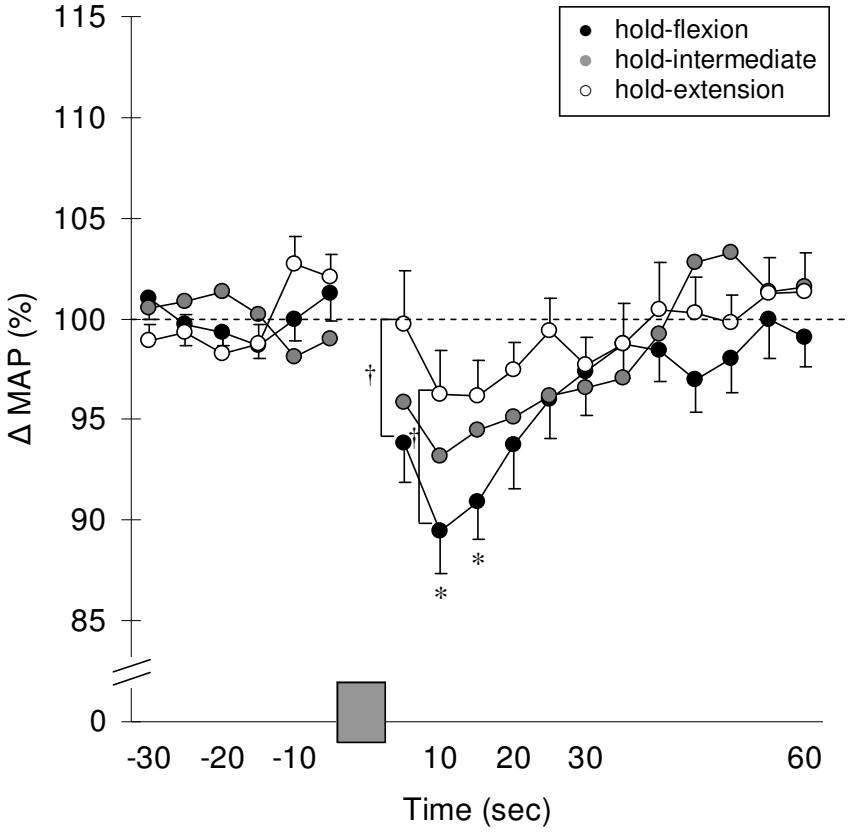

Figure 7.3. Different mean arterial pressure responses to head-up tilt following different forms of neck muscle conditioning.
The muscle conditioning manoeuvre and 20° head-up tilt were executed during the period of the shaded box. Data were averaged over 5 seconds and expressed as mean ± SEM 100% refers to the average of pre-tilt value (for 30 seconds) in each condition. MAP = mean arterial pressure, sec = second. Asterisk (*) indicates significant difference from pre-tilt value. Cross (†) indicates significant difference between hold-flexion and -extension conditioning. Following hold-flexion conditioning, MAP significantly decreased. Change in MAP for hold-extension and -intermediate conditioning was not significant.

There were significant time effects for MAP following hold-flexion [$\chi^2(3, N = 14) = 18.34$, $p < 0.001$], -intermediate [$\chi^2(3, N = 14) = 9.69$, $p = 0.021$], and -extension [Wilks'$\Lambda = 0.24$, $F(3,11) = 11.48$, $p = 0.001$, $\eta^2 = 0.76$] conditioning. Further comparisons revealed significant reductions in MAP at 10 and 15 seconds after HUT following hold-flexion conditioning [t (13) = 4.73, $p < 0.001$, $d = 1.26$ and t (13) = 3.76, $p = 0.002$, $d = 1.00$, respectively] but not hold-intermediate or -extension conditioning.

The result of the repeated measures ANOVA showed that there was no difference in MAP among the three different forms of muscle conditioning at baseline [Wilks'$\Lambda = 0.72$, $F(2,12) = 2.31$, $p = 0.14$, $\eta^2 = 0.28$]. Further comparisons showed that MAP was significantly different between hold-flexion and -extension conditioning at 5 and 10 seconds after HUT [z (14) = -2.61, $p = 0.009$ and t (13) = -2.91, $p = 0.012$, $d = 0.78$, respectively], but not at 15 seconds.

7.3.3. Other cardiovascular parameter responses to head-up tilt (< 15 seconds)

The results of other cardiovascular parameters (diastolic BP; DBP, systolic BP; SBP, and FiBF) are presented in Table 7.1. Changes in DBP were different between hold-flexion and -extension conditioning in the HUT position. Following those forms of muscle conditioning, DBP significantly reduced, but the reduction following hold-extension conditioning was significantly less than hold-flexion conditioning. Systolic blood pressure was higher following hold-extension conditioning than hold-flexion conditioning immediately after HUT (at 5 and 10 seconds), although SBP did not differ from the pre-conditioning value for any of the muscle conditioning interventions. While FiBF significantly reduced after all forms of muscle conditioning and immediately after HUT, there was no difference among the three forms of muscle conditioning.

Table 7.1 Cardiovascular responses to head-up tilt following each muscle conditioning

Parameters	Conditioning	Pre	head-up tilt		
			5 sec	10 sec	15 sec
DBP (%)	hold-flexion	101.19 ± 1.50	90.25 ± 1.72*	86.56 ± 1.80*	89.21 ± 1.59*
	hold-intermediate	98.86 ± 1.01	90.73 ± 3.04	89.04 ± 2.94	91.68 ± 2.69
	hold-extension	101.69 ± 1.10	95.50 ± 2.75	93.17 ± 2.03*†	93.65 ± 1.72*
SBP (%)	hold-flexion	101.37 ± 1.30	97.43 ± 2.35	92.38 ± 2.58	92.69 ± 2.36
	hold-intermediate	99.11 ± 0.95	100.77 ± 2.14	97.15 ± 2.38	97.09 ± 2.67
	hold-extension	102.44 ± 1.26	104.03 ± 2.90†	99.32 ± 2.76†	98.74 ± 2.26
FiBF (%)	hold-flexion	87.45 ± 6.75	56.60 ± 6.67*	81.52 ± 10.72	89.17 ± 10.59
	hold-intermediate	99.20 ± 5.88	49.84 ± 5.60*	81.91 ± 10.14	81.11 ± 9.80
	hold-extension	86.34 ± 6.59	51.00 ± 8.16*	69.80 ± 11.80	85.55 ± 14.02

Data are expressed as mean ± SEM (%). Asterisk (*) indicates a significant difference from pre-conditioning value ($p < 0.05$). Cross (†) indicates a significant difference between hold-flexion and -extension conditioning ($p < 0.05$). HR = heart rate, MAP = mean arterial pressure, DBP = diastolic blood pressure, SBP = systolic blood pressure, FiBF = finger blood flow, sec = second.

7.3.4. Heart rate variability (1 minute) and 1-minute heart rate analysis

The results of changes in normalised HF component and the other HRV parameters are presented in Figure 7.4 and Table 7.2, respectively. The results showed that the normalised HF component significantly decreased at the third and fourth minutes after hold-extension conditioning [t (13) = -4.49, p = 0.001, d = 1.20 and t (13) = -4.99, $p < 0.001$, d = 1.33, respectively]. Additionally, these reductions in the normalised HF component at the third and fourth minutes in the HUT position were significantly different from hold-flexion conditioning [t (13) = 3.46, p = 0.004, d = 0.92 and t (13) = 2.77, p = 0.016, d = 0.74, respectively].

Additionally, the absolute value of HF was significantly lower at the third minute after hold-extension conditioning [t (13) = -4.09, p = 0.001, d = 1.09] while hold-flexion and -

intermediate conditioning did not reveal significant changes in the absolute value of HF. On the other hand, there were no significant changes in TP after either form of muscle conditioning. Time domain analysis of HRV parameters was also included in the statistical analysis (i.e. SDNN and RMSSD). There were significant reductions in RMSSD at the third and fourth minutes in the HUT position following hold-extension conditioning [t (13) = -3.82, p = 0.002, d = 1.02 and t (13) = -3.53, p = 0.004, d = 0.94, respectively] while SDNN did not change after any forms of muscle conditioning.

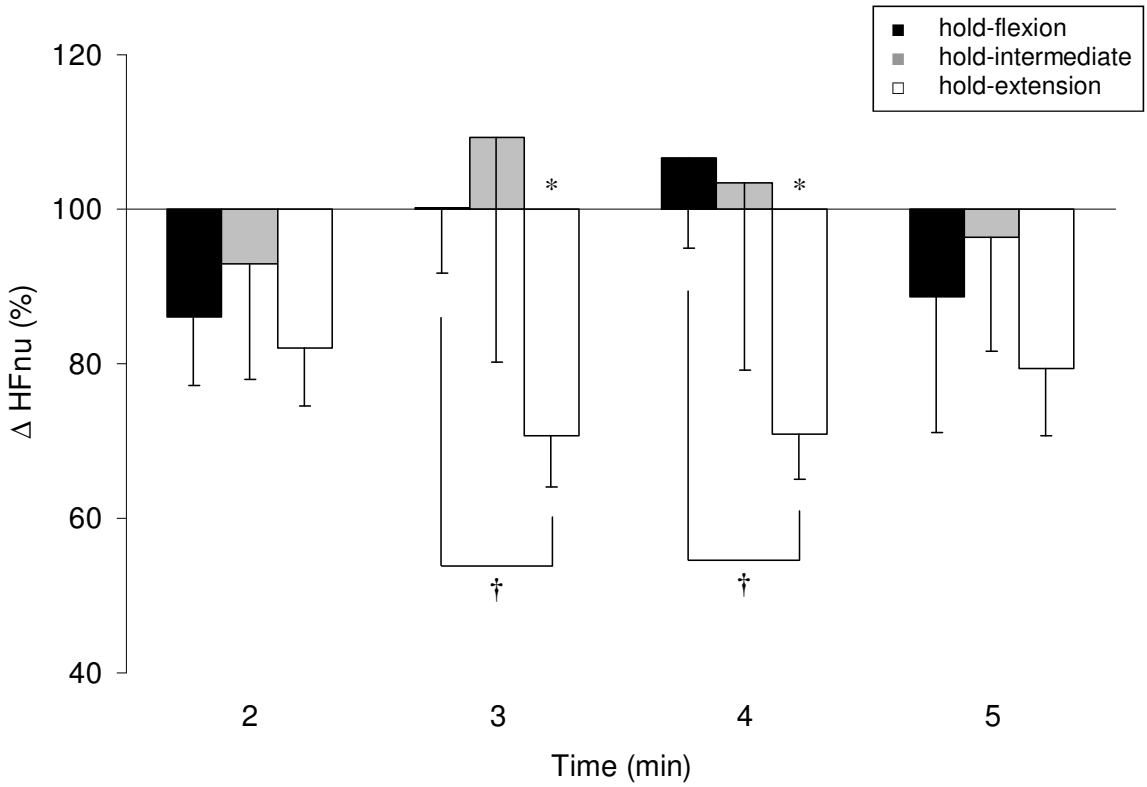

Figure 7.4. Different cardiac vagal responses to head-up tilt following different forms of neck muscle conditioning.
Data are expressed as mean ± SEM 100% refers to the average of pre-tilt value in each condition. HFnu = normalised high frequency power of heart rate variability, min = minute Asterisk (*) indicates significant difference from pre-tilt value ($p < 0.01$). Cross (†) indicates significant difference between hold-flexion and -extension conditioning ($p < 0.0167$). Following hold-extension conditioning, HFnu significantly decreased. Changes in HFnu following hold-flexion and -intermediate conditioning were not significant.

Table 7.2. Heart rate variability parameter changes in response to head-up tilt following each muscle conditioning.

Parameters	Conditioning	head-up tilt			
		2 min	3 min	4 min	5 min
TP (%)	hold-flexion	102.84 ± 19.60	93.59 ± 21.25	99.04 ± 17.22	106.27 ± 14.74
	hold-intermediate	127.53 ± 33.42	185.24 ± 77.00	186.42 ± 39.95	226.34 ± 62.34
	hold-extension	92.25 ± 13.04	167.28 ± 50.86	141.24 ± 39.66	140.35 ± 26.19
HFab (%)	hold-flexion	72.94 ± 11.53	86.87 ± 16.13	104.00 ± 24.36	76.75 ± 13.32
	hold-intermediate	145.52 ± 69.29	199.56 ± 121.20	172.40 ± 54.93	183.25 ± 69.24
	hold-extension	73.86 ± 14.81	65.46 ± 8.45*	70.62 ± 11.54	85.57 ± 15.16
SDNN (%)	hold-flexion	95.66 ± 6.89	91.54 ± 9.79	101.52 ± 8.57	103.08 ± 8.80
	hold-intermediate	98.92 ± 10.80	113.90 ± 18.29	120.55 ± 12.80	122.45 ± 15.63
	hold-extension	91.60 ± 5.62	109.69 ± 10.01	102.83 ± 12.57	111.03 ± 11.10
RMSSD (%)	hold-flexion	83.03 ± 6.29	82.23 ± 8.49	86.69 ± 7.43	86.18 ± 6.76
	hold-intermediate	93.12 ± 13.31	100.17 ± 15.61	111.74 ± 11.22	110.84 ± 12.22
	hold-extension	81.68 ± 7.89	79.77 ± 5.30*	78.78 ± 6.02*	83.82 ± 7.35

Data are expressed as mean ± SEM (%). Asterisk (*) indicates a significant difference from pre-conditioning value ($p < 0.01$). TP = total power, HFab = absolute high frequency power, SDNN = standard deviation of all NN intervals, RMSSD = the square root of the mean of the sum of the squares of differences between adjacent NN intervals, min = minute.

For 1-minute HR responses to HUT, there was a significant time effect following hold-extension conditioning [Wilks'$\Lambda = 0.25$, $F(5,9) = 5.35$, $p = 0.015$, $\eta^2 = 0.75$], but not hold-flexion [$\chi^2(5, N = 14) = 7.35$, $p = 0.20$] or -intermediate [Wilks'$\Lambda = 0.53$, $F(5,9) = 1.63$, $p = 0.25$, $\eta^2 = 0.48$] conditioning. Pairwise comparisons with Bonferroni correction showed that HR significantly increased and the increase was sustained during the HUT position following hold-extension conditioning.

The result of a repeated measures ANOVA showed that there was no difference in HR among the three different types of muscle conditioning during baseline [Wilks'Λ = 0.99, $F(2,12)$ = 0.055, p = 0.95, η^2 = 0.009]. Additionally, HR did not differ among the different forms of conditioning during the HUT position.

Mean HR responses following each form of muscle conditioning appeared similar but the statistical results were different. Thus, in order to examine differences in the extent of the variation of HR in response to HUT, the F value was calculated (see Appendix 21 for calculation). The results of the F statistics showed the extent of the variation of HR following hold-extension conditioning was significantly less than hold-flexion and -intermediate conditioning at the second minute ($p < 0.05$). Otherwise, the extent of the variation of HR was not significantly different following the different forms of muscle conditioning.

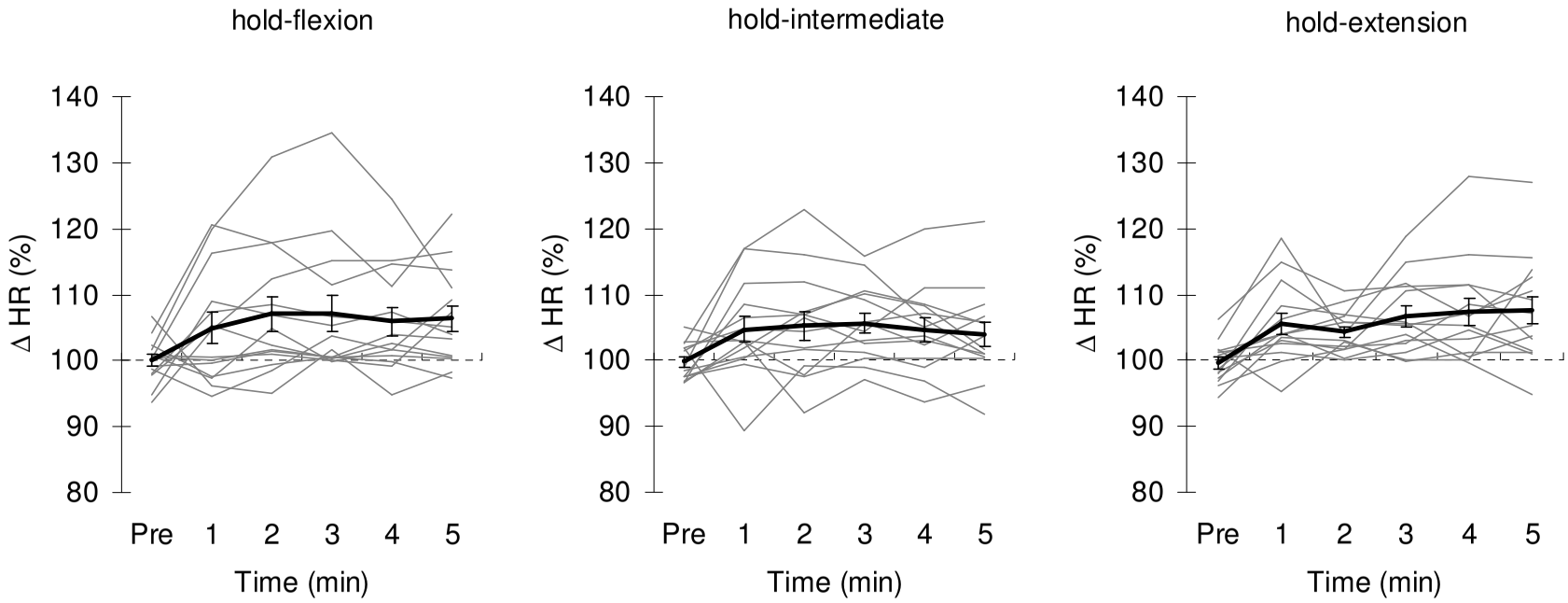

Figure 7.5. Differences in variation of heart rate response to head-up tilt following different forms of neck muscle conditioning.
Mean value of the group is expressed as mean ± SEM (thick line). Individual heart rate (HR) is also presented (thin line). 100% refers to the average of pre-tilt value in each conditioning.

7.3.5. Comparisons of heart rate variability parameters (5 minutes) between hold-flexion and -extension conditioning

The results of comparisons were summarised in Table 7.3 and showed that there were no significant differences in any 5-minute HRV parameters between hold-flexion and -extension conditioning.

Table 7.3. Comparisons of heart rate variability parameters (5 minutes) between hold-flexion and -extension conditioning

parameters	Δflex	Δext	*t* or *z*	*p*	*d*
TP (ms^2)	-622.42 ± 473.17	95.00 ± 460.32	-1.72	0.11	0.46
LF (ms^2)	-188.12 ± 93.42	-176.38 ± 126.77	-0.089	0.93	0.024
LF (nu)	0.81 ± 1.76	-4.82 ± 2.53	-1.48†	0.14	N/A
HF (ms^2)	-207.62 ± 107.24	27.83 ± 92.14	-1.54†	0.12	N/A
HF (nu)	-0.90 ± 1.67	5.11 ± 2.43	-1.76	0.10	0.47
LF/HF	0.00 ± 0.15	-0.24 ± 0.26	-1.54†	0.12	N/A

Data are presented as mean ± SEM Δflex = parameters following hold-flexion conditioning which was subtracted by following hold-intermediate conditioning. Δext = parameters following hold-extension conditioning which was subtracted by following hold-intermediate conditioning. Cross (†) indicates *z* score, *d* = Cohen's *d*, TP = total power, LF = low frequency power, HF = high frequency power, LF/HF = the ratio of low frequency power to high frequency, ms^2 = millisecond square, nu = normalised unit.

7.3.6. Comparisons of calf blood flow between hold-flexion and -extension conditioning

Data of calf blood flow were obtained from 10 participants. Calf blood flow was not significantly different between the two forms of muscle conditioning at both the first and second minutes [t (9) = -0.047, p = 0.96, d = 0.015 and t (9) = -0.48, p = 0.64, d = 0.15, respectively].

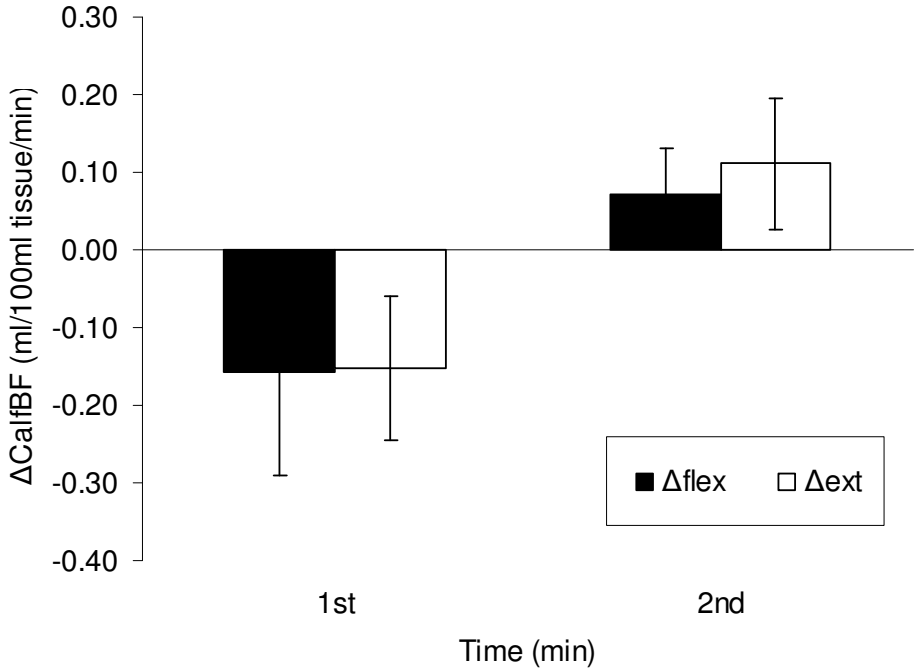

Figure 7.6. Comparisons of calf blood flow during the head-up tilt following hold-flexion and -extension conditioning.
Data are presented as mean ± SEM CalfBF = calf blood flow, Δflex = calf blood flow following hold-flexion conditioning which was subtracted by following hold-intermediate conditioning. Δext = calf blood flow following hold-extension conditioning which was subtracted by following hold-intermediate conditioning.

7.3.7. Comparisons of cardiovascular parameters with one leg rise

During calf blood flow measurement, the left leg was lifted. Although cardiovascular parameters during controlled breathing (HRV recording session) were significantly dependent on the form of muscle conditioning, this was not the case in calf blood flow. Condition during experiment was slightly different between the two sessions; such as left leg rise, respiratory rhythm control, and the number of participants. Thus, in order to investigate whether the same cardiovascular responses were observed despite of different experiment conditions, the investigation (*section 7.3.1* and *7.3.2*) was repeated on the cardiovascular parameters (HR, BP, and FiBF).

A one-way repeated measures ANOVA showed that there was a significant time effect on DBP [Wilks'Λ = 0.30, $F(3,7) = 5.45$, $p = 0.03$, $\eta^2 = 0.70$]. Pairwise comparisons found that

DBP significantly decreased at 15 seconds after the onset of HUT following hold-extension conditioning [t (9) = 3.92, p = 0.021, d = 1.24]. Otherwise, there were no significant changes in DBP following the other two forms of conditioning or no differences among the three forms of muscle conditioning. For FiBF, a one-way repeated measures ANOVA demonstrated a significant time effect [Wilks'Λ = 0.18, F(3,7) = 10.95, p = 0.005, η^2 = 0.82] and FiBF decreased at 5 second after the onset of HUT following hold-flexion and -intermediate conditioning [t (9) = 4.14, p = 0.015, d = 1.31 and t (9) = 3.47, p = 0.042, d = 1.10, respectively], but not hold-extension [t (9) = 2.90, p = 0.11, d = 0.92]. There were no significant differences in FiBF among all forms of muscle conditioning.

In addition, unlike HRV recording session, HR and MAP recorded during SGP recording session did not change in response to 20° HUT following either form of the muscle conditioning manoeuvre or differ between any forms of the manoeuvre. For example, HR in response to HUT following hold-extension conditioning was not differ from the baseline [at 5 seconds; t (9) = -0.47, p = 1.00, d = 0.15, at 10 seconds; t (9) = -0.29, p = 1.00, d = 0.092, at 15 seconds; t (9) = 0.81, p = 1.00, d = 0.26]. Differences in HR response between hold-flexion and -extension conditioning were not significant, either [at 5 seconds; t (9) =0.24, p = 1.00, d = 0.076, at 10 seconds; t (9) = 1.05, p = 0.96, d = 0.33, at 15 seconds; z (10) = -1.27, p = 0.20]. Also, the response of MAP to HUT following hold-flexion conditioning was not differ from the baseline [at 5 seconds; t (9) = 0.080, p = 0.94, d = 0.025, at 10 seconds; z (10) = -1.07, p = 0.29, at 15 seconds; t (9) = 0.39, p = 0.71, d = 0.12]. Differences in MAP response between hold-flexion and -extension conditioning were not significant, either [at 5 seconds z (10) = -1.17, p = 0.24, at 10 seconds z (10) = -1.68, p = 0.093, at 15 seconds t (9) = 2.31, p = 0.046, d = 0.73].

Table 7.4. Cardiovascular responses to head-up tilt following each muscle conditioning during left leg rise.

Parameters	Conditioning	Pre	head-up tilt		
			5 sec	10 sec	15 sec
DBP (%)	hold-flexion	101.15 ± 1.33	99.01 ± 2.28	97.57 ± 1.39	99.52 ± 2.08
	hold-intermediate	99.66 ± 1.40	91.21 ± 2.38	89.22 ± 3.44	87.17 ± 4.60
	hold-extension	98.84 ± 1.61	91.06 ± 3.92	90.13 ± 3.09	91.59 ± 1.44
SBP (%)	hold-flexion	102.68 ± 1.53	104.53 ± 2.58	102.53 ± 2.07	102.66 ± 2.40
	hold-intermediate	99.37 ± 1.39	96.51 ± 2.38	92.57 ± 3.85	90.25 ± 4.93
	hold-extension	98.00 ± 1.82	98.13 ± 4.29	95.86 ± 4.09	94.83 ± 2.68*
MAP (%)	hold-flexion	101.91 ± 1.41	101.73 ± 2.36	100.02 ± 1.69	101.05 ± 2.13
	hold-intermediate	99.51 ± 1.34	93.90 ± 2.21	90.91 ± 3.53	88.72 ± 4.65
	hold-extension	98.41 ± 1.62	94.58 ± 4.02	93.01 ± 3.45	93.22 ± 1.92
HR (%)	hold-flexion	99.12 ± 0.81	100.65 ± 2.28	101.33 ± 2.16	101.44 ± 2.37
	hold-intermediate	100.61 ± 1.07	101.96 ± 2.20	101.35 ± 2.05	98.53 ± 2.02
	hold-extension	99.68 ± 0.83	100.03 ± 1.35	98.57 ± 1.54	98.43 ± 0.97
FiBF (%)	hold-flexion	100.41 ± 7.91	58.65 ± 6.46*	67.44 ± 3.65	81.03 ± 7.81
	hold-intermediate	97.49 ± 4.37	70.62 ± 9.02*	116.97 ± 36.15	128.24 ± 39.30
	hold-extension	99.19 ± 9.14	74.66 ± 7.39*	76.92 ± 5.85	88.08 ± 7.97

Data were expressed as mean ± SEM Asterisk (*) indicates a significant difference from pre-conditioning value ($p < 0.05$). DBP = diastolic blod pressure, FiBF = finger blood flow, HR = heart rate, MAP = mean arterial pressure, SBP = systolic blood pressure, sec = second.

7.3.8. A change in skin potential following the completion of head-up tilt

A significant change in skin potential was found in all participants during both HRV and SGP recording sessions. Although it seemed that the occurrence of skin potential change slightly decreased over the three trials, there was no clear trend.

7.4. Discussion

This study examined whether alteration in the quantity of proprioceptive afferent inputs from dorsal neck muscles, presumably from muscle spindles, contributes to autonomic regulation of cardiovascular function in response to mild orthostatic stress, and primarily focused on the initial stage of cardiovascular regulation when the vestibulo-sympathetic reflex is thought to be influential (Olufsen et al. 2006). The central finding of the present study was that immediate responses (within 15 seconds) of HR and BP to 20° HUT were dependent on the form of muscle conditioning used to define the quantity of proprioceptive afferent inputs from the dorsal neck muscles in a systematic way. Following hold-extension conditioning, HR increased in response to HUT and BP did not change significantly from pre-tilt values. Conversely, hold-flexion conditioning resulted in a blunted increase in HR and a significant drop in BP. Additionally, both HR and BP responses following these two forms of muscle conditioning were significantly different. Muscle conditioning-dependent changes were also observed in HR and cardiac vagal activity over a longer time period (up to 5 minutes). This study may indicate an as yet undetermined contribution of neck muscle proprioceptive afferent inputs to autonomic cardiovascular regulation associated with postural changes in humans.

7.4.1. Effects of muscle conditioning on immediate cardiovascular response to head-up tilt

The usual cardiovascular response to postural change (from supine to standing) has been characterised as an initial increase in HR and a transient reduction in BP by the 15th heart beat (Appenzeller & Oribe 1997). Heart rate slows and BP increases by the 30th heat beat and both HR and BP gradually stabilise over the subsequent 1 to 2 minutes (Appenzeller & Oribe 1997). Milder levels of orthostatic stress seemed to induce a similar time course of cardiovascular regulation. Toska and Walloe (2002) found that cardiovascular adjustments, in

general, are completed by approximately 30 seconds after the onset of 30° HUT. In the present study, similar trends of cardiovascular responses were observed – that is, an initial increase in HR and a transient reduction in BP, and both parameters appeared to stabilise within 30 to 60 seconds after the onset of HUT. However, cardiovascular responses observed in the present study were clearly dependent on the form of dorsal neck muscle conditioning. More specifically, following hold-extension conditioning, which was thought to reflect a relative increase in proprioceptive afferent inputs from the dorsal neck muscles, HR significantly increased and BP did not change after the onset of HUT. These cardiovascular responses were consistent with cardiovascular responses characterised previously (Appenzeller & Oribe 1997) as well as the results of graded tilt studies (Saito et al. 1997; Shoemaker et al. 2001). On the other hand, following hold-flexion conditioning, which was presumed to reflect a relative decrease in proprioceptive afferent inputs from the dorsal neck muscles (the opposite to that following hold-extension conditioning), there was a muted increment in HR coupled with a more profound reduction in BP after the onset of HUT. The primary focus of the present study was to examine the interaction of proprioceptive afferent inputs from the dorsal neck muscles with the vestibulo-sympathetic reflex, so cardiovascular responses up to 15 seconds after HUT were investigated. An increase in proprioceptive afferent inputs from the dorsal neck muscles (a result of hold-extension conditioning) led to presumably desirable responses for cardiovascular adjustments to orthostatic stress. Therefore, these results suggest that proprioceptive afferent inputs from the dorsal neck muscles participate in cardiovascular regulation associated with posture changes presumably in a synergistic manner with the vestibulo-sympathetic reflex.

7.4.2. Effects of muscle conditioning on heart rate variability following head-up tilt

In addition to the immediate HR and BP responses to HUT, this study examined "conditioning-dependent" changes in HRV. Due to the nature of conventional HRV analysis

(i.e. fast Fourier Transform), a longer duration of recording was required. For determination of the lower frequency power component of the HRV, at least 2-minutes recording is required while a 1-minute recording is the minimum duration for calculation for the HF power component (Task Force 1996). The HRV analysis provided with an index of averaged autonomic nerve activity to the heart over a longer period in contrast to the immediate response examined using HR and BP measurements. Although cardiovascular function has presumably stabilised within 1 minute after the onset of HUT, "conditioning-dependent" changes in HRV parameters were evident over a more prolonged period. However, data averaged over longer periods may blunt the sensitivity to observe short duration changes in HRV parameters. Thus, the present study focused on the HF component of HRV (including the time-domain method of analysis), which required a minimum 1-minute of recorded data and is generally accepted as an index of vagal drive to the heart (Task Force 1996).

Graded tilt studies using HRV parameters have demonstrated progressive trends of sympathetic excitation and vagal withdrawal (Montano et al. 1994; Mukai & Hayano 1995; Yokoi & Aoki 1999). Further, vagal withdrawal becomes more evident at HUT angles greater than 30° (Mukai & Hayano 1995). In the present study, vagal withdrawal did not occur at 20° HUT following hold-flexion or -intermediate conditioning as shown by Mukai & Hayano (1995). In contrast, at 3 to 4 minutes after the onset of HUT following hold-extension conditioning, a significant reduction in normalised HF component (an index of cardiac vagal activity) was observed. This finding was supported by changes in other HRV parameters (i.e., the absolute value of HF and RMSSD) as well as the HR response averaged into one-minute bins. Following hold-extension conditioning, HR in the HUT position increased significantly and was sustained over the recording period (5 minutes). In addition, an evaluation of the variation of the HR response to HUT (Figure 7.5) showed that the HR response following hold-extension conditioning was less variable across individuals at the second minute when

compared with the other two forms of conditioning. Therefore, this observation suggest that increasing the quantity of neck proprioceptive inputs to the CNS serves to increase the sensitivity of cardiovascular adjustments in response to orthostasis, resulting in consistent cardiovascular response across individuals.

The impact of different head orientations on cardiac autonomic drives and cardiovascular function during orthostasis has been demonstrated. For example, Bouvette et al. (1996) observed that a neck flexion manoeuvre (chin to chest) improved orthostatic-related symptoms in patients with neurologic orthostatic hypotension during posture change. Ray (2000) demonstrated that head-down neck flexion (HDNF) induced an additional effect on cardiovascular regulation related to lower body negative pressure. More recently, Wilson et al. (2003) also employed a similar head position to the study by Ray (2000) and examined the effects of different head orientations on cerebrovascular responses during lower body negative pressure. The primary purpose of the HDNF was to alter vestibular afferent inputs. Although the changes in cardiovascular function observed in these studies (i.e., Ray 2000; Wilson et al. 2003) were attributed to vestibular activation, the involvement of other sensory afferent inputs such as baroreceptors and neck proprioceptors may be inevitable (Bolton et al. 2004; Wilson et al. 2006a). Importantly, in the present study, since HUT occurred after the head had been returned to its original position following each muscle conditioning procedure, other sensory inputs such as the vestibular, articular, and cutaneous receptors were presumably consistent in each trial in the HUT position. Therefore, it was concluded that the changes in cardiovascular responses seen in the present study were likely to be attributed to the alteration of proprioceptive afferent inputs from the dorsal neck muscles as a consequence of the neck muscle conditioning manoeuvre.

7.4.3. Possible mechanism of the effects of neck muscle proprioceptive afferent alteration on cardiovascular regulation during orthostasis

As well-known, baroreceptors play a significant role in cardiovascular regulation as a part of feedback mechanisms (see also *section 1.2.1*). In addition, the vestibular system is accepted to participate in cardiovascular regulation in a feedforward manner. For example, Doba and Reis (1974) first showed that transection of either the vestibular nerves or the fastigial nucleus led to orthostatic hypotension in anaesthetised and paralysed cats and a more recent study found similar outcomes in conscious cats (Jian et al. 1999). In humans, gravitational-related cardiovascular responses were blunted in patients following vestibulectomy compared to healthy controls (Yates et al. 1999). A recent review of vestibular-related autonomic nervous reflexes reported that the neural circuitry of the vestibulo-sympathetic reflex was quite complex (Balaban & Yates 2004). Afferents originating from the vestibular apparatus reach the cardiovascular control centre in multi brain stem areas including the parabrachial nuclei, nucleus tractus solitarius, caudal and rostral ventrolateral medulla, and dorsal motor vagal nucleus via the inferior and medial vestibular nuclei (Balaban & Yates 2004). The significance of the inferior and medial vestibular nuclei in the vestibulo-sympathetic reflex has been exemplified using conscious cats where damage of these vestibular nuclei resulted in orthostatic hypotension (Mori et al. 2005), which is consistent with previous studies (Doba & Reis 1974; Jian et al. 1999).

In addition to the vestibular system, the present study employed the muscle conditioning manoeuvre to define dorsal neck muscle proprioceptive inputs in a systematic way and demonstrated that BP and HR responses immediately after 20° HUT were dependent on the form of the manoeuvre (in turn, the quantity of dorsal neck muscle proprioceptive inputs). Since the vestibulo-sympathetic reflex is thought to be influential in the initial stage of cardiovascular regulation associated with posture change (Olufsen et al. 2006), the

cardiovascular responses indicated that the neck muscle proprioceptive inputs possibly contributed to immediate cardiovascular regulation in concert with the vestibulo-sympathetic reflex. Anatomical evidence suggests that neck sensory inputs including muscles have access to the vestibular nuclei. It has been shown that the inferior and medial vestibular nuclei receive projections from primary afferents of the upper cervical region in rats (Neuhuber & Zenker 1989; Prihoda et al. 1991). Also electrical stimulation of the central cervical nuclei, which receive primary afferent inputs from the upper neck muscles, are capable of activating the inferior, medial, and lateral vestibular nuclei (Sato et al. 1997b).

Neck muscle afferent inputs (i.e., from muscle spindles and Golgi tendon organs) are known to make connection to the "intermediate nucleus of the medulla". A recent investigative study showed that neruons from this nucleus make monosynaptic connections to the nucleus tractus solitarius (Edwards et al. 2007). Since head position did not alter once the body position had reached 20°, afferent inputs from the vestibular apparatus were presumed to be constant during data collection. Therefore, the finding of Edwards et al. (2007) may explain on-going suppression of the vagal drive to the heart (i.e., the HF power of HRV) in the tilted position.

Collectively, these neuroanatomical studies suggest that proprioceptive afferents from the neck muscles are capable of contributing to autonomic regulation of cardiovascular function through multiple pathways with and without synapsing in the vestibular nuclei.

7.4.4. Different results of cardiovascular data obtained during strain-gauge plethysmogram recording session

Data obtained from HRV recording session exhibited muscle conditioning-dependent changes in both cardiac autonomic drives and cardiovascular function. In contrast, cardiovascular parameters recorded during SGP recording session did not show the dependency of form of

the muscle conditioning manoeuvre. There are a few reasons to explain differences in results obtained in the two (HRV and SGP) recording sessions.

Firstly, during SGP recording session, the left leg was raised to measure calf blood flow. This physical condition might have resulted in different results from those in HRV recording session. The left leg rise during data collection may be associated with activation of mechanosensitive receptors and/or metaboreceptors connecting with group III and IV afferent fibres, which is known to influence autonomic drives and cardiovascular function (Wilson et al. 1994; Murata & Matsukawa 2001; Gladwell & Coote 2002; Gladwell et al. 2005; Cui et al. 2006). Although it was attempted to avoid excessive stretch of the leg muscles during leg rise, it was not possible to avoid a certain extent of muscle stretch. Potts and Mitchell (1998) demonstrated that both passive muscle stretch and electrically-induced muscle contraction induced a rapid resetting of baroreceptor setting point to a higher level in anaesthetised dogs. Similarly, electrical stimulation of the thigh muscles, which presumably activated mechanosensitive receptors, decreased the baroreceptor sensitivity in humans (Iellamo et al. 1997). Thus, mechanocensitive receptor activation by muscle stretch presumably might have induced baroreflex resetting and in turn, attenuated cardiovascular control in response to cardiovascular perturbations. It was reported that an attenuation in baroreflex sensitivity influenced cardiovascular regulation including HR control. For example, the attenuation of HR control by the baroreflex is common in aged population (Matsukawa et al. 1998). In response to the Valsalva's manoeuvre, an attenuation in HR increase was observed in the elder group (70-80 years old) but not in younger group (20-44 years old) (Matsukawa et al. 1998). It was also demonstrated that reduced baroreflex sensitivity, which was induced by nitrous oxide infusion, blunted HR control associated with orthostatic stress change (tilt-up from supine and tilt-back from upright posture) (Ostlund & Linnarsson 1999). In addition, it was found that an increase in HR in response to gravitational change (i.e. the vestibulo-sympathetic reflex) was impaired following the denervation of baroreceptors in conscious rats

(Zhu et al. 2007). In other words, vestibular-induced autonomic and cardiovascular responses are presumably influenced by the extent of baroreflex sensitivity. Since the effect of neck muscle conditioning on autonomic regulation of cardiovascular function during the supine position (i.e., constant vestibular activation and no significant baroreceptor change) in previous chapters, it could be assumed that the major role of neck muscle proprioceptive inputs in autonomic regulation of cardiovascular function is to tune the vestibulo-sympathetic reflex (Bolton et al. 2006) whereas its independent contribution was small (but statistically significant). Therefore, it was assumed that blunted baroreflex sensitivity induced by leg muscle stretch (in turn, presumably muscle mechanosensitive receptor activation) attenuated an immediate HR response to HUT, which may predominantly be attributed to the consequence of the vestibulo-sympathetic reflex, in SGP recording session.

Secondly, during SGP recording session, little BP response to HUT was observed, either. If baroreflex sensitivity was only attenuated as a consequence of leg muscle stretch during data collection as discussed above, BP reduction should be more pronounced. However, BP in response to HUT during one leg rise did not differ from the baseline (in the supine posture). Thus, it could be presumed that other factors were also involved in the one leg raise condition. One of the possibilities may be an insufficient blood shift following HUT and central volume during one leg rise (SGP recording session) was higher in the HUT position than without leg rise (HRV recording session). Consequently the extent of baroreceptor unloading was presumably lower during one leg rise. For example, Fu et al. (2001) employed lower body positive pressure to reduce the accumulation of the blood in the lower body associated with orthostatic stress. With the application of the lower body positive pressure, cardiovascular responses to 70° HUT was significantly decreased compared with without positive pressure; for example, less reduction in stroke volume and increases in HR and MAP (Fu et al. 2001). Thus, this suggests that a reduction in the extent of baroreceptor unloading during orthostasis

led to an attenuation in cardiovascular responses. As a result, one leg rise during SGP recording session in this chapter failed to induce sufficient body fluid shift and to observe muscle conditioning-dependent changes in cardiovascular function following the onset of moderate (20°) HUT as observed during HRV recording session.

Thirdly, the number of participants might have influenced the results obtained during HRV (n = 14) and SGP (n = 10) recording session. Since some of statistical analyses were performed using non-parametric test and no effect size of these parameters could be obtained, it is not possible to compare the effect sizes of all statistical analyses between HRV and SGP recording sessions. However, for instance, the effect size of HR response to HUT following hold-extension conditioning tends to be large in HRV recording session (Cohen's $d > 0.8$) but small ($d < 0.26$) in SGP recording session. In order to obtain 80% of statistical power for HR response following hold-extension conditioning during SGP recording session, approximately 224 participants were required (Portney & Watkins 2000).

Therefore, it seems that experiment conditions were different between the two recording sessions and the differences were significantly influenctial on study results. Consequently, larger number of participants was needed for SGP recording session to conclude the contribution of muscle proprioceptive inputs from the dorsal neck to cardiovascular responses to mild orthostatic stress.

In conclusion, this study demonstrated conditioning-dependent changes in autonomic regulation of cardiovascular function in response to 20° HUT. Muscle conditioning was assumed to alter the mechanical sensitivity of neck muscle proprioceptors which in turn altered the size of the incoming proprioceptive inputs to the CNS. Therefore, the results suggest that proprioceptive afferent inputs from the dorsal neck muscles participate in

cardiovascular regulation associated with posture changes presumably in a synergistic manner with the vestibule-sympathetic reflex. Also, the effects of an alteration of neck muscle proprioceptive inputs on HRV parameters were observed over a more prolonged period during the HUT position. The findings in this study indicate that large afferents originating from the dorsal neck muscles may participate in neural integration in the cardiovascular centre at least in the short term (< 5 minutes), while large afferents from limb muscles are generally agreed not to influence the autonomic nervous and cardiovascular systems (Sato et al. 1997a).

Chapter 8

General Conclusion & Recommendations

8.1. General conclusions

The purpose of this thesis was to investigate the contribution of somatosensory afferent inputs from the neck to autonomic regulation of cardiovascular function in awake humans. In particular, the type of neck sensory inputs focused in this thesis was characterised as "innocuous". Also head displacement, which results in vestibular activation, was minimised when the "interventions" were applied to the neck. Three interventions were utilised during the experimental work; an Activator® Instrument (which induces a phasic mechanical stimulus), a vibratory stimulus, and the muscle conditioning manoeuvre. Additionally, in experiments of Chapter 7, the role of neck sensory inputs in autonomic regulation of cardiovascular function associated with orthostasis was examined through the combination of the muscle conditioning manoeuvre and 20° head-up tilt.

The conclusions through this thesis regarding specific research questions are as follows;

- Cardiovascular function during the two horizontal postures (prone and supine) was not equivalent. For example, blood pressure (BP) and heart rate (HR) were significantly different between these postures, however, the differences in heart rate variability (HRV) parameters were negligible. This indicates that cardiovascular function is different between the prone and supine postures despite consistent gravity to the body as the posture was maintained in the horizontal plane. Therefore, the direction of horizontal posture needs to be taken into account where experiments are investigating cardiovascular function (Chapter 3).

- An innocuous mechanical stimulus applied to the neck is capable of evoking a BP response while HR remains unaffected. Psychological factors associated with the application of the mechanical stimulus (i.e., an arousal reaction) should be taken into account when obtained data are interpreted. Additional measurements to the main

interest parameters may be required to deal with this factor, depending on the operation mechanism of the measures. For example, BP measure from the finger artery (i.e., Portapres®) may be influenced by finger blood flow (FiBF), which is presumably affected by an arousal effect. In Chapter 4, although the influence of FiBF values is included in statistical analysis as a covariate, the results of BP responses induced by the mechanical stimulus were seldom altered. Therefore, an innocuous mechanical stimulus to the neck may be able to influence autonomic regulation of cardiovascular function, however, an arousal effect is unavoidable and may constitute a part of the observed responses.

- Techniques to alter the strength of neck muscle proprioceptive inputs may enable the investigation of their contribution to autonomic regulation of cardiovascular function. The muscle conditioning manoeuvre was applied to either the right rotator muscles of the head and neck in the sitting posture or the dorsal neck muscles in the supine. At the resting condition following the application of the manoeuvre, the effect of the alteration in neck muscle proprioceptive inputs on autonomic regulation of cardiovascular function was negligible. In contrast, cardiovascular effects were observed associated with a unilateral vibratory stimulus. Therefore, these results may indicate that neck muscle proprioceptive inputs contribute to autonomic regulation of cardiovascular function, however, its contribution is only minor under resting conditions (Chapter 5 & 6).

- With the addition of a mild orthostatic stress (20° head-up tilt), the contribution of neck muscle proprioceptive inputs to autonomic regulation of cardiovascular function became more evident. Responses of cardiac autonomic and cardiovascular parameters to the head-up tilt were dependent on the form of the muscle conditioning manoeuvre.

Therefore, the findings presented in Chapter 7 indicate that, associated with posture changes, proprioceptive inputs from the dorsal neck muscles may participate in cardiovascular regulation presumably in a synergistic manner with the vestibule-sympathetic reflex (< 10 seconds) as well as short-term autonomic regulation of cardiovascular function (< 5 minutes).

8.2. Study limitations

The limitations associated with this thesis are identified as follows;

- The study population in this thesis included generally healthy, young adults. Therefore, the results obtained in the work presented here may not be applicable to other groups such as younger and elder populations and patients with cardiovascular disorders or musculoskeletal dysfunctions

- The mechanical stimuli employed in Chapter 4 were partly simulated as spinal manipulation commonly used in chiropractic clinics. As the primary interest of the chapter was to examine whether a mechanical stimulus to the neck *per se* induced cardiac autonomic and cardiovascular responses rather than to deliver as a therapeutic procedure, the region of the neck where the stimuli were applied was not assessed for dysfunction. Therefore, the results of this thesis may not be applicable to dysfunctional regions of the neck.

- The muscle conditioning manoeuvre has been systematically investigated and well-defined (Proske et al. 1993). The manoeuvre used in this thesis was theoretically based on data obtained from the limb muscles because there has been little published data obtained from the neck. Although studies which applied the muscle conditioning manoeuvre to the neck muscles document that perceptions of the head orientation are

influence by the manoeuvre (Owens et al. 2006; Repka & Polus 2008), mechanisms underpinning this observation are not identified. Additionally, it is not known whether there is a difference in effects of the manoeuvre on the resting discharges of intramuscular receptors (including muscle spindles and Golgi tendon organs) between the limb and neck. Therefore, it is not possible to specify the exact origins of the observed cardiac autonomic and cardiovascular responses from the data of this thesis.

- A vibratory stimulus used in this thesis was aimed to unilaterally stimulate the dorsal neck muscles. The majority of participants in Chapter 5 perceived a head motion illusion during the application of the stimulus. Thus, this indicates that right or left dorsal neck vibration used in this chapter stimulated a certain group of neck muscles (i.e., right or left head-neck rotator muscles, respectively). In contrast, there are a few limitations related to vibratory stimulus use; 1) it is not possible to restrict vibrated muscles. The antagonist muscles might also be stimulated, 2) the stimulation of cutaneous receptors is not avoidable, 3) widespread vibratory stimulus might transmit to the vestibular apparatus or baroreceptor afferents. Thus, the involvement of these afferent inputs may need to be awared of.

8.3. Recommendations

In order to develop the body of knowledge in this area, further experiments, that take into account the limitations of the current work as listed above, are essential.

Firstly, it is important to further improve the knowledge on the muscle conditioning manoeuvre. In contrast to a vibratory stimulus, as demonstrated in the limbs, the manoeuvre is unlikely to influence the cutaneous receptors but alter the strength of muscle spindle afferent inputs in a systematic way. Thus, the muscle conditioning manoeuvre may be useful to

investigate the contribution of the somatosensory inputs from the neck to body functions including autonomic regulation of cardiovascular function. However, the effects of the manoeuvre have not been characterised with regard to somatosensory receptors in the neck. Therefore, several questions need to be clarified; 1) whether the muscle conditioning manoeuvre alters the mechanical state of muscle spindles in the neck in a systematic way as demonstrated in the limbs, 2) whether the types of sensory receptors influenced by the manoeuvre are different between the limbs and neck, and 3) whether there is a difference in effect of the manoeuvre between different (layers of) neck muscles.

Secondly, since a conventional analysis of HRV was used in this thesis (i.e., fast Fourier transform), a certain length of recording period is required to reliably determine power spectrum bands (Task Force 1996). Thus, a transient change in the modulation of cardiac autonomic nervous activity was unable to be tested using the method. Therefore, different analytical techniques of HRV may need to be used in order to further investigate continuous changes in the modulation of cardiac autonomic nervous activity within a shorter time window.

Thirdly, the origin of cardiovascular responses observed in Chapter 7 to 20° head-up tilt may be identified using a different orthostatic stress. For example, in contrast to head-up tilt, lower body negative pressure can be used for unloading the baroreceptors while constant vestibular inputs are maintained throughout data collection (Kitano et al. 2005). These sorts of experiments may clarify whether neck proprioceptive inputs independently influence cardiovascular function in response to orthostatic stress.

These potential extensions of this thesis may be helpful for understanding how alterations in neck sensory inputs (in particular, focusing on muscle proprioceptive inputs) influence

autonomic regulation of cardiovascular function in humans. Additionally, it is important to investigate whether physical therapy-types of interventions influence autonomic regulation of cardiovascular function in different populations such as patients with dysfunctional neck or with different classes of hypertensive condition (e.g., Chobanian et al. 2003) using a similar protocol of this thesis.

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Appendix 1

Used for a study outlined in Chapter 3

Dear participants

My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University.

I would like to invite you to participate in a study that will examine the effects of spinal manipulation on heart function. This project consists of three experiments. You are invited to participate in any or all of these experiments.

Project Title;
The effect of change in posture and mechanical stimulation of the spine on cardiovascular function in conscious humans.

Background Information;
Circulation problems are a serious issue all over the world. In Australia, high blood pressure was listed as the most common condition of the cardiovascular system and 10% of Australian people have reported a hypertensive condition. Effective treatment for cardiovascular problems may not only include taking medications but also manipulative therapies such as chiropractic, osteopathy and physiotherapy as an adjunct to medical care. The fundamental concept of these kinds of therapies is that the applied mechanical stimulation (spinal manipulation) results in an improved physical condition.

Requirements of Participants;
In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You should meet all of the following conditions;

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known arrhythmia, stroke and myocardial infarction
- No history of diabetes mellitus
- No history of cancer

Experimental procedures to be carried out
You will need to fast for at least 4 hours before participating in the experiment. Also you will need to abstain from any caffeine-containing beverages for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the experiment. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the experiment. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes.

We will measure your heart rate and blood pressure in each experiment. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the experimental session. Your heart rate will be measured using electrodes which consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period (10 minutes). This will modulate your breathing rhythm to a rate of 15 breaths a minute. At the end of the second session in this experiment we will ask you to soak your hand up to your wrist in a bucket of icy water for no

longer than 1 minute (cold pressor test). During this time your heart rate and blood pressure will be continuously monitored.

The followings are the details of the first experiment of this project.

In the first experiment, heart rate and blood pressure will be measured during sitting, lying on your back and then lying on your front. Once you have made yourself comfortable in each of these postures, you will be asked to keep your position, and remain relaxed and quiet for a period of 5 minutes. After 5 minutes relaxation, you will be asked to start to synchronise your breathing with a metronome rhythm for 5 minutes in each posture. The first experiment consists of two sessions. Each session in this experiment will take about 60 minutes and be conducted on different days.

a) You will be asked to firstly lie down on your front (nose down) and then on your back (nose up) on a chiropractic technique table.
b) You will be asked to lie down on your front (nose down) on chiropractic technique table and then sit on the specially designed chair. At the end of this session, the cold pressor test will be conducted.

This project will be conducted in the Aerospace laboratory on the ground floor of the building 203 at RMIT University Bundoora West Campus.

During some experiments we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the experiment for publication/presentation. Your face will be blacked out so you will not be identified in this photo.

All electrical equipment that comes into contact with you is approved for use with humans and will be connected to the mains power outlet via a core leakage detector.

Unpleasant symptoms may be caused by the cold pressor test, for example fainting, sweating, a prolonged pain sensation or itchiness of the hand. To minimise these risks, you will need to complete a cardiovascular risk questionnaire. Your answers to some questions on this questionnaire may exclude you from the study. We stress that it is very important that you complete this questionnaire as honestly as you can in order to minimise your risk of harm through involvement in this project. Your blood pressure and heart rate will be continuously monitored for the duration of this cold pressor test and the test will be terminated if you either do not want the test to continue or your blood pressure and/or heart rate drops significantly. First aid will be conducted by one of the investigators trained in first aid if necessary.

Guidelines on the classification of human research projects by the Human Research Ethics Committee suggest this project to be in the At Risk category. Participants maintain the right to withdraw from the study at any time. All information concerning individuals will remain confidential and no individual will be identified in any publications or presentations resulting from this study.

If you would like further information about this study you may contact me on (03) 9925 7655. Thank you for your support.

Yours faithfully,

Investigator; Nobuhiro Watanabe BAppSc(Chiro)

Supervisors; Barbara I. Polus BAppSc(Chiro), MSc, PhD
Brian S. Budgell DC, MSc

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 2

Used for studies outlined in Chapter 3 & 4

General Health Questionnaire

Date: ____/____/_____

ID: _____ Date of Birth: _____

Age: _____ years Weight: _____ kg Height: _____cm

Give a brief description of your average weekly activity pattern:

_____

_____

_____

Circle the most appropriate responses for the following questions:

1. Do you have a history of recent trauma (past three months), surgery, fracture and dislocation of your neck?

   Yes No Don't Know

2. Do you have an anatomical malformation of the bones in your neck?

   Yes No Don't Know

3. Are you engaged in litigation for spinal injury?

   Yes No Don't Know

4. Are you asthmatic? Yes No Don't Know

5. Are you (or Is your family) diabetic? Yes No Don't Know

6. Do you have a history of positional vertigo?

   Yes No Don't Know

7. Do you (or Does your family) have a history of cancer?

   Yes No Don't Know

8. Do you have a history of chronic or recurrent inflammatory disease?

   Yes No Don't Know

9. Are you receiving anticoagulant or steroid therapy?

   Yes No Don't Know

Appendix 3

Used for studies outlined in this thesis

CARDIOVASCULAR RISK FACTOR QUESTIONNAIRE

In order to be eligible to participate in the experiment you are required to complete the following questionnaire, designed to assess the risk of you having a cardiovascular event during the course of the trial.

ID: _____

Circle the most appropriate responses for the following questions:

1. Are you overweight? Yes No Don't Know
2. Do you smoke? Yes No Don't Know
3. Do you or your family have a history of premature cardiovascular problems (eg. heart attack, stroke)? Yes No Don't Know
4. Do you have high blood cholesterol levels? Yes No Don't Know
5. Do you have high blood pressure? Yes No Don't Know
6. Do you have an arrhythmia? Yes No Don't Know
7. Do you have a heart murmur? Yes No Don't Know
8. Do you have impaired circulation in the hands or feet when cold? Yes No Don't Know
9. Are you on any medication Yes No
   If so, what is the medication? _____
10. Do you think you have any medical complaint or any other reason which you know of which you think may prevent you from participating in this trial?
    Yes No
    If yes, please elaborate. _____

I, _____, believe that the answers to these questions are true and correct.

Signed: _____ Date: _____

Appendix 4

Used for studies outlined in Chapter 3 & 4

RMIT HUMAN RESEARCH ETHICS COMMITTEE

Prescribed Consent Form For Persons Participating In Research Projects Involving Questionnaires, Tests and/or Medical Procedures

School of	**Health Sciences**		
Division of	**Chiropractic**		
Name of participant:			
Project Title:	**The effect of change in posture and mechanical stimulation of the spine on cardiovascular function in conscious humans.**		
Name(s) of investigators: (1)	**Nobuhiro Watanabe**	Phone:	**9925-7655**
(2)	**Barbara I. Polus**	Phone:	**9925-7714**
(3)	**Brian S. Budgell**	Phone:	**+ 81-75-751-3925**

1. I have received a statement explaining the tests/procedures involved in this project.

2. I consent to participate in the above project, the particulars of which - including details of tests or procedures - have been explained to me.

3. I authorise the investigator or his or her assistant to use with me the tests or procedures referred to in 1 above.

4. I acknowledge that:

   (a) Having read Plain Language Statement, I agree to the general purpose, methods and demands of the study.
   (b) The possible effects of the tests or procedures have been explained to me to my satisfaction.
   (c) I have been informed that I am free to withdraw from the project at any time and to withdraw any unprocessed data previously supplied (unless follow-up is needed for safety).
   (d) The project is for the purpose of research and/or teaching. It may not be of direct benefit to me.
   (e) The privacy of the personal information I provide will be safeguarded and only disclosed where I have consented to the disclosure or as required by law.
   (f) The security of the research data is assured during and after completion of the study. The data collected during the study may be published, and a report of the project outcomes will be provided to RMIT University (researcher to specify). Any information which will identify me will not be used.

Participant's Consent

Name: _____ **Date:** _____
(Participant)

Name: _____ **Date:** _____
(Witness to signature)

Participants should be given a photocopy of this consent form after it has been signed.

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 5

Used for studies outlined in Chapter 3&4

Pre-experimental Questionnaire

Date: ____/____/_____

1. ID: _____

2. Gender: Male □ Female □

3. Age: _____

4. Height: _____cm Weight:_____kg

5. How long have you fasted, and abstained from caffeine-containing and alcoholic beverages?
foods;_____hours alcohol;_____hours caffeine;_____hours

6. Have you had neck or arm pain or stiffness in the past week?
Yes □ No □

7. Do you currently have neck or arm pain/stiffness?
Yes □ No □

If "yes", please indicate the degree of your pain or stiffness at this moment on the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

0_____10

Appendix 6

Used for a study outlined in Chapter 3

Post-experimental Questionnaire

Date: ____/____/_____

1. ID: _____

2. Do you currently have neck or arm pain/stiffness?
   Yes □ No □
   If "yes", please indicate the degree of your pain or stiffness at this moment on the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

   0_____10

3. Was the any part of the procedure today, unpleasant or painful?
   Yes □ No □

   If "yes", please indicate which procedure was unpleasant or painful and the degree of the pain or discomfort of the procedure the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

   Which procedure? _____.

   0_____10

4. Do you now have any unpleasant symptoms, such as nausea or dizziness, which you did not have before the experiment?
   Yes □ No □
   If "yes", please describe them below.

   _____.

Appendix 7

Used for a study outlined in Chapter 4

Dear participants

My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University.

I would like to invite you to participate in a study that will examine the effects of spinal manipulation on heart function. This project consists of three experiments. You are invited to participate in any or all of these experiments.

Project Title;
The effect of change in posture and mechanical stimulation of the spine on cardiovascular function in conscious humans.

Background Information;
Circulation problems are a serious issue all over the world. In Australia, high blood pressure was listed as the most common condition of the cardiovascular system and 10% of Australian people have reported a hypertensive condition. Effective treatment for cardiovascular problems may not only include taking medications but also manipulative therapies such as chiropractic, osteopathy and physiotherapy as an adjunct to medical care. The fundamental concept of these kinds of therapies is that the applied mechanical stimulation (spinal manipulation) results in an improved physical condition.

Requirements of Participants;
In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You should meet all of the following conditions;

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known arrhythmia, stroke and myocardial infarction
- No history of diabetes mellitus
- No history of cancer

Experimental procedures to be carried out
You will need to fast for at least 4 hours before participating in the experiment. Also you will need to abstain from any caffeine-containing beverages for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the experiment. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the experiment. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes.

We will measure your heart rate and blood pressure in each experiment. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the experimental session. Your heart rate will be measured using electrodes which consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period (10 minutes). This will modulate your breathing rhythm to a rate of 15 breaths a minute. At the end of one of four sessions in this experiment we will ask you to soak your hand up to your wrist in a bucket of icy water for no

longer than 1 minute (cold pressor test) if you have not been involved in this project before. During this time your heart rate and blood pressure will be continuously monitored.

The followings are the details of the second experiment of this project.

In the second experiment, heart rate and blood pressure will be measured throughout all procedures. Heart rate and blood pressure will be compared before and after a neck manipulation in two different situations – while you are sitting and while you are lying down. To manipulate your neck, an Activator instrument will be used. The Activator Instrument is a device that is routinely used in chiropractic clinical practice to manipulate the spine. The device will allow us to deliver a reproducible "tap" (spinal manipulation) to your neck without inducing any form of neck movement – particularly neck rotation. The Activator Instrument is similar in form to a reflex hammer that is routinely used by clinicians. The body of the hammer is made of metallic materials and the tip, which contacts your spine, is covered with rubber. The instrument is used to produce a brief mechanical impulse (tap) over specific parts of your spine. When a spinal manipulation is performed using the Activator Instrument you will hear a "clicking" sound from the instrument. You will also feel a tap that will feel firm but not painful.

Stroke due to vascular injury is very rare complication (estimated at 1 in 1.3 million neck manipulations) that can occur after neck movement or manipulation, including neck movements that occur during normal daily activities. Please note that the "risk of neck manipulation" information given above has been calculated for neck manipulation delivered by the therapist using his/her hands rather than for neck manipulation delivered using the Activator instrument as will be used in this project. There are no specific studies that have been done concerning adverse events occurring as a result of delivering a spinal manipulation using the Activator Instrument to our knowledge. It must be noted that spinal manipulation using the Activator Instrument does not involve any neck movement. However blood vessel injury has been reported in the literature even after simple neck movements during the course of normal daily activities. Therefore we need to inform you about this risk of injury. We will minimise any risks to you by asking you to accurately complete the cardiovascular risk questionnaire. It is very important that you complete this questionnaire as accurately as you can. We will also take your blood pressure and both these procedures will allow us to decide whether or you are able to participate in the experiment.

The "Activator instrument" is used routinely by some chiropractors in their clinical practice. However, if you feel any unpleasant symptoms after the neck manipulation, the experiment will be stopped immediately and you will be assessed by the attending chiropractors and appropriate management of you will be offered.

The second experiment consists of four sessions. Each session in this experiment will take about 50 minutes and will be conducted on different days. In each of the four sessions you will receive a spinal manipulation either while you are lying down or when you are in the seated position. However, the type of spinal manipulation delivered at each session will be different. The following paragraphs detail the actual procedure we will be using.

a) You will be asked to lie down on a chiropractic technique table. You will be asked to stay in this position for 5 minutes during which time your heart rate and blood pressure will be monitored. At the end of the first 5 minute period, you will be asked to synchronise your breathing to a metronome rhythm and your blood pressure and heart rate will be monitored for a further 5 minutes. At the end of this 5 minute period your neck will be manipulated using the Activator Instrument described above. We will then

again monitor your blood pressure and heart rate for the final 5 minute period while you continue timing your breathing rate to the metronome rhythm.

b) You will be asked to sit on a specially designed, straight-backed chair. After you have made yourself comfortable you will be securely strapped into the chair with a racing car harness. A modified bicycle helmet that is attached to the frame of the chair will be gently lowered over your head and will be loosely strapped around your head. The purpose of the helmet is to discourage you from turning your head. However, if you should feel uncomfortable at any time, you will be able to easily slip out of the helmet and move your head freely. Once we have set you up in the chair, you will be asked to stay in this position for 5 minutes during which time your heart rate and blood pressure will be monitored. At the end of the first 5 minute period you will be asked to synchronise your breathing to a metronome rhythm and your blood pressure and heart rate will be monitored for a further 5 minutes. At the end of this 5 minute period your neck will be manipulated using the Activator Instrument described above. We will then again monitor your blood pressure and heart rate for the final 5 minute period while you continue timing your breathing rate to the metronome rhythm. At the end of one of these sessions, the cold pressor test will be conducted.

Unpleasant symptoms may be caused by the cold pressor test, for example fainting, sweating, a prolonged pain sensation or itchiness of the hand. To minimise these risks, you will need to complete a cardiovascular risk questionnaire. Your answers to some questions on this questionnaire may exclude you from the study. Again, we stress that it is very important that you complete this questionnaire as honestly as you can in order to minimise your risk of harm through involvement in this project. Your blood pressure and heart rate will be continuously monitored for the duration of this cold pressor test and the test will be terminated if you either do not want the test to continue or your blood pressure and/or heart rate drops significantly. First aid will be conducted by one of the investigators trained in first aid if necessary

This project will be conducted in the Aerospace laboratory on the ground floor (level 2, room 5) of building 203 at RMIT University Bundoora West Campus.

During some experiments we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the experiment for publication/presentation. Your face will be blacked out so you will not be identified in this photo.

All electrical equipment that is contacted with you is approved for use with human and will be connected to the mains power outlet via a core leakage detector.

Guidelines on the classification of human research projects by the Human Research Ethics Committee suggest this project to be in the At Risk category. Participants maintain the right to withdraw from the study at any time. All information concerning individuals will remain confidential and no individual will be identified in any publications or presentations resulting from this study.

If you would like further information about this study you may contact me on (03) 9925 7655.

Thank you for your support.

Yours faithfully,

Investigator; Nobuhiro Watanabe BAppSc(Chiro)

Supervisors; Barbara I. Polus BAppSc(Chiro), MSc, PhD
Brian S. Budgell DC, MSc

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 8

Used for studies outlined in Chapter 4

Post-experimental Questionnaire

Date: ____/____/_____

1. ID: _____

2. During the experiment, was your neck manipulated?
   Yes □ No □ Not sure □

3. Do you currently have neck or arm pain/stiffness?
   Yes ☐ No ☐
   If "yes", please indicate the degree of your pain or stiffness at this moment on the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

   0_____10

4. Was the any part of the procedure today, unpleasant or painful?
   Yes ☐ No ☐
   If "yes", please indicate which procedure was unpleasant or painful and the degree of the pain or discomfort of the procedure the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

   Which procedure? _____.

   0_____10

5. Do you now have any unpleasant symptoms, such as nausea or dizziness, which you did not have before the experiment?
   Yes □ No □
   If "yes", please describe them below.

   _____.

6. Did you have difficulties in synchronising your breathing to the metronome rhythm?
   Yes □ No □

7. Did you have difficulties in keeping awake during the experiment?
   Yes □ No □

Appendix 9

Used for a study outlined in Chapter 4

(for a participant who newly attended in this study)

Dear participants

My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University.

I would like to invite you to participate in a study that will examine the effects of spinal manipulation on heart function. This project consists of three experiments. You are invited to participate in any or all of these experiments.

Project Title;
The effect of change in posture and mechanical stimulation of the spine on cardiovascular function in conscious humans.

Background Information;
Circulation problems are a serious issue all over the world. In Australia, high blood pressure was listed as the most common condition of the cardiovascular system and 10% of Australian people have reported a hypertensive condition. Effective treatment for cardiovascular problems may not only include taking medications but also manipulative therapies such as chiropractic, osteopathy and physiotherapy as an adjunct to medical care. The fundamental concept of these kinds of therapies is that the applied mechanical stimulation (spinal manipulation) results in an improved physical condition.

Requirements of Participants;
In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You should meet all of the following conditions;

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known arrhythmia, stroke and myocardial infarction
- No history of diabetes mellitus
- No history of cancer

Experimental procedures to be carried out
You will need to fast for at least 4 hours before participating in the experiment. Also you will need to abstain from any caffeine-containing beverages for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the experiment. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the experiment. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes.

We will measure your heart rate and blood pressure in each experiment. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the experimental session. Your heart rate will be measured using electrodes which consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period (10 minutes). This will modulate your breathing rhythm to a rate of 15 breaths a minute. At the end of one of four sessions in this experiment we will ask you to soak your hand up to your wrist in a bucket of icy water for no

longer than 1 minute (cold pressor test) if you have not been involved in this project before. During this time your heart rate and blood pressure will be continuously monitored.

The following are the details of the second experiment of this project.

In the second experiment, heart rate and blood pressure will be measured throughout all procedures. Heart rate and blood pressure will be compared before and after a neck manipulation while you are lying down. To manipulate your neck, an Activator instrument will be used. The Activator Instrument is a device that is routinely used in chiropractic clinical practice to manipulate the spine. The device will allow us to deliver a reproducible "tap" (spinal manipulation) to your neck without inducing any form of neck movement – particularly neck rotation. The Activator Instrument is similar in form to a reflex hammer that is routinely used by clinicians. The body of the hammer is made of metallic materials and the tip, which contacts your spine, is covered with rubber. The instrument is used to produce a brief mechanical impulse (tap) over specific parts of your spine. When a spinal manipulation is performed using the Activator Instrument you will hear a "clicking" sound from the instrument. You will also feel a tap that will feel firm but not painful.

Stroke due to vascular injury is very rare complication (estimated at 1 in 1.3 million neck manipulations) that can occur after neck movement or manipulation, including neck movements that occur during normal daily activities. Please note that the "risk of neck manipulation" information given above has been calculated for neck manipulation delivered by the therapist using his/her hands rather than for neck manipulation delivered using the Activator instrument as will be used in this project. There are no specific studies that have been done concerning adverse events occurring as a result of delivering a spinal manipulation using the Activator Instrument to our knowledge. It must be noted that spinal manipulation using the Activator Instrument does not involve any neck movement. However blood vessel injury has been reported in the literature even after simple neck movements during the course of normal daily activities. Therefore we need to inform you about this risk of injury. We will minimise any risks to you by asking you to accurately complete the cardiovascular risk questionnaire. It is very important that you complete this questionnaire as accurately as you can. We will also take your blood pressure and both these procedures will allow us to decide whether or you are able to participate in the experiment.

The "Activator instrument" is used routinely by some chiropractors in their clinical practice. However, if you feel any unpleasant symptoms after the neck manipulation, the experiment will be stopped immediately and you will be assessed by the attending chiropractors and appropriate management of you will be offered.

The second experiment consists of four sessions. Each session in this experiment will take about 50 minutes and will be conducted on different days. In each of the four sessions you will receive a spinal manipulation in lying down position. However, the type of spinal manipulation delivered at each session will be different. The following paragraph details the actual procedure we will be using.

You will be asked to lie down on a chiropractic technique table. You will be asked to stay in this position for 5 minutes during which time your heart rate and blood pressure will be monitored. At the end of the first 5 minute period, you will be asked to synchronise your breathing to a metronome rhythm and your blood pressure and heart rate will be monitored for a further 5 minutes. At the end of this 5 minute period your neck will be manipulated either by using the Activator Instrument described above or by applying gentle pressure to the top of your neck with a finger for a period of about 10 seconds. We will then again monitor your

blood pressure and heart rate for the final 5 minute period while you continue timing your breathing rate to the metronome rhythm.
At the end of one of these sessions, the cold pressor test will be conducted.

The Cold Pressor test
The cold pressor test is where you soak your hand up to your wrist in a bucket of icy water for no longer than 1 minute. We use this test to understand your body's normal response to this unpleasant stimulus. Unpleasant symptoms may be caused by the cold pressor test, for example fainting, sweating, a prolonged pain sensation or itchiness of the hand. To minimise these risks, you will need to complete a cardiovascular risk questionnaire. Your answers to some questions on this questionnaire may exclude you from the study. Again, we stress that it is very important that you complete this questionnaire as honestly as you can in order to minimise your risk of harm through involvement in this project. Your blood pressure and heart rate will be continuously monitored for the duration of this cold pressor test and the test will be terminated if you either do not want the test to continue or your blood pressure and/or heart rate drops significantly. First aid will be conducted by one of the investigators trained in first aid if necessary

This project will be conducted in the Aerospace laboratory on the ground floor (level 2, room 5) of building 203 at RMIT University Bundoora West Campus.

During some experiments we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the experiment for publication/presentation. Your face will be blacked out so you will not be identified in this photo.

All electrical equipment that is contacted with you is approved for use with human and will be connected to the mains power outlet via a core leakage detector.

Guidelines on the classification of human research projects by the Human Research Ethics Committee suggest this project to be in the At Risk category. Participants maintain the right to withdraw from the study at any time. All information concerning individuals will remain confidential and no individual will be identified in any publications or presentations resulting from this study.

If you would like further information about this study you may contact me on (03) 9925 7655.

Thank you for your support.

Yours faithfully,

Investigator; Nobuhiro Watanabe BAppSc(Chiro)

Supervisors; Barbara I. Polus BAppSc(Chiro), MSc, PhD
Brian S. Budgell DC, MSc

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 10

Used for a study outlined in Chapter 4

(for a participant who attended in a previous session)

Dear participants

My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University.

I would like to invite you to participate in a study that will examine the effects of spinal manipulation on heart function. This project consists of three experiments. You are invited to participate in any or all of these experiments.

Project Title;
The effect of change in posture and mechanical stimulation of the spine on cardiovascular function in conscious humans.

Background Information;
Circulation problems are a serious issue all over the world. In Australia, high blood pressure was listed as the most common condition of the cardiovascular system and 10% of Australian people have reported a hypertensive condition. Effective treatment for cardiovascular problems may not only include taking medications but also manipulative therapies such as chiropractic, osteopathy and physiotherapy as an adjunct to medical care. The fundamental concept of these kinds of therapies is that the applied mechanical stimulation (spinal manipulation) results in an improved physical condition.

Requirements of Participants;
In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You should meet all of the following conditions;

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known arrhythmia, stroke and myocardial infarction
- No history of diabetes mellitus
- No history of cancer

Experimental procedures to be carried out
You will need to fast for at least 4 hours before participating in the experiment. Also you will need to abstain from any caffeine-containing beverages for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the experiment. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the experiment. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes.

We will measure your heart rate and blood pressure in each experiment. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the experimental session. Your heart rate will be measured using electrodes which consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period (10 minutes). This will modulate your breathing rhythm to a rate of 15 breaths a minute.

The following are the details of the additional sessions in the second experiment of this project.

In the second experiment, heart rate and blood pressure will be measured throughout all procedures. Heart rate and blood pressure will be compared before and after a neck manipulation while you are lying down. To manipulate your neck, an Activator instrument will be used. The Activator Instrument is a device that is routinely used in chiropractic clinical practice to manipulate the spine. The device will allow us to deliver a reproducible "tap" (spinal manipulation) to your neck without inducing any form of neck movement – particularly neck rotation. The Activator Instrument is similar in form to a reflex hammer that is routinely used by clinicians. The body of the hammer is made of metallic materials and the tip, which contacts your spine, is covered with rubber. The instrument is used to produce a brief mechanical impulse (tap) over specific parts of your spine. When a spinal manipulation is performed using the Activator Instrument you will hear a "clicking" sound from the instrument. You will also feel a tap that will feel firm but not painful.

Stroke due to vascular injury is very rare complication (estimated at 1 in 1.3 million neck manipulations) that can occur after neck movement or manipulation, including neck movements that occur during normal daily activities. Please note that the "risk of neck manipulation" information given above has been calculated for neck manipulation delivered by the therapist using his/her hands rather than for neck manipulation delivered using the Activator instrument as will be used in this project. There are no specific studies that have been done concerning adverse events occurring as a result of delivering a spinal manipulation using the Activator Instrument to our knowledge. It must be noted that spinal manipulation using the Activator Instrument does not involve any neck movement. However blood vessel injury has been reported in the literature even after simple neck movements during the course of normal daily activities. Therefore we need to inform you about this risk of injury. We will minimise any risks to you by asking you to accurately complete the cardiovascular risk questionnaire. It is very important that you complete this questionnaire as accurately as you can. We will also take your blood pressure and both these procedures will allow us to decide whether you are able to participate in the experiment.

The "Activator instrument" is used routinely by some chiropractors in their clinical practice. However, if you feel any unpleasant symptoms after the neck manipulation, the experiment will be stopped immediately and you will be assessed by the attending chiropractors and appropriate management of you will be offered.

The second experiment consists of two sessions. Each session in this experiment will take about 50 minutes and will be conducted on different days. In each of the two sessions you will receive a spinal manipulation while you are lying down on a bench. However, the type of spinal manipulation delivered at each session will be different.
The following paragraph details the actual procedure we will be using.

You will be asked to lie down on a chiropractic technique table. You will be asked to stay in this position for 5 minutes during which time your heart rate and blood pressure will be monitored. At the end of the first 5 minute period, you will be asked to synchronise your breathing to a metronome rhythm and your blood pressure and heart rate will be monitored for a further 5 minutes. At the end of this 5 minute period, your neck will be manipulated using the Activator Instrument described above. We will then again monitor your blood pressure and heart rate for the final 5 minute period while you continue timing your breathing rate to the metronome rhythm.

This project will be conducted in the Aerospace laboratory on the ground floor (level 2, room 5) of building 203 at RMIT University Bundoora West Campus.

During some experiments we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the experiment for publication/presentation. Your face will be blacked out so you will not be identified in this photo.

All electrical equipment that is contacted with you is approved for use with human and will be connected to the mains power outlet via a core leakage detector.

Guidelines on the classification of human research projects by the Human Research Ethics Committee suggest this project to be in the At Risk category. Participants maintain the right to withdraw from the study at any time. All information concerning individuals will remain confidential and no individual will be identified in any publications or presentations resulting from this study.

If you would like further information about this study you may contact me on (03) 9925 7655.

Thank you for your support.

Yours faithfully,

Investigator; Nobuhiro Watanabe BAppSc(Chiro)

Supervisors; Barbara I. Polus BAppSc(Chiro), MSc, PhD
Brian S. Budgell DC, MSc

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 11

Used for a study outlined in Chapter 5

(for the muscle conditioning study)

DIVISION OF CHIROPRACTIC
SCHOOL OF HEALTH SCIENCES
PH: +61 3 9925 7596 FAX: +61 3 9467 2794

INVITATION TO PARTICIPATE IN A RESEARCH PROJECT
PROJECT INFORMATION STATEMENT

Project Title:

- The contribution of muscle spindles in the dorsal neck to autonomic regulation of cardiovascular function in humans

Investigators:

- Mr Nobuhiro Watanabe (Chiropractic PhD degree student)
- Associate Professor Barbara I Polus (Project Supervisor: RMIT University, barbara.polus@rmit.edu.au, 9925-7714)

Dear participants

This research project consists of three studies, and you are invited to take part in one, two or all three studies of this project being conducted by RMIT University. This information sheet describes the project in straightforward language, or 'plain English'. Please read this sheet carefully and be confident that you understand its contents before deciding whether to participate. If you have any questions about the project, please ask one of the investigators.

Who is involved in this research project? Why is it being conducted?

- My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University. I am conducting this research project as a part of my PhD thesis, and Dr. Barbara I Polus (my supervisor) and I (primary investigator) are involved in the project.
- This research project has been approved by the RMIT Human Research Ethics Committee.
- This study is partially funded by the Australian Spinal Research Foundation.

Why have you been approached?

- Generally healthy young adults at RMIT University have been invited to this study.

What is the project about? What are the questions being addressed?

- Human beings living on the earth must regulate various body functions in response to changes in body posture relative to gravity. For example, lying to standing. In healthy humans, changes in body posture must be accompanied by adjustments in blood pressure so that adequate blood flow to the brain is maintained. There is evidence that the vestibular system (balance control system of the inner ear) contributes to blood pressure adjustments that occur with changes in posture. However, there are a few studies which have examined the

interaction between neck muscle activity and the inner ear in the regulation of blood pressure control with posture changes.

- Through this research project, the following questions will be answered.
  - Does a change in neck muscle spindle discharge affect autonomic and cardiovascular activity?
  - Can manipulation of neck muscle spindle discharge in the presence of a constant vestibular stimulus alter the response pattern of autonomic and cardiovascular parameters to posture change?
- Up 20 participants will be involved in this study.

If I agree to participate, what will I be required to do?

- **Requirements of Participants**

In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You should meet all of the following conditions;

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known arrhythmia, stroke and myocardial infarction
- No history of vestibular diseases such as vestibular neuronitis and Meniere's disease
- No history of claustrophobia
- No history of diabetes mellitus
- No history of cancer

- **Study procedures to be carried out**

You will need to fast for at least 4 hours before participating in the study. Also you will need to abstain from any caffeine-containing beverages (e.g. coffee, tea, and coke) for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the study. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the study. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes (this 20 minute moderate exercise restriction only applies for the 48 hours prior to the experiment day).

We will measure your heart rate, blood pressure, sweat response in the hand, finger and forearm blood flow and neck muscle activity in each experimental session. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the study session. Your heart rate will be measured using electrodes which consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. Sweating response in your hand will be recorded through three electrodes placed on the hand and forearm, and finger blood flow will be taken with an instrument gently clipped

on the finger tip. In addition, forearm blood flow will be measured by loosely fastening a thin mercury-in-ruber tube around your forearm. This device gives us changes in forearm blood flow. In addition, neck muscle activity will be measured though each experimental session. To record muscle activity, several electrodes mentioned above will be attached on the front and back sides of your neck.
You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period (6 minutes in the first session and 9 minutes in the second session). This will modulate your breathing rhythm to a rate of 15 breaths a minute. Additionally you will be asked to wear a blindfold to exclude visual input.

The following is the details of the first study of this project.

In the first study, you will be asked to seat yourself comfortably in a custom-built chair with a racing car harness strapped over your shoulders. Your feet will rest on footplates that can be adjusted to any distance from the chair to your comfort. A helmet-frame will be lowered over your head and will fit across your forehead and base of your head. This helmet-frame is designed to keep your head steady as your body turns around it. You will be able to easily remove your head from this helmet if you experience any discomfort. The chair and footplate are designed to be rotated in both directions in 5 degree increments to a maximum of 60 degrees. Heart rate, blood pressure, sweating response in the hand, finger blood flow and neck muscle activity will be recorded throughout the study session, and changes in forearm blood flow will be measured after the intervention in the sitting posture.
Once you have made yourself comfortable in this posture, you will be asked to remain relaxed and quiet for a period of 5 minutes. After 5 minutes relaxation, you will be asked to start to synchronise your breathing with a metronome rhythm. The first study consists of two sessions. Each session in this study will take about 30 minutes and be conducted on different days.

a) After the 3 minutes controlled breathing, you will be asked to rotate your head to the right side against a resistance applied by the investigator's hand positioned over the side of your lower face (jaw area) for 3 seconds. You will then be asked to relax your neck muscles and keep your neck relaxed (it will be supported by the helmet and frame) for the rest of the experiment. The chair will be turned towards your right side up to 20 degrees (Test position), and your body will be kept in this position for 3 minutes while blood pressure, heart rate, sweating response in the hand, and finger and forearm blood flow will be recorded.
b) After the 3 minutes controlled breathing, you will be asked to complete the same procedures as the entire first session. After that, we will turn the chair further to 30 degrees. Again, you will be asked to rotate your head to the right side against a resistance applied by the investigator's hand positioned over the side of your lower face (jaw area). You will then be asked to relax your neck muscles and keep your neck relaxed (it will be supported by the helmet and frame) for the rest of the experiment. After a period of 5 seconds the chair will be returned back to the "Test position", and your body will be kept in this position for 3 minutes while

blood pressure, heart rate, sweating response in the hand, and finger and forearm blood flow will be recorded.

What are the risks or disadvantages associated with participation?

- Synchronising breathing with a metronome may cause dizziness and difficulty in breathing at the desired rate for some participants. If you experience any unpleasant symptoms during data recording, please notify the investigator immediately.
  The experiment will be terminated.
- Electrodes applied to the skin may cause rash and/or itchy sensation during and/or after placing on the body due to one or any of the followings; skin cleansing swab (70 % alcohol containing), and adhesive and gel of the electrode.
- The intervention in this project (muscle contraction and moderate stretch of the neck muscles) may cause muscle soreness or discomfort during and/or after the experimental session. If you experience any symptoms in the neck, you need to report this to the investigator immediately and the experiment will be cancelled. One of the investigators (Barbara I Polus) is a registered chiropractor within the state of Victoria. You will be examined by the investigator and advised on appropriate management.

What are the benefits associated with participation?

- There may not be direct benefit to the participants.

What will happen to the information I provide?

- All information concerning individuals will remain confidential and no individual will be identified in any publications and presentations resulting from this study. The information will be securely stored in a lockable office, retained for 5 years, and then destroyed. Only my supervisor (Barbara I Polus) and I will have access to the information.
- During some studies we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the study for publication and presentation only. Your face will be blacked out so you will not be identified in this photo.

What are my rights as a participant?

- Participants in this study will have the following rights;
  - ✓ The right to withdraw their participation at any time, without prejudice.
  - ✓ The right to have any unprocessed data withdrawn and destroyed, provided it can be reliably identified, and provided that so doing does not increase the risk for the participant.
  - ✓ The right to have any questions answered at any time.

Whom should I contact if I have any questions?

- For inquiry, please do not hesitate to contact with us
- Mr Nobuhiro Watanabe (s9812566@student.rmit.edu.au; 9925-7655)
- Dr Barbara I Polus (barbara.polus@rmit.edu.au; 9925-7714)

What other issues should I be aware of before deciding whether to participate?

- We believe that there are no other issues related to your involvement in this study.

Thank you very much for your support.

Yours Sincerely,

Nobuhiro Watanabe BAppSc(Chiro)

Barbara I Polus BAppSc(Chiro), MSc, PhD

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745. Details of the complaints procedure are available fom the above address.

Appendix 12

Used for studies outlined in Chapter 5, 6&7

General Health Questionnaire

Date: ____/____/_____

ID: _____ Date of Birth: _____

Age: _____ years Weight: _____ kg Height: _____ cm

Give a brief description of your average weekly activity pattern:

_____

_____

_____

Circle the most appropriate responses for the following questions:

1. Do you have a history of recent trauma (past three months), surgery, fracture and dislocation of your neck? Yes No Don't Know
2. Do you have an anatomical malformation of the bones in your neck? Yes No Don't Know
3. Are you engaged in litigation for spinal injury? Yes No Don't Know
4. Are you asthmatic? Yes No Don't Know
5. Are you (or Is your family) diabetic? Yes No Don't Know
6. Do you have a history of positional vertigo? Yes No Don't Know
7. Do you have a history of vestibular disease? Yes No Don't Know
8. Do you have a history of claustrophobia? Yes No Don't Know
9. Do you (or Does your family) have a history of cancer? Yes No Don't Know
10. Do you have a history of chronic or recurrent inflammatory disease? Yes No Don't Know
11. Are you receiving anticoagulant or steroid therapy? Yes No Don't Know

I, _____, believe that the answers to these questions are true and correct.

Signed: _____ Date: _____

Appendix 13

Used for studies outlined in Chapter 5, 6&7

(for the muscle conditioning studies)

Prescribed Consent Form For Persons Participating In Research Projects Involving Tests and/or Medical Procedures, and Involving Interviews, Questionnaires or Disclosure of Personal Information

Portfolio **Science, Engineering and Technology**
School of **Health Sciences**
Name of participant:
Project Title: **The contribution of muscle spindles in the dorsal neck to autonomic regulation of cardiovascular function in humans**

☐ study1 ☐ study2 ☐ study3

Name(s) of investigators: **(1)** **Nobuhiro Watanabe** Phone: **9925-7655**
(2) **Barbara I Polus** Phone: **9925-7714**

1. I have received a statement explaining the tests/procedures and the interview/questionnaire involved in this project.

2. I consent to participate in the above project, the particulars of which - including details of tests or procedures and of the interviews or questionnaires - have been explained to me.

3. I authorise the investigator or his or her assistant to use with me the tests or procedures, and to interview me or administer a questionnaire, referred to in 1 above.

4. I acknowledge that:

   (e) Having read Plain Language Statement, I agree to the general purpose, methods and demands of the study.
   (f) The possible effects of the tests or procedures have been explained to me to my satisfaction.
   (g) I have been informed that I am free to withdraw from the project at any time and to withdraw any unprocessed data previously supplied (unless follow-up is needed for safety).
   (h) The project is for the purpose of research and/or teaching. It may not be of direct benefit to me.
   (i) The privacy of the personal information I provide will be safeguarded and only disclosed where I have consented to the disclosure or as required by law.
   (j) The security of the research data is assured during and after completion of the study. The data collected during the study may be published, and a report of the project outcomes will be provided to RMIT University. Any information which will identify me will not be used.

Participant's Consent

Participant: _____ **Date:** _____
(Signature)

Witness: _____ **Date:** _____
(Signature)

Participants should be given a photocopy of this consent form after it has been signed.

Any complaints about your participation in this project may be directed to the Executive Officer, RMIT Human Research Ethics Committee, Research & Innovation, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 2251. Details of the complaints procedure are available from the above address.

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Appendix 14

Used for studies outlined in Chapter 5, 6&7

Pre-experimental Questionnaire

Date: ____/____/_____

1. ID: _____
2. Gender: Male ☐ Female ☐
3. Age: _____
4. Height: _____cm Weight:_____kg
5. How long have you fasted, and abstained from caffeine-containing and alcoholic beverages?
   foods;_____hours alcohol;_____hours caffeine;_____hours
6. Have you had neck or arm pain or stiffness in the past week?
   Yes ☐ No ☐
7. Do you currently have neck or arm pain/stiffness?
   Yes ☐ No ☐

If "yes", please indicate the degree of your pain or stiffness at this moment on the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

0_____10

Appendix 15

Used for studies outlined in Chapter 5, 6&7

Post-experimental Questionnaire

Date: ____/____/_____

1. ID: _____

2. Do you currently have neck or arm pain/stiffness?

   Yes ☐ No ☐

If "yes", please indicate the degree of your pain or stiffness at this moment on the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

0_____10

3. Was any part of the procedure today, unpleasant or painful?

   Yes ☐ No ☐

If "yes", please indicate which procedure was unpleasant or painful and the degree of the pain or discomfort of the procedure the scale below. "0" represents complete comfort. "10" represents the worst pain imaginable.

Which procedure? _____.

0_____10

4. Do you now have any unpleasant symptoms, such as nausea or dizziness, which you did not have before the experiment?

   Yes ☐ No ☐

   If "yes", please describe them below.

   _____.

5. Did you have difficulties in synchronising your breathing to the metronome rhythm?

   Yes ☐ No ☐

6. Did you have difficulties in keeping awake during the experiment?

   Yes ☐ No ☐

Appendix 16

Used for studies outlined in Chapter 5

(for the vibration studies)

DIVISION OF CHIROPRACTIC
SCHOOL OF HEALTH SCIENCES
PH: +61 3 9925 7596 FAX: +61 3 9467 2794

INVITATION TO PARTICIPATE IN A RESEARCH PROJECT
PROJECT INFORMATION STATEMENT

Project Title:

- The effect of neck vibration on autonomic regulation of cardiovascular function in humans

Investigators:

- Mr Nobuhiro Watanabe (Chiropractic PhD degree student)
- Associate Professor Barbara I Polus (Project Supervisor: RMIT University, barbara.polus@rmit.edu.au, 9925-7714)

Dear participants

This research project consists of three studies, and you are invited to take part in one, two or all three studies of this project being conducted by RMIT University. This information sheet describes the project in straightforward language, or 'plain English'. Please read this sheet carefully and be confident that you understand its contents before deciding whether to participate. If you have any questions about the project, please ask one of the investigators.

Who is involved in this research project? Why is it being conducted?

- My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University. I am conducting this research project as a part of my PhD thesis, and Dr. Barbara I Polus (my supervisor) and I (primary investigator) are involved in the project.
- This research project has been approved by the RMIT Human Research Ethics Committee.
- This study is partially funded by the Australian Spinal Research Foundation.

Why have you been approached?

- Young adults believed to be of good general health are being invited to participate in this study.

What is the project about? What are the questions being addressed?

- Human beings are constantly exposed to the force of gravity and therefore must regulate various body functions in response to changes in body posture relative to gravity. For example, moving from lying to standing. In healthy humans, changes in body posture must be accompanied by adjustments in blood pressure so that adequate blood flow to the brain is maintained. There is evidence that the vestibular system (balance control system of the inner ear) contributes to blood pressure adjustments that occur with changes in posture. However, the inner ear

can only detect changes in head position relative to gravity special nerves in the neck inform the nervous system about whether the whole body has shifted relative to gravity or whether just the head has moved. There are no studies which have examined the interaction between neck muscle length which monitors neck position and the inner ear in the regulation of blood pressure control with posture changes.

- Through this research project, the following questions will be answered.
  - Does a change in neck muscle length and activation history affect autonomic and cardiovascular activity?
  - Can manipulation of neck muscle length and activation history in the presence of a constant head position alter the response pattern of autonomic and cardiovascular parameters to posture change?
- Up to 20 participants will be involved in this study.

If I agree to participate, what will I be required to do?

- **Requirements of Participants**

In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You must meet all of the following conditionsG

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma including a car accident (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known heart murmur, stroke and heart attack
- No history of thrombosis or high cholesterol levels
- No history of inner year disease and problems like dizziness, loss of balance or ringing in the ears
- No history of claustrophobia
- No history of diabetes mellitus
- No history of cancer

- **Preparing for the experiment**

You will need to not eat or drink anything (except water) for at least 4 hours and not consume any alcohol for at least 12 hours before participating in the study. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the study. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes (this 20 minute moderate exercise restriction only applies for the 48 hours prior to the experiment day).

During the recording period, we will measure your heart rate, blood pressure, sweat response in the hand, and finger and forearm blood flow. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the study. Your heart rate will be measured using electrodes which

consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. The sweating response of your hand will be recorded through three electrodes placed on the hand and forearm, and finger blood flow will be taken with an instrument gently clipped on the finger tip. In addition, forearm blood flow will be measured by loosely fastening a thin mercury-in-ruber tube around your forearm. This device gives us a measure of changes in the size of your forearm associated with changes in forearm blood flow. During the blood flow measurement period, two cuffs will be fitted around your upper arm and wrist. Before the recording period starts, the cuff around your wrist will be inflated and maintained at a pressure above your systolic blood pressure until the end of the experimental session (a pressure of approximately 200 mm Hg – equivalent to someone squeezing your wrist tightly). The second cuff which is fitted around your upper arm will be periodically inflated and deflated through the recording period. The pressure in this second cuff will be approximately 50 mm Hg – roughly equivalent to someone moderately squeezing your arm.
You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period. This will modulate your breathing rhythm to a rate of 15 breaths a minute.
Additionally you will be asked to wear a blindfold to exclude visual input.

The following are the details of the first study of this project.

First of all, you will be asked to seat yourself comfortably in a custom-built chair with a racing car harness strapped over your shoulders. Your feet will rest on footplates that can be adjusted to any distance from the chair to your comfort. A helmet-frame will be lowered over your head and will fit across your forehead and base of your head. This helmet-frame is designed to keep your head steady as your body turns around it. You will be able to easily remove your head from this helmet if you experience any discomfort. The chair and footplate are designed to be rotated in both directions in 5 degree increments to a maximum of 60 degrees. Heart rate, blood pressure sweating response in the hand and finger blood flow will be recorded throughout the study session, and changes in forearm blood flow will be measured during the intervention in the sitting posture.
Once you sit down on the chair, the chair will be rotated up to 30 degrees. Then you will be asked to make yourself comfortable in this posture, and to remain relaxed and quiet for a period of 5 minutes. After 5 minutes relaxation, you will be asked to start to synchronise your breathing with a metronome rhythm for 6 minutes. After the first 3 minutes of controlled breathing, a vibrator apparatus, which will be attached on the right back (or side) of your neck, will be turned on and the vibration will be delivered to your neck for 3 minutes while heart rate, blood pressure, sweat response in the hand, and finger and forearm blood flow will be recorded.
This experimental session will take about 40 minutes including preparation time.

What are the risks or disadvantages associated with participation?

- Synchronising breathing with a metronome may cause dizziness and difficulty in breathing at the desired rate for some participants. If you experience any unpleasant symptoms during data recording, please notify the investigator immediately.
  The experiment will be terminated.
- Electrodes applied to the skin may cause rash and/or an itchy sensation during and/or after placing on the body due to one or any of the followingsG skin cleansing swab (70 % alcohol containing), and adhesive and gel of the electrode.
- The intervention in this project (sustained neck rotation with a vibration stimulus) may cause an itchy sensation, neck muscle soreness or discomfort during and/or after the experimental session. If you experience any intolerable symptoms in the neck, you need to report this to the investigator immediately and the experiment will be cancelled. One of the investigators (Barbara I Polus) is a registered chiropractor within the state of Victoria. You will be examined by the investigator and advised on appropriate management.
- It is known that prolonged vibration (applied over a period of hours) when applied to the hand may cause damage or constriction of finger blood vessels. These blood vessels are located very close to the skin. Further the size of the vibratory stimulus used in these reports is of a larger amplitude and lower frequency than what we will be using in our study. There are no reported adverse effects associated with a vibratory stimulus applied either to the neck or even when a vibratory stimulus is applied to the forearm where a major forearm blood vessel travels very close to the skin.

What are the benefits associated with participation?

- There may not be direct benefit to the participants. However, some people may find interest to contribute to developing the general body of knowledge in this field.

What will happen to the information I provide?

- All information concerning individuals will remain confidential and no individual will be identified in any publications and presentations resulting from this study. The information will be securely stored in a lockable office, retained for 5 years, and then destroyed. Only my supervisor (Barbara I Polus) and I will have access to the information.
- During some studies we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the study for publication and presentation only. Your face will be blacked out so you will not be identified in this photo.

What are my rights as a participant?

- Participants in this study will have the following rightsG
  - ✓ The right to withdraw their participation at any time, without prejudice.
  - ✓ The right to have any unprocessed data withdrawn and destroyed, provided it can be reliably identified, and provided that so doing does not increase the risk for the participant.

- ✓ The right to have any questions answered at any time.

Whom should I contact if I have any questions?

- For inquiry, please do not hesitate to contact with us
- Mr Nobuhiro Watanabe (s9812566@student.rmit.edu.au 9925-7655)
- Dr Barbara I Polus (barbara.polus@rmit.edu.au 9925-7714)

What other issues should I be aware of before deciding whether to participate?

- We believe that there are no other issues related to your involvement in this study.

Thank you very much for your support.

Yours Sincerely,

Nobuhiro Watanabe BAppSc(Chiro)

Barbara I Polus BAppSc(Chiro), MSc, PhD

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745. Details of the complaints procedure are available fom the above address.

Appendix 17

Used for studies outlined in Chapter 5

(for the vibration studies)

Prescribed Consent Form For Persons Participating In Research Projects Involving Tests and/or Medical Procedures, and Involving Interviews, Questionnaires or Disclosure of Personal Information

Portfolio	**Science, Engineering and Technology**	
School of	**Health Sciences**	
Name of participant:		
Project Title:	**The effect of neck vibration on autonomic regulation of cardiovascular function in humans**	
	☐ study1 ☐ study2 ☐ study3	
Name(s) of investigators:	**(1) Nobuhiro Watanabe**	Phone: **9925-7655**
	(2) Barbara I Polus	Phone: **9925-7714**

5. I have received a statement explaining the tests/procedures and the interview/questionnaire involved in this project.

6. I consent to participate in the above project, the particulars of which - including details of tests or procedures and of the interviews or questionnaires - have been explained to me.

7. I authorise the investigator or his or her assistant to use with me the tests or procedures, and to interview me or administer a questionnaire, referred to in 1 above.

8. I acknowledge that:

   (k) Having read Plain Language Statement, I agree to the general purpose, methods and demands of the study.
   (l) The possible effects of the tests or procedures have been explained to me to my satisfaction.
   (m) I have been informed that I am free to withdraw from the project at any time and to withdraw any unprocessed data previously supplied (unless follow-up is needed for safety).
   (n) The project is for the purpose of research and/or teaching. It may not be of direct benefit to me.
   (o) The privacy of the personal information I provide will be safeguarded and only disclosed where I have consented to the disclosure or as required by law.
   (p) The security of the research data is assured during and after completion of the study. The data collected during the study may be published, and a report of the project outcomes will be provided to RMIT University. Any information which will identify me will not be used.

Participant's Consent

Participant: _____ **Date:** _____
(Signature)

Witness: _____ **Date:** _____
(Signature)

Participants should be given a photocopy of this consent form after it has been signed.

Any complaints about your participation in this project may be directed to the Executive Officer, RMIT Human Research Ethics Committee, Research & Innovation, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 2251. Details of the complaints procedure are available from the above address.

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Appendix 18

Used for studies outlined in Chapter 5

(for the vibration studies)

- **Do you feel as though your head is turning?**

1. **NO**
2. **MILD**
3. **MODERATE**
4. **STRONG**
5. **VERY STRONG**

- **If no, do you feel as though you are moving in any way?**

Appendix 19

Used for a study outlined in Chapter 6

DIVISION OF CHIROPRACTIC
SCHOOL OF HEALTH SCIENCES
PH: +61 3 9925 7596 FAX: +61 3 9467 2794

INVITATION TO PARTICIPATE IN A RESEARCH PROJECT
PROJECT INFORMATION STATEMENT

Project Title:

- The contribution of muscle spindles in the dorsal neck to autonomic regulation of cardiovascular function in humans

Investigators:

- Mr Nobuhiro Watanabe (Chiropractic PhD degree student)
- Associate Professor Barbara I Polus (Project Supervisor: RMIT University, barbara.polus@rmit.edu.au, 9925-7714)

Dear participants

This research project consists of three studies, and you are invited to take part in one, two or all three studies of this project being conducted by RMIT University. This information sheet describes the project in straightforward language, or 'plain English'. Please read this sheet carefully and be confident that you understand its contents before deciding whether to participate. If you have any questions about the project, please ask one of the investigators.

Who is involved in this research project? Why is it being conducted?

- My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University. I am conducting this research project as a part of my PhD thesis, and Dr. Barbara I Polus (my supervisor) and I (primary investigator) are involved in the project.
- This research project has been approved by the RMIT Human Research Ethics Committee.
- This study is partially funded by the Australian Spinal Research Foundation.

Why have you been approached?

- Generally healthy young adults at RMIT University have been invited to this study.

What is the project about? What are the questions being addressed?

- Human beings living on the earth must regulate various body functions in response to changes in body posture relative to gravity. For example, lying to standing. In healthy humans, changes in body posture must be accompanied by adjustments in blood pressure so that adequate blood flow to the brain is maintained. There is evidence that the vestibular system (balance control system of the inner ear) contributes to blood pressure adjustments that occur with changes in posture. However, there are a few studies which have examined the

interaction between neck muscle activity and the inner ear in the regulation of blood pressure control with posture changes.

- Through this research project, the following questions will be answered.
  - Does a change in neck muscle spindle discharge affect autonomic and cardiovascular activity?
  - Can manipulation of neck muscle spindle discharge in the presence of a constant vestibular stimulus alter the response pattern of autonomic and cardiovascular parameters to posture change?
- Up 20 participants will be involved in this study.

If I agree to participate, what will I be required to do?

- **Requirements of Participants**

In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You should meet all of the following conditions;

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known arrhythmia, stroke and myocardial infarction
- No history of vestibular diseases such as vestibular neuronitis and Meniere's disease
- No history of claustrophobia
- No history of diabetes mellitus
- No history of cancer

- **Study procedures to be carried out**

You will need to fast for at least 4 hours before participating in the study. Also you will need to abstain from any caffeine-containing beverages (e.g. coffee, tea, and coke) for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the study. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the study. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes (this 20 minute moderate exercise restriction only applies for the 48 hours prior to the experiment day).

We will measure your heart rate, blood pressure, sweat response in the hand, finger and forearm (or calf) blood flow and neck muscle activity in each experimental session. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the study session. Your heart rate will be measured using electrodes which consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. Sweating response in your hand will be recorded through three electrodes placed on the hand and forearm (or calf), and finger blood flow will be taken with an

instrument gently clipped on the finger tip. Forearm (or calf) blood flow will be measured by loosely fastening a thin mercury-in-ruber tube around your forearm (or calf). This device gives us changes in forearm (or calf) blood flow. In addition, neck muscle activity will be measured though each experimental session. To record muscle activity, several electrodes mentioned above will be attached on the front and back sides of your neck.
You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period (6 minutes). This will modulate your breathing rhythm to a rate of 15 breaths a minute.
Additionally you will be asked to wear a blindfold to exclude visual input.

The followings are the details of the second study of this project.

In the second study, heart rate, blood pressure, sweating response in the hand, finger blood flow and neck muscle activity will be recorded throughout the study session, and changes in forearm (or calf) blood flow will be measured after the intervention. You will be asked to lay on a bench with your body positioned in either the face down or face up position. Your head will be positioned over the head-edge of the bench and will be supported on a stand that is at the same height as the bench. Once you have made yourself comfortable, you will be asked to keep still and remain relaxed for a period of 5 minutes. After 5 minutes relaxation, you will be asked to start to synchronise your breathing with a metronome rhythm. The second study consists of two sessions. Each session in this study will take about 30 minutes and be conducted on different days.
After the 3 minute controlled breathing period, the stand supporting your head will be removed and your head will be gently lowered (bent forward or backward) as far as possible but only within your level of comfort. Once we have determined this position we will raise your head by approximately 10 degrees. For this experiment, this is the "test position" for your head and neck. From this position you head will be either bent forwards or backwards. This is the position at which we will condition your neck muscles. You will be asked to contract the muscles on the back of your neck against the resistance of the investigator's hands which will cup the back of your head. The contraction will last 3 seconds. You will then be asked to relax your neck muscles and rest your head on a support that will be provided for your head. After 5 seconds the support will be raised or lowered so that your head is returned to the "test position". After this intervention, you will be asked to remain completely relaxed while continuing the controlled breathing. You will remain in the test position for a further 3 minutes while your blood pressure, heart rate, sweating response in the hand, finger blood flow and forearm (or calf) blood flow are recorded.

What are the risks or disadvantages associated with participation?

- Synchronising breathing with a metronome may cause dizziness and difficulty in breathing at the desired rate for some participants. If you experience any unpleasant symptoms during data recording, please notify the investigator

immediately.
The experiment will be terminated.

- Electrodes applied to the skin may cause rash and/or itchy sensation during and/or after placing on the body due to one or any of the followings; skin cleansing swab (70 % alcohol containing), and adhesive and gel of the electrode.
- The intervention in this project (muscle contraction and moderate stretch of the neck muscles) may cause muscle soreness or discomfort during and/or after the experimental session. If you experience any symptoms in the neck, you need to report this to the investigator immediately and the experiment will be cancelled. One of the investigators (Barbara I Polus) is a registered chiropractor within the state of Victoria. You will be examined by the investigator and advised on appropriate management.

What are the benefits associated with participation?

- There may not be direct benefit to the participants. However, some people may find interest to contribute to developing the general body of knowledge in this field.

What will happen to the information I provide?

- All information concerning individuals will remain confidential and no individual will be identified in any publications and presentations resulting from this study. The information will be securely stored in a lockable office, retained for 5 years, and then destroyed. Only my supervisor (Barbara I Polus) and I will have access to the information.
- During some studies we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the study for publication and presentation only. Your face will be blacked out so you will not be identified in this photo.

What are my rights as a participant?

- Participants in this study will have the following rights;
  - ✓ The right to withdraw their participation at any time, without prejudice.
  - ✓ The right to have any unprocessed data withdrawn and destroyed, provided it can be reliably identified, and provided that so doing does not increase the risk for the participant.
  - ✓ The right to have any questions answered at any time.

Whom should I contact if I have any questions?

- For inquiry, please do not hesitate to contact with us
- Mr Nobuhiro Watanabe (s9812566@student.rmit.edu.au; 9925-7655)
- Dr Barbara I Polus (barbara.polus@rmit.edu.au; 9925-7714)

What other issues should I be aware of before deciding whether to participate?

- We would like to reimburse you for your travel and time associated with participating in this research project.

Thank you very much for your support.

Yours Sincerely,

Nobuhiro Watanabe BAppSc(Chiro)

Barbara I Polus BAppSc(Chiro), MSc, PhD

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available fom the above address.

Appendix 20

Used for a study outlined in Chapter 7

DIVISION OF CHIROPRACTIC
SCHOOL OF HEALTH SCIENCES
PH: +61 3 9925 7596 FAX: +61 3 9467 2794

INVITATION TO PARTICIPATE IN A RESEARCH PROJECT
PROJECT INFORMATION STATEMENT

Project Title:

- The contribution of muscle spindles in the dorsal neck to autonomic regulation of cardiovascular function in humans

Investigators:

- Mr Nobuhiro Watanabe (Chiropractic PhD degree student)
- Associate Professor Barbara I Polus (Project Supervisor: RMIT University, barbara.polus@rmit.edu.au, 9925-7714)

Dear participants

This research project consists of three studies, and you are invited to take part in one, two or all three studies of this project being conducted by RMIT University. This information sheet describes the project in straightforward language, or 'plain English'. Please read this sheet carefully and be confident that you understand its contents before deciding whether to participate. If you have any questions about the project, please ask one of the investigators.

Who is involved in this research project? Why is it being conducted?

- My name is Nobuhiro Watanabe. I am a research student in the Division of Chiropractic, School of Health Sciences, RMIT University. I am conducting this research project as a part of my PhD thesis, and Dr. Barbara I Polus (my supervisor) and I (primary investigator) are involved in the project.
- This research project has been approved by the RMIT Human Research Ethics Committee.
- This study is partially funded by the Australian Spinal Research Foundation.

Why have you been approached?

- Generally healthy young adults at RMIT University have been invited to this study.

What is the project about? What are the questions being addressed?

- Human beings living on the earth must regulate various body functions in response to changes in body posture relative to gravity. For example, lying to standing. In healthy humans, changes in body posture must be accompanied by adjustments in blood pressure so that adequate blood flow to the brain is maintained. There is evidence that the vestibular system (balance control system of the inner ear) contributes to blood pressure adjustments that occur with changes in posture. However, there are a few studies which have examined the

interaction between neck muscle activity and the inner ear in the regulation of blood pressure control with posture changes.

- Through this research project, the following questions will be answered.
  - Does a change in neck muscle spindle discharge affect autonomic and cardiovascular activity?
  - Can manipulation of neck muscle spindle discharge in the presence of a constant vestibular stimulus alter the response pattern of autonomic and cardiovascular parameters to posture change?
- Up 20 participants will be involved in this study.

If I agree to participate, what will I be required to do?

- **Requirements of Participants**

In order to be eligible for this study, you need to be aged between 18 and 35 years and of good general health. You should meet all of the following conditions;

- Non-smoker
- Non-medication user
- No history of neck abnormality such as recent trauma (past three months), surgery, fracture, dislocation or known anatomical malformation of the spine
- No history of cardiovascular diseases such as known arrhythmia, stroke and myocardial infarction
- No history of vestibular diseases such as vestibular neuronitis and Meniere's disease
- No history of claustrophobia
- No history of diabetes mellitus
- No history of cancer

- **Study procedures to be carried out**

You will need to fast for at least 4 hours before participating in the study. Also you will need to abstain from any caffeine-containing beverages (e.g. coffee, tea, and coke) for at least 4 hours and from any alcoholic beverages for at least 12 hours prior to the study. To ensure that you are relaxed when you come to the laboratory we will ask you to stop any exercise 12 hours prior to the study. However, before the exercise restriction period, you will be able to exercise moderately for up to 20 minutes (this 20 minute moderate exercise restriction only applies for the 48 hours prior to the experiment day).

We will measure your heart rate, blood pressure, sweat response in the hand, finger and forearm (or calf) blood flow and neck muscle activity in each experimental session. Blood pressure will be measured by placing 2 finger cuffs around the fingers of one of your hands. This instrument (Portapres) will give us a continuous measure of your blood pressure throughout the study session. Your heart rate will be measured using electrodes which consist of metal discs surrounded by an adhesive plastic material onto both the outer sides and upper part of the front of your chest. Sweating response in your hand will be recorded through three electrodes placed on the hand and forearm (or calf), and finger blood flow will be taken with an

instrument gently clipped on the finger tip. Forearm (or calf) blood flow will be measured by loosely fastening a thin mercury-in-ruber tube around your forearm (or calf). This device gives us changes in forearm (or calf) blood flow. In addition, neck muscle activity will be measured though each experimental session. To record muscle activity, several electrodes mentioned above will be attached on the front and back sides of your neck.
You will also be asked to synchronise your breathing to a metronome rhythm during the entire recording period (6 minutes). This will modulate your breathing rhythm to a rate of 15 breaths a minute.
Additionally you will be asked to wear a blindfold to exclude visual input.

The followings are the details of the third study of this project.

First of all, you will be asked to lie on your back comfortably on a bench with tilting function. This bench is equipped with a foot plate as well as a racing car harness strapped over your shoulders for your safety. In the third study, heart rate, blood pressure, sweating response in the hand, finger blood flow and neck muscle activity will be recorded throughout the study session, and changes in forearm (or calf) blood flow will be measured after the intervention. You will be asked to lay on a bench with your body positioned in either the face down or face up position. Your head will be positioned over the head-edge of the bench and will be supported on a stand that is at the same height as the bench. Once you lie on the bench, you will be moderately tilted up to 20 degrees. Then you will be asked to make yourself comfortable in this posture, and to remain relaxed and quiet for a period of 5 minutes. After 5 minutes relaxation, you will be asked to start to synchronise your breathing with a metronome rhythm. The third study consists of two sessions. Each session in this study will take about 30 minutes and be conducted on different days.
After the 3 minute controlled breathing period, the stand supporting your head will be removed and your head will be gently lowered (bent forward or backward) as far as possible but only within your level of comfort. Once we have determined this position we will raise your head by approximately 10 degrees. For this experiment, this is the “test position” for your head and neck. From this position you head will be either bent forwards or backwards. This is the position at which we will condition your neck muscles. You will be asked to contract the muscles on the back of your neck against the resistance of the investigator’s hands which will cup the back of your head. The contraction will last 3 seconds. You will then be asked to relax your neck muscles and rest your head on a support that will be provided for your head. After 5 seconds the support will be raised or lowered so that your head is returned to the “test position”. After this intervention, you will be asked to remain completely relaxed while continuing the controlled breathing. You will remain in the test position for a further 3 minutes while your blood pressure, heart rate, sweating response in the hand, finger blood flow and forearm (or calf) blood flow are recorded.

What are the risks or disadvantages associated with participation?

- Synchronising breathing with a metronome may cause dizziness and difficulty in breathing at the desired rate for some participants. If you experience any unpleasant symptoms during data recording, please notify the investigator immediately.
  The experiment will be terminated.
- Electrodes applied to the skin may cause rash and/or itchy sensation during and/or after placing on the body due to one or any of the followings; skin cleansing swab (70 % alcohol containing), and adhesive and gel of the electrode.
- The intervention in this project (muscle contraction and moderate stretch of the neck muscles) may cause muscle soreness or discomfort during and/or after the experimental session. If you experience any symptoms in the neck, you need to report this to the investigator immediately and the experiment will be cancelled. One of the investigators (Barbara I Polus) is a registered chiropractor within the state of Victoria. You will be examined by the investigator and advised on appropriate management.

What are the benefits associated with participation?

- There may not be direct benefit to the participants. However, some people may find interest to contribute to developing the general body of knowledge in this field.

What will happen to the information I provide?

- All information concerning individuals will remain confidential and no individual will be identified in any publications and presentations resulting from this study. The information will be securely stored in a lockable office, retained for 5 years, and then destroyed. Only my supervisor (Barbara I Polus) and I will have access to the information.
- During some studies we may ask you to have your photo taken. These photos are taken to demonstrate procedures of the study for publication and presentation only. Your face will be blacked out so you will not be identified in this photo.

What are my rights as a participant?

- Participants in this study will have the following rights;
  - ✓ The right to withdraw their participation at any time, without prejudice.
  - ✓ The right to have any unprocessed data withdrawn and destroyed, provided it can be reliably identified, and provided that so doing does not increase the risk for the participant.
  - ✓ The right to have any questions answered at any time.

Whom should I contact if I have any questions?

- For inquiry, please do not hesitate to contact with us
- Mr Nobuhiro Watanabe (s9812566@student.rmit.edu.au; 9925-7655)
- Dr Barbara I Polus (barbara.polus@rmit.edu.au; 9925-7714)

What other issues should I be aware of before deciding whether to participate?

- We would like to reimburse you for your travel and time associated with participating in this research project.

Thank you very much for your support.

Yours Sincerely,

Nobuhiro Watanabe BAppSc(Chiro)

Barbara I Polus BAppSc(Chiro), MSc, PhD

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745. Details of the complaints procedure are available fom the above address.

Appendix 21

The calculation of *F* statistics

Used for a study outlined in Chapter 7

For the *F* value determination, the standard deviations of HR following the two forms of muscle conditioning to be compared were squared, and the larger value was divided by the smaller value. The *F* value was compared with the critical *F* value calculated as follows. The numerator's degrees of freedom were calculated as "the number of forms of muscle conditioning, which were compared" minus 1, so in this study, 2 - 1 = 1. The denominator's degrees of freedom were calculated as "sample size minus 1 for one form of muscle conditioning" plus "sample size minus 1 for another form of muscle conditioning", so in this study, (14-1) + (14-1) = 26. When statistical significance level was set at $p < 0.05$, the critical *F* value for this study was 4.22 (Munro 2001). Therefore, when the calculated *F* value was larger than 4.22, the extent of the variation of HR was significantly different between the two different forms of compared muscle conditioning.