CHANGES IN VENOMOTOR TONE DURING SYMPATHOEXCITATION: INFLUENCE OF GENDER AND FEMALE CYCLE PHASE

by

CHRISTIEN PHILLIPS

A thesis submitted to
The University of Birmingham
for the degree of
DOCTOR OF PHILOSOPHY

School of Sport and Exercise Sciences
The University of Birmingham
February 2010

ABSTRACT

Moving from the supine to the upright posture presents a significant challenge to the human body since the lower limb veins become distended due to gravitational pooling and venous return is decreased. An increase in leg venomotor tone would assist in countering pooling and help to maintain a pressure gradient for forward flow. Quantifiable proof of active venoconstriction in humans is sparse with the majority of work in this field having been done on forearms. The studies within this thesis have looked directly at lower limb responses to venoconstriction in young healthy volunteers and have compared different venous vascular beds (deep and superficial) and also the influence of female hormones. The data show that increases in venomotor tone in the calf are relatively modest and are specific to superficial rather than deep veins and that calf limb responses are attenuated in females. Venous function in women appears to be modified during oral contraceptive use compared to normally menstruating women. It is unlikely that the degree of venoconstriction observed in these experiments is sufficient on its own to maintain venous return during orthostatic challenge.

ACKNOWLEDGEMENTS

First and foremost my many and grateful thanks must go to my PhD Supervisor Dr Maggie Brown who has mentored me throughout the course of my research studies. Without her tireless support, encouragement and guidance I am sure that I would not have seen this work through to its successful conclusion. I also offer my thanks to Dr Gemma Bishop, at the time a fellow PhD student, for teaching me the basics of ultrasound machine operation, for assisting me with the data collection during my first experimental study and for generally being around to act as a sounding board for my ideas and frustrations. Jenny Horsburgh (a fellow PhD student), Beth Clabburn, Amy Smith, Greg Freeman, Paul Saunders, Ruth Lawler and Kate Witham (all undergraduate project students) have also assisted me with my data collection at various points throughout the course of my studies. I am also grateful to the many subjects who volunteered to participate in my experimental studies – undergraduates, postgraduate colleagues and members of the academic staff - without them giving up their time I would not have had any data to report. I thank my family for their continuous support, both emotional and financial, throughout. I must thank our cat, Daisy, for her company throughout the entire period of time that it has taken me to write my thesis and also for her occasional “assistance” with typing. Finally, I would like to offer a special thank you to my wife Sally for all of her support and encouragement throughout the past four years. Sally has been there throughout the good points and the numerous periods of frustration that have been associated with the completion of this work and she has always managed to say the right thing at the right time to encourage and motivate me to keep going.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF ABBREVIATIONS

AT	Anterior tibialis
BMI	Body mass index
CHF	Chronic heart failure
CON	Control distension
CPT	Cold pressor test
CSA	Cross sectional area
FL	Female luteal phase (group)
FM	Female menstrual phase (group)
GTN	Glyceryl trinitrate
HR	Heart rate
HUT	Head up tilt
ILE	Isometric leg exercise
IPAQ	International physical activity questionnaire
LBNP	Lower body negative pressure
LC	Lateral calf
LS	Long saphenous
M	Male (group)
MAP	Mean arterial pressure
MCFP	Mean circulatory filling pressure
MHG	Medial head of gastrocnemius
MRI	Magnetic resonance imaging
MSNA	Muscle sympathetic nerve activity
MST	Mental stress task
MVC	Maximal voluntary contraction
N	Non-OC female (group)
NL	Non-OC luteal phase (group)
NM	Non-OC menstrual phase (group)
NO	Nitric oxide
O	OC female (group)
OC	Oral contraceptive
OI	Orthostatic intolerance
OHH	OC high hormone phase (group)
OLH	OC low hormone phase (group)

PASAT	Paced auditory serial addition test
POP	Popliteal vein
POTS	Postural orthostatic tachycardia syndrome
P - V	pressure-volume
SNA	Sympathetic nerve activity
SSNA	Skin sympathetic nerve activity
TAVM	Time averaged velocity mean
VAR	Veno-arteriolar response
VIA	Vascular image analysis

OVERVIEW OF THESIS

The venous system is a high volume, low pressure, low resistance capacitance system. While its primary function is as a series of conduits to facilitate the return of blood to the heart (venous return), the veins have a number of other physiologically significant functions in relation to blood volume, capacitance, temperature regulation, post capillary resistance and fluid filtration that render them important contributors to cardiovascular homeostasis. This thesis investigates specifically the effects of sympathetic nervous system activation on venous tone in the lower legs of healthy humans. On moving to an upright posture (orthostasis), blood pools in the lower body and extremities contributing to a reduction in venous return, smaller stroke volume, lower cardiac output and fall in blood pressure. Reflex mechanisms to compensate for this include aortic and cardiopulmonary baroreceptor stimulation resulting in sympathoexcitation and increased heart rate and peripheral vasoconstriction. The latter applies not only to arterial resistance vessels but also to venous vessels and the increase in venomotor tone helps to counter the blood pooling.

Recently there has been debate as to the significance of venoconstriction within the splanchnic capacitance bed during orthostasis in terms of the contribution it can make to venous return (Rothe / Drinkhill & Hainsworth 2006). Although the amount of pooling that occurs in the lower limbs on standing is less than in the abdominal region (Taneja *et al.* 2007), the ability to increase leg venous tone is also considered an important factor in tolerance to orthostasis (Streeten, 1999; Smit *et al.* 1999). The majority of studies examining venoconstriction in humans have focused on the forearm circulation as this is most accessible for the range of techniques available. There is therefore a lack of information about the responses of leg veins, which are more relevant in orthostasis, to reflex sympathetic activation.

With regard to orthostasis, women are known to be less tolerant and more prone to syncope than men. The main reason for this appears to be their smaller heart size and consequent lower capacity to compensate for reduced venous return. However, it has also been suggested that blood pooling in the capacitance vessels may differ in women and men, potentially because of greater venous distensibility and / or compliance or because of reduced venoconstriction. The female reproductive hormones oestrogen and progesterone are known to act as dilators for arterial resistance vessels (Sarrel, 1999) and there is evidence that they have similar effects on venous vessels. Venous distensibility is reported to increase during the high hormone phases of the menstrual cycle, oral contraceptive use and pregnancy, and women are more prone to venous disease than men potentially because of hormonal effects weakening vein wall structure. Whereas these effects would facilitate blood pooling during orthostasis, oestrogen can also modify release or reuptake of noradrenaline to

enhance venous adrenergic constriction. It is not clear therefore whether venoconstriction differs in women from men under the influence of female hormones.

To address this question, this thesis examines the responses of leg veins to sympathoexcitation by several different interventions (exercise, mental stress, cold pressor test) in young healthy men and women either with normal menstrual cycles or taking an oral contraceptive. The data reported will demonstrate that in males, increases in venomotor tone in the calf are relatively modest and are specific to superficial rather than deep veins. Females show little venoconstriction in the calf, consistent with evidence that constriction of arterial vessels to sympathetic activation is also attenuated in comparison with males. This could potentially contribute to their orthostatic intolerance. Venous function in women was not modified by varying levels of endogenous female reproductive hormones during the menstrual cycle. However, oral contraceptive use led to calf venodilation in response to a mental stress test and significant venoconstriction to a cold pressor test, whereas women with normal cycles showed no response to either intervention. The interaction of exogenous female reproductive hormones with the reactivity of leg veins to sympathoexcitation merits further investigation in the context of the prevalence of venous disease in women.

Chapter 1:
GENERAL INTRODUCTION

1.1 The venous circulation

The venous system contains around two thirds (~ 3 l) of the body's total blood volume (adults ~ 5 l total) and shifts in blood volume within it are dependent on either changes in arterial inflow, the effects of gravity due to changes in posture, changes in surrounding skeletal muscle tension or changes in venous tone but usually a combination of all. Thus the venous system is a reservoir of variable capacity, which is capable of redistributing blood (either actively or passively) in quantities that are sufficient to alter cardiovascular function. Within the venous system there are two major circuits, which are the splanchnic circuit and the peripheral circuit. The splanchnic circuit contains around one-fifth (~ 1 l) of the body's total blood volume (Shepherd & Vanhoutte, 1975) and refers to the circulation of blood through the blood vessels supplying the abdominal viscera, including liver, gastrointestinal tract, spleen and pancreas. The peripheral circulation refers to all other venous vasculature except that within the pulmonary circulation and includes all veins that originate from a capillary bed and ultimately drain into the right atrium.

The system is a low pressure system. At rest in the supine position, venous blood pressure in the venules is ~ 12 – 20 mmHg and in named veins (e.g. femoral) is ~ 8 – 10 mmHg compared to ~ 95 mmHg in the arterial resistance vessels, but because venous resistance is small, the resultant low central venous pressure (~ 0 – 10 mmHg) is sufficient to drive the cardiac output back to the right atrium, where pressure at the veno-arterial junction is ~ 0 – 10 mmHg.

1.1.1 Gross anatomy of the venous circulation in the human lower limb

In addition to cadaver studies, the gross anatomy of the leg veins is now available *in vivo* for assessment using modern ultrasound imaging methods. For clinical purposes, there are consensus guidelines for ultrasound examination (Cavezzi *et al.* 2006). Within the lower limb, vein sizes range from 10 µm for the smallest post capillary venules to many times that for the large conduit veins e.g. the femoral vein (~ 10 mm supine resting diameter). The work presented later in this thesis has been performed on the veins of the lower limb and therefore it is the structures of the vein systems in that part of the body that are detailed in this section. However, the principals of these structure and systems within the lower limb can also be applied to the upper extremities.

Within the lower limb compartment there are two venous systems, the deep and the superficial. As the name suggests, the deep veins are found deep within the skeletal muscle of the limb and unlike superficial veins are generally found next to an artery of the same name. The deep veins transport the majority of the blood within the limb and are connected to the superficial veins by communicating or perforator veins, which transport blood either

from the superficial veins into the deep veins for drainage or from the deep veins into the superficial veins for cooling. Superficial veins are found close to the surface of the skin and are not paired with an artery.

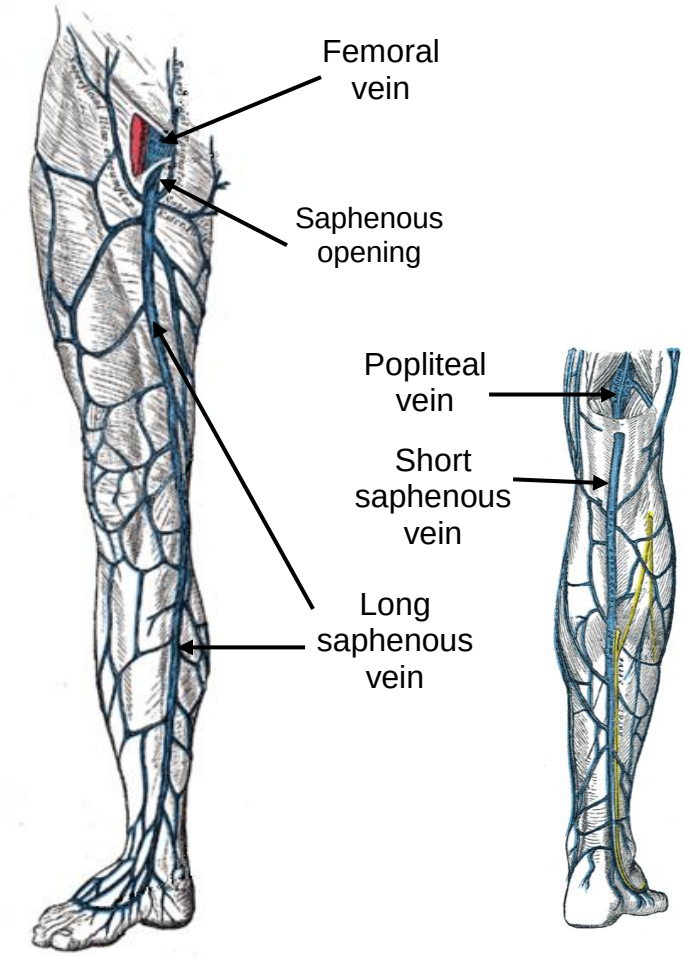


Figure 1.1*: Vein anatomy of the leg. Figure identifies some of the key deep (femoral and popliteal) and superficial (long saphenous and short saphenous) veins of the human lower limb. Illustrations adapted from Gray's anatomy.*

The main deep veins of the lower limb that are responsible for transporting blood towards the heart are the anterior and posterior tibial veins and the peroneal vein of the lower/mid portion of the calf, which drain into the popliteal vein (figure 1.1), the main vessel within the calf portion of the lower limb, which in turn drains into/becomes the femoral vein (figure 1.1) at or slightly above the knee line and courses within the thigh towards the pelvis and is the main route for blood out of the lower limb. The superficial veins are generally very small and as such are often unnamed. The two most well known superficial veins of the lower limb are the long or great saphenous vein and the small saphenous vein (figure 1.1). The long saphenous vein runs the entire length of the leg from the dorsal venous arch of the foot, up the medial side of the lower leg; above the knee line it then courses up the anterior surface of the thigh moving deeper through the fascia lata before joining with the femoral vein in the region of the

femoral triangle near the pelvis. The small saphenous vein originates at around the same point as the long saphenous vein (dorsal venous arch of foot) and courses up the posterior aspect of the leg before draining into the popliteal vein either at or slightly above the level of the knee.

Both the long and small saphenous veins are typical of all superficial veins in that they drain the cutaneous tissue. Most other superficial veins either drain into the two saphenous veins or drain into the deep system via perforator veins, which penetrate through the deep fascia on the medial side of the leg (Craven, 2004). Venous valves generally lie immediately below the point of entry of major tributaries and are found in almost all of the deep, superficial and perforating vessels (Alexander, 1963; Shepherd and Vanhoutte, 1975; Maros, 1981); they are even present in vessels as small as 20 µm in diameter (Marin *et al.* 1994; Aharinejad *et al.* 2001; Phillips *et al.* 2004; Caggiati *et al.* 2006). The valves are more prevalent in the legs than in the arms, more numerous in the lower than in the upper leg compartments, and in deep than superficial veins (Shepherd and Vanhoutte, 1975).

1.1.2 Vein morphology

In contrast to arteries, veins have thinner walls, higher compliance and valves to prevent retrograde flow. The vein wall is constructed of three layers: the tunica intima (inner-most), the tunica media and the tunica adventitia (outer-most). The tunica intima consists of a thin layer of connective tissue on the vessel wall's inner-most surface called the lamina propria surrounded by a layer of endothelial cells, which are the main barrier to the escape of plasma from within the vessel (Levick, 2003) and secrete vasoactive agents such as nitric oxide. The tunica media is predominantly made up of smooth muscle, arranged around the circumference of the tunica intima to provide mechanical strength and contractile ability. Dispersed within the smooth muscle fibre layer are collagen and elastin fibres, which provide the passive properties of the vessel wall (see below). The tunica media is separated from the inner and outer layers of the vessel wall by two thin elastic fibre membranes: the internal and external elastic membranes. The tunica adventitia is the outer-most and thickest of the three layers in the vein wall and consists of connective tissue, which primarily serves to anchor the vein to the surrounding tissue.

Intrinsic control of venous tone is achieved locally via both the active and passive components of the vein wall: collagen (passive), elastin (passive) and smooth muscle (active). Relatively small increases in active tension of the venous smooth muscle can substantially reduce vessel capacity (see below) (Monos, 1993). The passive behaviour of the vein wall is due to the viscoelastic properties of the collagen and elastin components, which unload to produce a net fall in wall tension as the smooth muscle contracts to reduce

vessel CSA. This unloading is thought to make the act of constriction a more stable process (Levick, 2003). The proportions of collagen, elastin and smooth muscle differ depending on limb and vein type and the veins near or above heart level also have thinner walls and are more distensible (Rowell, 1993). It is suggested (Shepherd & Vanhoutte, 1975) that differences in composition might be due to the hydrostatic pressures that the respective vessels are subject to during orthostasis and also to the presence or absence of supporting structures. Furthermore, in adults it has been reported that the proximal end of the long saphenous vein contains more collagen and elastin than the distal end, whereas the latter has more smooth muscle (von Kugelgen, 1955, Svejcar *et al.* 1962, Kresse *et al.* 1970). Stooker *et al.* (2003) studied upper and lower long saphenous leg vein segment suitability for graft and found that the lower vein segment was more distensible than the upper leg vein segment at low pressure (< 50 cm H_2O), which is consistent with the lower segment containing more smooth muscle. These proportional differences were not found in the long saphenous veins of non-walking infants (von Kugelgen 1955, Svejcar *et al.* 1962, Kresse *et al.* 1970). This evidence would appear to suggest that changes in composition of the distal segments of the vein wall from infancy to adulthood may be due to exposure to the upright posture.

1.1.3 Relationship between pressure and vein shape and size

At low pressures vein CSA becomes markedly reduced (figure 1.2). At low transmural pressure (~0 mmHg, e.g. legs raised above heart level) the veins collapse into a dumb-bell shape and blood flow is restricted to the marginal channels at each side. At 1 mmHg transmural pressure, the vein passively assumes a narrow elliptical shape (Levick, 2003) and as the pressure rises towards 10 mmHg it will become more rounded. At low pressure, where the vessel is the most distensible, the slope of the pressure volume curve is steep and the compliance is therefore high (figure 1.2, upper curve). As the vessel becomes less distensible at higher pressures, the compliance becomes lower due to the complex relationship of the two characteristics. The total limb venous compliance is dependent on the size, relative number and the wall structure and will also vary depending on the location of the limb (upper or lower limb). It can also be affected by the pressure exerted on the vein by contraction of the surrounding muscle (Olsen & Lanne, 1998). After the vein has become fully rounded through passive filling, any further increase in the CSA is determined by the elastic properties of the collagen and elastin within the vessel wall (assuming that the smooth muscle tone is not actively increased). If the vascular smooth muscle tone is active increased, i.e. increasing tension within the vessel wall, the capacity can be markedly reduced (figure 1.2, lower curve).

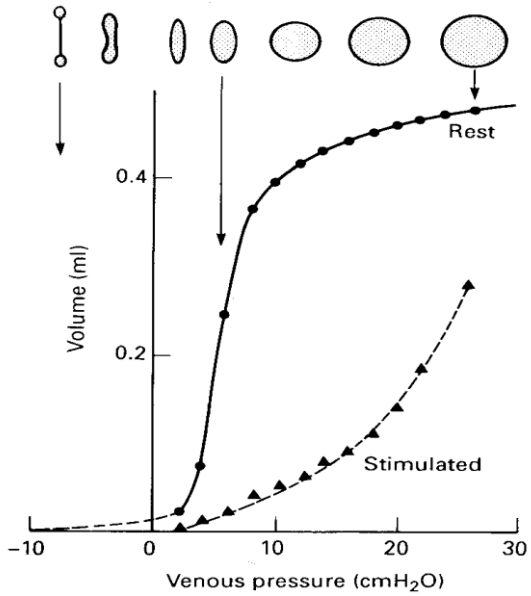


***Figure 1.2**: Illustration of vein distensibility in a relaxed (upper curve) and maximally contracted (lower curve) state. Figure adapted from Vanhoutte and Leusen (1969).*

The presence of elastin and collagen with the vein wall impart viscoelasticity and allow for the property of 'creep' or what is called stress relaxation or delayed compliance. When under applied pressure this allows the vein to display hysteresis, i.e. a smaller degree of stretch during increasing pressure than during decreasing pressure (Hasegawa, 1983, figure 1.4). This has implications for the methods to determine venous compliance (see later).

In terms of stress-strain relationships, veins are not dissimilar to arteries (Fung, 1993), but because they operate at a lower pressure range they are more sensitive to external pressure. Also application of agents that increase venous smooth muscle tone such as noradrenaline decrease stress relaxation. The biomechanical properties of veins become important when they are used as graft replacements for arteries and experience higher pressures (Wesly *et al.* 1975), and have been considered to account for a proportion of the increase in intrinsic vascular volume during prolonged volume expansion (Prather *et al.* 1969). Wall properties can also be altered in diseases associated with arterial hypertension (Journo *et al.* 1992) and venous hypertension leading to venous disease (Beebe-Dimmer *et al.* 2005), thereby modifying venous pressure-volume relationships.

1.1.4 Venous return

Venous return (volume flow) is dependent on the difference in pressure between mean circulatory filling pressure (MCFP) and central venous pressure, divided by venous resistance (Gelman, 2008). MCFP – the equalized pressure within the circulatory system

assuming that the heart is no longer beating (Pang, 2001; Gelman, 2008) - depends primarily on the 'stressed' volume of blood in the venous system. Because of its large capacitance and compliance, venous volume can be defined in two compartments as 'stressed' or 'unstressed' relating to the way in which they do or do not contribute to venous transmural pressure.

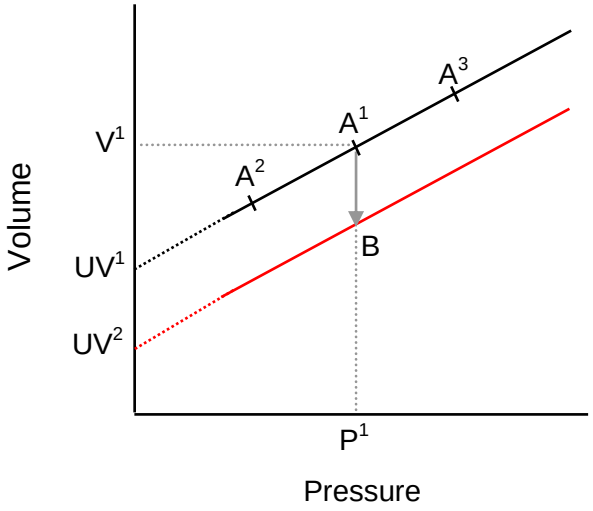


***Figure 1.3**: Illustration of the effects of changes in venous pressure and tone on the pressure–volume relationship (compliance) of a venous vessel. Point A^1 represents total volume of blood (v^1) in the vein at transmural pressure P^1. Points A^2 and A^3 show how changes in the transmural pressure affect the total volume of blood within the vein. Figure shows how unstressed volume (UV^1) can be estimated by extrapolating the pressure – volume relationship back to the volume at pressure 0. Point A^1 to point B illustrates how an increase in venous tone can reduce capacitance and the unstressed volume (UV^2) in a vessel without altering the compliance (slope of line does not alter). Figure adapted from Gelman 2008*

1.1.5 Unstressed and stressed venous volumes

Largely based on Guyton's work on the venous system, the unstressed volume (approximately 70 % of total volume) can be defined as the portion of the blood volume that fills the venous system without contributing to venous distension. At low blood volumes and therefore low transmural pressure, venous vessels tend to collapse because of their thin and highly-compliant walls. As additional volume is added, a critical volume is reached (~ 4000 ml, Guyton & Hall, 2006) whereby any further volume added causes the internal pressure of the vessel to increase, causing the vessel to change its cross-sectional profile and become more rounded. This critical volume is often reported as the value for unstressed volume. Unstressed volume can also be estimated by extrapolating the venous pressure-volume relationship derived experimentally back to the volume at pressure zero (Figure 1.3). The size of unstressed volume depends on the capacity of the blood vessels, level of venomotor

tone, rate of arterial inflow and transmural pressure (Pang, 2001; Gelman, 2008). Unstressed volume acts as a reserve that can be mobilized by venoconstriction and converted into stressed volume during situation such as haemorrhage or exercise.

The stressed volume is the difference between total blood volume in the venous system and the unstressed volume. Around one third of the total blood volume (~ 1.5 l) is in the stressed volume compartments of the peripheral circulation (Rothe, 2006). Adding volume to the unstressed volume distends the venous reservoir and generates a hydrostatic pressure gradient that drives venous return back to the right atrium. This driving pressure is also dependent on active venous tone, which can increase blood volume by adding from the unstressed volume hence passively increasing transmural pressure, and on vein compliance.

1.1.6 Venous capacitance, capacity and compliance

There is some confusion in the literature over the use of the terms venous capacitance and capacity. Capacitance defines the pressure–volume relationship of the venous vessels at a given pressure over the normal physiological range, and is altered by changes in venous tone (figure 1.3). Capacity (often reported as the capacitance in much of the literature) is the maximum capacitance for a given vessel, i.e. the point at which the vessel is full, the view in cross section is round in shape, the 'passive' collagen and elastin fibres within the vessel wall have reached their elastic limit and the smooth muscle is inactive (thus the tension in the vessel wall is reached purely through passive means). Capacity is a volume expressed in $ml.100ml^{-1}$.

Venous compliance describes the ability of a blood vessel to distend and increase volume with increasing transmural pressure i.e. it describes the pressure–volume (p-v) relationship. Although in the context of venous return this is often depicted as a straight line (Figure 1.3; Tyberg, 2002; Schmitt *et al.* 2002; Gelman, 2008), the p-v plot is in fact non-linear (Figure 1.4). Because of the nature of the venous wall composition, compliance decreases as transmural pressure and volume increase i.e. the vessel becomes stiffer as pressure and volume increase. At higher pressures and volumes, venous compliance is similar to arterial compliance, making them suitable for arterial by-pass grafts (von Kugelgen 1955, Svejcar *et al.* 1962, Kresse *et al.* 1970). At low pressures, vein compliance is up to 50 times greater than artery compliance (Levick, 2003). Compliance is expressed as $\Delta v/\Delta p$, where v = volume and p = pressure, over a defined pressure range.

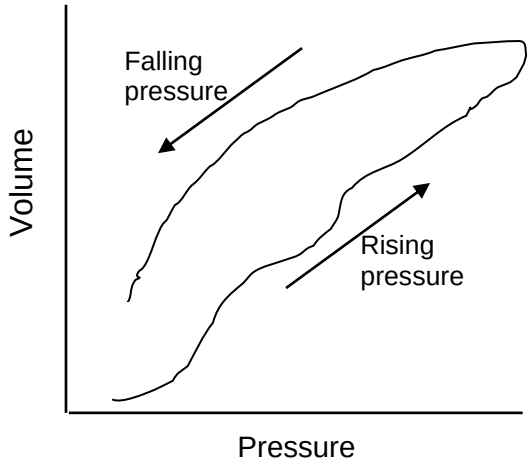


Figure 1.4*: A typical venous p-v curve for assessment of capacity or compliance showing the non-linear relationship during rising and falling transmural pressure. During falling pressure, it can be seen that venous compliance decreases as transmural pressure and volume increase. Also illustrated is hysteresis, i.e. vein shows a smaller degree of stretch during increasing pressure than during decreasing pressure. Adapted from Halliwill et al. (1999).*

1.1.7 Venous tone

Venous tone refers to the state of contractile tension in the vessel wall. At rest there is a low level contraction of the vascular smooth muscle in the tunica media layer (middle) of the blood vessel wall, which is mediated by circulating catecholamines and basal postganglionic sympathetic neural activity within the vessel wall itself. Most venules and veins have little basal tone in the absence of sympathetic activity. An increase in the venous tone reduces the venous capacitance (figure 1.3) and converts unstressed volume into stressed volume, thereby increasing venous return. Similarly, an increase in venous tone may reduce vascular compliance, thereby increasing central venous pressure and thus venous return. It is, however, possible for venomotor tone to reduce capacitance (shift the p - v relationship downward to a lower intercept on the volume axis) without altering compliance (the slope of the relationship) as shown in Figure 1.3 (Rothe, 1986; Schmitt *et al.* 2002). An increase in venous tone may also increase venous resistance (in addition to raising venous pressure), which, although low compared to arterial resistance, could reduce venous flow and potentially venous return. The effects of this differ depending on which compartment of the venous system is examined, as explained below. Increased venous post-capillary resistance will elevate hydrostatic pressure within the proximal microcirculation favouring net fluid filtration.

1.1.8 Venous Compartments

In considering the capacitance role of the venous circulation and its contribution to cardiovascular control, it is common to define two compartments: splanchnic and peripheral,

the latter comprising cutaneous and muscular venous systems. The main blood reservoirs of the splanchnic and cutaneous venous circulations are more compliant than the muscular veins of the extremities. Splanchnic venous circulation in particular contains about 20-30% of total blood volume and, because is lies 'outside' of the main circulation and is highly compliant (Gelman, 2008), makes the major contribution to maintaining blood volume during e.g. blood loss, orthostasis or exercise. Splanchnic venous volume is mobilised either passively by a reduction in arterial inflow that transiently decreases venous pressure and volume, and / or actively by increased venous tone (Rothe, 1986; Pang, 2001; Gelman, 2008).

Cutaneous veins respond predominantly to thermoregulatory challenges, with dilation on heating and constriction on cooling, and contribute less to blood volume shifts (Rothe, 1983; Hainsworth, 1986). Muscular veins in the extremities are not as compliant as splanchnic veins (0.48 $ml.mmHg^{-1}.kg^{-1}$ in skeletal muscle compared to 20.0 $ml.mmHg^{-1}.kg^{-1}$ in liver; Rothe, 1986) and have been considered to play little role in maintenance of cardiovascular capacitance because of their minimal response to reflex autonomic activation. Hainsworth (1986, 1990) has made a convincing case from experiments on reflex regulation of the splanchnic venous circulation in both animals and humans that only this compartment contributes to the body's capacitance control. Much of the evidence comes from studying how both arterial and venomotor tone alter during physiological and pathological conditions that modify blood volume distribution, e.g. haemorrhage, lower body negative pressure, head-up or head-down tilt, microgravity, exercise, temperature changes and mental stress, and the fact that peripheral, especially muscular veins, appear to respond little to sympathetic neural activation. There are several excellent reviews on venous function that concur with this point of view (Gauer & Thron, 1965; Rothe, 1983).

1.1.9 Peripheral venous circulation and orthostasis

Yet there is one condition for humans in which the peripheral venous circulation is undoubtedly important, and that is orthostasis. Pooling of blood in the lower limb veins in humans in the upright posture can play a role in limiting venous return, and compensatory mechanisms to counter this, e.g. the presence of venous valves, respiratory pump, muscle pump contractions and venoconstriction, are necessary to maintain venous return (Rowell, 2006).

In humans in the upright posture, there is an increase in pressure in all vessels below heart level of 0.8 mmHg for each cm below the right atrium. Moving from the supine to the upright posture causes the translocation of 500 – 700 ml of total blood volume from the thoracic (central) compartment into the lower body, i.e. abdomen (splanchnic) and lower limb veins

(Mathias, 2002; Robertson, 2008; Rothe 1983). Using impedance plethysmography, Taneja *et al.* (2007) showed that during incremental head-up tilt (HUT) from 20-70 degrees, blood volume decreased 12% in the thoracic region but increased 18% in splanchnic, 10% in pelvic, and 8% in calf regions. Lower limb veins contain a larger total volume (500 ml) when tilted than when supine (250ml) (Gauer & Thron, 1965). Within the different compartments of the leg, about 80% of lower limb blood is contained in the upper leg (130 ml thigh, buttocks) with less in calf (80 ml) and foot (40 ml, Ludbrook, 1966). It should be noted that venous pooling in the feet and calf comes primarily from arterial inflow from the thoracic compartment and not from venous backflow whereas in thighs, buttocks, pelvis and abdomen it arises from backflow (Smit *et al.* 1999), due to fewer valves in these areas.

The smaller blood volume in the thoracic veins leads to reductions in venous return, central venous pressure, impairment of stroke volume through the Frank-Starling mechanism, a 20% fall in cardiac output and a decrease in mean arterial pressure. Reflex compensatory actions to maintain cardiac output and blood pressure include increased heart rate and increased arterial resistance to flow via baroreflex-mediated peripheral vasoconstriction. The latter acts to buffer capillaries against the hydrostatic rise in pressure and prevent oedema, and is aided by local myogenic or veno-arteriolar constriction. Taneja *et al.* (2007) reported that, when upright, blood flow decreased in all regions and vascular resistance therefore increased 145%, 90%, ~100% and 140% in thoracic, splanchnic, pelvic and calf regions respectively. In addition, reflex sympathetic activation will increase venous tone to reduce capacity and limit pooling.

To help maintain venous return, valves situated periodically within the veins of the limbs close shut to prevent any backflow from the resultant increase in pressure when upright. Respiratory activity helps to increase venous return by increasing thoracic and hence right atrial pressure during inspiration. Venous flow velocity in the lower limbs increases and decreases in phase with respiration, although the pattern of breathing does not impact on steady state venous flow (Miller *et al.* 2005). The muscle pump is considered a key factor in maintenance of venous return from the legs when upright. Compression of capacitance vessels by contraction of the surrounding musculature acts to propel blood towards the heart and on muscle relaxation the tethering of vessels to surrounding tissues opens the veins and, because backflow is prevented by valves, reduces venous pressure (Pollack & Wood, 1949). The resultant increase in arterio-venous pressure difference has been considered an important contributor to muscle hyperaemia during rhythmic muscle contractions and there has been much debate as to the magnitude of this effect in dynamic exercise (Laughlin, 1987; Laughlin & Schrage, 1999; Sheriff / Clifford *et al.* 2005; Tschakovsky & Sheriff, 2004). Decreases in pressure of ~60 mmHg have been recorded in foot veins during walking

(Pollack & Wood, 1949; Kugler *et al.* 2001) with smaller decreases or even increases in deeper calf veins (Neglen & Raju 2000a & b), but effective functioning of the calf muscle pump is dependent upon competent valves and can be modified by surrounding muscle mass - it has been estimated that muscle contraction can expel ~ 35 ml of blood from the thigh and ~ 60 ml from the calf (Raju *et al.* 1999).

1.1.10 Role of venomotor tone in the upright posture

Although the splanchnic venous circulation is the main contributor to blood volume regulation, pooling in the legs can match that of abdominal and pelvic pooling in determining haemodynamic consequences. Halliwill *et al.* (1998) used medical antishock trousers to negate specifically either leg or legs plus abdominal / pelvic pooling during lower body negative pressure in healthy subjects and saw considerably attenuated reflex heart rate rise and vasoconstriction even when only leg pooling was prevented. The extent of leg venous pooling in humans, i.e. filled vein capacity, is therefore a factor when considering reduced venous return in orthostasis, and is dependent on:

1) The physical size and capacity of the capacitance vessels.
2) The distensibility and compliance of the vein wall related to its composition in terms of elastic and non-elastic components.
3) The support provided by the surrounding tissue and interstitial fluid pressure.
4) The degree of venomotor tone.

Factors such as body size and composition (in terms of muscle mass), levels of physical activity, venous disease alterations to vein wall composition, female reproductive hormones, and any change in the ability to regulate venous tone will thus have an impact on leg pooling.

Excessive leg pooling has been considered as a factor in orthostatic intolerance and in conditions of autonomic dysfunction such as neurally-mediated syncope and postural orthostatic tachycardia syndrome (POTS). Orthostatic intolerance (OI) is characterised by an inability to maintain blood pressure on standing which may present as syncope (fainting). The clinical definition of OI is either a systolic (fall of at least 20 mmHg) or diastolic (fall of at least 10 mmHg) hypotension within 3 minutes of standing (Schatz *et al.* 1996). Prevalence of OI and OI-related dizziness in the population, corrected for age, has been estimated as 16% and 5% respectively, with women being more susceptible than men (Wu *et al.* 2008). There are many causes and predisposing circumstances for OI (Smit *et al.* 1999) that include excessive venous pooling, impaired baroreflex sensitivity, inadequate heart rate increase, defective arterial vasoconstriction and changes to autonomic function / orthostatic neural reflex pathways.

There is, however, good evidence that a failure of reflex resistance vessel constriction plays an important role in OI by allowing increased downward pooling of venous blood (Smit *et al.* 1999). In adolescents with POTS, calf blood flow during HUT increased far more and was associated with double the increase in calf volume compared to control subjects (Stewart & Weldon, 2001). Likewise, calf volume measured by radionuclide technique (^{99m}Tc labelled red cells) on HUT was higher in patients with unexplained syncope than without (Hargreaves & Muir, 1992) although another study did not find this difference in a similar patient cohort (Bellard *et al.* 2003). Stewart (2002) investigated calf volume responses to venous occlusion and to HUT in POTS patients, and argued that as p-v curves were not different between POTS and controls, there was no evidence of a difference in venous capacitance/pooling. However, in this study, a normalisation procedure whereby calf volumes during tilt were scaled according to those during distension by occlusion may have disguised differences.

Even if the capacity for leg pooling based on volume measurement is not different in OI, there remains the possibility that any active venoconstriction that occurs alongside the sympathoexcitation and resistance vasoconstriction evoked by orthostasis may be attenuated. Sympathetically mediated venoconstriction has been suggested as one of the compensatory adjustments during standing (orthostasis) to counter venous pooling (Sammueloff *et al.* 1966a; Stewart *et al.* 2004). There is evidence of impaired venoconstriction in conditions associated with OI. Manyari *et al.* (1996) recorded forearm p-v relationships using radionuclide plethysmography before and during sympathoexcitation by mental stress test. Venoconstriction was evident in control subjects as a 15% decrease in venous volume whereas over half the patients with neurally-mediated syncope did not show this response. In contrast, Thomson *et al.* (1996) found no difference in the decrease forearm venous volume in response to LBNP when using a similar method in controls or patients with vasovagal syncope. This discrepancy may be because failure of venomotor reactions is likely to be more evident in the lower limbs themselves rather than forearms. Indeed, Streeten (1990) found that whereas dorsal hand veins responded normally to infused noradrenaline in patients with OI, superficial veins in the foot were supersensitive, suggested to be the result of impaired sympathetic innervation. It was proposed that this would lead to subnormal venoconstriction and excessive leg pooling as a significant non-cardiac mechanisms of OI (Streeten & Scullard, 1996). However, p-v curves constructed by strain gauge plethysmography during venous emptying were found to be shifted to a lower level indicative of increased venous tone during sympathoexcitation by handgrip exercise in the legs of both controls and OI patients (Freeman *et al.* 2002).

This conflicting evidence as to the role of venomotor tone in the lower limbs as a counter to

orthostatic pooling may reflect in part different experimental approaches to the assessment of active vein constriction in humans. It raises the question that forms the focus of this thesis, namely to what extent is there active venoconstriction in the lower leg venous system. The evidence that reflex activation of the autonomic nervous system by arterial or cardiopulmonary baroreceptor input or other cardiovascular afferent signals affects venomotor tone will be reviewed in brief below and in detail in subsequent chapters. In addition, aside from any effects of centrally mediated sympathoexcitation on lower limb veins in humans, there are other local mechanisms that can regulate venous tone e.g. myogenic, endothelial and veno-arteriolar responses, and these may also contribute when orthostatic venous pooling occurs. Before considering mechanisms that control of venous tone, the properties of venous circulation of the lower limbs will be described.

1.2 Mechanisms of peripheral venous tone control

1.2.1 Neural control of venous tone

1.2.1.1 Vein innervation

The anatomical innervation of veins has been studied in animals and humans by light microscopy and histology, immunohistochemistry, electron microscopy, and the functional effects of neural activation by electrical and pharmacological stimulation. Evidence from animal studies (Burnstock *et al.* 1970) using cholinesterase staining, fluorescent histochemistry and electron microscopy to examine vessel segments, has shown that the postganglionic fibers can usually be found abundantly within the tunica media layer of the vein wall, whereas these nerves are not found to penetrate deeper than the tunica adventitia in the arteries (Shepherd & Vanhouttte, 1975). Based on fluorescence histology for catecholamines, veins of the splanchnic / mesenteric system receive sympathetic constrictor nerves with vessels greater than 40 μm diameter responding to nerve stimulation with constriction (Furness & Marshall, 1974) consistent with their role in responding to blood volume changes. Although it is generally held that veins are less densely innervated than arteries (Bevan, 1975), electron microscopy studies of human mesenteric vessels showed greater nerve density in vein than artery (Birch *et al.* 2008) emphasising the importance of neural control of capacitance in this circulation.

In the peripheral venous system, differences in innervation exist between the deep (skeletal muscle) veins and the superficial (cutaneous) veins since animal studies have shown that there are relatively sparse adrenergic / sympathetic nerves supplying the deep veins within the skeletal muscles (Burnstock *et al.* 1970, Ehinger *et al.* 1967; Fuxe & Sedvall, 1965). Marshall (1982) used fluorescence histology to show that venules and veins from 9 up to 130 microns diameter in rat spinotrapezius muscle had no innervation. On the other hand, Chen & Ta (1994) used horseradish peroxidise as a retrograde tracer from the lumbar

paravertebral sympathetic chain to study limb veins in cats and found projections from L4 and 5 to the vein to the semimembranosus muscle and a density of 10 ± 1 labelled neurones per mm^2, although this was less than in the saphenous and femoral veins (44 ± 5 per mm^2, 25 ± per mm^2, mean ± SEM). For the peripheral venous circulation, functional studies seem to support these findings by indicating sympathetic effects mainly on superficial and cutaneous rather than deep muscular veins.

Ahluwalia & Vallance (1997) investigated the role of sensory C-fibres in venous tone. Exposure of precontracted small mesenteric rat veins to calcitonin gene-related peptide and Substance P failed to relax the fibres. However, capsaicin, a C-fibre activator, caused relaxation of small veins which was reduced when C-fibres were destroyed and abolished by removal of endothelium, but not affected by NOS inhibitors or indomethacin. These findings demonstrated that the venous side of the circulation responds directly to sensory stimulation.

There are surprisingly few anatomical or histological studies of the innervation of human veins but functional responses have received more attention. Most investigations have focused on the human saphenous vein because of its use as a bypass graft. Loesch & Dashwood (2009) recently reviewed the innervation of the greater saphenous vein, describing sympathetic nerve fibres positive for tyrosine hydroxylase together with parasympathetic cholinergic perivascular nerves and sensory fibres positive for substance P and calcitonin-gene-related peptide (CGRP) (Amenta *et al.* 1983; Herbst *et al.* 1992). Human mesenteric veins also contain these three types of nerves (Birch *et al.* 2008). Patterns of innervation of the femoral or deep veins of the lower leg in humans are hardly described in the literature and the assumption is that they will be similar to those in animals.

1.2.1.2 Vein responses to neural activation

Donegan (1921), following work done at the end of the 19th century showing venous constriction to nerve stimulation, performed similar experiments on cat internal and external saphenous in lower limb, dorso-radial in upper limb, external jugular, large intestine and mesenteric and vena cava. He observed vessels that reacted immediately or with delay depending on whether the vein was filled or not. Macroscopically, nerve fibres were observed to pass into veins and stimulation caused mesenteric veins to constrict. In response to adrenaline, superficial veins contracted but larger vessels did not. Reflex responses to stimulation of vagus, sciatic and sensory nerves were also studied; no response in superficial veins was observed. Donegan concluded that superficial and mesenteric veins constrict to nerve stimulation and adrenaline but muscle veins and the vena cava do not.

Webb-Peploe & Shepherd (1968) stimulated the lumbar sympathetic nerves and measured

pressures along the constant-flow perfused venous tree of the dog hindlimb. Venous pressure increased in relation to stimulus frequency and this effect was not sensitive to atropine. By measuring pressures below and above the knee, they found that the vein segments above the popliteal fossa were not sensitive to sympathetic stimulation or noradrenaline while the below knee veins were. In the below knee segment, the distal veins were more sensitive to stimulation (response to 0.5 – 1 Hz) than the proximal (4-6 Hz). Vanhoutte & Leusen (1969) stimulated isolated dog saphenous vein segments showing a frequency response relationship with biggest constrictions to frequencies below 2 Hz. However, Gero & Gerova (1975) observed that the dog femoral vein did constrict to low frequencies (1-2 Hz) of sympathetic stimulation with less response at 4-8 Hz and relaxation at 15 Hz or higher. They suggested that loss of constriction at higher frequencies may be due to transmitter depletion because vessels still were able to constrict to noradrenaline.

These data suggest that sympathetically-mediated venoconstricton does occur in the large veins of the hindlimb, albeit with decreasing effect in the more proximal vessels. By contrast, small venules and veins (from 9 – 130 microns diameter) directly observed within the spinotrapezius muscle of rats did not respond to sympathetic nerve stimulation at frequencies between 1-20Hz (Marshall, 1982). This, together with their lack of innervation, is taken to indicate that there is little or no sympathetic adrenergic control over small muscle veins. In contrast, Bjornberg & Maspers (1991) stimulated sympathetic nerves to cat hind limb skeletal muscle (gastrocnemius) (1 – 16 Hz) and measured the capacitance and venous resistance responses. Graded stimulation resulted in a similarly graded increase in venous vascular resistance and decrease in regional blood volume in the post capillary vessels.

Functionally the human saphenous vein constricts to transmural stimulation of sympathetic nerves at frequencies of 0.5 – 8 Hz and to application of noradrenaline (Fabi *et al.* 1996). The predominant adrenergic receptors in human saphenous vein are α_1 and α_2 (Steen *et al.* 1986; Sjoberg *et al.* 1989; Weinstein *et al.* 1989) with some evidence for a predominance of α_2 (Steen *et al.* 1984; Giessler *et al.* 2002). Based on radio-ligand binding studies in autopsy specimens, the density of α receptors is similar on human saphenous vein and vena cava, and not different than in arteries (Rudner *et al.* 1999). The affinity of α receptors for noradrenaline is enhanced by cold in human and canine saphenous vein (Shepherd *et al.* 1983), indicating the importance of venoconstrictor responses in superficial veins in thermoregulation. There are also pre-junctional receptors in human saphenous veins that respond to muscarinic stimulation by a reduction and to β stimulation by an increase in noradrenaline release (Vanhoutte & Shepherd, 1985). This β-mediated facilitation of noradrenaline may involve local angiotensin II synthesis (Molderings *et al.* 1988). Sympathetic nerve activation in veins releases not only noradrenaline but also the co-

transmitters neuropeptide Y and ATP (Racchi *et al.* 1999) and it may be inhibited by nitric oxide but enhanced by thromboxane (Fabi *et al.* 1996, 2004).

1.2.2 Hormonal control of venous tone

In veins as in arterial resistance vessels, adrenaline acts on α and β adrenoceptors but the α-adrenergic component dominates such that the typical response is a venoconstriction (Shepherd & Vanhoutte, 1975). Noradrenaline causes venoconstriction via both α_1 and α_2 adrenoceptors. Beta adrenoceptor stimulation with noradrenaline can cause venodilation through post-junctional activation (in contrast to the increased noradrenaline release described above) but to a lesser extent than with adrenaline. Because of the sparse sympathetic innervation of smaller veins, circulating catecholamines may actually be more important for venoconstriction in these vessels (Marshall, 1982). Systemic infusion of noradrenaline at concentrations similar to those reached during sympathoexcitation was reported to evoke a decrease in blood flow in the cat hindlimb, whereas adrenaline infusion produced an increase (Marshall, 1991). Hindlimb volume fell following infusion, even when blood flow was maintained, suggesting that catecholamines exert an active constrictor influence on the venous vasculature that is greater than that induced by sympathetic nerve stimulation. In an earlier experiment, Marshall (1982) found that noradrenaline in concentrations of greater than 6×10^{-8} $g.ml^{-1}$ constricted all rat arterial and venous vasculature. The required dose to constrict the venous vessels progressively increased away from the capillary bed with 6×10^{-8} $g.ml^{-1}$ being the required dose for collecting venules, while 10^{-7} $g.ml^{-1}$ for main veins.

Other hormonal agents that influence venous tone are atrial natriuretic peptide (ANP) and prostaglandins. ANP dilates arterial vessels in the human forearm (Hughes *et al.* 1988; Doorenbos *et al.* 1991; Ando *et al.* 1992). It is reported to have a weak venodilator effect based on increases in forearm volume measured by plethysmography (Ando *et al.* 1992) or venous vascular volume (Schmitt *et al.* 2003). This may be due to its arterial effects leading to greater filling because venous distensibility during filling was unchanged by ANP (Groban *et al.* 1990, Schmitt *et al.* 2003) and had no relaxant effects on dorsal hand veins precontracted with noradrenaline (Webb *et al.* 1988). Other types of natriuretic peptide (C-type and brain) also appear to be venodilator (Banks *et al.* 1996, Protter *et al.* 1996). Venodilator prostaglandins modulate adrenergic venoconstriction based on enhanced responses in dorsal hand vein to noradrenaline (Callow *et al.* 1998, Dzeka *et al.* 2000) or sympathoexcitation (Dzeka *et al.* 2000, 2003) after indomethacin. Likewise, inhibition of prostaglandins by aspirin potentiates venoconstriction to endothelin-1 in the dorsal hand vein (Webb & Haynes, 1993). Venoconstrictor prostaglandins directly affect the smooth muscle of the larger veins to cause constriction (Ducharme *et al.* 1968; Mark *et al.* 1971). They have

been shown to cause contractions of isolated strips of canine skeletal muscle veins, rabbit portal-mesenteric veins and umbilical veins (Dyer, 1970, Hughes & Vane, 1970, Dyer *et al.* 1972, Greenberg *et al.* 1973).

1.2.3 Myogenic control of venous tone

Local mechanisms of regulation of venous tone have been reviewed by Monos *et al*. (1995). Intrinsic myogenic tone has been demonstrated in animals (bat wing, rat portal vein, mesenteric vein of guinea pig and facial vein). Monos and colleagues have shown that in the rat saphenous vein, myogenic tone controls 30% of vascular capacity (Monos, 1993). Within the rat gracilis muscle, first order venules demonstrate a myogenic response to pressure that is enhanced by noradrenaline (Dornyei *et al.* 1996), so that during exercise, increases in venous pressure and circulating catecholamines could interact to produce significant myogenic constriction. This would increase the pressure drop from post-capillary to central vessels and limit the increase in capacitance, aiding venous return.

Berczi *et al.* (1992) compared the active and passive mechanical properties of isolated human saphenous and dog femoral and saphenous vein segments. In response to intraluminal pressure increases, human saphenous veins showed greater myogenic tone than dog veins and it was suggested that this was because of the greater orthostatic load imposed in the human leg. This agrees with data from humans showing that myogenic responses of conduit arteries are greater in legs than arms (Lott *et al.* 2008). Myogenic responses can be enhanced by prolonged exposure to pressure (Monos, 2003), which may require some consideration when planning longer bouts of venous occlusion (e.g. > 2 hours).

1.2.4 Metabolic control of venous tone

While the precapillary vessels respond to local metabolic changes such as anoxia and acidosis, the veins are little affected by such changes (Vanhoutte & Janssens, 1978). Severe oxygen deprivation and substrate depletion must be combined before venous smooth muscle reactivity is markedly altered (Shepherd & Vanhoutte, 1975). This suggests that the usual fluctuations in oxygen in the venous blood do not affect the reactivity of the veins. In the limbs, contraction of the skeletal muscle and temporary arrest of the circulation lead to dilation of arterial vessels as a consequence of local metabolic changes, while the veins are less affected (Lewis & Mellander, 1962, Sharpey-Schafer *et al.* 1965).

1.2.5 Endothelial control of venous tone

Small venules in skeletal muscle dilate to increases in flow and shear stress via endothelial-dependent production of nitric oxide and prostaglandins which can also dilate nearby arterioles (Boegehold, 1996, Racz *et al.* 2009). However, levels of shear in the venous

circulation are lower than in arterial vessels (Kim & Sarelius, 2003) and the significance of flow-induced responses in overall control of venous tone is unclear. Dornyei *et al.* (1997) applied low doses of acetycholine to rat gracilis muscle venules (225 μm in diameter) and induced dilations that were eliminated by removal of endothelium and reduced by NO synthase inhibition. Conversely, high doses of acetylcholine caused constrictions, which were also abolished by endothelial removal and cyclooxygenase inhibition. In humans, Blackman *et al.* (2000) showed that forearm venous volume was reduced by nitric oxide synthase inhibition and increased when nitric oxide production was stimulated by carbachol, indicating endothelial control of both resting and stimulated venous tone.

1.2.6 Local veno-arteriolar response (VAR)

The veno-arteriolar response (VAR) is triggered by stretch receptors located in the walls of small veins and involves changes in arteriolar vascular tone upstream of the veins. When venous pressure is elevated to pressures greater than 25 mmHg through manoeuvres such as postural change (increase in the hydrostatic load) or inflation of a venous occluding cuff, a localised VAR increases arterial resistance in cutaneous, subcutaneous and skeletal muscle tissues, resulting in ~ 40 % decrease in blood flow (Andersen *et al.* 1986; Henriksen, 1991; Crandall *et al.* 2002). The VAR attenuates the pressure in the capillaries, limiting the rate of fluid filtration from the capillaries into the surrounding tissue. Although the mechanism(s) for VAR are not fully understood, local nerve fibres appear to play a part as the response is abolished following sympathectomy (Henriksen *et al.* 1983), anesthetic block (Okazaki *et al.* 2005) or spinal chord transection (Skagen *et al.* 1982; Theisen *et al.* 2000). Okazaki *et al.* (2005) compared VAR responses to limb dependency with and without local anaesthetic blockade. Skin blood flow decreased by 45 ± 9 % in the forearm and by 40 ± 20% in the calf (both $p < 0.01$) during limb lowering in the control condition (without neural blockade). However during local anaesthetic blockade conditions, skin blood flow remained unchanged (-5 ± 23 % and -2 ± 32 % for forearm and calf respectively, both $p > 0.05$), confirming that VAR is also associated with local neural control mechanisms.

In summary, there are multiple mechanisms that can regulate venomotor tone:

1. Veins are responses to sympathetic stimulation at low frequencies, but response varies being more pronounced in more distal veins, and greater in superficial. Smaller intramuscular veins and venules appear to be unresponsive.
2. Veins are responsive to circulating catecholamines by constriction and this is particularly evident in smaller venous vessels.
3. Veins respond to increases in transmural pressure by myogenic constriction which could be important in orthostasis to counter venous pooling.
4. Increases in venous stretch and / or pressure can also trigger a local neural

venoarteriolar (VAR) response that leads to pre-capillary constriction.

5. Veins are relatively insensitive to metabolic conditions but do show a degree of endothelial control.

1.3 Methods for measuring peripheral venous properties and venomotor tone in humans

There are a number of ways in which peripheral venous tone can be assessed in humans (Pang, 2000). Because veins may not adopt a fully circular profile at ambient venous pressure (see above), it is usual to induce distension in order to examine changes in venous size or volume in the filled state. Filling may be achieved by congestion or occlusion with a pressure cuff fitted to the upper part of the arm or leg inflated above venous but below diastolic pressure. Other procedures such as standing, sitting, head-up tilt or Valsalva's manoeuvre also result in increased venous pressure and filling. In clinical practice, head-down tilt (the Trendelenburg position) is commonly used to expand large central veins such as the jugular to make catheterisation easier (Prince *et al.* 1976, McGee & Gould, 2003, Lewin *et al.* 2007).

1.3.1 Dorsal hand or foot vein

One approach to study venous tone is to measure diameter changes of an individual vein with pressure held constant by occlusion. This can be achieved for superficial veins of the hand or foot either by observing stereoscopic movement of a marked point on the skin surface above the distended vein, or by using the linear differential differential transformer (LVDT) technique as described by Aellig (1994). Direct local infusion, intravenous or subcutaneous injection, microdialysis or oral administration can then be used to apply various vasoactive substances to study venomotor responses of specific human veins in situ (Aellig, 1994, Pang, 2000). For example, Streeten (2001) used this method to show supersensitivity to noradrenaline in the foot veins of patients with orthostatic hypotension.

1.3.2 Isolated forearm vein

Another method for studying individual human superficial veins is to isolate a segment of forearm vein with pressure clamps applied at either end to hold volume constant and record pressure within the segment via a catheter and pressure transducer. Increases in pressure indicate vein constriction and vice versa. Although invasive, this technique was used in several early studies to investigate reflex venomotor responses to manoeuvres such as a deep breath, cold, exercise and postural changes (Duggan *et al.* 1953, Page *et al.* 1955).

1.3.3 Limb vascular volume

The above methods are only applicable for superficial veins and the majority of studies on

peripheral venous function in humans have recorded limb volume during distension by inflation of a proximal cuff to above 30 mmHg but below diastolic pressure as a surrogate for venous vascular volume. Scintography of red cells labelled with ^{99m}Tc (radionuclide plethysmography method) shows that at maximum filling, venous blood volume accounts for about 10% of the total calf segment volume (Vissing & Nielsen, 1988) and ~65% of total blood volume in the forearm (Todo *et al.* 1986). Vascular volume of the deep veins was measured from MRI scans of the calf during venous occlusion and estimated to account for ~70% of the increase in limb volume (Buckey *et al.* 1988). Using ultrasound, Cirovic *et al.* (2006) measured the cross-sectional area (CSA) of all the major deep and superficial large veins in the calf as 0.68 ± 0.17 cm^2 compared to the whole calf CSA of 108 ± 20 cm^2 in the unfilled state, but these large veins accounted for 30% of the increase in calf CSA during venous congestion, the remained presumed to be filling of smaller veins and venules. Mutinga & Visser (1992) used a bio-impedance method to determine arterial and venous fluid shifts on release of a cuff occluding flow to the arm and estimated that 49% of the total blood compartment was venous blood. Although these data show variations in the proportion of a limb volume that can be attributed to venous vascular volume, the fact that the values are relatively large enables limb vascular volume to be used as a surrogate for venous volume when assessing venous characteristics and venous tone during e.g. the occluded limb method and equilibration technique.

1.3.4 Venous distension and limb volume changes during filling

Limb volume response to venous occlusion has two phases – a rapid filling phase due to arterial inflow and a slower increase due to fluid filtration and, potentially, venous 'creep'. Plethysmography by air, water or strain gauge records the rate of volume increase during rapid filling which is taken to indicate blood flow provided that the limb venous circulation is elevated above heart levels and therefore has spare capacitance (Joyner *et al.* 2001; Gamble *et al.* 2005). However, strain gauge plethysmography has recently been used to determine calf blood flow during head-up tilt and showed good agreement with ultrasound measures of superficial femoral artery flow (Kooijman *et al.* 2007), although it is not possible to say whether changes in blood flow under these conditions are due to altered resistance vessel tone or an increase in venous pressure leading to decreased arterio-venous pressure gradient and perfusion. For further discussion on this, see introduction to Chapter 2.

1.3.5 Venous capacity

Since the definition of capacity is the maximum filled venous volume of a limb, plethysmography can also record peak percentage changes in limb volume during venous occlusion as venous capacity. Typical values obtained by strain gauge measurements are 2-4 $ml.100ml^{-1}$ (%) for calf and 3-4% for forearm (Boutcher & Boutcher, 2005). There is good

correlation between changes in vascular volume measured by radiolabelled red cells and plethysmographic limb volume during alterations in occluding cuff pressure and vasodilator administration (Clements *et al.* 1982; Schmitt *et al.* 2002) but periods of venous occlusion for more than one or two minutes also result in fluid filtration out from the vascular compartment into the tissues, at approximately 0.2% per min at 50 mmHg, and measured limb volume will include this component. The duration of venous occlusion will therefore obviously influence the extent to which filtration rate contribute to limb volume (Halliwill *et al.* 1999). It is important to define the measurement criteria for determining venous capacity. In many studies this is taken as the point at which the rapid filling phase deviates from a straight line extrapolated backwards from the plateau filtration phase (Sejersen *et al.* 1981, Olsen and Lanne, 1998, figure 1.5). Gamble *et al.* (1993) gave the mean time constant of the rapid filling phase in response to a single venous occluding pressure step of 50 mmHg as 77 seconds. Brown *et al.* (1966) noted disparate rates of filling of forearm veins during upper arm cuff inflation to 40 mmHg but concluded that it was complete after 120 seconds while Halliwill *et al.* (1999) estimated forearm filling to be complete by 147 seconds after cuff inflation to 60 mmHg. In practice, for the calf, venous capacity can be taken from the volume after 3 minutes by which time filling will be complete but fluid filtration minimal, and values at 3 minutes differ little (~ 3%) from those at the inflection point between filling and filtration (Phillips *et al.* unpublished observations).

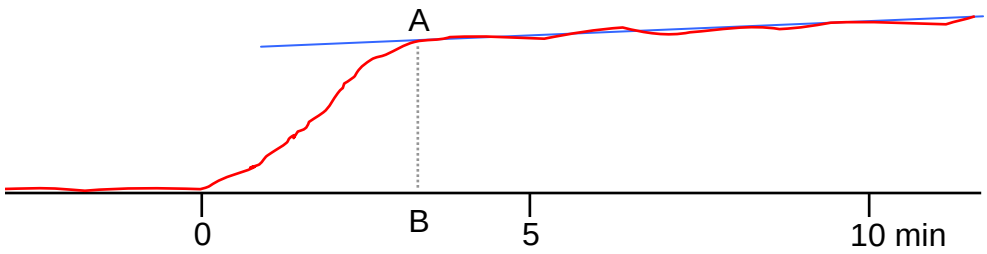


Figure 1.5*: Example of limb volume trace showing increase following inflation of an occluding cuff at time 0. Venous capacity can be taken as the point (A) at which the rapid filling phase deviates from a straight line (blue line) extrapolated backwards from the plateau filtration phase or as the value at a given time point (B). At 3 minutes, filling will be complete but fluid filtration minimal, and values differ little (~ 3%) from those at the inflection point between filling and filtration. Illustration adapted from Sejresen et al. 1981.*

The distribution of venous filling between superficial and deep tissues is difficult to estimate. Zelis & Mason (1969) compared forearm venous volume with an occluding cuff inflated to 30 mmHg before (4.16 ± 0.3 ml.min^{-1}) and after (2.54 ± 0.3 ml.min^{-1}) iontophoresis of the entire forearm with adrenaline to eliminate skin and subcutaneous tissue flow. Thus, approximately 40% of the forearm venous volume (1.62 ml.min^{-1}) could be attributed to muscle. It is not

known whether these proportions are similar for the calf or how they are influenced by differences in limb and body composition.

Jorfeldt *et al.* (2003) used strain gauge plethysmography and examined the rate of volume expansion in the leg (thigh, and three segments of the calf – top, mid and lower) during inflation of a proximal thigh cuff to 50 mmHg at different ambient temperatures and leg positions. They concluded that the strain gauge may measure not only arterial inflow but displacement of venous blood volume from other regions distal to the cuff. Heating significantly increased the rate of volume expansion, more so at the calf than thigh, most likely because of the larger proportion of skin tissue in the former than latter. Leg position had an effect on filling rates in that they increased with elevation and decreased with lowering. Variations in sympathetic tone of superficial vessels impacted on the volume response because heating reduced superficial venous tone and led to greater rates of filling. Yamazaki *et al.* (2002) also measured rate of calf volume increase during cuff inflation at pressures between 20-80 mmHg during whole body heating / cooling (water-perfused suit) or local calf heating (warm air box). Heating increased and cooling decreased the rate of calf volume increase but the magnitude of volume change was not altered.

1.3.6 Venous distensibility and compliance

Venous compliance, as explained earlier, describes the volume or diameter - pressure relationship over a range of cuff inflation pressures above ambient venous but below diastolic arterial pressure. In the literature, compliance is often used in this way and derived from limb p-v plots during increases in cuff pressure and filling. Due to the nature of vein wall composition and its viscoelasticity, the filling and emptying curves of venous vasculature display pronounced non-linearity and hysteresis (figure 1.4). Therefore, expansion of vein diameter or volume during filling should more properly be called distensibility. Halliwill *et al.* (1999) discuss this issue in a detailed methodological paper on how to measure vein compliance, pointing out that compliance should rather be measured during vein emptying. This allows a more accurate determination of the passive elastic recoil properties of the vein wall and how they impact upon compliance (figure 1.4). Halliwill *et al.* (1999) modelled the curvilinear decline in limb volume during emptying after cuff deflation by an exponential, and derived beta coefficients to describe the relationship that could be used to reflect both capacity and compliance, and show whether venous tone was altered. By plotting compliance calculated from these coefficients at each pressure, a compliance-pressure plot was obtained showing a linear inverse relationship between the two parameters.

Risk *et al.* (2003) applied several different models to the plot of compliance (derived from p-v curves during emptying) against pressure in arms and legs. They concluded that a bi-phasic

model provided the best fit, in agreement with the known non-linear features of vein compliance. High compliance at low pressures would allow for large translocations of blood from the venous system in response to small changes in pressure when central blood volume is low in order to maintain central venous pressure. Low compliance at high pressures would minimise pooling in the upright position. Risk *et al.* (2003) found that the break-point between high and low compliance occurred at different pressures in the arms (29 ± 5 mmHg) than the calf (34 ± 4 mmHg), consistent with the calf veins supporting higher pressures during orthostasis. They also considered that the break-point may be related to differing proportions of elastin and collagen within the vein wall. Because of discrepancies in the literature over the use of the terms distensibility and compliance, it will be made clear in further references which protocol was used, filling or emptying, and data based on the former will be termed distensibility and on the latter, compliance.

1.3.7 Occluded limb technique

The isolated vein segment method has been adapted for the whole limb by using a venous occlusion cuff inflated to below diastolic pressure so that the veins were filled, followed by rapid cuff inflation to suprasystolic pressure. This effectively isolates the limb at constant volume so that a catheter inserted into a deep or superficial vein could be used to register pressure changes as indicators of venomotor tone in response to reflex activation (Gauer & Thron, 1962; Samueloff *et al.* 1966a). This method was described by Samueloff et al. (1966a) and has been used by these (Samueloff *et al.* 1966b) and many other authors to demonstrate reflex venoconstriction (Epstein *et al.* 1968, Zitnik & Lorenz, 1969, Delius 1971). The disadvantages of the technique are that it is invasive and that although different veins can be cannulated, it is difficult to distinguish superficial from deep vein responses since pressure will be able to equilibrate between the two venous compartments.

1.3.8 Equilibration technique

Analogous to the measurement of superficial vein diameter or volume with pressure held constant is the equilibration technique for the whole limb. Venous occlusion is applied at pressures between 30 – 60 mmHg to induce filling and when a steady state has been achieved, changes in limb volume registered by plethysmography are taken to represent constriction or dilation of the veins because the contribution of arterial volume is relatively much smaller (Wood & Eckstein, 1958, Mason *et al.* 1964, Zelis & Mason, 1969).

1.3.9 Ultrasound imaging

The more recent development of ultrasound imaging techniques has led to significant advances in being able to track changes in the size of individual veins, deep as well as superficial (Coleridge-Smith *et al.* 2006, Cavezzi *et al.* 2006). This approach has been used

to compare the anatomy of different leg veins in relation to body size, age, gender (Mortensen *et al.* 1990, Jeanneret *et al.* 1999, Fronek *et al.* 2001, Kroger *et al.* 2003, Berczi *et al.* 2005, Haenen *et al.* 1999) and presence of venous disease (Hertzberg *et al.* 1997, Danielsson *et al.* 2005, Jeanneret *et al.* 2007), to establish the relationship between vein CSA and pressure during cuff inflation (Planken *et al.* 2006) or tilting (Chauveau *et al.* 2006), to evaluate vein distensibility (Eiken & Kolegard, 2004) or compliance (Neglen & Raju, 1995, De Groot *et al.* 1998, Young *et al.* 2008, Delaney *et al.* 2008) and to study modifications to venous function during exercise (Ooue *et al.* 2008, van Diujnhoven *et al.* 2008), inactivity (de Chantemele *et al.* 2004, Arbeille *et al.* 2008, van Diujnhoven *et al.* 2008) and heat stress (Abraham *et al.* 1994, Ooue *et al.* 2007).

1.4 Reflex regulation of venomotor tone in peripheral veins

Venous tone directly impacts blood pooling, venous return and cardiac filling. There are several pathways by which venous tone can be modified including baroreflex (carotid, cardiopulmonary), cortical (exercise, mental stress) and thermoregulatory (exercise, passive heat stress)**.** A number of experimental paradigms can be used to induce sympathetic activation and elicit changes in venomotor tone in human limb(s), such as deep breath (Zelis & Mason, 1969, Samueloff *et al.* 1966b), Valsalva's manoeuvre (Page *et al.* 1955, Robertson *et al.* 1979), cold pressor test (Dzeka & Arnold, 2003, Zelis & Mason, 1969, Page *et al.* 1955, Samueloff *et al.* 1966b, Robertson *et al.* 1979), mental stress (Dzeka & Arnold, 2003, Zelis & Mason, 1969, Samueloff *et al.* 1966b), exercise (Zelis & Mason, 1969, Page *et al.* 1955, Samueloff *et al.* 1966b, Robertson *et al.* 1979), lower body negative suction (Brown *et al.* 1966, Dzeka & Arnold, 2003, Browse *et al.* 1966, Samueloff *et al.* 1966a & b) and tilt (Samueloff *et al.* 1966a, Page *et al.* 1955, Stewart & Weldon, 2001a). Most studies have investigated venomotor tone in the upper limb (forearm or hand). During a venous distension, interventions are best incorporated into the procedure after the vein has reached capacity so that it is certain that any observed change in limb volume / vessel diameter will be through sympathoexcitation rather than through changes in the transmural pressure caused by venous filling.

Early studies (Page *et al.* 1955, Gauer & Thron, 1962, Sharpey-Schafer, 1965) showed Valsalva's manoeuvre to produce the largest response in an isolated forearm vein compared with exercise, a cold pressor test and hyperventilation. Samueloff *et al.* (1966a) used the occluded forearm technique in conjunction with 70^{o} head-up tilt (HUT) and reported a reduction in forearm blood flow (3.6 ± 0.8 $ml.100ml^{-1}.min^{-1}$ (control) vs. 2.5 ± 0.9 $ml.100ml^{-1}.min^{-1}$ (tilt)), while forearm venous pressure increased by 7.9 ± 1.2 mmHg, suggesting that venoconstriction had occurred. The same authors (Samueloff *et al.* 1966a & b) and Browse *et al.* (1966) examined the reflex effects of blood volume shifts using LBNP again observing

reductions in forearm blood flow in association with increased intravenous pressure.

Samueloff *et al.* (1966a) noted that deep and the superficial veins of the occluded forearm exhibited the same time-course of reactivity to the same stimulus e.g. a deep breath, which was a sudden, transient rise in pressure of ~ 19 mmHg returning to control levels after 70 – 80s. Zelis & Mason (1969) established that reflex forearm venoconstriction during deep breath, cold pressor test, lower limb exercise and mental stress was predominantly occurring in cutaneous tissue since elimination of skin blood flow by adrenaline iontophoresis abolished the response. Samueloff *et al.* (1966a) compared venoconstriction in hand, foot, forearm and calf during various stimuli using the occluded limb method. Although data values were not given, they reported that venous pressure increase during a deep breath was greatest in the hand and foot veins, slightly less in the forearm veins and least in the long saphenous vein of the leg. In the contralateral forearm, radial artery pressure did not increased and forearm circumference remained unchanged indicating venous but not arterial constriction.

Lower limb reactivity to HUT has recently been studied by Stewart and colleagues on a number of occasions in patients with postural orthostatic tachycardia syndrome (POTS) and control subjects. They found significantly reduced calf blood flow in POTS patients at 35° HUT compared to their control subjects (-70 % (POTS) vs. -42 % controls, Stewart & Weldon, 2001b, Stewart, 2003, Stewart *et al.* 2004). Although venoconstriction was implied from observed reductions in calf volume, whether these changes reflect active venoconstriction or just passive reductions in venous volume is unclear by this whole limb plethysmography method. In another study Stewart (2002) reported reduced calf capacity (3.8 ± 0.4 ml.100ml^{-1} at 0° vs. 0.16 ± 0.17 ml.100ml^{-1} at 35°) during 35° head up tilt but this was not found to be significant. Similarly, in an earlier study (Stewart & Weldon, 2000), reductions in calf blood flow (2.3 ± 0.7 ml.100ml^{-1}.min^{-1} at 0° vs. 2.0 ± 0.6 ml.100ml^{-1}.min^{-1} at 70°) and calf diameter (-2.4 ± 0.5 %) were also not found to be significant.

Much of the above evidence of venoconstriction is measured as limb volume decrease or vein pressure increase, illustrating the downward shift of the p-v relationship and reduction in unstressed volume and venous capacity as depicted in Figure 1.3. With regard to the shape and slope of the p-v relationship and venous compliance, it appears that there is no change in this parameter during sympathoexcitation. Halliwill *et al.* (1999) used isometric handgrip to elevate sympathetic activity and showed the downward shift of the p-v curve during vein emptying in both contralateral forearm and calf (i.e. decreased capacity) but no change in the relationship between compliance and pressure. Monahan & Ray (2004) also found no change in calf compliance during emptying combined with ischemic handgrip or a cold

pressor test, and Delaney *et al.* (2008) saw no change in compliance with the same interventions in forearm or calf. More recently, studies have employed ultrasound to examine individual leg veins and determine filling and emptying properties and whether they are affected by sympathoexcitation. De Groot *et al.* (2005) described measurement of popliteal vein compliance from pressure-diameter curves obtained during cuff deflation but did not provide quantitative values for normal controls while Young *et al.* (2008) derived quadratic regression coefficients for the pressure-popliteal vein cross-sectional area curve during emptying in the same way as Halliwill *et al.* (1999) did for calf volume. Young *et al.* (2008) showed that popliteal compliance was unchanged during a cold pressor test to evoke sympathoexcitation.

1.5 Active or passive regulation of venous tone?

Because of the complex nature of interactions between pressure and flow within the venous system and the importance of arterial resistance in regulating forward flow into the venous system (Rothe, 1983, Gelman, 2008), there has been debate as to whether changes in venous volume measured during venoconstrictor stimuli represent true changes in venous tone or passive changes as a result of pressure differences when arterial resistance alters. For example, arterial constriction will reduce pressure within the capillary and venous circulations and the latter could show a passive reduction in volume. Conversely, arterial dilation will increase pressure potentially leading to a passive increase in venous volume. This issue is important especially when inferring venoconstriction from changes in venous vascular volume in response to interventions that are likely to alter arterial resistance as well as venous smooth muscle tone.

Active venoconstriction will result from activation of the vascular smooth muscle, whereas passive venoconstriction or more precisely - passive reduction in the venous volume - will result from a decrease in distending pressure (Rothe, 2006). “An active venoconstriction, causing a reduction in volume, will simultaneously cause a proportionally much larger change in resistance to flow resulting in an increase in upstream pressure and distended volume provided that the inflow does not decrease” (Rowell, 1993). On the other hand, since active constriction is dependent on contraction of the venous smooth muscle and since an increase in sympathetic activity not only constricts veins but also constricts arterioles, the reduction of venous capacitance during sympathoexcitation could also be as a result of reduced inflow from the arterioles i.e. a passive reduction in blood volume. Under these circumstances venous return is more dependent on external influences on the veins such as skeletal muscle pump (Hainsworth & Drinkhill, 2006). However, since Hainsworth & Drinkhill pointed out that “sympathetic activity not only constricts veins but also arterioles”, then the opposite must be true that sympathetic activity not only constricts arterioles but also veins. In

which case, if active venoconstriction does take place during orthostasis then the more precise question must be “to what extent does active venoconstriction occur and in which vessels”? Although sympathetically mediated (active) venoconstriction has been demonstrated in the isolated vein segments of animals, evidence of active venoconstriction during sympathoexcitatory challenge in humans is inconsistent and particularly sparse in the case of the lower limb, thus warranting further investigation.

Despite this, there is evidence to show that arterial and venous vasomotor responses may occur independently. Browse *et al.* (1969) determined venous tone from pressure within a large forearm vein with the limb circulation occluded (isolated / occluded limb method) and blood flow by strain gauge plethysmography from the contralateral forearm during LBNP (-60 mmHg). Forearm blood flow was reduced by over half and there was a transient rise in vein pressure i.e. venoconstriction. When LBNP was release, blood flow increased 4-fold but this coincided with another transient venoconstriction. Thus, the resistance and both superficial and deep venous vessels displayed opposite responses.

Coffman & Lempert (1975) applied a thigh cuff at 30 mmHg and used plethysmography to examine calf venous volume (equilibration method) and blood flow, and venous flow velocity as the transit time of radio labeled albmunin travelling from calf to inguinal region. A catheter inserted into the saphenous vein at the ankle was pushed up the limb into the mid calf area. Responses to adrenaline, isoprenaline, angiotensin and heating to 40 ^{o}C were studied. Adrenaline did not alter blood flow but decreased venous volume. Isoprenaline and heating increased and angiotensin decreased blood flow without change in venous volume. Again, this demonstrates dissociation between changes in resistance vessel tone and venomotor responses.

Peters *et al.* (1997) used the occluded forearm method during positive pressure breathing and LBNP and noted changes in forearm blood flow before any changes in venous tone. LBNP increased forearm vascular resistance without change in venomotor tone. Active venoconstriction was transient, in line with observations by Samueloff *et al.* (1966). Tripathi *et al.* (1984) also used the occluded limb method in forearm and recorded superficial venous pressure as an indicator of venomotor tone in response to LBNP (-20 to -60 mmHg) and neck suction to activate cardiopulmonary and carotid baroreceptors. They also used the equilibration method, measuring forearm volume with strain gauge and cuff inflated to 30 mmHg. Both methods showed venoconstriction to LBNP but not neck suction, indicating a role for the low pressure cardiopulmonary baroreceptors. They did observe that venous volume increased if ambient temperature was increased, and decreased if it was lowered, consistent with effects on superficial venous tone. Schmitt *et al.* (2000) used hydralazine, a

selective arterial vasodilator in the forearm, during venous occlusion and did not see any effect on venous volume.

These studies have confirmed independent effects of vasoactive agents on resistance arterial vessels and on venous tone. It is possible that when veins are filled under high pressure and at the top of the p-v curve, effects of sympathetic activation may be obscured. However, Sumner *et al.* (1989) measured forearm venous volume by plethysmography and showed that glyceryl trinitrate (GTN), a known arterial dilator, also increased venous volume, indicating venodilation, but the venoconstriction to ice applied to the forehead was similar before and after GTN.

1.6 Gender and female hormones

1.6.1 Effects on venous function

The evidence for venoconstriction above is almost exclusively obtained from studies on men. Yet there is sound evidence that venous function differs in women and men, an effect that has been attributed to the action of female reproductive hormones oestrogen and progesterone. In the context of orthostasis, women are less tolerant than men and several studies have shown that on being tilted to the upright position or when LBNP is applied, women are more prone to syncope than men. During both tilt and LBNP, women show bigger decreases in stroke volume and greater heart rate increases than men (Convertino *et al.* 1998; Frey & Hoffler, 1988; Shoemaker *et al.* 2001; Fu *et al.* 2004; Fu *et al.* 2005).

It has been suggested that increased pooling in the female lower body is a factor in their orthostatic intolerance, relating to venous capacity and compliance in the legs. However, men were found to have greater calf capacity than the females when venous volume was increased by occlusion (Monahan & Ray, 2004) or LBNP (Lindenberger & Lanne, 2007). These studies also demonstrated that men had greater venous distensibility, measured from the p-v relationship during the filling phase of LBNP (Lindenberger & Lanne, 2007), and compliance, measured from the venous emptying curve after release of venous occlusion (Monahan & Ray, 2004). The latter finding was confirmed by others (Hernandez & Franke, 2004, Meendering *et al.* 2005) using similar methodology. One factor determining venous capacity is the anatomical size of the capacitance vessels and the smaller leg size of women may account for lower capacity.

This evidence does not support gender differences in leg pooling as a factor in female orthostatic intolerance. Furthermore there are no apparent differences in tolerance to upright posture when women are tested across follicular, mid-cycle and luteal menstrual cycle phases (Meendering *et al.* 2005, Claydon *et al.* 2006) and no effect of oral contraceptive use

on responses to LBNP (Franke *et al.* 2003), implying that the female reproductive hormones are not important. Despite this, oestrogen and progesterone have significant effects on venous function and are linked with the higher incidence of venous disease such as varicose veins and chronic venous insufficiency in women than men (Beebe-Dimmer *et al.* 2005). Receptors for oestrogen and progesterone are present in human saphenous veins (Perrot-Applanat, 1996, Masiah *et al.* 1999) and the hormones have non-genomic vasoactive effects on vascular smooth muscle. In arterial vessels, the relaxant action of oestrogen via endothelial nitric oxide is well known (Review papers: Du *et al.* 1995, Sarrel, 1999, Suzuki *et al.* 2003, Orshal & Khalil, 2004) while progesterone can have both dilator and constrictor actions (Sarrell, 1999) and can act as an antagonist to oestrogen (Kuhl, 1999, Jespersen *et al.* 1990). Oestrogen is also a dilator in isolated porcine femoral (Bracamonte *et al.* 2002) and human saphenous veins (Babaei & Azarmi, 2008), and oestrogen receptor activation by raloxifene caused endothelial-dependent dilation in rat pulmonary veins (Chan *et al.* 2005). In rats, chronic oestrogen treatment lowered mean circulatory filling pressure indicative of reduced venous tone (Nekooeian & Pang, 2000). Progesterone also dilated isolated rabbit jugular veins via an endothelial-dependent mechanism (Herkert *et al.* 2000).

The relaxant action of oestrogen and progesterone in veins can account for the early observations in women of increased venous distensibility in the finger, forearm or calf during filling when hormone levels are raised during the natural menstrual cycle (McAusland *et al.* 1964, Fawer *et al.* 1978), oral contraceptive use (Goodrich & Wood, 1964, Fawer *et al.* 1978), administration of 17β-oestradiol (Goodrich & Wood, 1966) or pregnancy (McAusland *et al.* 1961, Goodrich & Wood, 1964, Barwin & Roddie, 1975). Later studies confirmed that p-v curves for the calf were raised to higher volumes during the ovulatory and luteal compared to follicular phases of the menstrual cycle, although compliance during emptying was unchanged (Meendering *et al.* 2005). In contrast, exogenous female hormones administered via the oral contraceptive did not appear to modify either venous capacitance or compliance in the calf (Meendering *et al.* 2005).

Since orthostatic intolerance in women versus men is not associated with increased leg capacity or compliance and there is no change in OI across the menstrual cycle or with oral contraceptive use despite differences in leg capacity, it can be concluded that the capacitance volume available for blood pooling is not a major factor. There is, however, another mechanism that could differ between genders and, under the influence of female hormones, impact upon leg pooling, namely venoconstriction. If this were attenuated in women, the ability to counter pooling would be impaired. To our current knowledge, no studies have examined gender differences in venoconstriction or whether *in vivo* it is attenuated in women compared to men.

Evidence from animal studies indicates that female hormones do interact with adrenergic constrictor mechanisms but is conflicting. It is difficult to predict because of the differing ways in which oestrogen could potentially modify adrenergic constriction. It could attenuate noradrenaline release by inhibition of synthesis (Bengtsson, 1978) or enhancement of α_2-mediated presynaptic inhibition of release (Du *et al.* 1995). Alternately, oestrogen could enhance constriction by increasing post-synaptic α_2 receptor density or inhibition of neuronal noradrenaline reuptake (Du *et al.* 1995). Rorie & Muldoon (1979) showed that the constrictor response of isolated rabbit saphenous veins to noradrenaline was enhanced by oestrogen and progesterone. In rats, removal of female hormones by ovariectomy blunted the constrictor response of the saphenous vein to noradrenaline but this was restored by hormone replacement treatment (Varbiro *et al.* 2002). *In vivo* chronic administration of 17β-oesetradiol to rats enhanced venoconstriction to noradrenaline (Nekooeian & Pang, 2000) and Dhawan *et al.* (2005) observed increased effects of noradrenaline on whole body and isolated mesenteric vessel venous tone as a result of pregnancy in rats. Taken together, this evidence suggests that adrenergic venoconstriction would be enhanced rather than attenuated in women under the influence of female hormones, possibly to counter their relaxant effects on basal tone. This effect may be specific to venoconstriction induced by circulating catecholamines since in rat mesenteric circulation, 17β-oestradiol enhanced constriction to exogenous noradrenaline but not to that released by sympathetic neural stimulation (Chu & Beilin, 1997). In contrast, Gisclard *et al.* (1987) showed a depressed constriction to noradrenaline in rabbit saphenous vein after 17β-oestradiol treatment, and Ceballos *et al.* (2000) found less constriction to noradrenaline in the dorsal hand vein in post-menopausal women receiving hormone replacement therapy. It is therefore not clear how female hormones will actually impact upon venoconstriction during sympathoexcitation.

To date, there are hardly any data from human studies to indicate if venoconstriction is modified in women compared to men. Monahan & Ray (2004) showed that calf venous capacitance was reduced similarly (15-19%) in men and women by LBNP, but whereas in women the p-v curve was shifted downward and compliance remained unchanged, the shape of the curve was altered in men so that compliance was reduced. This differs from studies using exercise or a cold pressor test in which calf compliance was not altered (Halliwill *et al.* 1999; Young *et al.* 2008) and it is not clear why LBNP should result in different responses for men and women. There is no information about venoconstriction in women and men during upright posture. There are, of course, other factors that could account for the gender difference in orthostatic tolerance including lower cardiovagal baroreflex sensitivity, blunted increase in muscle sympathetic nerve traffic to the limbs (MSNA), and attenuated transduction of MSNA into increases in arterial resistance. The latter in particular has

relevance for impacting upon passive venous function, and differences in cardiovascular responses to tilt in women and men will therefore be discussed in brief.

1.6.2 Differences in orthostatic responses between men and women

Women may be less tolerant to orthostasis because of lower baroreflex sensitivity. Tank *et al.* (2005) showed that women had a lower MSNA output at a given MAP than men during administration of vasoactive drugs, and Christou *et al.* (2005) demonstrated similar blood pressure responses to phenylephrine in men and women but women were less able to buffer against the decreases in blood pressure following ganglion blockade. Together these studies indicate that females have a lower sympathetic outflow under normal conditions. Despite this, gender effects on MSNA responses to tilt are contradictory, having been reported to be smaller in women than men (Shoemaker *et al.* 2001) and also similar (Fu *et al.* 2005). Recently, Fu *et al.* (2009) noted lower increases in total MSNA during the follicular than luteal cycle phase in women during HUT although overall, there was no difference between women and men. However, findings are more consistent for catecholamine increases, which are similar during HUT in men and women (Geelen *et al.* 2002, Fu *et al.* 2005).

Based on studies using LBNP to mimic tilt, women show attenuated increase in vascular resistance than men in the calf (Frey & Hoffler, 1988) and forearm (Convertino, 1998, Franke *et al.* 2003). However, during LBNP, blood volume decreases in thoracic and splanchnic regions whereas during tilt, it increases in both these regions and the increase in leg volume is greater (Taneja *et al.* 2007) and this may evoke different arterial resistance responses. Forearm vasoconstriction to brachial artery infusion of noradrenaline was less in women than men although similar for women across menstrual cycle phases and those taking the oral contraceptive (Bowyer *et al.* 2001). Freedman & Girgis (2000) on the other hand saw greater forearm adrenergic constriction during the luteal compared to follicular phase in women.

There are no studies that directly compare peripheral arterial constrictor responses in men and women during tilt but similar gender increases in total peripheral resistance, diastolic blood pressure and mean arterial pressure have been reported (Geelen *et al.* 2002, Fu *et al.* 2005) although smaller increases in systolic blood pressure in women were observed by Fu *et al.* (2005). In women, HUT resulted in similar vasoconstrictor responses in the forearm across all phases of the menstrual cycle (Meendering *et al.* 2005; Claydon *et al.* 2006). If, during tilt, MSNA output is comparable between men and women but the increase in vascular resistance blunted, this could also apply to venoconstriction.

1.7 Conclusions and aims of thesis

The venous system is a highly responsive system that can readily react to changes in blood

pressure via sympathoexcitation to alter venous tone, maintaining blood flow and therefore venous return. Orthostasis is one of the greatest challenges for the venous system, as the effects of gravity cause blood to pool in the veins of the lower limbs and sympathetically-mediated venoconstriction is one of the compensatory adjustments that is suggested acts to counter venous pooling. The ability of the lower limb veins to actively constrict at sufficient magnitude to cause large blood volume shifts, i.e. increase venous return significantly, has been questioned and it has been proposed that skeletal muscle pump plays the more significant role in the context of orthostasis (Hainsworth & Drinkhill, 2006). On the other hand, significant α-receptor mediated venoconstriction has been demonstrated in the veins of the forearm (Zelis & Mason, 1969) during cold pressor stimulation but the question is whether a similar mechanism is present in the lower limb veins during sympathoexcitation as relatively little work has been done to study lower limb vein reactivity to such stimuli. There are two venous beds contained within the lower limb, deep and superficial, which are interconnected with perforator veins. The veins are termed the capacitance vessels and shift the large volumes of blood that they contain towards the heart by using a system of valves and increases in tone brought about through contraction via sympathoexcitation of the smooth muscle found within their intima media (middle) layer of the vessel wall. Histological evidence gained largely through animal studies has provided evidence that the deep veins contain greater numbers of valves and are less well innervated than their superficial counterparts and direct measurement of individual vein reactivity *in vivo* has come generally through the invasive technique of vein isolation and catheterization. Of the methods for studying venous behaviour, the equilibration technique for strain gauge plethysmography appears to allow the venous pressure to be equalised within the limb without completely occluding or impeding the blood flow, thus enabling non-invasive observations of whole limb characteristics. Doppler ultrasound imaging is a relatively new technique and similarly would allow continuous non-invasive observation of an individual vein during venous occlusion and potentially could also be used to observe reactivity to sympathoexcitation. Therefore, the main objective of this thesis is to establish if significant lower limb venoconstriction occurs following sympathoexcitation. Of particular interest, is the question of the relative contributions of the different venous beds, deep and superficial, to whole limb venoconstriction and it is expected that the use of ultrasound imaging will help to answer this.

As highlighted earlier in this Chapter, gender has also been shown to influence venous function, with the observed differences having been attributed to the female hormones oestrogen and progesterone, which have been reported to have a relaxant effect on the vessel wall. However, evidence of the exact influence of the female hormones during sympathoexcitation *in vivo* in humans is somewhat unclear, with contradictory reports of attenuated or no difference compared to males and altered or unchanged reactivity during

different phases of the normal menstrual cycle or in normal menstrual cycles vs. oral contraceptive use. Therefore, the second aim of this thesis is to look at the responses of males and females to sympathoexcitatory challenges and to attempt to identify if the female hormones play a role in responses. This will be done by comparing males to females during both the low and high hormone phases of their menstrual cycle and also to women taking higher levels of exogenous hormones in the form of oral contraceptives. The specific hypotheses for this thesis are:

1) **Significant whole limb constriction will occur in the calf limb during sympathoexcitation.**

2) **Doppler ultrasound imaging will help to identify that the deep and superficial venous vascular beds react differently to sympathetic stimuli; with the superficial veins showing significant venoconstriction while the deep veins show little response.**

3) **Large quantities of the female hormones oestrogen and progesterone, particularly during exogenous hormone use (oral contraceptive), will modify the responses to sympathoexcitation.**

Chapter 2:

THE EFFECTS OF VENOUS OCCLUSION ON ARTERIAL FLOW AND VENOUS VOLUME IN THE CALF IN MEN AND WOMEN

2.1 Introduction

Venous occlusion plethysmography has long been used in research to make observations of peripheral arterial blood flow and venous reactivity on the basis that the veins are occluded and the arteries are "left open" (Hewlett & van Zwaluwenburg (1909). However, Hiatt *et al.* (1989) observed that the cuff inflation during the technique caused a reduction in diameter and flow velocity in the superficial femoral artery. If venous occlusion is to be used in this present thesis to distend the veins in order to make observations of reactivity to sympathoexcitation, then it is important to gain an insight into what happens during the procedure with respect to the different vascular beds within the lower limb, so that any reactions to venous distension can be accounted for when comparing these responses to those during the sympathoexcitatory challenges. Venous occlusion plethysmography was first described for the measurement of blood flow in humans by Hewlett & van Zwaluwenburg in 1909. Later the use of mercury-in-silastic strain gauges to make these measurements was devised by Whitney (1953). The technique allows *in vivo* observations of volume changes within a limb segment and is commonly used on both upper and lower limbs (forearm and calf) to measure blood flow.

2.1.1 Venous distension

For assessment of venomotor reactivity in either of these segments, the two most common methods employed are the occluded or isolated limb which involves a fixed volume and measured changes in venous pressure (Samueloff *et al.* 1966a & b) and the equilibration technique which involves constant pressure and measured changes in venous volume (Zelis & Mason, 1969). As the equilibration technique will be used in this thesis it is described in more detail as follows. It involves induction of venous distension with a pressure cuff in a forearm or calf and observing the change in venous vascular volume during some protocol to induce sympathetic activation or a pharmacological intervention (see chapter 1, p26). Venous distension by cuff occlusion evokes two phase response in measured limb volume, rapid vascular filling followed by slower fluid filtration (e.g. figure 1.5, p24). The volume or capacity of the limb taken during the control distension condition will therefore be changing over time and it is important to define the temporal conditions under which measurements are made for comparison with any intervention. It is possible that decreases in limb vascular volume could occur passively, secondary to reductions in arterial inflow in response to sympathoexcitation. Arterial inflow can alter (either increase or decrease) without concomitant changes in venous volume or may remain unaltered while venous volume changes (See chapter 1, Browse *et al.*1966, Coffman & Lempert, 1975). Aside from the effects of sympathetic activity on resistance vessels, there is evidence that venous distension by itself may lead to a reduction in arterial inflow either 1) by eliciting myogenic constriction, 2) by evoking the venoarteriolar response (VAR) or 3) mechanically, by elevating venous

relative to arterial pressure.

Greenfield & Patterson (1954) proposed schematically the venous volume and pressure changes within a limb during venous occlusion, demonstrating the gradual rise in venous pressure to that of the occluding cuff within 1-2 minutes. Others have recorded venous pressure during occlusion (Brown *et al.* 1966, Halliwill *et al.* 1999) noting equilibration with cuff pressure within 1-2 minutes for superficial veins, while in a deep forearm vein Ireland *et al.* (1983) found venous and cuff pressure to be equal within 20-30 sec. Superficial vein pressure matches occluding cuff pressure over the range from 8 to 50-70 mmHg in the forearm (Mahy *et al.* 1995) and calf (Christ *et al.* 1997). Gamble *et al.* (1993) observed that calf blood flow was decreased when thigh cuff pressure was increased rapidly to 50 mmHg. With occluding cuff pressures of 40-50 mmHg, the arterio-venous pressure gradient will be reduced and, without any accompanying change in vascular resistance, blood flow will decrease. This could be an active decrease brought about by myogenic constriction or via the venoarteriolar local axon reflex, or a passively-determined decrease.

2.1.2 Myogenic constriction

The application of a venous occluding pressure cuff to the upper part of a limb leads to a rise in venous pressure that is transmitted back to the capillaries (Mahy *et al.* 1995). This could potentially lead to myogenic constriction of the pre-capillary arterioles which would reduce flow in the microcirculation. Studies have shown that application of suction to human limbs so as to increased transmural pressure resulted in a reduction in flow velocity in the conduit artery without change in vessel diameter (Lott *et al.* 2002). This was attributed to myogenic constriction of the downstream resistance vessels in the limb and responses were greater in the leg than in the arm (Lott *et al.* 2009a).

2.1.3 The veno-arteriolar response (VAR) and local vasoconstriction

As described in chapter 1, the VAR is a response to venous distension and leads to pre-capillary vasoconstriction, mediated by a local neural pathway (de Graaff *et al.* 2003). Okazaki *et al.* (2005) addressed the issue of whether blood flow in skin of the forearm and calf is reduced by VAR-mediated vasoconstriction during venous distension by measuring laser Doppler perfusion signals during cuff inflation with and without local anaesthetic. Blood flow decreased in both conditions, while skin vascular resistance, calculated by taking into account the reduced arterio-venous pressure, increased by 35-45% at both sites in the control condition but was unchanged after local anaesthetic. It was estimated that during cuff inflation, the contribution of the VAR to vasoconstriction was 23% for forearm and 17% for calf, leaving about 35% of the decrease in flow due to the local decrease in perfusion pressure. Whilst this suggests that some of the reduction in blood flow during venous

occlusion derives from the VAR in skin, the situation for the whole limb is different. Oldfield & Brown (2006) measured calf blood flow plethysmographically during 5 min of venous distension by cuff inflation and found no evidence of VAR-mediated vasoconstriction, reduced blood flow being accounted for by the decrease in perfusion pressure alone. Since plethysmographic blood flow represents mainly skeletal muscle, this would appear not to exhibit a VAR whereas the skin does.

2.1.4 Reduction of perfusion pressure gradient by cuff occlusion

The VAR operates to bring about constriction of pre-capillary vessels of the microcirculation but there is also evidence that venous congestion by cuff inflation can reduce large artery flow into the limb. Hiatt *et al.* (1989) examined calf blood flow by plethysmography and flow in the superficial femoral artery by ultrasound during thigh cuff inflation to 40 mmHg. Directly under the cuff, the diameter of the artery was reduced by 30-40% during inflation although it did not change above the cuff. Neither did the diameters of the common femoral (proximal to cuff) or popliteal artery (distal to cuff) change on inflation. However, flow velocity was significantly lower in all three arteries during cuff inflation. Tschakovsky *et al.* (1995) made a similar comparison between ultrasound measures of brachial artery flow and forearm blood flow by strain gauge plethysmography, showing reduced brachial flow velocity while the upper arm cuff was being inflated to 50 mmHg to measure forearm blood flow. They attributed the reduction, which increased in magnitude throughout the 4-5 cardiac beats of the duration of cuff inflation, to progressive reduction in arterio-venous pressure gradient as venous volume filled.

2.1.5 Gender differences in the response to cuff occlusion

Myogenic constrictions of resistance arteries and arterioles are attenuated in female compared to male animals through the action of oestrogen (Huang & Kaley, 2004). Lott *et al.* (2009b) also showed that women had attenuated myogenic constriction in their legs compared to men when transmural pressure was altered by limb suction. Blood flow reductions during venous distension may therefore also be smaller in women.

VAR responses to venous distension also differ between men and women. In their study on young healthy male subjects, Oldfield & Brown (2006) found reduced calf blood flow during venous distension that was due to the reduction in perfusion pressure rather than active VAR constriction. In a similar study, Bishop *et al.* (2004) examined young healthy females, both taking and not taking oral contraceptives (OC), in both phases of their menstrual cycle. The reduction in calf blood flow during cuff inflation was significantly greater in females (-70%) than in males (-16%) but there were no differences in flow reduction between non-OC and OC females or between cycle phases within those groups. This could imply that females, in

contrast to males, do demonstrate a VAR-mediated constriction during cuff occlusion in addition to a decrease in perfusion pressure. Alternatively, it could indicate that the venous filling volume is smaller is females and pressure equates with cuff pressure sooner. Another possibility is that because females have a greater proportion of skin and subcutaneous tissue relative to muscle mass within the calf than males (Fuller *et al.* 2008), and calf skin has a significant VAR constriction (Okazaki *et al.* 2005), the reduction in whole limb blood flow during venous distension is more evident in the female population. Lastly, lower leg composition in terms of muscle versus skin and subcutaneous tissue might impact on venous distension volume by providing differing amounts of support from surrounding tissue.

2.1.6 Summary and Chapter aims

While this evidence is supportive of a reduction in arterial inflow to a limb during cuff inflation to induce venous distension, volume flow in the popliteal artery, the main supply to the lower leg, was not estimated in the study by Hiatt *et al.* (1989). In later chapters of this thesis, venous distension by thigh cuff inflation will be used in the equilibration technique to assess venomotor responses to reflex sympathoexcitation. It is therefore necessary to establish whether there are changes in arterial inflow that could impact upon venous volume. Ultrasound will be used to measure popliteal artery flow during venous distension, and men and women will be tested because any reduction in arterial inflow could be due to myogenic constriction which is attenuated in females compared to males, to the venoarteriolar response which is more pronounced in females, or related to limb composition and venous capacity. As described in chapter 1, application of a venous occlusion cuff allows filling of the capacitance vessels but there are discrepancies in estimates of how much large veins contribute to the measured increased in limb volume (70%, Buckey *et al.* 1988, 30% Cirovic *et al.* 2006). To clarify the venous response, calf volume will be recorded plethysmographically and the long saphenous (LS) vein will be imaged using ultrasound. The specific hypotheses for this Chapter are:

1) **Any reduction in popliteal artery blood flow during venous distension (equilibration technique) will not directly impact upon plethysmographic measurements of whole limb volume changes.**
2) **The reduction in calf blood flow during venous distension will be greater in females than in males.**
3) **Whole limb filling during venous distension will reflect long saphenous vein filling.**

2.2 Methods

2.2.1 Subjects and groups

27 young healthy subjects (14 males and 13 females) volunteered to participate in testing

during which two venous distensions were applied to investigate 1) lower limb artery and 2) vein responses. All procedures were approved by the Local Research Ethics Subcommittee, School of Sport and Exercise Sciences, University of Birmingham, UK in accordance with the declaration of Helsinki 1964. Following informed consent, all subjects were screened by a general health questionnaire (see Appendix A) in order to ensure that they were in good health and injury free. Exclusion criteria were: current illness, consumption of 'over the counter' anti-inflammatory drugs in the previous 24 hour period, cardiovascular or venous disorders, pregnancy (either prior to or at time of study), injury to limbs (either during or at any time over 6 months prior to study) and immune, metabolic or kidney condition(s) (established by the questionnaire).

The female group consisted of 10 women who were taking a combined oral contraceptive pill (primarily microgynon30), which contained both a synthetic oestrogen (e.g. 30 µm ethinylestradiol) and a progestin (e.g. 150 µm levonorgestrel). The remaining 3 women displayed normal menstrual cycle patterns (~ 28 days) and were not taking (and had not previously taken) oral contraceptives, as established by self-report questionnaire (see Appendix B). Volunteers were excluded from the study if they displayed an irregular menstrual cycle pattern i.e. markedly longer or shorter (± 3 days) than 28 days or were taking a form of oral contraceptive other than a "combined" pill e.g. a progesterone only pill or the contraceptive injection. The women were tested as one group and were examined during both the menstrual phase (days 1 – 5) and the high hormone pill-taking/luteal phase (days 16 – 28) of their respective cycles (day 1 was taken to be the first day of bleeding) to investigate the influence of the female hormones oestrogen and progesterone. Men were tested on one occasion only.

All subjects were asked to refrain from eating or drinking anything other than water in the 4 hours prior to their testing session and to avoid caffeine or alcohol in the 12 hours prior to the session to ensure that there were no major blood volume shifts as a direct result of digestion and also to prevent external factors (e.g. caffeine) from influencing basal venous tone (Sato *et al.* 1988). A modified euhydration protocol (Veldhuijzen van Zanten *et al.* 2005) was used to prevent dehydration from reducing blood plasma volume (de Boer *et al.* 2006) and its associated effects (Guyton & Richardson, 1961), whereby subjects were asked to consume 250 ml of water 4 hours prior to the start of the testing session and a further 250 ml of water 30 minutes prior to the start of the session. Metabolic rate has been shown to differ throughout the day in both men and women (Aschoff & Heise, 1972, Kleitman & Ramsaroop, 1948, Wenger *et al.* 1976, Stephenson & Kolka, 1985) but it does not significantly differ between menstrual cycle phases (Stephenson & Kolka, 1985). It is unclear whether circulating catecholamine levels differ throughout the day (Sowers, 1981, Stephenson &

Kolka, 1985). Therefore, all subjects were tested in the afternoon to avoid any possible differences due to circadian effects.

Both the venous and arterial distensions were performed on the same day and were performed in a random order by the subjects within each group so that half of the subjects performed the distension with the arterial measurements first and half with the venous measures. The study was conducted in the Vascular laboratory at the School of Sport and Exercise Sciences, University of Birmingham, UK. The laboratory is situated along a quiet corridor and a sign was put outside the laboratory requesting silence from passers by so as to not distract the experimental subjects. The environmental temperature within the laboratory was maintained at 21 °C to keep room temperature fluctuations and its associated effects on calf volume measurements by strain gauge plethysmography to a minimum. Jorfeldt *et al.* (2003) showed calf volume expansion to increase approximately 3-fold over the temperature range from 10 to 50°C. Lighting inside the laboratory was dimmed to ensure subjects remained relaxed and to facilitate viewing of ultrasound images.

2.2.2 Examination procedures

Prior to testing, subjects' height was recorded in centimeters by a stadiometer and weight in kilograms by Seca scales in order to calculate body mass index (BMI). Harpenden calipers were used to take skinfold thickness at four sites - biceps, triceps, subscapular and suprailiac – in order to calculate % body fat (see below). Subjects then lay on a couch in the supine position with lower limbs raised slightly above heart level to aid venous emptying (figure 2.1). All measurements were made on the dominant calf, which in most cases was the right leg. Using a hand-held tape measure, limb circumferences at the knee, largest calf region and ankle were taken and limb length measured between those points.

The combined skin and subcutaneous tissue thickness at calf levels was measured using Doppler ultrasound at four sites around the largest circumference of the test limb. A suitable image of the soft tissue was found using B mode (ultrasound) at each of the four sites: medial head of gastrocnemius (MHG), the long saphenous region (LS), anterior tibialis (AT) and the lateral calf (LC). Images clearly showing the adjoining line of the superficial and deep fascias were frozen and the in-built caliper tool was used to measure tissue depth. A mean of three measurements was used for analysis and during measurement collection only measurements that were within ± 1 mm of each other were accepted.

A small inflatable cuff (Hokanson Ltd.) of 6cm width was placed around the ankle of the test limb to eliminate foot blood flow, which has previously been shown to be different to that of the calf (Kerslake, 1949). A large inflatable cuff (Hokanson Ltd.) of 18 cm width was placed

around the mid-thigh of the test limb. A mercury in silastic strain gauge (measuring 2 cm smaller than the largest circumference of the calf, Hokanson Ltd.) was placed around the belly of the calf. Subjects were instructed to breathe at normal rhythm throughout testing and to avoid sudden gasps or deep inspirations.

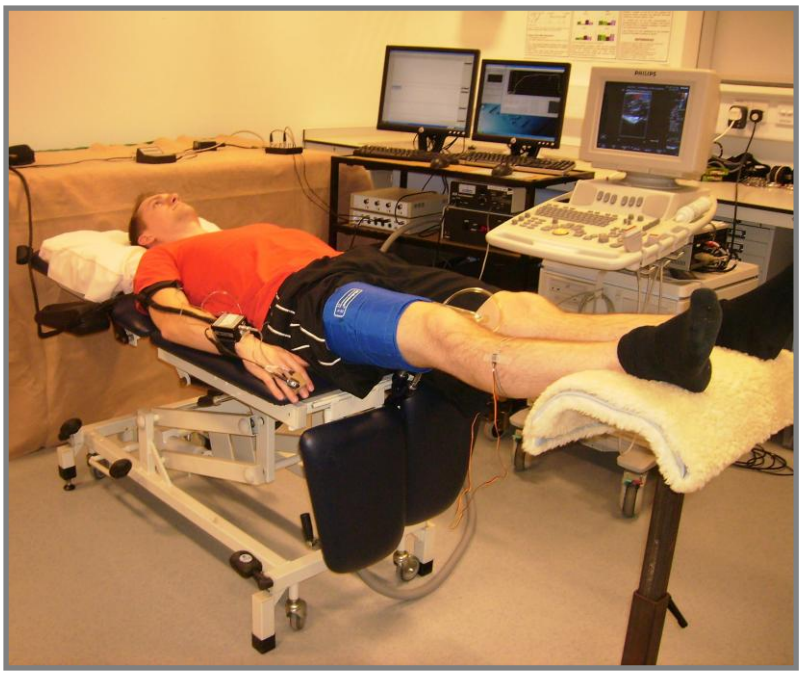


Figure 2.1*: Photograph of experimental setup showing subject lying on a couch in the supine position with lower limbs raised slightly above heart level to aid venous emptying. Figure also shows positioning of the strain gauge around the calf, thigh cuff and Portapress photoplethysmograph on the right middle finger.*

2.2.3 Measurement of variables

Beat-to-beat blood pressure, from which mean arterial pressure (MAP, calculated as diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure) and heart rate (HR) values were derived, was detected using a Portapress photoplethysmograph (Finapres Medical Systems, The Netherlands) incorporating height correction, placed around the right middle finger (figure 2.1). The analogue signal from the Portapress control console was input via a Powerlab A/D converter to a PC where it was sampled at a rate of 40 Hz using Chartlab software (ADInstruments Ltd.).

Calf blood flow, venous capacity and fluid filtration rate were obtained from calf volume changes detected using strain-gauge plethysmography. A mercury in silastic strain gauge, placed around the calf (figure 2.1), was connected to a plethysmograph (Hokanson Inc.) and the output signal was connected to the same PC running Chartlab software via a Powerlab A/D converter. Limb volume changes were continuously recorded simultaneously with

cardiovascular variable changes so that changes throughout the experiment were time-matched.

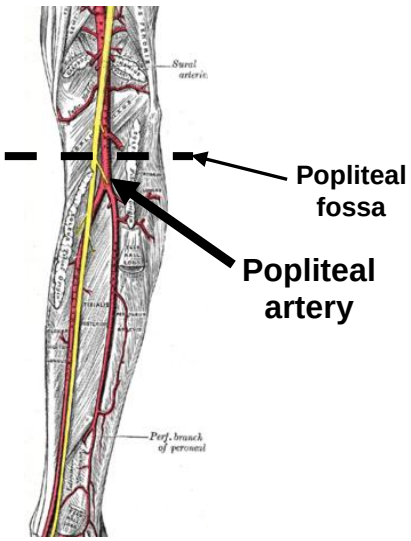


Figure 2.2*: Location of imaging area for popliteal artery. Figure also identifies the line of the popliteal fossa. Images were found 5 – 7 cm below the line of the popliteal fossa. Picture adapted from Gray's anatomy.*

Arterial and venous diameters and arterial blood flow velocity were all obtained using Doppler ultrasound imaging. To measure vessel diameters, the popliteal artery or long saphenous vein were imaged in longitudinal section in either colour Duplex mode (artery) or B mode (vein) with a Philips Envisor ultrasound machine. A linear 3 - 12 MHz probe was positioned by hand 5 - 7 cm below the line of the popliteal fossa over the respective artery or vein (figures 1.3 and 2.2) and the transducer head was manipulated carefully, ensuring to not press into the leg tissue, in order to obtain optimum images. The video output from the ultrasound machine was input to a second PC running on-line edge-detection software, VIA (Newey & Nassiri, 2002). This uses two artificial neural networks to detect anterior and posterior walls of the vessel and produces a digital display tracking vessel diameter at a sample rate of ~ 25 Hz (figure 2.3c).

Popliteal artery flow velocity was measured at rest and during venous distension so that volume flow and resistance could later be calculated. The artery was located (figure 2.2) and imaged in colour Duplex mode so that a live image of the vessel borders was always available for on-line edge-detection to provide a continuous recording of vessel diameter change throughout each procedure, while at the same time being able to perform velocity measurements on the same vessel segment. A large sample volume length was set to cover

the entire width of the chosen popliteal artery segment in order to account for any differing flow velocities within the measurement area (i.e. in the likely event of a parabolic flow pattern). The ultrasound machine's in-built angle correction cursor was used to set the angle of insonation between the Doppler ultrasound beam and the blood flow to 60° (optimum angle for flow measurements). Blood velocity readings (see below) were reported on the ultrasound machine's monitor next to the live blood vessel images and these were recorded onto VHS tape for later analysis.

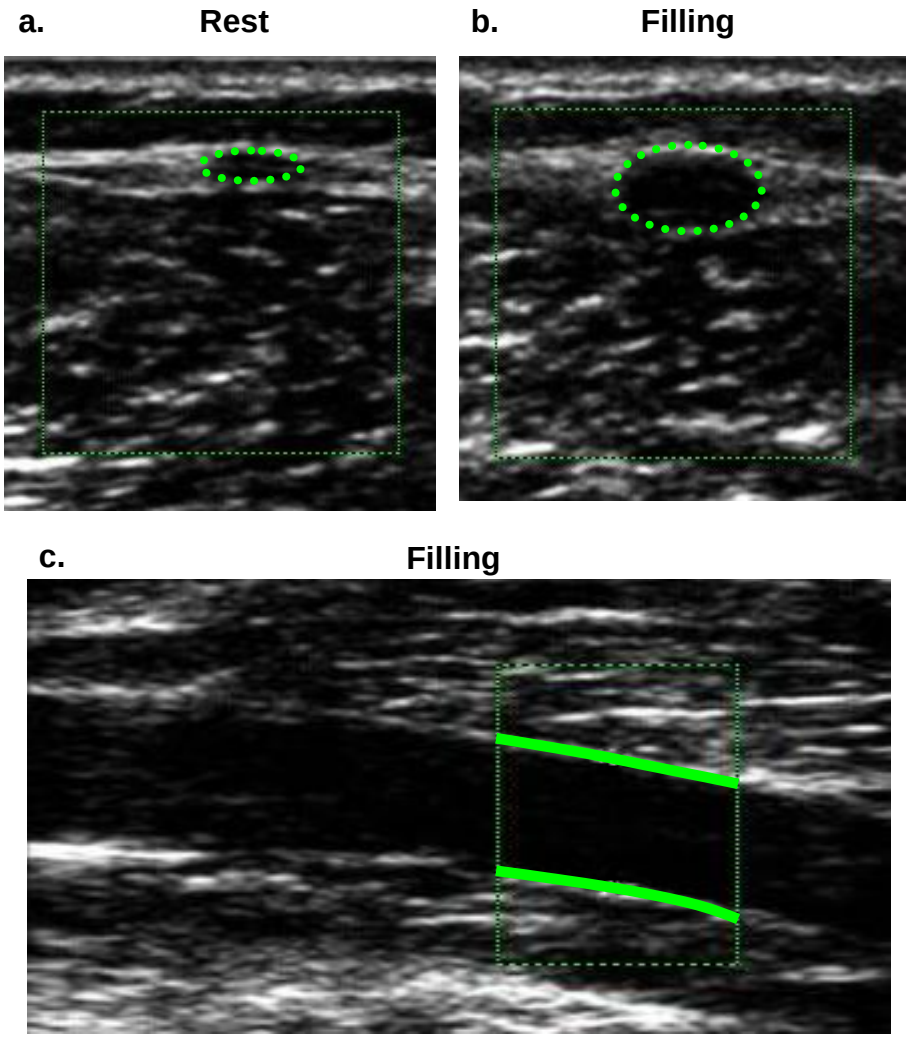


Figure 2.3*: Image of leg vein in cross section during rest (a) and filling (b) and the same vein in longitudinal section during filling (c) as imaged by Doppler ultrasound and displayed by VIA software. Vein circumferences are highlighted by green dots to illustrate difference in cross section shape during rest and filling respectively. Green box and lines in "c" illustrate the region of interest (ROI) enclosing the section of vein and the respective "near" and "far" borders of the vessel that are measured by the edge-detection software.*

2.2.4 Distension protocol

The same protocol was used for the distensions for arterial and venous measures. Subjects rested in the supine position for 20 minutes prior to the distension procedure. An ankle cuff was inflated to > 50 mmHg above systolic pressure to eliminate foot blood flow. A baseline measurement period of 2 minutes was undertaken during which MAP, HR, calf volume (strain gauge) and vessel diameter were recorded. Calf arterial inflow rate was then measured by inflating the thigh cuff to 50mmHg for 10 seconds while the increase in calf volume was measured by the strain gauge. Following a ~ 30 s recovery period for variables to return to baseline values, the procedure was repeated until a total of 3 calf blood flow measurements had been produced for analysis.

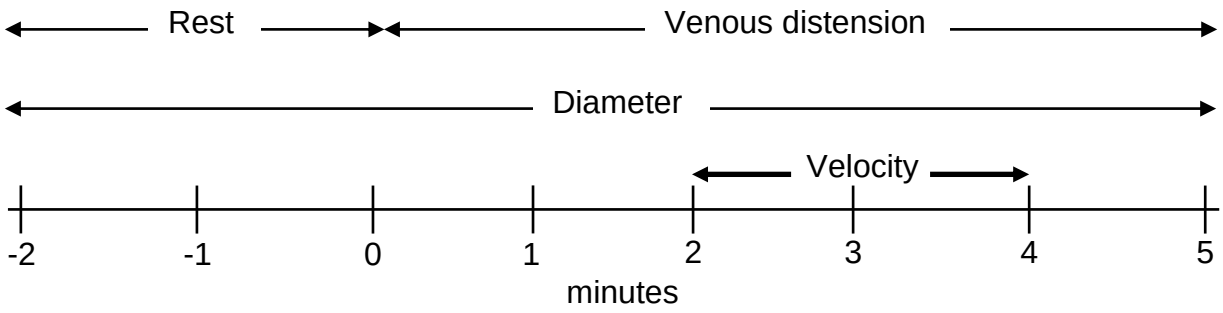


Figure 2.4*: Time line for arterial measurements. Figure shows time during which artery diameter (mm) and flow velocity (cm.s^{-1}) were measured during venous distension.*

Following a further 2 minute rest period, venous distension was then induced by thigh cuff inflation to 50 mmHg for 6 minutes while measurements of MAP, HR, calf volume and blood vessel diameter changes (either arterial or venous) were continuously recorded throughout. During the distension incorporating the arterial measurements, arterial flow velocity was measured between minutes 2 and 4 of the cuff inflation period (figure 2.4) so that arterial volume flow and resistance could be calculated later.

2.2.5 Data analyses

2.2.5.1 Anthropometric data

Body mass index was calculated as weight in kg divided by [height in cm]^2. Body fat % was calculated using the Durnin & Womersley (1974) regression equation for estimation of body density from skinfold measures and the Siri equation:

$$\text{Body fat \%} = [(495/\text{body density}) - 4.5] * 100 \qquad \text{(equation 1)}$$

Lower leg segment volume was calculated by using a truncated cone model (Karges *et al.* 2003), whereby measurements of circumference and length were used in the equation:

Segment volume (l) = 1/3 π x (radius of the base^2 (m^2) + radius of the base (m) x radius of the top (m) + radius of the top^2 (m^2)) x height (m) (equation 2)

To provide information on calf composition, the lean calf circumference at the level of the strain gauge (the largest calf circumference) was estimated by subtracting the mean of the 4 tissue thicknesses from calf radius. This was then used to give a lean calf diameter which could be put back into the formula:

Circumference = π x diameter (m). (equation 3)

The calculated lean calf circumference was then re-applied to the volume formula for the truncated cone model to give a "corrected" segment volume. This approach does not give a completely accurate representation of the lean calf volume since it made the assumptions that calf limb circumference is a circle and also that skin thickness remained the same at each level along the length of the limb segment. However, it did serve the purpose of providing an indication of the differences in lean calf volume between and within the groups.

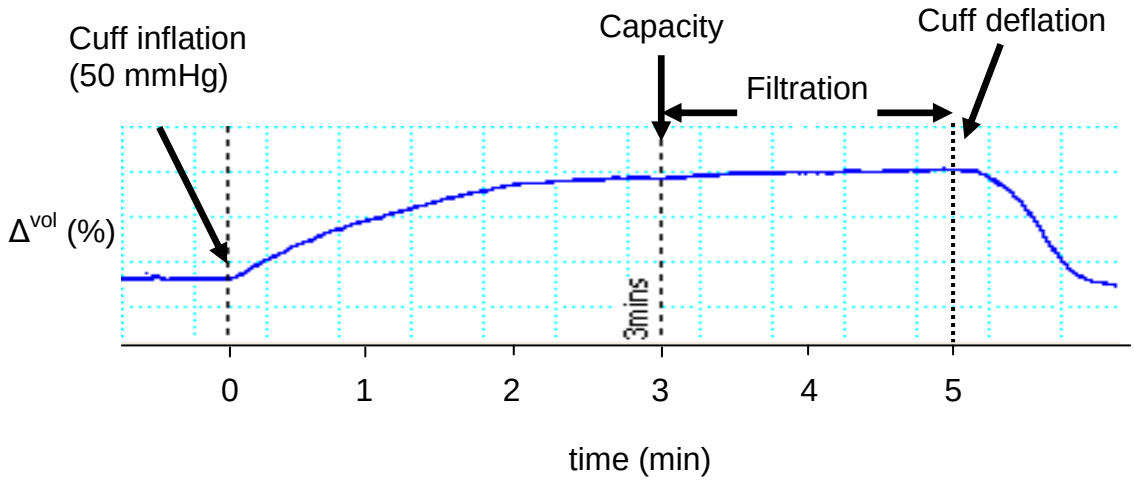


***Figure 2.5**: Example of an original calf volume trace during inflation of a thigh cuff to 50 mmHg for 5 minutes. Time points for determination of venous capacity (ml.100ml^{-1}) and fluid filtration (ml.100ml^{-1}.min^{-1}) are marked by large arrows.*

2.2.5.2 Distension procedure measures

All cardiovascular measurements recorded using Chart and VIA edge-detection software were initially analysed using the Microsoft Excel software package. MAP and the derived HR were averaged every 10 sec in Chart and baseline values taken as the mean of these over the 2 min rest period. The effects of venous distension on MAP and HR were shown by

plotting 10 sec averages throughout the 5 min period of cuff inflation. Calf blood flow was calculated from the slope of volume increase per unit time ($ml.100ml^{-1}.min^{-1}$) during the three 10 s cuff inflations and averaged to give a single resting value.

An Excel macro was used to reduce the 25 data points.s^{-1} of ultrasound vessel diameter values into 10 sec averages. Filling of the long saphenous vein was shown by plotting these averages against time. Baseline vein and artery diameters were taken as the mean values over the 2 min rest period. Changes in artery and vein diameter during distension were expressed as % of baseline diameter.

In order to calculate arterial volume flow, artery diameter was examined during the 2 min of distension that corresponded to the flow velocity measurement. Since artery diameter did not significantly vary throughout venous distension (see results), it is reported as the value at 3 minutes (figure 2.5). In offline analysis of the video images recording flow velocity, it was recorded in both peak velocity and time-averaged velocity mean (over 10 cardiac cycles) formats. Peak velocity is a measure of the flow at its maximum peak systolic velocity and therefore does not account for pulsatile variations in flow or slower moving blood nearer the vessel wall. On the other hand, time-averaged velocity accounts for all variations in flow at a given time point and can be used to report average blood velocity over a complete cardiac cycle; on that basis, time-averaged velocity was deemed more appropriate for use in this present study. To account for fluctuations in flow during systole and diastole, the time-averaged velocity was averaged over 10 cardiac cycles to create a mean time averaged velocity (TAVM). The TAVM was recorded onto VHS tape for 2 minutes at rest and between minutes 2 and 4 of venous distension. To account for heart rate variation between subjects, the middle 10 TAVM readings were taken from each recording and averaged to provide a mean TAVM reading for calculation of blood flow and resistance. Popliteal artery blood flow was calculated by multiplying the vessel cross sectional area (CSA = π x $radius^2$, cm^2) by the measured blood velocity ($cm.sec^{-1}$):

$$\text{Blood flow } (ml.min^{-1}) = \text{CSA } (cm^2) \times \text{velocity } (cm.s^{-1}) \times 60 \qquad \text{(equation 4)}$$

Popliteal artery resistance was calculated by dividing mean arterial pressure (MAP) by the calculated blood flow:

$$\text{Resistance } (mmHg.ml^{-1}.min^{-1}) = \text{MAP } (mmHg) / \text{blood flow } (ml.min^{-1}) \qquad \text{(equation 5)}$$

2.2.6 Statistical analyses

All statistical analyses were conducted using the Statistical Package for the Social Sciences,

version 13 (SPSS, Inc., Chicago, IL). A one-sample Kolmogorov-Smirnov test was firstly performed to check for normal distribution of the data to determine the most appropriate (parametric or non-parametric) statistical analysis. All data were revealed to be normally distributed.

A hierarchical approach was used for statistical analysis, first for comparison of gender differences, and secondly for menstrual cycle phase difference. For the first, the data for the male group were compared to those of the female group obtained from testing during their menstrual phase since the levels of oestrogen and progesterone are reported to be at their lowest at this point in the cycle (Heitz *et al.* 1999). Therefore any gender differences observed would more likely be as a result of physiological than hormonal differences. Gender differences were established using independent t-test. To investigate the influence of the female hormones oestrogen and progesterone, female data obtained from testing during their luteal phase were compared to the respective data for the menstrual phase using a paired samples t-test. Within-group responses between rest and venous distension were compared using repeated measures ANOVA with Bonferroni post-hoc tests. Data are presented as means ± S.E.M. unless otherwise stated. Differences were deemed significant if $p < 0.05$.

2.3 Results

2.3.1 Anthropometry

The subjects' physical characteristics are shown in table 2.1. Males were significantly taller, heavier and leaner than the females (table 2.1, all $p < 0.01$).

Table 2.1*: Basic physical characteristics of subjects. Values are mean ± SD. Table shows data for males and females.* * *= p < 0.01 males vs. females.*

	Males (n=14)		Females (n=13)	
	Mean	± SD	Mean	± SD
Age (years)	20	2	19	1
Height (cm)	179	6 *	165	5
Weight (kg)	75.1	7.3 *	61.3	4.4
Body Mass index (kg/m^2)	23	2	23	1
Body fat (%)	14.0	3.5 *	24.6	3.2

Lower limb segment volume as calculated by the truncated cone model was significantly greater in males than females (Table 2.2). The superficial tissue thickness determined by ultrasound was significantly less in males than in females at all 4 sites around the largest circumference of the calf (Table 2.2). The smallest difference between the genders for these measurements was at the medial head of gastrocnemius (MHG) site ($p = 0.03$). Corrected lean segment volume, calculated after removing tissue thickness, was significantly smaller compared to the calculated total calf volume for both genders. This difference was greatest in the females who had a smaller lean proportion of calf (66.8 ± 1.8 %) than the males (78.6 ± 4.4 %, $p < 0.01$ males vs. females, table 2.2).

Table 2.2*: Skin thickness and calf segment volume. Table shows mean ± SD for superficial fascia thicknesses at the 4 sites (Medial head of Gastrocnemius (MHG), the long saphenous region (LS), Anterior Tibialis (AT) and the lateral calf (LC)) measured around the largest circumference of the calf and calf segment volume calculated using the truncated cone method for the whole limb and corrected for lean tissue only. * = p < 0.05 males vs. females. † = p < 0.05 calf limb volume vs. corrected calf limb volume.*

	Males (n=14)		Females (n=13)	
	Mean	± SD	Mean	± SD
MHG (mm)	5.7	0.3 *	8.2	0.3
LS (mm)	5.7	0.2 *	9.3	0.4
AT (mm)	4.8	0.1 *	8.4	0.2
LC (mm)	6.0	0.1 *	9.4	0.3
Mean “skinfold” thicknesses (mm)	5.6	0.2 *	8.8	0.3
Calf segment volume (l)	2.7	0.5 *	2.3	0.4
Corrected calf segment volume (l)	2.1	0.4 *†	1.5	0.2 †
Lean % of total calf volume (%)	78.6	4.4 *	66.8	1.8

***Table 2.3**: Values for variables at rest and during distension. Values are mean ± SEM for all subjects. Data for females is shown during both menstrual (FM) and luteal (FL) Phases. * = p < 0.05 M vs. FM group. † = p < 0.05 distension vs. rest.*

	M (n=14)		FM (n=13)		FL (n=13)	
	Mean	± SEM	Mean	± SEM	Mean	± SEM
Resting MAP (mmHg)	89	4	88	4	96	5
Distension MAP (mmHg)	88	1	87	1	97	1
Resting HR (beats.min^{-1})	61	2	62	3	60	3
Distension HR (beats.min^{-1})	60	1	61	1	61	1
Resting artery diameter (mm)	5.46	0.17 *	4.76	0.24	4.82	0.17
Distension artery diameter (mm)	5.46	0.23 *	4.71	0.26	4.89	0.22
Resting artery blood flow (ml.min^{-1})	112.9	16.9	103.2	15.6	63.1	7.3
Distension artery blood flow (ml.min^{-1})	71.0	17.5 †	56.5	11.9 †	73.1	15.7
Distension filled vein diameter (mm)	2.65	0.26	2.15	0.15	2.59	0.26
Limb blood flow (ml.100ml^{-1}.min^{-1})	2.96	0.32	3.55	0.59	3.27	0.52
Calf fluid filtration (ml.100ml^{-1}.min^{-1}.mmHg^{-1} x 10^{-3})	1.66	0.25	2.04	0.32	2.19	0.45

2.3.2 Resting values for variables

During the initial rest period, no significant differences were found between males and females for MAP or HR. While popliteal artery diameter was larger in males than in females ($p < 0.03$, table 2.3), popliteal artery blood flow (table 2.3) and resistance (figure 2.11) were similar between the genders ($p > 0.05$). In the luteal phase, MAP was slightly raised and popliteal artery blood flow attenuated compared to the menstrual phase in the females, although these differences were not significant (table 2.3). Popliteal artery resistance was larger in the luteal phase compared to the menstrual phase (1.27 ± 0.29 mmHg.ml.min^{-1} FM vs. 2.06 ± 0.50 mmHg.ml.min^{-1} FL) but these differences did not reach significance ($p > 0.05$, figure 2.11). Overall there were no significant phase based differences in variables at rest. Mean limb blood flow calculated from the rapid cuff inflations was not significantly different between the genders or between the cycle phases for the females ($p > 0.05$, table 2.3).

2.3.3 Time course patterns of responses to venous distension

During the rest periods prior to the distensions, all variables remained at a steady level. MAP (figure 2.6), HR (figure 2.7) and popliteal artery diameter (figure 2.9) were unaffected by venous distension and did not significantly alter from their baseline values at any time point. Calf venous volume (figure 2.8) and long saphenous vein diameter (figure 2.10) both increased markedly during venous distension and both became full, i.e. reached their inflection point (capacity) at approximately 3 minutes into the distension procedure. The difference in diameter increase between the genders (M vs. FM) was related to the relative baseline values and when these increases were calculated as a percentage, these increases were not significantly different between the genders or between cycle phases ($p > 0.05$) although, the baseline vein diameter and diameters throughout filling were larger in the FL phase.

Popliteal artery blood flow was significantly attenuated during venous distension compared to rest in the males and female group in their menstrual phase ($p < 0.02$) but was not significantly altered during distension in the females in their luteal / high hormone phase ($p > 0.05$, table 2.3). Despite resting artery blood flow being lower at rest in their luteal phase compared to the menstrual phase the difference was not significant ($p > 0.05$). Vascular resistance in the popliteal artery during venous distension was twice that at rest in males and FM females ($p < 0.05$) but similar between the two conditions in the females during the luteal phase (FL, figure 2.11). Although during their menstrual phase the females displayed smaller filled vein diameter, this was not significantly different from that of the males or during their luteal phase ($p > 0.05$, table 2.3). Calf venous capacity was almost identical between males and females and between cycle phases ($p > 0.05$, figure 2.12). Calf fluid filtration was less but not significantly smaller in males than in females ($p > 0.05$, table 2.3). Overall there was

no significant gender or female cycle phase difference for any of the variables measured ($p > 0.05$).

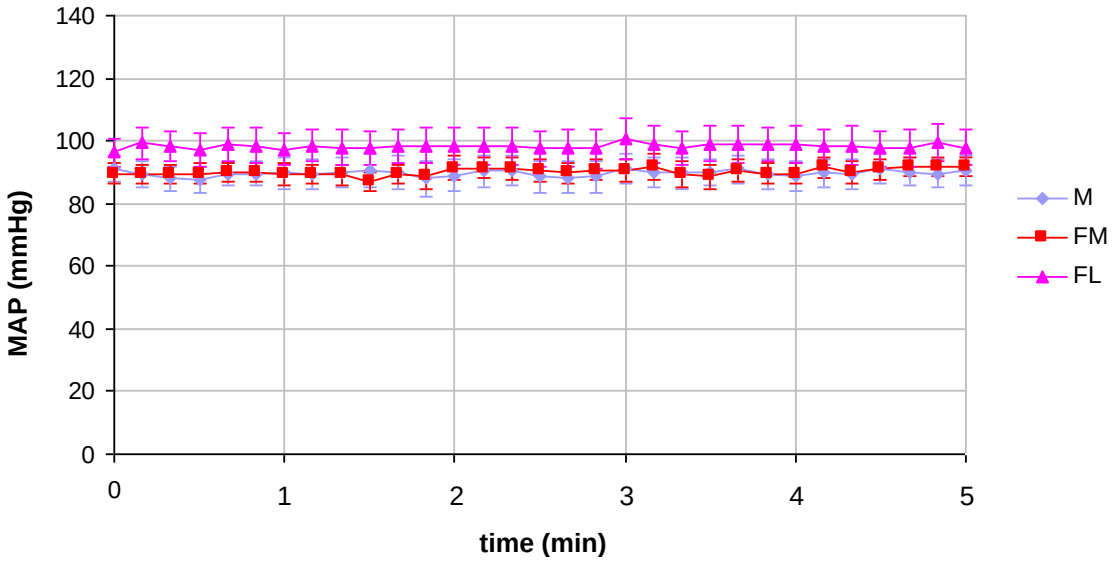


***Figure 2.6**: MAP (mean ± SEM) throughout 5 min of venous distension (thigh cuff 50 mmHg). Female values are shown for both cycle phases (FM and FL). Data are presented here as the mean of the two venous distensions combined since there were no significant differences at any time point between the two interventions.*

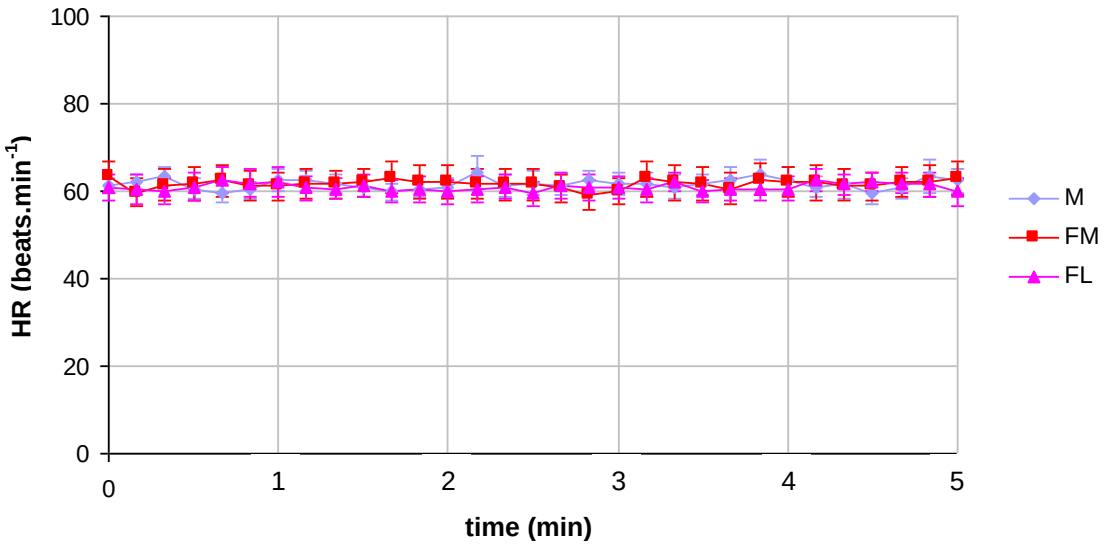


***Figure 2.7**: HR (mean ± SEM) throughout 5 min of venous distension (thigh cuff 50 mmHg). Female values are shown for both cycle phases (FM and FL). Data are presented here as the mean of the two venous distensions combined since there were no significant differences at any time point between the two interventions.*

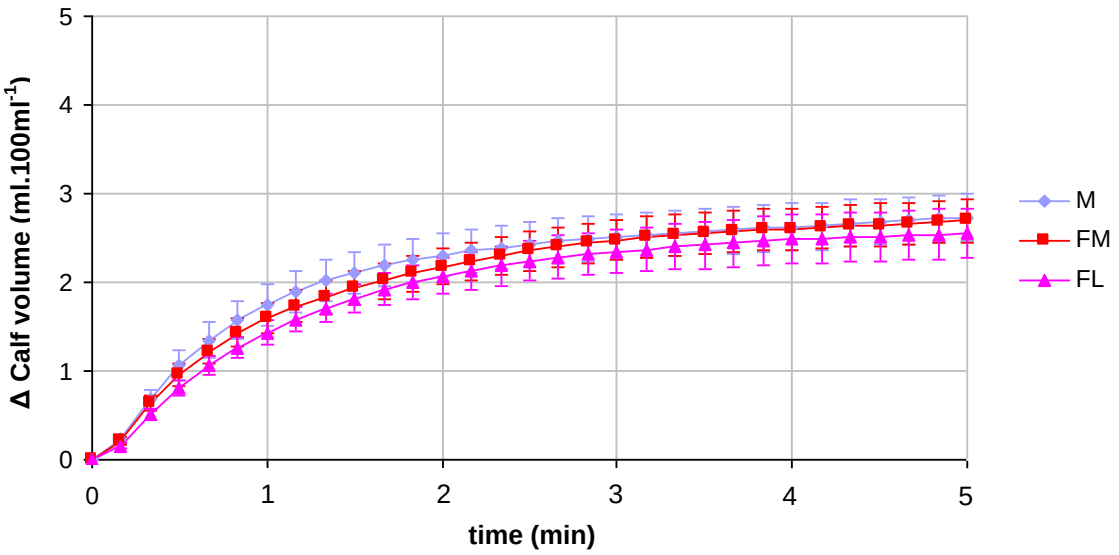


***Figure 2.8**: Calf limb responses to venous distension. Figure shows mean ± SEM volume changes for males and females during both cycle phases. Data are presented here as the mean of the two venous distensions combined.*

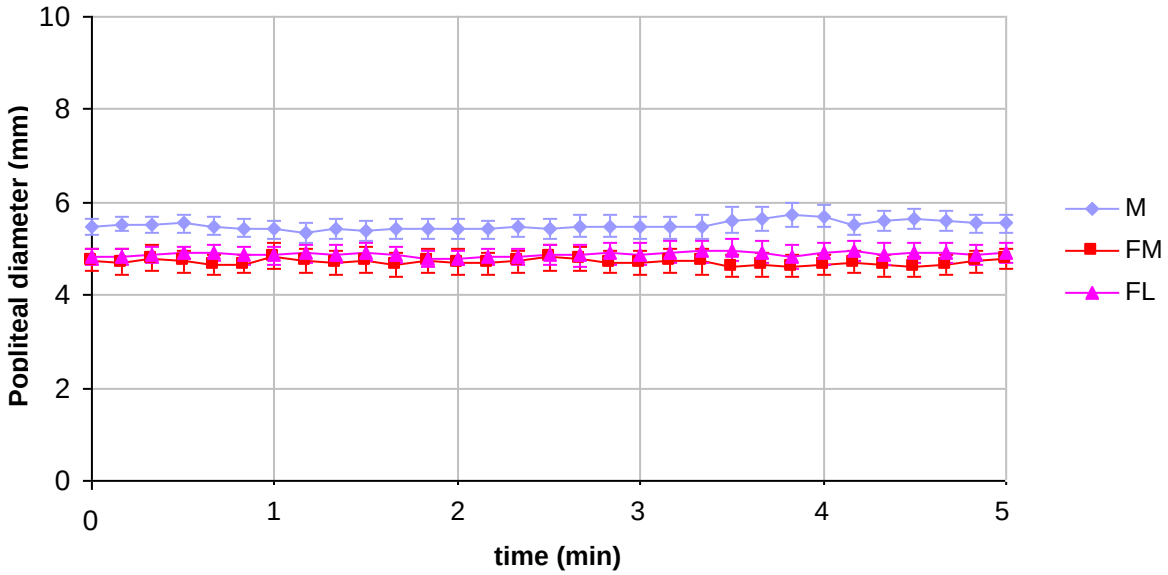


***Figure 2.9**: Popliteal artery diameter during venous distension. Figure shows mean ± SEM actual diameter changes for males and females during both cycle phases.*

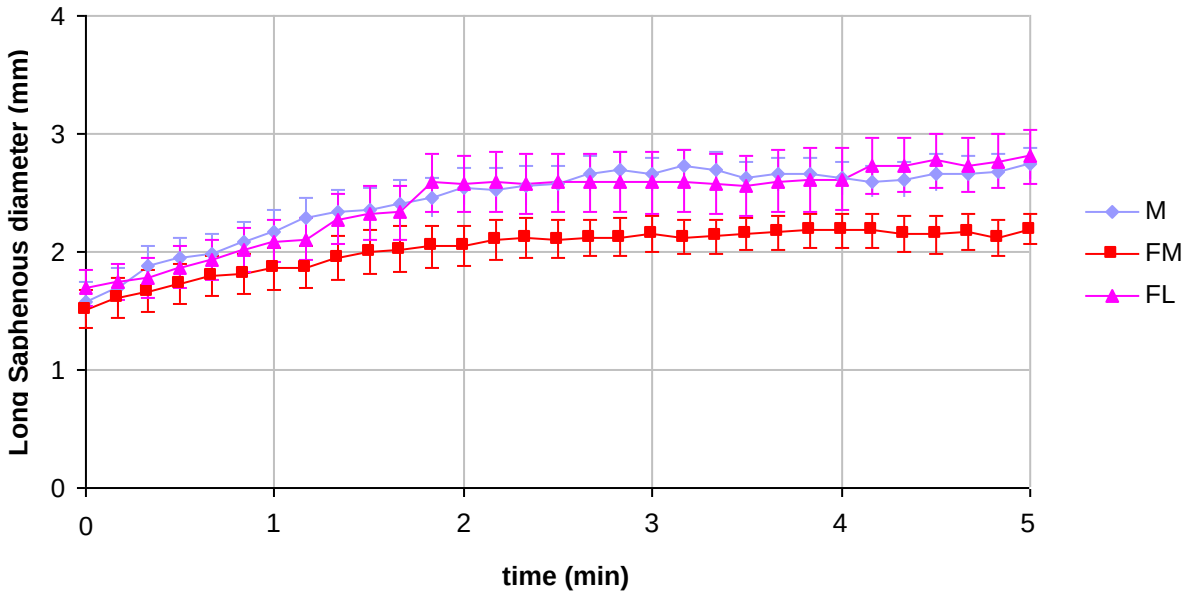


***Figure 2.10**: Long saphenous vein diameter during venous distension. Figure shows mean ± SEM actual changes in diameter for males and females during both cycle phases.*

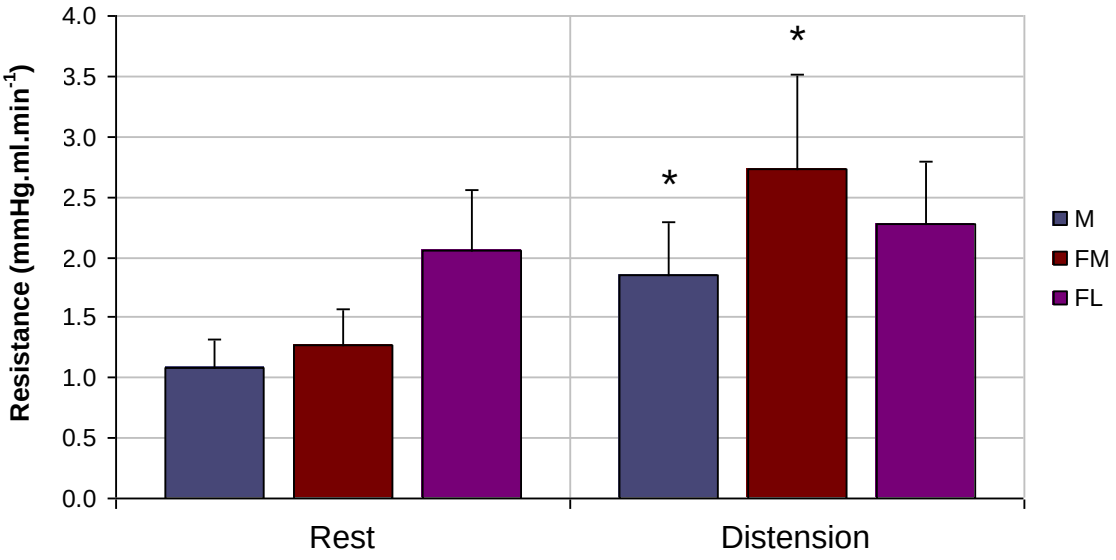


***Figure 2.11**: Popliteal artery resistance during rest and venous distension. Figure shows mean ± SEM values for resistance (mmHg.ml.min^{-1}) for males and females during both FM and FL phases. ⋆ = $p < 0.05$ for venous distension vs. rest.*

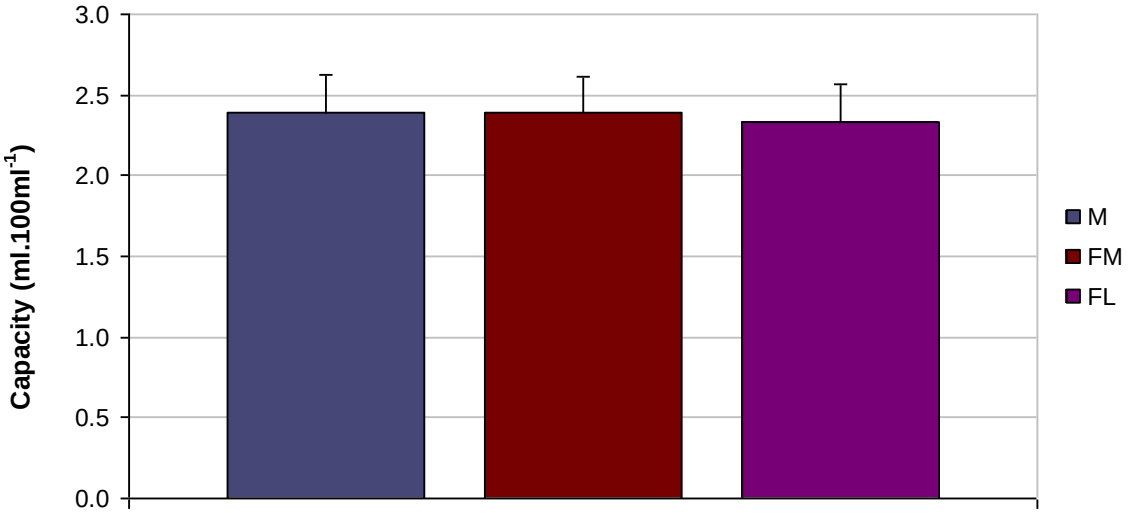


***Figure 2.12**: Calf venous capacity for males (M, n = 14) and females (F, n=13). Figure shows mean ± SEM for capacity (ml.100ml^{-1}) taken as the limb volume increase at 3 minutes during venous distension. Female group data is shown during both the menstrual (FM) and luteal (FL) phases.*

2.4 Discussion

The aim of this study was to investigate whether there are changes in arterial inflow during (50 mmHg) thigh cuff inflation that could impact upon venous volume during venous distension measurement(s). Ultrasound was used to measure popliteal artery flow during venous distension, and men and women were tested because any reduction in arterial inflow could be due to myogenic constriction (attenuated in females), to the venoarteriolar response (more pronounced in females), or related to limb composition and venous capacity. Furthermore, calf volume was recorded plethysmographically and the long saphenous (LS) vein was imaged using ultrasound in an attempt to also clarify the size of the venous response, which has been reported differently (70%, Buckey *et al.* 1988, 30% Cirovic *et al.* 2006).

2.4.1 Popliteal artery flow during venous distension

The main findings are that popliteal artery flow decreased significantly during venous distension without change in artery diameter. This was evident both in males and in women (tested during the menstrual phase of their cycle), with no difference between genders (flow reduction of -36 ± 8% males, -40 ± 10% females). Hiatt *et al.* (1989) also saw no change in popliteal artery diameter when a venous occlusion cuff was inflated around the thigh although they did not calculated volume flow. The values obtained for baseline popliteal artery flow were calculated from measured diameter and flow velocity and were similar (107.2 ± 11.3 ml.min^{-1} for all subjects) to those reported in other studies in supine males and females of mixed age range (110 ± 43 ml.min^{-1} - Delis *et al.* 2000; 72 ± 34 ml.min^{-1} - Holland

et al. 1998).

With mean arterial pressures of ~ 90 mmHg, it seems unlikely that the reduction in popliteal artery flow would be due to any impairment of flow from the more proximal upper leg by a thigh cuff inflated to 50 mmHg. However, inflation of a thigh cuff to venous occluding pressures did cause a reduction of femoral artery diameter directly under the cuff (Hiatt *et al.* 1989). This would reduce flow velocity in downstream arteries below the cuff, as was seen in the present study. More recently, Lott *et al.* (2002, 2009a & b) reported that alteration of transmural pressure by applying suction or positive pressure to a limb did not change diameter of the conduit artery but did reduce blood flow through myogenic mechanisms operating most likely in the downstream resistance vessels. The conditions of these experiments where the whole limb segment and all vessels are exposed to pressure changes are different from those where an occluding cuff applies pressure to a specific region of the upper limb. In the latter situation, however, it is possible that resistance vessel myogenic constriction could be triggered by the back pressure from venous occlusion. If pressure exceeds 40 mmHg, venous valves become incompetent (Brown *et al.* 1966) and pressure in the capillary bed rises in line with venous pressure (Mahy *et al.* 1995), with the potential for reaching arterial vessels (Bram *et al.* 1989). Although myogenic constriction is attenuated in women compared to men (Lott *et al.* 2009b), and in animal studies resistance arteries from females show smaller myogenic constrictions (Huang & Kaley, 2004), no gender differences were observed in flow reduction in the present study, which may indicate that other mechanisms were involved in the flow reduction.

Vasoconstriction downstream of the arterial measuring site could be caused by the VAR. Vascular resistance calculated from popliteal artery flow and mean arterial pressure increased 2-fold during venous distension ($p<0.05$). However, it is also possible that popliteal flow was reduced because the pressure difference between artery and vein was decreased by the occluding cuff around the thigh. Therefore, vascular resistance was also calculated using mean arterial pressure minus cuff pressure. When this was done and the change in resistance from rest to distension was compared, there was no significant difference for males (1.1 ± 0.2 vs. 0.9 ± 0.2 $mmHg.ml^{-1}.100ml^{-1}.min^{-1}$, $p=0.34$) or females (1.3 ± 0.3 vs. 1.0 ± 0.2 $mmHg.ml^{-1}.100ml^{-1}.min^{-1}$, $p=0.25$). Therefore the reduction in artery flow could be accounted for by the smaller arterio-venous pressure difference. This was also the conclusion arrived at by Levy *et al.* (1979) and Tschakovsky *et al.* (2004) who compared arterial flow velocity with plethysmographic blood flow in the forearm and observed reductions in velocity during venous occlusion.

It was notable that there was no difference between males and females in the reduction in

flow or increase in vascular resistance during venous distension. It had been postulated that women might show a greater constriction due to the VAR than men. This was based on previous findings that calf blood flow measured by plethysmography decreased more during a 5 min venous distension in women than men (Bishop *et al.* 2004). No such difference was observed in popliteal artery flow in the current study. It is still possible, however, that the downstream resistance vasculature in the calf might respond differently to venous distension in women and men, particularly if the VAR is more evident in skin and subcutaneous tissue which comprise a greater proportion of the female calf. The VAR is also dependent on a rise in venous pressure of greater than 25 mmHg during distension (Henriksen, 1991) and a smaller limb size in women may be linked with lower venous volume and faster rise in pressure during filling. The characteristics of calf and vein filling were therefore investigated in the genders.

Although popliteal artery flow and calf blood flow measured by plethysmography did not differ in women tested in their menstrual phase compared to men, in their luteal phase popliteal vascular resistance at rest tended to be higher and there was therefore no further increase during cuff inflation (Figure 2.11). Minson *et al.* (2000a) reported higher MSNA activity and noradrenaline levels during the luteal vs. menstrual phase which could lead to higher vascular resistance but this was in women with natural menstrual cycles and not taking oral contraceptives, which most of the current female cohort were. Minson *et al.* (2000b) also found no phase difference in MSNA or noradrenaline in women taking oral contraceptives. On the other hand, Arangino *et al.* (1998) found that 6 months administration of oral contraceptive did elevate noradrenaline levels although it was without effect on blood pressure. The reason for the higher vascular resistance therefore remains unexplained but the lack of change during venous distension is consistent with luteal attenuation of myogenic vasoconstriction in skin of the lower leg in OC users (Bishop & Brown, 2006).

2.4.2 Calf volume changes and filling during venous distension

As shown in Figure 2.8, calf volume increased rapidly throughout the first 3 min after thigh cuff inflation and thereafter the increase was slower and more linear. The first phase of rapid filling is dependent on the rate of arterial inflow and when blood flow was derived from brief cuff inflations prior to the prolonged venous distension, the values were similar for men and women (Table 2.3). This agrees with other data comparing resting limb blood flow between the genders. Although some studies have reported higher resting blood flow in women than men (Kneale *et al.* 2000), this is for the forearm whereas for the calf, resting blood flows are usually similar between genders (Hogarth *et al.* 2006, Schank *et al.* 2007).

The time course of filling of the calf and the increase in venous volume during distension in

men and women yielded similar values for capacity (around 2 - 3 ml/100mls) as other data published for young healthy individuals using strain gauge plethysmography (Stewart, 2003, Lindenberger & Lanne, 2007). Other studies have reported bigger calf venous capacity in men than women (Monahan & Ray, 2004; Lindenberger & Lanne, 2007). However, the subjects participating in the current study were all young fit sports science students most of whom were regularly undertaking either recreational or competitive level physical activities. Calf venous volume is higher in athletes than non-athletes (Boutcher & Boutcher, 2005) and in those with higher fitness levels, both men and women (Hernandez & Franke, 2004, 2005) and is decreased by inactivity (Bleeker *et al.* 2004). Although levels of activity were not recorded for this study, it is possible that differences between the genders are less evident in fitter individuals.

In women, there was also no difference in calf venous capacity between phases of the menstrual cycle. Significant hormonal effects on female calf have been demonstrated as increased capacity during the luteal phase of the menstrual cycle, OC use, pregnancy and oestrogen administration (Goodrich & Wood 1964, 1966, Barwin & Roddie, 1976). However, Meendering *et al.* (2005) showed no difference between calf volumes during venous occlusion between the menstrual and luteal cycle phases in women taking oral contraceptives, similar to most of the women in the present study population. They did find a small increase in calf capacity in normally menstruating women between menstrual and luteal phases, but these were the minority in the present study (3 out of 13) and may not have been sufficient to influence the data.

The second phase of the calf volume response to venous occlusion represents fluid filtration. Data on gender differences in filtration have mostly shown no difference (Gamble *et al.* 1998) and no effect of female hormones (Gooding *et al.* 2005, Stachenfeld & Taylor, 2007). In contrast, Lindenberger & Lanne (2007) found higher net filtration in women than men, but this was from calf volume changes obtained during application of lower body negative pressure which may result in different adjustments of blood volume between upper and lower body compartments (Taneja *et al.* 2007) than venous occlusion alone.

Another reason for discrepancies relating to gender and hormonal differences in calf venous volume may be the conditions under which it was measured. Many early studies applied cuff pressure to induce venous filling and distension and reported volumes after relatively short periods (~1-2 min), e.g. Barwin & Roddie (1976) used 2 min. Such brief occlusion may not allow time for full filling (Brown *et al.* 1966) and, because of the hysteresis present in venous filling and emptying curves (see chapter 1), may also underestimate true capacity. Indeed, Lindenberger & Lanne (2007) compared both rapid filling and slow filtration phases of the calf

response to venous distension and since capacity was less and filtration was greater in women than men, the total venous volume response after 8 min was similar between the genders. Likewise, Meendering *et al.* (2005) saw no difference in male and female calf volume responses to 8 min of venous occlusion. The present study also shows that with 5 min of occlusion, the volume responses are independent of gender and cycle phase.

2.4.3 Long saphenous vein filling during venous distension

The time course and pattern of filling of the LS vein was very similar to that of the whole calf (compare Figures 2.8 and 2.10). Baseline diameters of the vein were similar for men (1.73 ± 0.19 mm) and women (1.41 ± 0.12 mm, p=0.16) and were slightly smaller than those previously reported for the long saphenous vein (males 3.4 mm, females 3.2 mm, Jeanneret *et al.* 1999). The latter values, however, were obtained in subjects ranging in age from 20 to 66 years and vein size increases with age (Kroger *et al.* 2003). It also depends on where diameter is measured along the length of the vessel as diameter decreases from proximal to distal (Haenen *et al.* 1999). Filled vein diameter (in Jeanneret *et al.* study) when scaled according to body size, tended to be smaller in women than men, but the relative increases compared to baseline were not different between the genders (72% males, 73% females). These values are comparable to the 59% increase reported for the saphenous vein (baseline diameter 2.0 ± 0.7 mm) when distended by pressure in males (Eiken & Kolegard, 2004).

During distension there was no evidence of venous 'creep' in the saphenous vein, i.e. further increase in size once filled due to stress relaxation. In all groups, diameter at the end of distension (5 min) was within 1-2% of that recorded at filled capacity (3 min). This is in contrast to the whole limb which continued to increase in volume throughout. The slower phase of limb volume increase once filling is complete has been attributed to both fluid filtration and venous creep (Halliwill *et al.* 1999) but if the latter does contribute, it can only do so if it applies to small venous vessels.

2.4.4 Body size and calf composition

The male subjects in this study were taller, heavier and leaner than the females and have greater lower limb segment size. The proportions of skin and subcutaneous fat to lean tissue were also different between genders with women having a smaller % lean tissue. This is consistent with studies using other methods to assess composition e.g. MRI (Abe *et al.* 2003). The conventional means of evaluating skin and adipose tissue is by skinfold caliper but ultrasound has also been used. The average values for tissue thickness obtained in the medial head of gastrocnemius region in the present study (Table 2.2) are similar to those reported for the same site using ultrasound by others (males, 6.2 ± 2.1 mm, mean ± SD, Eston *et al.* 1994, males 5.6 mm, females 7.9 mm, Weiss & Clark, 1985). Body size

differences between men and women are also reflected in the diameters of the popliteal artery and the LS vein when filled. Despite these differences, the venous capacities were similar. Since strain gauge plethysmography registers relative venous vascular volume increase as a % change, blood volume must be distributed differently between lean versus adipose tissues in the legs of men and women.

2.4.5 Limitations

The use of ultrasound to continuously measure vein size is a new technique and there are important operating issues that the user should consider when performing continuous measurements: Firstly, in this present study the ultrasound probe was held by hand. In this instance great care must be taken in positioning the probe to optimise the image since exerting excessive pressure on the skin will compress the vein. Conversely, poor contact with the skin can result in a “bad” image, which lacks good resolution of the vessel walls, resulting in inaccurate measurement. Great concentration is also required with this technique so as to not move from the point of interest while performing the scan as this could result in inaccurate changes in diameter, which may not occur. However, the specific advantage of the hand-held technique over using a fixture for the probe (such as a clamp) is that the hand-held technique allows subtle movements to correct the image quality, which is particularly important when dealing with moving tissues during venous distension (i.e. limb swelling and movement of the vein). With regard to the edge-detection (VIA) software: This was designed to assess arterial diameters during a flow-mediated dilation protocol. Although ultrasound settings were optimized to provide best contrast for the software to perform edge-detection, vein vessel borders are not as bright as arterial borders, largely due to the difference in wall thickness of the respective vessels – arterial vessels have a much thicker tunica adventitia (outer layer of the vessel wall) in comparison to their venous counterparts. To counter this, a number of image processing settings were adjusted to optimize the image: The dynamic range on the ultrasound machine was reduced to create a more contrasted (black and white) image to assist the VIA software. Additionally, the black and white gain control was increased when necessary, the harmonic image processing on the ultrasound machine was used to provide better resolution when imaging the long saphenous vein as it is near the surface (within 2-3 cm) of the skin. When imaging the popliteal artery (5-6 cm below skin), although its borders were easier to detect than its venous counterpart, the frequency that the probe was operating at was reduced to provide greater penetration through the tissues. Although, as much as possible was done to ensure image quality, brief intermittent failure of the software to capture vessel borders did occasionally occur and could have resulted in spurious values for diameter which were being sampled at 25Hz. To correct for this, visual inspection of the raw VIA diameter data was performed to remove obvious outliers before the values were grouped into 10 s averages.

The extent of venous vascular filling is influenced by a) rate of arterial inflow, b) limb position relative to heart level and c) ambient / body temperature. Limb position and temperature were controlled as best as possible by ensuring the couch and the leg support (figure 2.1) remained at the same height throughout the course of the study and that the environmental temperature setting within the laboratory was set to 21°C but slight differences from test session to test session may account for some variability in measures such as capacity and filtration. Cuff inflation was to 50 mmHg. Blood pressures are expressed as mean values but it is possible that in some cases 50 mmHg exceeded diastolic pressure and if so, the cuff would have mechanically impeded femoral, and hence, popliteal artery flow. However, analysis of the original Chart Lab raw data revealed that in only 3 cases of the total of 27 subjects did diastolic blood pressure fall below 50 mmHg during venous distension and at no time point did any of these 3 cases fall below 45 mmHg. Analyses for arterial blood flow measurements were checked with these 3 subjects removed from the data, but the recalculated results were not significantly altered compared to the results presented in this Chapter showing findings for all subjects.

The methodology used in this study cannot distinguish between mechanisms leading to the reduction in popliteal artery flow. A measurement of calf blood flow by plethysmography during the distension would have helped to confirm vasoconstriction, either myogenic or VAR-mediated, in downstream microcirculation. It was not possible to make this measure due to lack of personnel to operate equipment.

Although women were tested during the menstrual phase of their cycle in order to ensure lowest levels of both endogenous and exogenous oestrogen and progesterone, the cycle phase was established by self-report only. A more accurate assessment could have been made if either ovulation kits were used to determine mid-cycle days, or blood hormone levels had been measured. These were not carried out due to cost constraints. Further to this, the ratio of women taking oral contraceptives to those with normal cycles was 10:3. The purpose of this study was to compare the men to the women at the lowest hormone levels of their cycle (menstrual) in order to identify differences at the physiological level without the influence of the female hormones, and then to observe the same women during their highest hormone phase (luteal) to identify the specific influence of these hormones. It was on this basis that the women were grouped together regardless of their hormone type (endogenous or exogenous) since levels of oestrogen and progesterone are similarly low during the menstrual phase in both the normal and oral contraceptive menstrual cycles and, in both cases, significantly higher during their respective luteal phases.

2.4.6 Summary and conclusions

The main findings of this present study are that popliteal artery flow decreased significantly during venous distension without change in artery diameter. It is unlikely that these reductions are due to any impairment of flow by the more proximal thigh cuff since it was only inflated to 50 mmHg, which was below mean arterial pressure (~ 90 mmHg) and in all but 3 cases was also below diastolic pressure. Any myogenic effect is also unlikely as no gender affect was noted. However, this does not mean that it did not occur. The most likely explanation for the reductions in flow is that these could be due to the smaller arterio-venous pressure difference following cuff inflation, which resulted in back-flow. However, regardless of the exact mechanism the reduction in flow does not result in a reduction in artery diameter, which is therefore not reflected in whole limb volume measurements. This is an important finding with regard to the later experiments within this thesis and the methods used to detect venoconstriction following sympathoexcitation.

In terms of venous filling, the long saphenous vein displayed a similar pattern of filling to the whole calf limb, with both reaching “capacity”, i.e. the point of inflection at a similar time point (~ 3 minutes). This finding may suggest that examination of an individual vein during venous distension and intervention could provide insight into venous reactivity as a whole in the calf limb without the need for using plethysmography, however, further work may need to be done to validate this method. Having said that, the use of ultrasound imaging in conjunction with edge-detection software would appear, from the evidence presented here, to be a reliable method for observing vein reactivity to volume changes since events which occurred at specific time points within the long saphenous vein (e.g. initial filling period and capacity) matched the time points for those same events within the whole limb plethysmographic measurements.

Males and females displayed similar responses to venous distension in popliteal artery blood flow and calf and long saphenous vein filling. Despite females having smaller quantity of lean tissue and smaller artery and vein size, venous capacities were also similar between the genders.

Finally, the response to 50 mmHg thigh cuff inflation (venous distension) does not appear to be influenced by gender or female hormone status (as determined by cycle phase). Therefore, any observed differences within parameters during an intervention (in later chapters) are likely to be due to hormonal differences in response to the stimuli rather than to the specific effects of venous distension.

Chapter 3:
ULTRASOUND ASSESSMENT OF VENOMOTOR RESPONSES TO SYMPATHOEXCITATION BY ISOMETRIC LEG EXERCISE AND MENTAL STRESS TASK IN MEN AND WOMEN

3.1 Introduction

An increase in venous tone through active constriction would help to counter pooling of blood within capacitance vessels. As has been pointed out in Chapter 1, this assumes importance in humans especially for the peripheral leg veins during upright posture. Different protocols for evaluation of venoconstriction that involve venous distension and measurement of limb venous volume or a specific superficial vein (occluded vein / limb technique, equilibration method) were also described in Chapter 1. In Chapter 2, it has been established that although the calf consists of less lean mass in women than men, venous distension by thigh cuff occlusion results in similar calf venous vascular volume in young men and women, and that the genders show similar decreases in popliteal artery flow. The popliteal artery and long saphenous vein dimensions are scaled according to body size and limb composition, being larger in men than women. The similarity of profile of both calf volume and single vein distension in men and women will allow the equilibration technique to be used alongside ultrasound to evaluate the venoconstrictor effects of two different sympathoexcitatory interventions, exercise and mental stress. The effects of exercise and mental stress on venomotor tone have been assessed in the literature using the occluded vein / limb and equilibration protocols and this evidence will now be reviewed.

3.1.1 Evidence for venoconstriction during exercise

As discussed in the General Introduction, animal studies observing venomotor tone during sympathoexcitation in exercise have reported no effect (Donegan, 1921, Marshall, 1982) or constriction in large (Webb-Peploe & Shepherd, 1968, Gero & Gerova, 1975) and small (Bjornberg & Maspers, 1991) venous vessels. For example, graded stimulation of the lumbar sympathetic chain at frequencies of 1-16 Hz results in a 4-fold increase in venous vascular resistance with maximum effect observed after 30 sec compared to 60 sec required for arterial resistance to reach steady state (Bjornberg & Maspers, 1991).

In humans, there is also evidence for exercise-induced venoconstriction. Sharpey-Schafer (1963, 1965) reported that 'the veins of the exercising forearm were conspicuously constricted after exercise' but did not see such a post-exercise effect in the contralateral limb. Page *et al.* (1955) recorded increases in pressure of 13 mmHg in an isolated segment of forearm vein without change in central venous pressure during knee extension exercise, although the intensity and duration of exercise were not specified. Bevegard & Shepherd (1965) also found pressure increases of 12-14 mmHg in the occluded forearm which peaked after 30-40 sec during several minutes of supine leg cycle exercise. In addition, they used plethysmography and reported a 1-2 $ml.100ml^{-1}$ decrease in filled forearm volume (equilibration method) during the exercise, with similar changes in calf volume during arm exercise. Volume decreases were graded with exercise intensity, assessed as heart rate

rise, and were of similar magnitude even when the limb blood flow had been elevated by prior handgrip exercise. Forearm venomotor responses to leg exercise were abolished by heating to 44^{o}C and were absent in a patient with sympathectomy, indicating neural involvement. They concluded that sympathetically-mediated venoconstriction during exercise acts to counter pooling in post-capillary capacitance vessels when resistance vessels dilate.

Bevegard & Shepherd (1966) extended these studies by measuring oxygen uptake during the exercise and comparing the capacitance with resistance vessel responses to exercise by measuring forearm blood flow with plethysmography and radial artery pressure in the contralateral arm. There was transient forearm vasodilation when leg exercise commenced, followed by vasoconstriction. This was confirmed by sampling blood from a superficial and a deep forearm vein simultaneously and showing in the former no change in oxygen saturation but in the latter, a transient increase followed by a decrease, suggesting that the vasodilation occurred in muscle tissue rather than skin. Despite these changes in arterial resistance vessels, venoconstriction, detected as a decrease in plethysmograph volume, was, as before, graded with exercise intensity, maintained for 3-4 min, abolished by heating and absent after sympathectomy. It also occurred when exercise was superimposed upon a breath-hold manoeuvre, showing that exercise-induced chest movements were not involved, and when occlusion cuffs were applied to prevent metabolite transfer from the working legs.

Samueloff *et al.* (1966b) examined forearm venomotor responses by the occluded limb technique during supine cycle ergometer exercise. They reported a sustained increase in measured vein pressure throughout exercise but the duration and intensity of the latter were not described, and the magnitude of the venous response was only shown illustratively, not quantitatively. Using the same technique, these authors did compare venomotor responses from the forearm, calf, hand and foot to a single stimulus, a deep breath, with the biggest responses in the hand and foot. It was not reported whether venous reactivity to an exercise stimulus differed likewise between regions.

The elimination of exercise-induced changes in limb venomotor tone by heating would suggest that it is the more superficial veins that contribute to decreases in venous vascular volume. Zelis & Mason (1969) used the equilibration method to show decreases of ~14 % in forearm venous volume during supine leg cycle exercise for 1-3 min in male subjects aged between 21 and 39 years. They then attempted to partition out the response between superficial and deep vessels by iontophoretic application of adrenaline to the whole forearm region. When skin circulation was constricted in this way, the forearm venous volume was unresponsive to leg exercise, confirming the importance of superficial capacitance vessels to the response. It was noted in some of the above studies that continued dynamic leg exercise

for several minutes that the initial venoconstriction waned over time. Wenger & Roberts (1980) suggested that this was due to thermal effects of rising core temperature. They reduced core temperature by consumption of ice and controlled forearm skin temperature by airflow and showed that during prolonged exercise (30-40 min) at 45% VO_2max there was a progressive increases in filled forearm venous volume as body temperature rose but that this was still less than at the same temperatures without exercise, indicating venoconstriction. From the same group, Fortney *et al*. (1983) also studied venomotor tone in 5 healthy males performing seated cycling at 65% VO_2max for 30 minutes. Forearm plethysmographic volume was repeatedly measured by inflating a cuff to 30 mmHg and showed a continual decrease throughout the exercise despite rising core body temperature. They attributed this persistent venoconstriction to the higher exercise intensity, and considered that after long duration of exercise, part of the increase in venous tone occurred as a result of reduction in plasma volume because they showed that tone was enhanced by hypovolaemia.

Since cycling is a dynamic exercise modality, Bevegard & Shepherd (1965) also examined vein pressure changes in an occluded forearm and noted increases when isometric contractions were performed by contralateral handgrip or by the legs. In one subject, percutaneous stimulation of the popliteal nerve to cause involuntary calf contractions also resulted in forearm venoconstriction although it was not established whether this was due to the contractions or activation of any afferent fibres. Seaman *et al.* (1973) examined the effects of graded isometric forearm handgrip (20, 30 and 40% MVC for 6-8, 3-5, 2-3 min respectively) on venomotor responses in the calf (equilibration method) and contralateral forearm (occluded limb technique) of male subjects. Calf volume decreased by 8, 10 and 12% at the different handgrip force intensities, and forearm vein pressure increased by 12 mmHg at 30% MVC. The authors concluded that such venoconstriction may be particularly important during static exercise when the muscle pump is non-functional as an aid to venous return. Stewart *et al.* (2007) used impedance plethysmography to examine blood volume changes during 2 min of handgrip at 35% MVC. Thoracic blood volume increased ~ 5% while splanchnic volume decreased by 2.5% although splanchnic flow remained unchanged, and calf volume decreased by just over 1% while calf vascular resistance increased by > 10%. The increase in cardiac output during isometric exercise was aided by both splanchnic venoconstriction, which increased central blood volume by 4.5%, and arterial vasoconstriction. Taken together, these data demonstrate increases in venomotor tone during both dynamic and isometric exercise, independent of changes in arterial tone.

In summary, studies using techniques that examine superficial veins either individually (isolated vein segment) or within the limb (occluded limb), and those that assessed whole limb volume plethysmographically (equilibration method) have all shown some degree of

venoconstriction in response to exercise. Most of the studies have tested forearm responses, and those that have examined the calf have seldom provided adequate quantitative data, let alone investigated differences between superficial and deep veins in the lower leg. Furthermore, the time course of venoconstrictor responses during exercise is unclear from these papers because very often individual original traces are presented and there are no group data. Most notably, all the studies have been carried out on male subjects so that there is no information about gender differences.

3.1.2 Evidence for venoconstriction during mental stress

There have been far fewer studies investigating the effects of mental stress on venomotor tone. In animal studies, Martin *et al.* (1996) inferred an increase in integrated venomotor tone from the rise in mean circulatory filling pressure in rats subjected to air-jet stress. They also showed involvement of the paraventricular nucleus of the hypothalamus in regulation of venous tone, in addition to circulating catecholamines (Martin *et al.* 2006). Early studies in humans tended to mention venomotor responses to mental stress as an aside rather than as an intentional intervention. For example, Samueloff *et al.* (1966b) commented that forearm venous pressure showed a constrictor increase of 6 mmHg to 'an emotional stimulus', the suggestion that the subject was about to faint. At the same time, blood flow in the contralateral forearm increased, again showing the opposite reflex changes that can occur in resistance and capacitance vessels. Zelis & Mason (1969) asked subjects to calculate a mathematical problem but the effect that this had on cardiovascular variables such as blood pressure or heart rate was not reported, and the magnitude of the decrease in forearm venous volume (equilibration method) was not given.

Robinson *et al.* (1989) applied the then newly-developed ^{99m}Tc blood pool scintography method for assessment of forearm venous vascular volume and constructed pressure-volume curves during cuff occlusion over the range of 10-30 mmHg (Manyari *et al.* 1988). The subjects, middle aged patients being investigated for cardiac problems, carried out a mental arithmetic test involving serial subtractions and the same pressure-volume curves were obtained. A pressure-volume plot based on vascular volume indicates venous capacity, and this was reduced by 13.5% during mental stress. Systolic and diastolic blood pressures rose 26 and 10 mmHg respectively and heart rate rose 10 beats.min^{-1} during the stress test. Although this was one of the first studies to assess venomotor tone responses to mental stress in a quantitative fashion, the subject cohort were not representative of a normal healthy population.

Dzeka & Arnold (2003) compared venoconstrictor responses in chronic heart failure (CHF) patients and age-matched healthy subjects by studying the dorsal hand vein dimensions with

the LDVT method and measuring distension during cuff occlusion of 45 mmHg. They also applied a serial subtraction arithmetic test that raised mean arterial pressure by ~ 12 mmHg and heart rate by ~12 beats.min^{-1} in the age-matched controls. Hand vein distension was reduced by 4.9% during the stress test in controls. Baseline venous distension in CHF patients tended to be smaller than in controls but the reduction during mental stress (6.6%) was similar. Having previously shown that hand vein constriction to infused alpha adrenergic agents could be enhanced by blocking prostaglandin synthesis with indomethacin in young healthy individuals (Callow *et al.* 1998), Dzeka & Arnold (2003) confirmed that indomethacin also increased distension to 19.2% and 19% in age-matched controls and CHF patients respectively. They concluded that dilator prostaglandins contribute to the response to mental stress and by their removal, adrenergic venoconstrictor effects were exaggerated.

Despite the relative lack of data on venomotor responses to mental stress, it is evident that constriction occurs, at least in forearm and hand veins. To the best of the author's knowledge, no study has investigated venomotor responses to mental stress in the leg. It is also apparent that there has been no systematic examination of whether there are gender differences in stress-induced venoconstriction. While evidence presented above indicates venoconstriction in upper limbs to both exercise and mental stress, and in lower limbs to exercise, there is no quantitative comparison of leg venomotor responses to these two interventions.

3.1.3 Cardiovascular responses to sympathoexcitation by exercise and mental stress

There are distinct differences in the characteristics of the sympathetic neural and catecholamine response to exercise and mental stress that lead to divergent cardiovascular and resistance vessel effects. In order to appreciate possible passive effects on venous volume and potential variations these will be considered.

During laboratory based research on humans, isometric exercise is generally performed in the upper limb by handgrip exercise or in the lower limb by either knee extension exercise or calf exercise (plantar flexion of the foot). Exercise is usually carried out at a calculated percentage of a predetermined maximal voluntary contraction (MVC) for a set period of time (e.g. 30% MVC for 2 min duration). Due to the mechanics of performing an isometric contraction, in particular the limb movement associated with the action, cardiovascular measurements are commonly taken from the contralateral limb. Mental stress can be administered to the participant in many forms. The two most common mental stress stimuli used in modern day studies are the Stroop colour conflict test or mental arithmetic task such as the PASAT (Tombaugh, 2006). Callister *et al.* (1992) compared the two types of task at varying levels of difficulty and reported that at similar levels of stress perception there were

no differences in neural output between the two tasks in their subjects. Most human observations of sympathoexcitation evoked from mental stress have been made on the forearm vasculature.

3.1.4 Blood pressure and heart rate responses

3.1.4.1 Isometric exercise

Significant increases in blood pressure and heart rate – the pressor response - have been reported during experiments involving isometric hand grip exercise (Eklund & Kaijser, 1976, 1978, Dietz *et al.* 1997, Reed *et al.* 2000, Leuenberger *et al.* 2003, Davies *et al.* 2007), lower limb isometric exercise (Mitchell & Wildenthal, 1974, Ray & Mark, 1993, Fisher & White, 2003, Bell & White, 2005, Fisher *et al.* 2005) or both (Eklund & Kaijser, 1974, Ray & Wilson, 2004). Both variables increase within seconds of exercise onset, and increases are graded with exercise intensity and enhanced by ischemic conditions. They represent the combined effects of 'central command' drive to and muscle afferent stimulation of the efferent autonomic pathways that increase cardiac output (via heart rate and stroke volume) and elevate peripheral vascular resistance (Fisher & White, 2004, Williamson *et al.* 2005, Smith *et al.* 2006, Green & Patterson, 2008). Evidence from the studies above would suggest that increases in MAP and heart rate in the range of 17 – 28 mmHg and 8 – 25 beats.min^{-1} would be observed in subjects performing isometric exercise at 30 – 40 % MVC intensity.

3.1.4.2 Mental stress

Like other sympathoexcitatory challenges, application of a mental stress intervention has also been shown to result in marked increases in blood pressure and heart rate of ~ 8 – 16 mmHg and ~ 12 – 25 beats.min^{-1} respectively (LeBlanc *et al.* 1979, Robinson *et al.* 1989, Herd, 1991, Manyari *et al.* 1996, Halliwill *et al.* 1997, Carter *et al.* 2002, Carter *et al.* 2004, Carter *et al.* 2005, de Boer *et al.* 2006). The increase in MAP during a stress test of 2 min duration follows a similar pattern to that of isometric exercise with significant increase during the first minute that continues during the 2nd minute (Herd, 1991). An increase in heart rate and hence cardiac output contributes to the rise in MAP, but the pattern of heart rate change is different from that during exercise. With continuous beat-by-beat monitoring, it is obvious that during isometric exercise lasting several minutes heart rate rises immediately and continues to do so throughout the intervention duration (Ferguson & Brown, 1997, Hisdal *et al.* 2004). In contrast, during mental stress, there is an initial rapid increase in heart rate that peaks during the first minute, following by a decline (Herd, 1991, Wasmund *et al.* 2002, Carter *et al.* 2005, Carter & Ray, 2009). This difference is due in part to the neuroendocrine responses whereby mental stress evokes marked increases in the circulating catecholamines adrenaline and noradrenaline (LeBlanc *et al.* 1979, Heidbreder *et al.* 1982; Ward *et al.* 1983, Morris *et al.* 1997) but with greater increases in circulating adrenaline than

exercise but similar levels of noradrenaline (Herd, 1991).

3.1.5 Sympathetic neural activation

3.1.5.1 Isometric exercise

It takes approximately 30-60 sec for muscle sympathetic nerve activity (MSNA) to show an increase of 3-fold with isometric handgrip contraction (Mark *et al.* 1985; Vissing *et al.* 1991), and with calf muscle contractions evoked by electrical stimulation i.e. involuntary, there was no increase in MSNA during the first minute (Fisher *et al.* 2005). Wallin *et al.* (1989) showed that the increase in MSNA in the second minute of contraction was similar in the peroneal nerve (leg) as in the radial nerve (arm). In all cases the increase in MAP and HR to an isometric contraction preceded the increase in MSNA, demonstrating the more rapid cardiac than vascular effects. A review of the MSNA responses to exercise (Seals & Victor, 1991) concluded that they were fairly uniform between different skeletal muscle nerves, consistent over time within a particular subject and correlated strongly with changes in vascular resistance. In contrast to MSNA, skin sympathetic nerve activity (SSNA) increases early during an isometric contraction. Vissing *et al.* (1991) compared MSNA and SSNA in the peroneal nerve during handgrip, showing a doubling of SSNNA within the first 5 sec that increased 5.7-fold compared to baseline after 2 min. MSNA, as expected, did not change until the 2nd min when it was increased by 2.7-fold. Other studies have found similar increases in SSNA with isometric contraction of both arms and legs (Ray & Wilson, 2004, Wilson *et al.* 2005).

3.1.5.2 Mental stress

Compared with isometric exercise, evidence for increases in MSNA during mental stress tests is inconsistent with reports of decreases, no change or an increase. Studies showing a decrease in MSNA have considered it the result of sympathetic withdrawal. For example, Halliwill *et al.* (1997) recorded a decrease in radial (forearm) MSNA from 5113 ± 788 to 1509 ± 494 total integrated activity.min^{-1} during the colour word test in conjunction with a significant decrease in forearm vascular resistance. Indeed, sensory stimulation causing arousal (electrical pulses to the finger) can inhibit MSNA in humans (Donadio *et al.* 2002). On the other hand, Carter *et al.* (2005a, b) found radial nerve MSNA remained at baseline level during the mental arithmetic challenge (10 ± 3 bursts.min^{-1} at baseline vs. 12 ± 3 bursts.min^{-1} during intervention). Studies reporting simultaneous upper and lower limb observations of neural activity during mental stress have given conflicting findings on the two limbs. Anderson *et al.* (1987) found in male subjects, that mental stress increased peroneal but not radial nerve MSNA although both were elevated by lower body negative suction (LBNP). Likewise Hjemdahl *et al.* (1989) and Carter *et al.* (2002) both recorded increased peroneal MSNA during mental stress although neither measured vascular resistance in their

studies. Kamiya *et al.* (2000) also noted significant increases in tibial nerve (leg) MSNA associated with decreased calf vascular resistance during mental arithmetic in healthy male subjects. In contrast, others have reported no significant change in peroneal MSNA during mental stress (Wasmund *et al.* 2002) and Kuipers *et al.* (2008) made the same observation although calf vascular conductance was increased. Thus it is not clear exactly how sympathetic neural activity alters during mental stress particularly in the leg, and the evidence that any increase in MSNA translates into a change in vascular resistance is equivocal (see below). Evidence for what happens to skin SNA during mental stress is limited, however, Iwase *et al.* (1997) did report that it increased markedly (1003.3 ± 457.4 %) in their control subjects during mental arithmetic stress.

3.1.6 Catecholamine response

3.1.6.1 Isometric exercise

Levels of circulating catecholamines, a combination of adrenal production and spillover from active muscle, increase to a smaller extent with isometric than dynamic exercise (Christensen & Galbo, 1983, Stratton *et al.* 1983). Reed *et al.* (2000) found that circulating adrenaline increased from 42.1 ± 12.7 $pg.ml^{-1}$ to 155.6 ± 28 $pg.ml^{-1}$ during handgrip, and Lake *et al.* (1976), Kopin *et al.* (1978) and Palmer *et al.* (1978) have all reported increased plasma noradrenaline levels during isometric handgrip in their experiments. Ward *et al.* (1983) reported increased levels of both adrenaline (+ 22%) and noradrenaline (+8%) taken from a brachial artery catheter in the contralateral forearm during 2 minutes of isometric handgrip exercise in 8 male subjects and Wallin *et al.* (1987) reported increases in circulating noradrenaline of 21% after a similar protocol. Robertson *et al.* (1979) performed a number of sympathoexcitatory challenges including a 3 minute isometric handgrip task on 15 subjects (14 male and 1 female). They also reported that both adrenaline and noradrenaline were significantly increased by 67% and 27% respectively during the exercise. Although the direction and magnitude of the catecholamine changes and those for the cardiovascular variables (MAP and HR both significantly increased during isometric exercise) were similar to the changes observed during Valsalva manoeuvre and cold pressor test, isometric handgrip was a less potent stimulus than the cold pressor test (Robertson *et al.* 1979).

3.1.6.2 Mental stress

LeBlanc *et al.* (1979) reported raised adrenaline (increase of ~ 110 $pg.ml^{-1}$) and noradrenaline (increase of ~ 60 $pg.ml^{-1}$) during 2 minutes mental arithmetic stress compared to resting levels (66.8 ± 6.7 $pg.ml^{-1}$ and 292.5 ± 18.4 $pg.ml^{-1}$ respectively) in 12 male subjects. Blood samples were taken via catheter in the arm and the adrenaline increase during mental stress was found to be significantly greater than in the same subjects during cold pressor test. These findings are supported by Heidbreder *et al.* (1982) and Morris *et al.*

(1997). In studies comparing catecholamine responses to mental stress with those to exercise, it is a universal finding that the former increase adrenaline more than noradrenaline, while the reverse is true for the latter (Lenders *et al.* 1988, Murakami *et al.* 1996, Ng *et al.* 1994).

3.1.7 Peripheral vascular resistance responses

3.1.7.1 Isometric exercise

Since methods to measure venomotor tone require a non-contracting limb, it is what happens to blood flow in a resting limb when exercise is performed by another limb that is of interest for the current study in so far as it could determine passive change in venous volume. When blood flow is measured in the non-contracting limb during isometric exercise, there is an initial vasodilation before vasoconstriction is established. For example, during 2 minutes of voluntary handgrip (33% MVC), contralateral forearm blood flow increased between 50 and 450% and vascular resistance decreased, with marked vasodilation in the first 30s before constriction became evident (Eklund *et al.* 1974). The delay in development of vasoconstriction is associated with the time taken for increases in sympathetic neural traffic to occur. The transient vasodilation was reduced significantly by β-adrenergic blockade (Eklund & Kaijser, 1976). A similar increase in contralateral non-exercising forearm blood flow noted by Dietz *et al.* (1997) was enhanced by α-blockade by phentolamine and suppression of noradrenaline release by bretylium, but attenuated by a nitric oxide synthase inhibitor or by atropine. The same group showed later that contralateral forearm vasodilation during exercise occurred even when the stellate ganglion was blocked by local anaesthetic and sympathetic neural activity thereby eliminated, and could be virtually abolished by combined administration of a nitric oxide (NO) synthase inhibitor and β-blocker propranolol (Reed *et al.* 2000). It is possible to conclude from such studies that transient vasodilation in the resting limb at the onset of isometric exercise, which can occur as early as 10 seconds (Fisher & White, 2003) arises through a combination of β-mediated effects due to circulating adrenaline and locally released NO. Several studies have confirmed that it is not due to inadvertent EMG activity in the resting limb (Eklund *et al.* 1974; Rusch *et al.* 1981).

By contrast with the forearm, blood flow to the resting calf during the first minute of isometric hand grip increased very slightly in line with MAP such that calf vascular resistance was unchanged (Eklund *et al.* 1974), and similar findings were reported by Rusch *et al.* (1981) during a 30% MVC handgrip. However, by the end of the second minute of handgrip, calf vascular resistance was 30-40% above baseline level (Eklund *et al.* 1974; Rusch *et al.* 1981). The lower and upper limbs therefore show different resistance vessel responses to isometric exercise with a more prominent initial vasodilation in the forearm before constriction is established.

3.1.7.2 Mental stress

Whereas forearm constriction is commonly observed during other sympathoexcitatory challenges, mental stress has been shown consistently to produce dilation in the forearm, (Wilkins & Eichna, 1941, Golenhofen & Hildebrandt, 1957, Blair *et al.* 1959, Rusch *et al.* 1981, Dietz *et al.* 1994, 1997, Halliwill *et al.* 1997, Joyner & Halliwill, 2000, Carter *et al.* 2004, Carter *et al.* 2005, Santos *et al.* 2005, Hamer *et al.* 2006, Seddon *et al.* 2008). Forearm conductance may be almost doubled during stress tests lasting several minutes (Lindqvist *et al.* 1996, Halliwill *et al.* 1997, Carter *et al.* 2005, Kuipers *et al.* 2008). Early studies (Wilkins & Eichna, 1941, Blair *et al.* 1959, Barcroft *et al.* 1960, Heidbreder *et al.* 1982) attributed the vasodilation to neural mechanisms, i.e. an active neurogenically mediated vasodilation. Blair *et al.* (1959) observed significant increases in forearm blood flow in 11 subjects (male and female) following 2 – 3 minutes mental stress by various means including mental arithmetic. During severe stress, involving an arterial puncture hoax, they reported that forearm blood flow reached 50 ml.100ml^{-1}.min^{-1} and that since there was no corresponding increase in forearm arterial pressure (measured by intra-arterial catheter) the authors suggested that the increase in flow was due to forearm vasodilation. On the basis that (1) the size of the forearm dilation that they had produced through severe stress exposure was much greater than had previously been produced by adrenaline infusion, (2) adrenaline infusion produces a marked reduction in flow in the hand, which had not been observed in their experiment, (3) they had observed different arterial pressure changes to those usually produced by adrenaline infusion, (4) the return of flow to normal levels following mental stress was much faster than that observed following adrenaline infusion, (5) the size of response (vasodilation) was greatly reduced following sympathectomy or deep nerve block, and (6) blood flow remained similar in cutaneous nerve-blocked forearms (3.4 ml.100ml^{-1}.min^{-1}) compared to their normal (non-blocked) counterparts (3.5 ml.100 ml^{-1}.min^{-1}) during mental arithmetic task, Blair *et al.* (1959) reasoned that the increase in forearm blood flow during stress is mainly due to activation of cholinergic vasodilator nerves to muscle.

Subsequent work has focused on whether mental stress-induced increases in blood flow are mediated via withdrawal of sympathetic vasoconstrictor nerve activity and / or β-mediated vasodilation. Studies investigating the involvement of neural mechanisms by axillary blockade show that this intervention had no effect on the response (Lindqvist *et al.* 1996). Halliwill *et al.* (1997) also examined the increase in forearm blood flow to mental stress following α-adrenergic neurotransmitter blockade by bretylium, stellate ganglion blockade by local anaesthetic or β-adrenergic blockade with propranolol following stellate ganglion blockade. The authors found that the marked increase in forearm blood flow during the mental stress task continued to occur during α-adrenergic neurotransmitter blockade and stellate ganglion blockade but was greatly attenuated during β-adrenergic blockade following

stellate ganglion blockade. Since the forearm dilation observed during mental stress following stellate block was blunted by the propanolol administration, the authors concluded that β-adrenergic mediated vasodilation makes a large contribution, along with sympathetic withdrawal, to the observed forearm dilation during mental stress. Halliwill *et al.* (1997) found no evidence for an active neurogenic vasodilatory mechanism but did not discount mechanisms other than those for which they had tested from contributing to the observed forearm dilation, namely the stimulation of other (cholinergic) nerves not accounted for in their study and more importantly the local release of nitric oxide, which had previously been shown by Dietz *et al.* (1994, 1997) to contribute to forearm vasodilation during sympathoexcitation (isometric exercise and mental stress) in part via cholinergic stimulation of the vascular endothelium.

With regard to the effects of mental stress on blood flow in the leg, the data are inconsistent, with reports of no significant change in vascular resistance (Rusch *et al.* 1981, Carter *et al.* 2005) or decreases (Freyschuss *et al.* 1988, Linde *et al.* 1989, Butt *et al.* 1999, Kuipers *et al.* 2008). Rusch *et al.* (1981) studied forearm and calf responses to 90 s mental arithmetic task in 6 male subjects, reporting that a mental arithmetic task caused increases in MAP, HR and forearm blood flow and a decrease in forearm vascular resistance while no significant changes in calf blood flow or vascular resistance were noted. In conjunction with the disparate data on MSNA to the leg during mental stress, it is evident that there is no clear relationship between levels of sympathetic activation and their transduction into changes in arterial resistance in the lower limb.

3.1.8 Effects of gender and female hormonal status on responses to isometric exercise and mental stress

3.1.8.1 Isometric exercise

Jones *et al.* (1996) compared young (~30 year old) men and women performing a 30% maximum voluntary contraction handgrip and saw similar increases in systolic and diastolic blood pressure and heart rate. Levels of peroneal nerve MSNA at baseline were lower in women than men and the absolute increase in neural activity during exercise was higher in men, attributed to the greater muscle mass involved at the same relative workload. The effects of MSNA responses on vascular resistance were not determined in this study. Ettinger *et al.* (1996) also compared responses to a 30% MVC handgrip contraction in men and women and found smaller increases in MSNA in women than men but also smaller rises in heart rate and blood pressure in the women. However, in this study, there was a much greater age range of subjects, some of whom were post-menopausal women, and some receiving hormone replacement treatment. More recently, Hogarth *et al.* (2007) also compared middle-aged men and women, including post-menopausal females, during

isometric handgrip at 30% MVC for 2 min and showed smaller increases in heart rate and blood pressure in the females but there was no difference in the absolute increase in MSNA. There was a relationship between peroneal MSNA and calf vascular resistance in men but this did not exist in women. Although responses to isometric exercise in these studies may well be confounded by aging which, in addition to hormonal status, is known to modify SNA levels, this finding is similar to the attenuated increase in leg resistance in women during tilt despite similar rises to men in MSNA (Frey & Hoffler, 1988). Noradrenaline responses to isometric exercise were reported as similar in men and women (75% and 64% increases above baseline respectively) but men showed bigger increases in adrenaline (140%) compared to women (39%, Jones *et al.* 1996).

Ettinger *et al.* (1998) found bigger increases in MSNA during the menstrual than follicular phase of the female cycle during isometric handgrip (305 MVC for 2 min) although blood pressure and heart rate rises were similar between phases. This was deemed not due to differences in muscle force generation, which has been shown repeatedly to be unaffected by menstrual cycle phase or oral contraceptive use (Petrofsky *et al.* 1976, de Jonge *et al.* 2001, Elliott *et al.* 2003). Minson *et al.* (2000a) found higher baseline levels of MSNA and noradrenaline in the luteal than follicular cycle phase in young women but no difference in the relationship of sympathetic activity to calf vascular resistance during ischemic rhythmic handgrip exercise.

3.1.8.2 Mental stress

Several studies have examined cardiovascular responses to mental stress (usually mental arithmetic test) in men and women. Increases in blood pressure are reported as similar between genders (Jones *et al.* 1996, Litschauer *et al.* 1998, Dishman *et al.* 2002, Farag *et al.* 2008) or greater in women (Carter & Ray, 2009) while heart rate rises are comparable (Jones *et al.* 1996, Litschauer *et al.* 1998, Dishman *et al.* 2002, Farag *et al.* 2008, Carter & Ray, 2009). Jones *et al.* (1996) saw no change in peroneal nerve MSNA during the stress test in either men or women and the very small, but significant, increases seen by Carter & Ray (2009) were no different between genders. In response to an emotional stress (visual orientation task), men and women both showed higher forearm conductance but responses in the calf were variable with men tending to show constriction and women dilation (Butt *et al.* 1999). On the other hand, in another study calf vascular resistance did not change from baseline in either men or women during mental arithmetic (Dishman *et al.* 2002). Noradrenaline was found to increase similarly in men and women (~ 50%) whereas only men showed a rise in plasma adrenaline during mental stress (Jones *et al.* 1996). In contrast, another study found increases in adrenaline of 118% and 217% in men and women respectively while only men showed a rise in noradrenaline (68%) compared to women (-3%)

(Ross *et al.* 2001). Litschauer *et al.* (1998) reported similar mental stress-induced increases in both catecholamines in men and women, and throughout the menstrual cycle. With regard to female hormonal status, there was no difference in blood pressure or heart rate responses to mental stress across phases of the menstrual cycle (Tersman *et al.* 1991, Litschauer *et al.* 1998, Sato & Miyake, 2004) and the small but significant increases in peroneal MSNA were also similar between menstrual and luteal cycle phases (Carter & Lawrence, 2007).

3.1.9 Summary and Chapter aims

Isometric exercise and mental stress tests are sympathoexcitatory manoeuvres that increase heart rate and blood pressure. They differ in mechanisms of sympathetic outflow because isometric exercise elevates muscle and skin sympathetic neural activity and increases circulating noradrenaline levels more than those of adrenaline whereas mental stress hardly alters neural activity but elevates circulating adrenaline more than noradrenaline. The effects of these different patterns on peripheral arterial resistance in a non-active limb are also contrasting. Isometric exercise leads to vasoconstriction in both forearm and calf and a reduction in blood flow while mental stress induces forearm vasodilation but little change in calf vascular resistance. In the face of arterial vasoconstriction, venoconstriction to sympathoexcitation by isometric exercise has been demonstrated in the forearm and, to a lesser extent, in the calf. Mental stress on the other hand causes forearm venous constriction despite arterial vasodilation. The response of leg veins to mental stress is as yet unexplored. It is possible that differences in catecholamine responses to these two means of sympathoexcitation may evoke diverse changes in venomotor tone due to the larger increase in noradrenaline to isometric exercise than mental stress, allied with the greater sensitivity of venous smooth muscle to non-neural (circulating) than to neural adrenergic stimulation. It is thus hypothesized that:

1) **Venoconstriction will be observed in the leg in response to isometric exercise and will be of greater magnitude than to mental stress.**

The majority of data relating to venoconstrictor responses has been obtained in male subjects. Gender differences in sympathetic outflow have been reported during isometric exercise, although not consistently, but not during mental stress. Even if increases in sympathetic neural activity are similar between men and women, their transduction into arterial constrictor or dilator responses suggest attenuation of vasoconstriction in women. Whilst this could in theory be related to levels of the female hormones oestrogen and progesterone, cardiovascular responses are similar across different phases of the menstrual cycle despite varying hormonal levels.

The aim of the study described in this chapter is to investigate the effects of isometric exercise and mental stress on venous tone in the legs of men and women, the latter tested during menstrual and luteal phases of their normal menstrual cycle and during low and high hormone phases of oral contraceptive use. Venoconstriction to these stimuli has largely been evaluated in forearms using plethysmographic techniques. The present aim is to study calf volume responses using the equilibration method and to compare this with ultrasound imaging of the popliteal vein which is the main deep vein draining the lower leg. In so far as gender differences in arterial vascular reactivity may impinge upon venous responses to sympathoexcitation, it is hypothesised that:

2) **Women will show reduced venoconstriction compared to men.**

If oestrogen and progesterone are contributors to this, then it is also hypothesized that :

3) **Women will show smaller venoconstriction when hormonal levels are high, e.g. during the luteal cycle phase or high hormone phase of oral contraceptive use.**

3.2 Methods

3.2.1 Subjects and groups

7 males and 14 females volunteered to participate in two protocols to investigate venous filling and venous reactivity to sympathoexcitation. Subjects were screened (general health questionnaire (Appendix B) and IPAQ, short last 7 days self-administered format (Appendix D)) to ensure good health, injury free and similar fitness levels. Exclusion criteria were: current illness, consumption of 'over the counter' anti-inflammatory drugs in the previous 24 hour period, cardiovascular disorders, pregnancy (either prior to or at time of study), injury to limbs (either during or at any time over 6 months prior to study) and immune, metabolic or kidney conditions. All experiments were carried out following approval from the Local Research Ethics Subcommittee, School of Sport and Exercise Sciences, University of Birmingham, UK in accordance with the declaration of Helsinki 1964. The subjects' anthropometric characteristics are shown in table 3.1.

The females consisted of two groups of 7 subjects; who displayed either a normal menstrual cycle pattern and were not taking (and had not previously taken) oral contraceptives (N) and females who were taking a "combined" oral contraceptive pill (O). The "combined" pill contained both a synthetic oestrogen (e.g. (30µm) ethinylestradiol) and a progestin (e.g. (150µm) levonorgestrel). Subjects were most commonly using the "Microgynon30" pill and had been using it for between 1 and 5 years (questionnaire, Appendix C). Assignment to a (female) group was established by menstrual cycle questionnaire and volunteers were excluded from the study if they displayed an irregular menstrual cycle pattern i.e. markedly

longer or shorter (± 3 days) than an average of 28 days or were taking a form of oral contraceptive other than a “combined” pill e.g. a progesterone only pill or the contraceptive injection. As in the previous chapter, all women were tested during both the menstrual phase (days 1 – 5) and the luteal / high hormone pill-taking phase (days 16 – 28) of their respective cycles (day 1 was taken to be the first day of bleeding, established via questionnaire) to investigate the influence of the female hormones oestrogen and progesterone.

3.2.2 Other considerations

As in Chapter 2, subjects were tested in a quiet laboratory with controlled temperature (21^{o}C) and lighting. Subjects were fasted before each testing session which took place at the same time of day (afternoon) to minimise circadian effects on cardiovascular and metabolic variables, and undertook the same modified euhydration protocol used previously to ensure fluid balance (Veldhuijzen van Zanten *et al.* 2005).

3.2.3 General experimental setup

On arrival at the laboratory, subjects’ height, weight, skinfold thicknesses and calf segment circumferences and lengths were measured as before. Subjects then lay in a semi-recumbent position on a Biodex dynamometer throughout testing. The dynamometer’s double-footplate was positioned so that the legs below the knees were parallel to the floor (horizontal position) and slightly higher than heart level. The knees were set at an angle of 160°, which, from pilot testing, was found to be a comfortable position in which subjects could remain relaxed without having to contract their leg muscles to maintain position. Arms, although not being studied for haemodynamic responses, were also supported at heart level to minimize gravitational pooling or postural constrictor effects within the arm vasculature. A small inflatable cuff (Hokanson Ltd.), 6cm width, was placed around the ankle of the test (non-dominant) limb. A mercury-in-silastic strain gauge (Hokanson Ltd.), measuring 2 cm smaller than the largest calf circumference, was placed around the lower leg so that it did not interfere with the ultrasound probe positioning. A large inflatable cuff (Hokanson Ltd.), 18cm width, was placed around the mid-thigh of the same limb. Subjects were instructed to breathe at normal rhythm throughout testing and to avoid sudden gasps or deep inspirations. Calf volume and vein measurements (see below) were made on the non-dominant limb.

3.2.4 Measurement of variables

Beat-to-beat blood pressure, from which mean arterial pressure (MAP, calculated as diastolic pressure + 1/3 (systolic pressure - diastolic pressure) and heart rate (HR) values were derived, was detected using a Portapress photoplethysmograph incorporating height correction (Finapres Medical Systems, The Netherlands) placed around the right middle finger.

***Table 3.1**: Physical characteristics, anthropometric data and activity levels of subjects. Values are mean ± SD. Table shows data for combined female group (n = 14) and for individual N and O female groups (both n = 7). * = p < 0.05 males vs. females.*

	Males (n=7)		Females (n=14) (combined N & O)		N (n=7)		O (n=7)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Age (years)	19	1	20	1	20	1	20	1
Height (cm)	180.6	9.0 *	166.2	7.6	166.3	7.1	166.1	8.6
Weight (kg)	78.7	11.8	62.2	13.3	65.5	17.1	58.8	7.9
Body Mass Index (kg/m^2)	24	2	22	4	24	5	21	2
Body Fat (%)	13.0	3.6 *	24.0	5.6	26.7	6.8	21.4	2.5
Calf segment volume (l)	3.79	0.65 *	2.95	0.79	3.14	1.00	2.75	0.51
Physical activity (MET-minutes/week)	2662	1068	2807	756	3674	1862	2623	925

The analogue signal from the Portapress control console was input via a Powerlab A/D converter to a PC where it was recorded at a rate of 40Hz using Chartlab software (ADInstruments Ltd.).

Blood-flow, calf venous filling, venous capacity and filtration were detected using strain-gauge plethysmography. The strain gauge was connected to a plethysmograph (Hokanson Inc.) from which the output signal was connected to the same PC running Chartlab software via a Powerlab A/D converter. Calf volume changes were recorded simultaneously with cardiovascular variable changes so that changes throughout the experiment were time-matched.

The popliteal vein diameter was imaged in longitudinal section by Doppler ultrasound in B mode with a linear 3 - 12MHz probe positioned by hand 5 - 7cm below the popliteal fossa (figure 1.3) and was manipulated, ensuring not to press into the leg tissue, in order to obtain an optimum image of the vessel borders. As in the previous chapter, the ultrasound image signal was relayed to a second PC running the on-line edge-detection software (Newey & Nassiri, 2002) at a rate of ~25Hz to continuously measure vessel diameter changes.

3.2.5 Venous distension procedures

Subjects were rested for 20 minutes prior to the testing procedure. The ankle cuff was inflated to supra-systolic pressure (> 50mmHg above systolic pressure) to eliminate foot blood flow and a baseline measurement period of 2 minutes was then undertaken during which MAP, HR, calf volume and vessel diameter were recorded. Calf blood flow was then measured by inflating the thigh cuff to 50mmHg for 10 seconds while the increase in calf volume was measured by strain gauge. Following a ~ 30 s recovery period for variables to return to baseline values, the procedure was repeated until a total of 3 calf blood flow measurements had been produced for analysis.

Following a further 2 minute baseline rest period, the thigh cuff was then inflated to 50mmHg for 6 minutes to occlude the venous outflow. Measurements of MAP, HR, calf volume and popliteal vein diameter were continuously recorded throughout. Values for venous capacity, fluid filtration and vein diameter were extracted from the filling data at specific time points (see later).

Two further venous distensions were performed during this protocol. On each occasion, following the 2 minute baseline rest period, venous occlusion was performed as described above with the addition of a 2 minute intervention of either isometric leg exercise (ILE) or a mental stress task (MST) performed between minutes 3 and 5 of the distension period (figure

3.1).

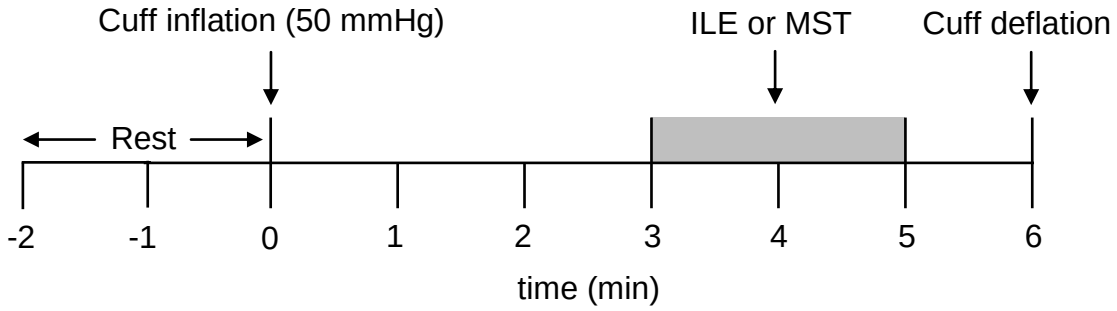


Figure 3.1*: Time line for sympathoexcitation protocol: Figure identifies the 2-minute baseline rest period and the point at which the 2-minute interventions were performed (shown by grey shaded area) within a venous distension period (50mmHg venous occlusion cuff inflation). The intervention was either 40% MVC isometric leg exercise or PASAT mental stress task.*

3.2.6 Protocols for sympathoexcitation by isometric leg exercise and mental stress task

The first venous distension was used as the control (CON) for comparison with the interventions and was always performed by the subject first. However, the other two venous distensions incorporating the interventions (ILE or MST) were presented to the subjects in a randomised order so that half of the subjects in each group (M, N or O) received the ILE intervention first and the MST intervention second and vice versa. For the female groups (N and O), the order of presentation of the interventions in the first study session was reversed for the second study session.

3.2.6.1 Isometric leg exercise

Isometric leg exercise was performed with the dominant leg by plantar flexion of the foot against the Biodex dynamometer footplate. At the start of the testing session each subject was asked to perform 3 maximal voluntary calf contractions (MVC's) by plantar flexing their foot as forcefully as possible and sustaining the contraction for approximately 2 s without contraction of thigh and/or other muscles or also inadvertently performing a Valsalva's manoeuvre. The subject was verbally encouraged to perform to the best of their ability during each effort and visual feedback was given by displaying the force output trace from the footplate on a computer monitor. Subjects were allowed to rest for 2 minutes between MVC's in order to enable them to produce their best efforts. Only efforts producing values that were within 5% of each other were accepted and once 3 of these had been recorded, the highest value was taken to be their MVC value. 40% of the subject's MVC value was then calculated and a "target" line representing this level was set on the display monitor for the subject to aim at during the 2 minute intervention period. Subjects were verbally encouraged to reach and maintain the 40% MVC throughout the entire ILE intervention period.

3.2.6.2 Mental stress task

Mental stress task was applied in the form of a 2 minute paced auditory serial addition test (PASAT), whereby 60 numbers were delivered to the subject over the 2 minute intervention period, i.e. one number every 2 s, and the subject was required to add each new number to the number previously given (Tombaugh, 2006). For example, if the subject was presented with the following numbers: 2, 7, 4 and 11 the subject should respond with the following answers after each new number: “9”, “11” and “15” respectively. All subjects were presented with the same 2 minute segment (and therefore were presented with the same numbers), i.e. minutes 3 and 4 from the full 8 minute PASAT. This segment was chosen as data from initial pilot testing suggested that significant increases in MAP and HR could be produced from numbers presented at this speed, while the first 2 minute segment (50 numbers over the 2 minute segment) could not produce these increases as it appeared too easy. Furthermore, although the later 2 minute segments (75 and 100 numbers over the 2 minute segment) also produced marked increases in MAP and HR, it was established that the pilot subjects who were subjected to these segments produced unacceptably low numbers of correct responses and some subjects were even unable to complete the task. During the MST intervention, subjects were informed by being told they were “wrong” when a mistake had been made and were instructed to “keep going” if they either hesitated with an answer or missed out an answer.

3.2.7 Data analyses

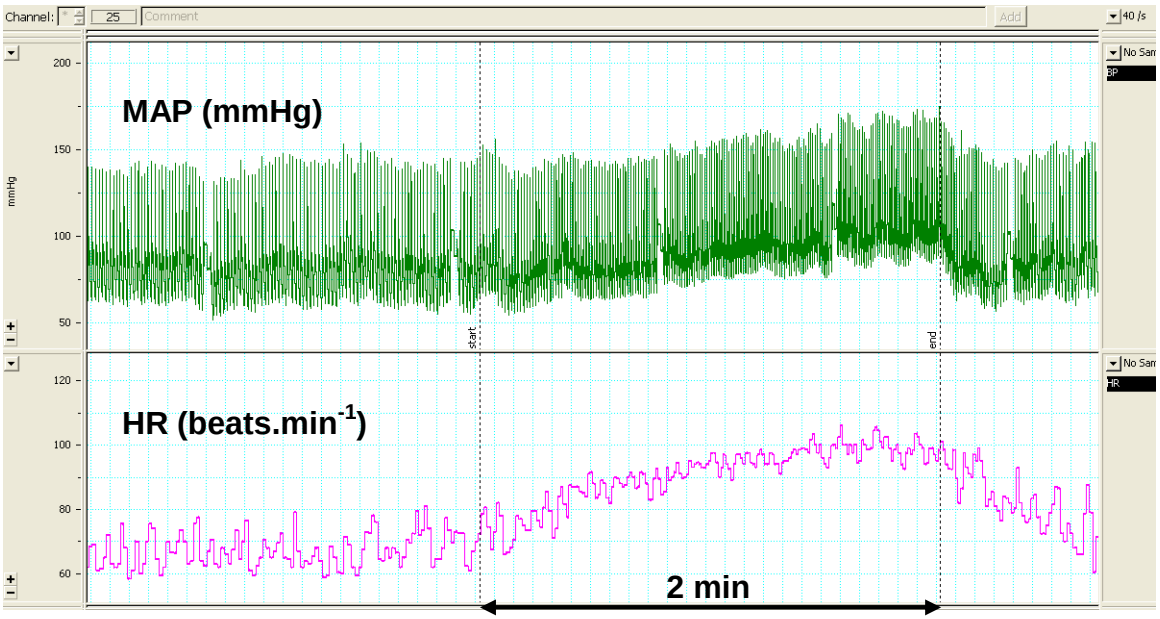
Anthropometric data for subjects' BMI, % body fat and calf segment size were calculated as described in Chapter 2. All measurements recorded onto the Chartlab and VIA edge-detection software were initially analysed using the Microsoft Excel software package. For each of the 3 venous distensions (CON, ILE and MST) baseline values for MAP, HR, calf volume and vein diameter were averaged over the 2 minute rest period.

3.2.7.1 Cardiovascular variables

Pilot study findings and evidence from the literature (LeBlanc *et al.* 1979, Rusch *et al.* 1981, Ray & Wilson, 2004, Carter *et al.* 2005, de Boer *et al.* 2006) suggested that the time course of heart rate response to mental stress stimulation is different from that typically displayed during other sympathoexcitatory challenges, while the time course of the blood pressure response is similar. Figure 3.2 shows original chart trace segments of actual MAP and HR responses to isometric leg exercise (figure 3.2a) and mental stress task (figure 3.2b) for one subject (responses were typical of all of subjects tested) and figure 3.3 shows the group mean MAP and HR responses to isometric leg exercise and mental stress task for the male group. The figures clearly illustrate that while MAP and HR for isometric leg exercise and MAP for mental stress task display a steady increase from the onset, which tended to plateau during the second minute, the HR response during mental stress task was different with the subject showing a much more marked initial increase which peaked at ~ 40s into the intervention period and then displayed a steady decrease back towards baseline values for the remaining 80s of the intervention.

In view of these differing time-course patterns, the change in MAP or HR during both intervention periods are expressed as the peak changes in relation to the respective value found at 10 s prior to the intervention period (i.e. the value for either MAP or HR at 3 minutes into venous distension, not the value found at baseline).

a. Isometric leg exercise



b. Mental stress task

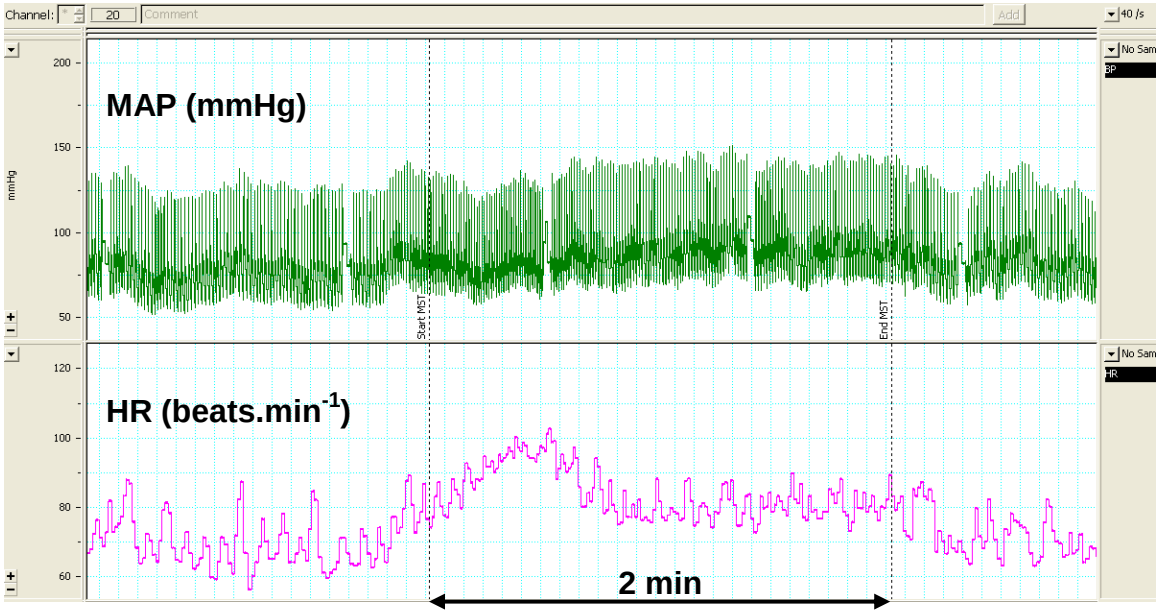


***Figure 3.2**: Original chart trace segments showing actual MAP and HR responses to isometric leg exercise (a) and mental stress task (b) for one subject. Figure shows 2 minute intervention periods between dotted lines. Steady increases in MAP and HR during isometric leg exercise (a) and MAP during mental stress task (b) are observed as well as a sharp increase in HR during mental stress task (b) which peaks during first minute.*

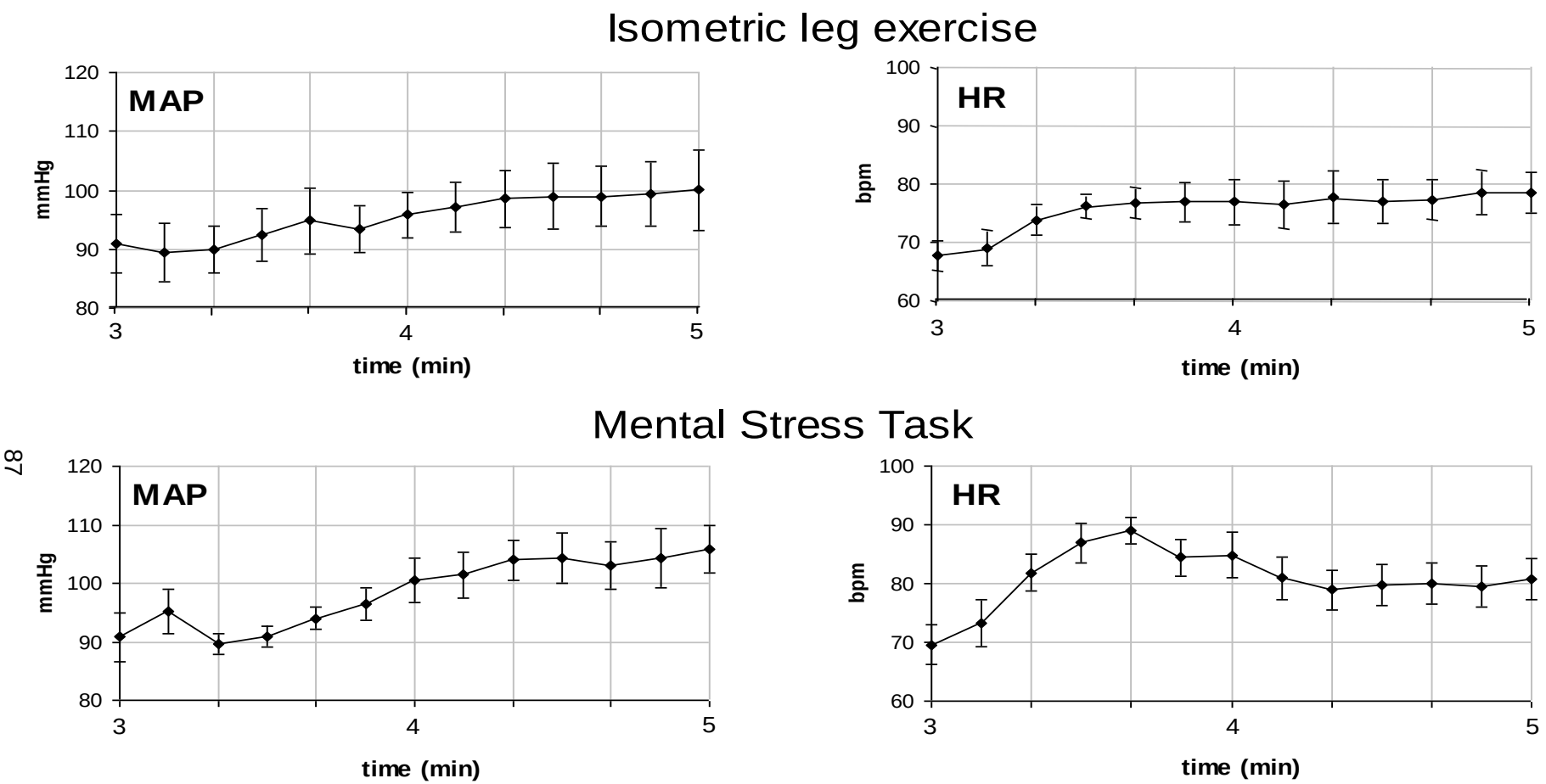


Figure 3.3*: Mean MAP and HR responses to isometric leg exercise and mental stress task for the male group (n = 7). Figure shows the mean ± SEM responses over the duration of the 2 minute intervention periods between minutes 3 and 5 of the venous distension period.*

3.2.7.2 Whole limb and vein responses

Data for calf volume change and popliteal vein filling were averaged over 10s periods throughout rest and distension as mls.100mls^{-1} (equivalent to % change in volume) and diameter in mm respectively. For each of the 3 venous distensions (CON, ILE and MST) baseline values for calf volume and vein diameter were averaged over the 2 minute rest period. Mean ± SEM group values for the different variables were plotted against time for each distension for comparison.

From the calf, blood flow was calculated as the mean of the three 10 s cuff inflations, venous capacity was reported as the volume increase at 3 minutes (Gamble *et al.* 1993, Lanne & Olsen, 1997, Halliwill *et al.* 1999, figure 2.5) and fluid filtration calculated from the rate of volume increase from capacity to the end of the distension period in ml.100ml^{-1}.min^{-1}.mmHg^{-1}. The shape of the popliteal vein filling curve was plotted (figure 3.8) and assessed to determine the most appropriate data/points at which to report filled vein diameter. Vein diameter was also expressed as % change from baseline.

Figure 3.4 shows calf volume data for one subject, which illustrates the typical volume responses to the control and intervention distensions. For each individual subject, the 2 minute intervention segment was taken from the respective whole limb trace (figure 3.4a) and plotted as change in relation to the volume prior to the intervention, i.e. at capacity (3 minutes, figure 3.4b). From this, the peak difference between the control distension and the respective intervention was identified (in this case - peak difference occurred at 5 minutes, figure 3.4b) and the limb volumes at those time points (figure 3.4c) were taken for further analysis. To provide a single value of volume change response for each subject (and thus group) from these values, the calf volume response to either isometric leg exercise or mental stress task intervention was expressed as the % difference between the peak control value and the respective peak intervention value found in figure 3.4c (figure 3.4d). Changes in popliteal vein diameter during the isometric leg exercise and mental stress task intervention periods compared to the control venous distension period were calculated and reported in the same way as for the calf volume.

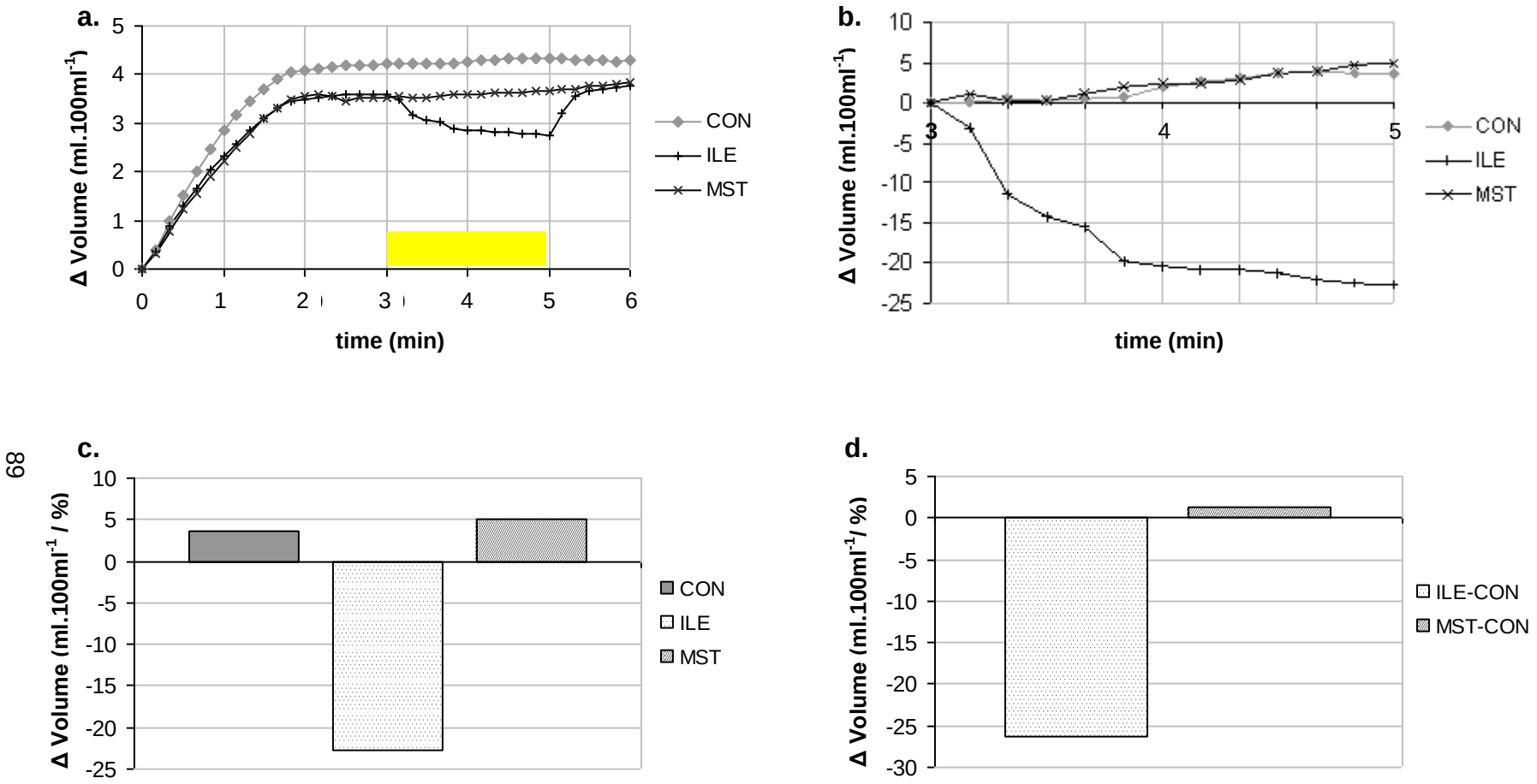


***Figure 3.4**: Step by step process for analysing whole limb data. Figure illustrates process by which whole limb reactivity to intervention segments (b) are taken from entire venous distension plot (a) and broken down into peak change during intervention period (c) and then peak difference between control and intervention values (d).*

3.2.7.3 Gender and female cycle phase influences

As in the previous chapter for gender comparison; the data for the male group was compared to those of the female groups during their menstrual phase (FM). Since oestrogen and progesterone levels are similar during the menstrual phase between normally menstruating women and synthetic hormone oral contraceptive users (as ingestion of the high dose of exogenous hormones is stopped completely to induce bleeding), the two female groups data were combined to make one large group (FM, n = 14) for comparison with the males (M, n = 7). Since the pharmacological content of oestrogen and progesterone can be up to ten times higher in oral contraceptives compared to endogenous hormones (Stachenfeld *et al.* 1999), the two female groups were assessed separately (both groups n = 7) during their luteal / high hormone phases for comparison with their respective menstrual phase. For the remainder of this chapter, the following abbreviations will be used to refer to the respective groups: NM = normal menstrual cycle – menstrual phase; NL = normal menstrual cycle – luteal phase; OLH = oral contraceptive user – low hormone (menstrual) phase; OHH = oral contraceptive user – high hormone (luteal) phase.

3.2.8 Statistical analyses

All statistical analyses were conducted using the Statistical Package for the Social Sciences, version 13 (SPSS, Inc., Chicago, IL). A one-sample Kolmogorov-Smirnov test was firstly performed to check for normal distribution of the data to determine the most appropriate (parametric or non-parametric) statistical analysis. Analysis revealed that the data were not normally distributed (most likely due to a low n) and therefore non-parametric tests were applied:

The male and female groups' anthropometric measurements were compared using a Mann-Whitney test.

Blood flow and venous distension characteristics were compared using a Mann-Whitney test to identify gender differences. Cycle phase related effects were established by using a Wilcoxon signed ranks test for NM vs. NL and OLH vs. OHH.

Within-group comparison of variables measured during control venous distension, isometric leg exercise and mental stress task was performed using Friedman's test. A Wilcoxon signed ranks test was used to identify specific differences between CON vs. ILE, CON vs. MST and ILE vs. MST. As for protocol 1, a Mann-Whitney test was used to identify differences between the genders and cycle phase related effects were established by using a Wilcoxon signed ranks test for NM vs. NL and OLH vs. OHH. Differences were taken to be significant if $p < 0.05$ and data are reported as means ± SEM unless otherwise indicated.

3.3 Results

3.3.1 Anthropometry

The male subjects were significantly taller, heavier, leaner and had significantly greater calf limb segment volume than the female subjects (table 3.1 (methods section), all $p < 0.05$). IPAQ (short last 7 days self-administered format) showed that all subjects were moderate to highly active and that no group was significantly more or less active than either of the other groups ($p > 0.05$).

3.3.2 Baseline data: Resting calf blood flow, MAP and heart rate

Calf blood flows were not significantly different between the male and combined female groups (table 3.2, $p > 0.05$). Neither endogenous (NL) or exogenous (OHH) female hormone caused calf blood flow to significantly alter compared to values found in their respective menstrual phase ($p > 0.05$ for NM vs. NL and OLH vs. OHH). Resting MAP and HR were not significantly different between the genders or between female cycle phases (table 3.2, $p > 0.05$).

3.3.3 Control venous distension

3.3.3.1 Time course patterns of responses to venous distension

In agreement with the findings from Chapter 2, calf limb volume (figures 3.5 and 3.7) and popliteal vein diameter (figures 3.6 and 3.8) both increased markedly during venous distension. The profile of filling suggested that the popliteal veins became full earlier than the whole limb (between 2 and 3 minutes) but unlike the previous Chapter, the response to distension during the high hormone phase of either the normal or oral contraceptive cycles was not larger ($p > 0.05$) than the menstrual phase. Again, the differences in the gender responses, were related to the relative starting baseline diameters and the % increases from those baselines were not significantly different ($p > 0.05$).

***Table 3.2**: Values for cardiovascular, whole limb and vein measures at rest and during venous distension. Values are mean ± SEM for all subjects. Table shows the separated female groups (N and O) in both observed phases of their menstrual cycles: menstrual (NM) and luteal (NL) for non-oral contraceptive group (N) and low-hormone (OLH) and high-hormone (OHH) for oral contraceptive using group (O).*

	Males (n=7)		NM (n=7)		NL (n=7)		OLH (n=7)		OHH (n=7)	
	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
Resting MAP (mmHg)	82	4	90	5	85	4	85	4	84	3
Distension MAP (mmHg)	84	5	92	6	84	4	87	4	85	3
Resting HR (beats.min^{-1})	64	4	61	3	70	4	63	3	62	2
Distension HR (beats.min^{-1})	63	3	63	3	64	4	64	3	64	3
Resting blood flow (ml.100ml^{-1}.min^{-1})	3.85	0.74	3.57	0.62	3.83	0.56	2.78	0.38	2.46	0.77
Fluid filtration (ml.100ml^{-1}.min^{-1}.mmHg^{-1} x 10^{-3})	3.30	0.54	3.72	1.40	3.75	1.01	5.33	0.77	5.39	0.57
Filled vein diameter (mm)	9.26	0.67	8.34	0.53	8.53	0.73	7.98	0.54	7.50	0.38

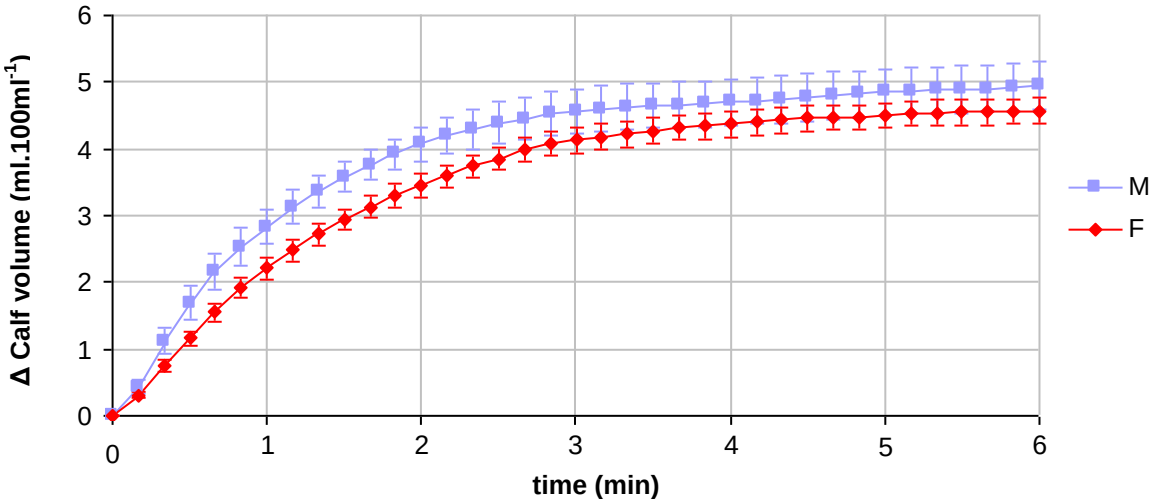


***Figure 3.5**: Male (n=7) and combined female (n=14) calf volume responses to venous distension. Figure shows mean ± SEM changes.*

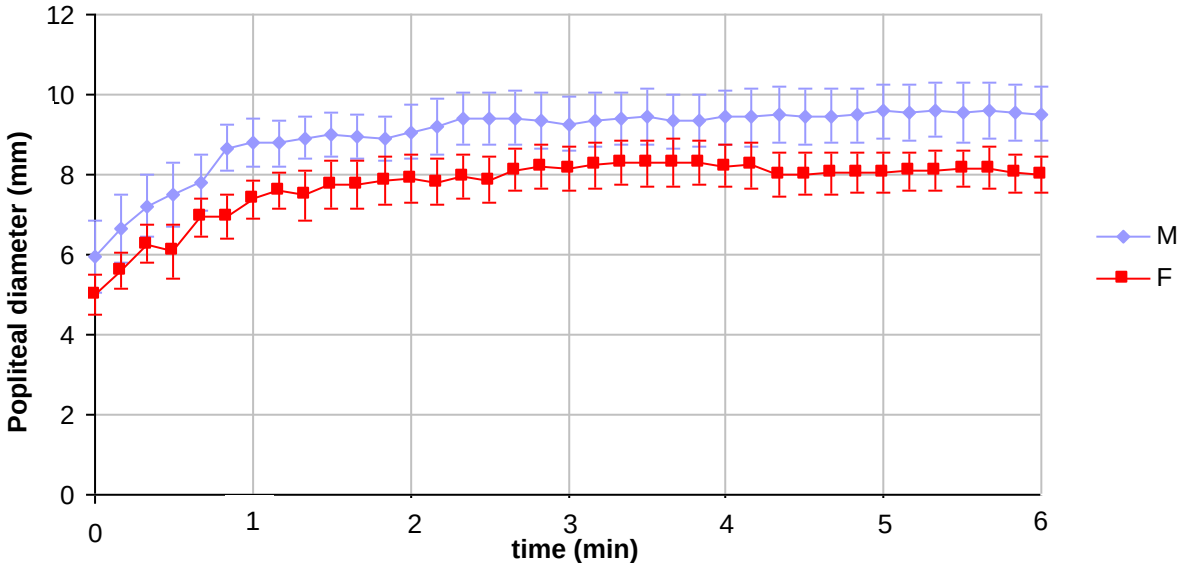


***Figure 3.6**: Male (n=7) and combined female (n=14) popliteal vein responses to venous distension. Figures show mean ± SEM actual diameter changes.*

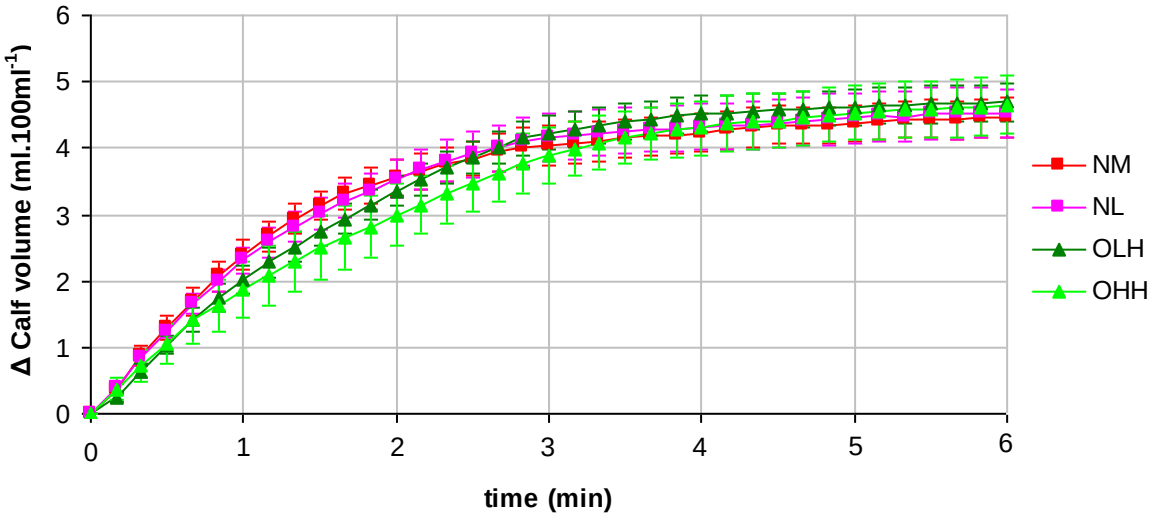


***Figure 3.7**: Calf volume responses to venous distension in the female groups (where N = normally menstruating women and O = oral contraceptive users, M and L = menstrual and luteal cycle phases respectively and LH and HH = low hormone and high hormone respectively). Figure shows mean ± SEM volume changes.*

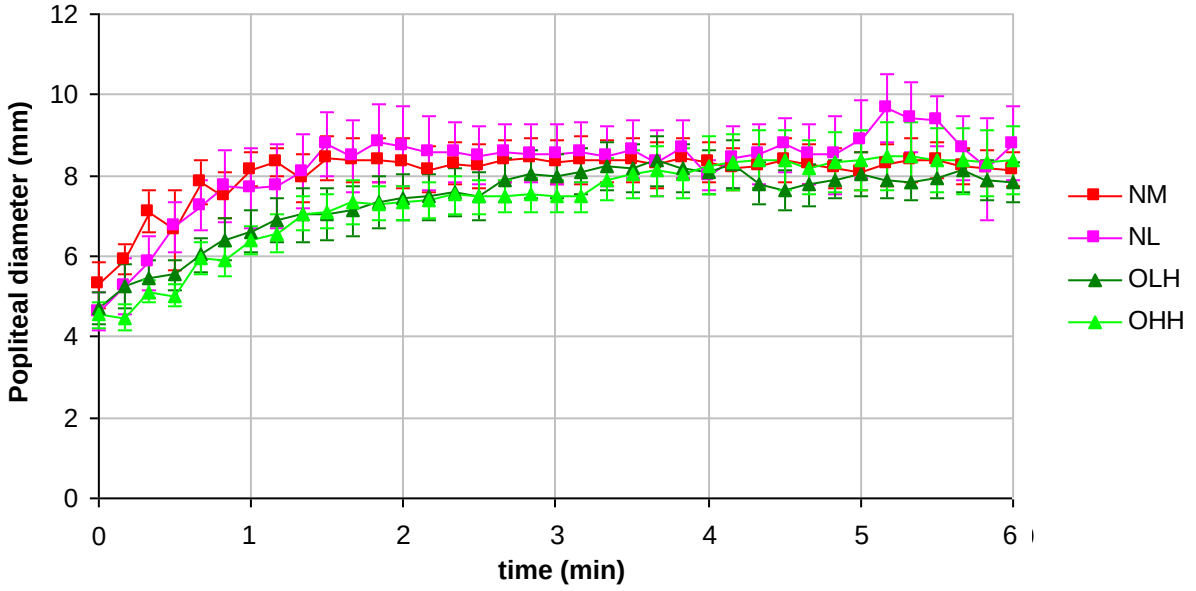


***Figure 3.8**: Female popliteal vein responses to venous distension. Figure shows mean ± SEM actual diameter changes.*

3.3.3.2 Venous distension

MAP and HR were not altered by venous distension (table 3.2, $p > 0.05$ distension vs. rest). Calf venous capacity was similar in males and females (figure 3.9, $p > 0.05$) despite the female group having a significantly smaller resting calf limb segment volume (table 3.1) and neither were there any cycle phase related differences ($p > 0.05$ for NM vs. NL and OLH vs. OHH). Fluid filtration was larger in the O group during both cycle phases than the other groups but not significantly (table 3.2, $p > 0.05$). Filled popliteal vein diameter was ~ 1 mm larger in the males than in the females (table 3.2) and when comparing the female groups across cycle phases, the O group women tended to have slightly smaller veins but again these differences were not significant ($p > 0.05$ for NM vs. NL and OLH vs. OHH).

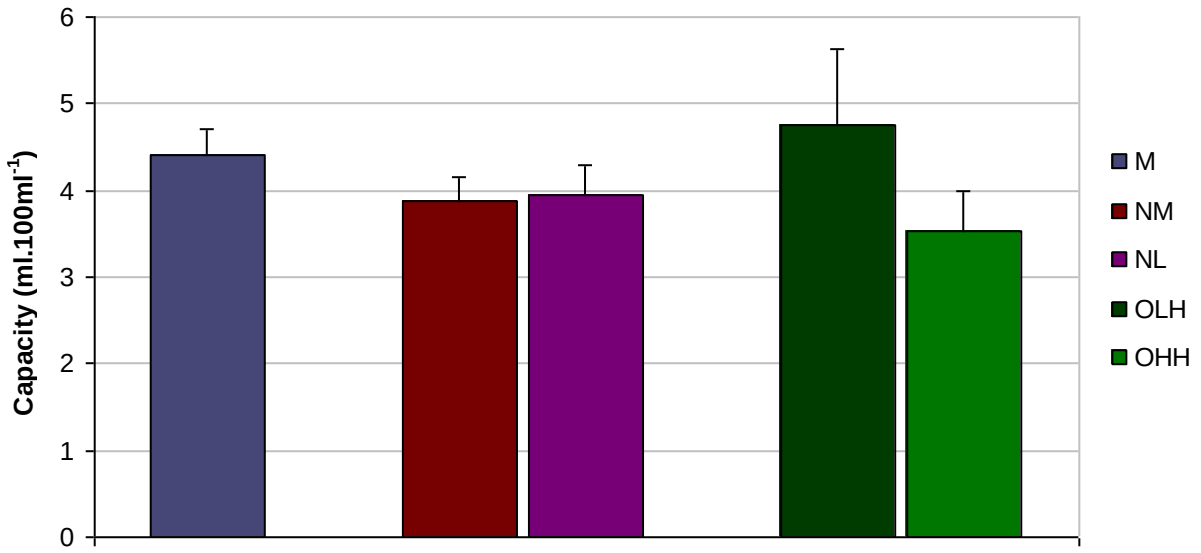


Figure 3.9*: Calf venous capacity. Figure shows mean ± SEM for capacity (ml.100ml^{-1}) taken as the limb volume increase at 3 minutes during venous distension. Data are shown for separated female group during both their menstrual (NM and OLH) and luteal / high hormone (FL / OHH) phases.*

3.3.4 Sympathoexcitation protocol

3.3.4.1 Responses to isometric leg exercise

MAP and HR increased in all groups during the 2 minute isometric calf exercise intervention compared to the control distension (figures 3.10 and 3.11), indicating that sympathoexcitation had occurred. For MAP, these increases were not found to be significant in the luteal / high hormone phases in the female groups, where the responses were only half those found in the menstrual phase. Calf limb volumes at 3 minutes into the isometric exercise distension were not different from those during the respective control distensions and although there was some evidence of venoconstriction in the calf limb during ILE, the decreases were not

significant (p > 0.05, figure 3.12). Male and female responses were not found to be different (p > 0.05) despite some evidence of dilation in the O group women during the menstrual phase and any calf limb venoconstriction that there was, appeared to be enhanced during the luteal / high hormone phases in the N and O groups respectively (p > 0.05 vs. menstrual phase). There was little evidence of any popliteal diameter change during isometric leg exercise with mean diameter changes being less than 4 % in all groups (p >0.05).

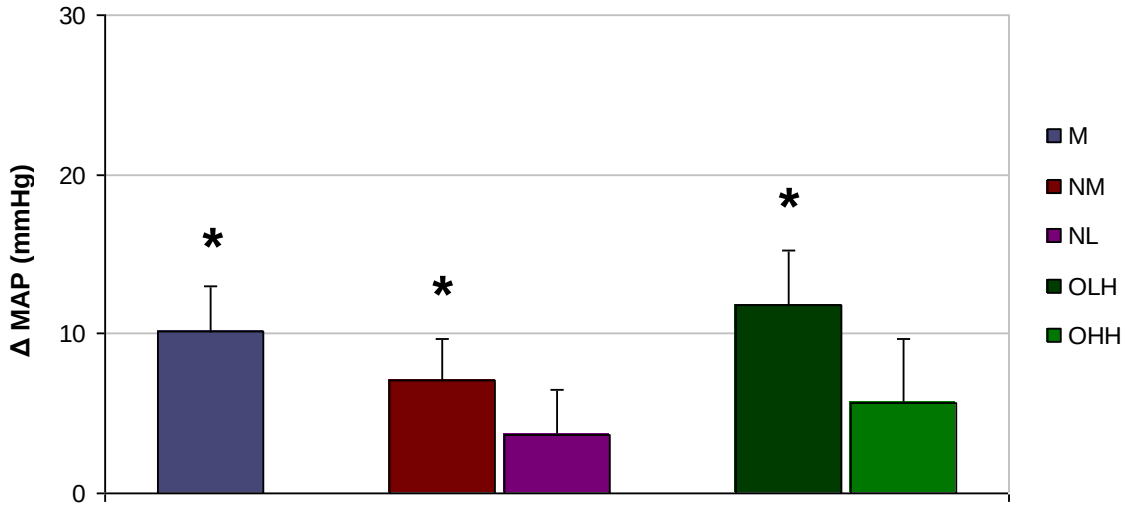


***Figure 3.10**: MAP responses to isometric leg exercise. Figure shows mean ± SEM peak responses for males and separate female groups. * = p < 0.05 vs. control distension value.*

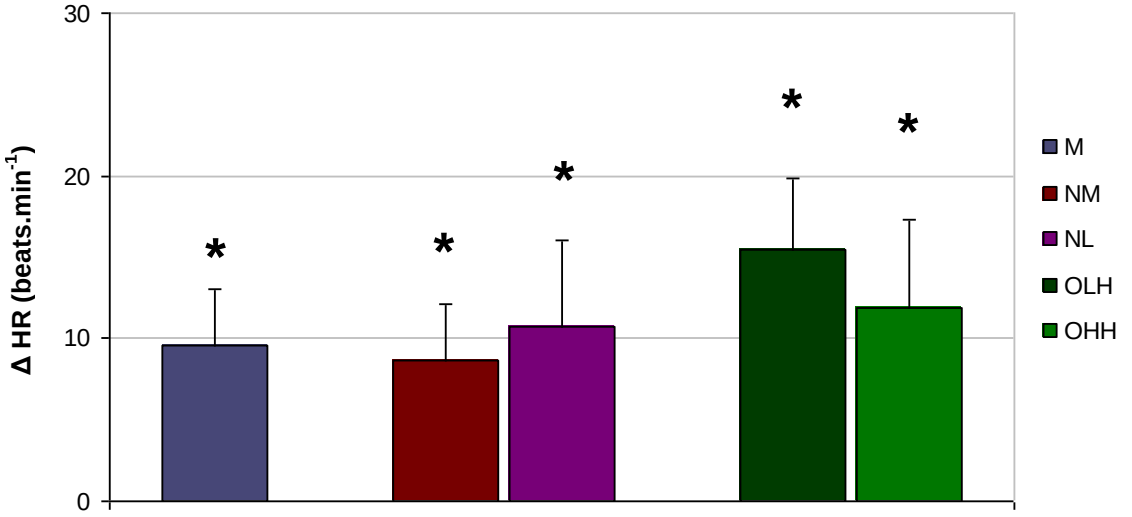


***Figure 3.11**: HR responses to isometric leg exercise. Figure shows mean ± SEM peak responses for males and separate female groups. * = p < 0.05 vs. control distension value.*

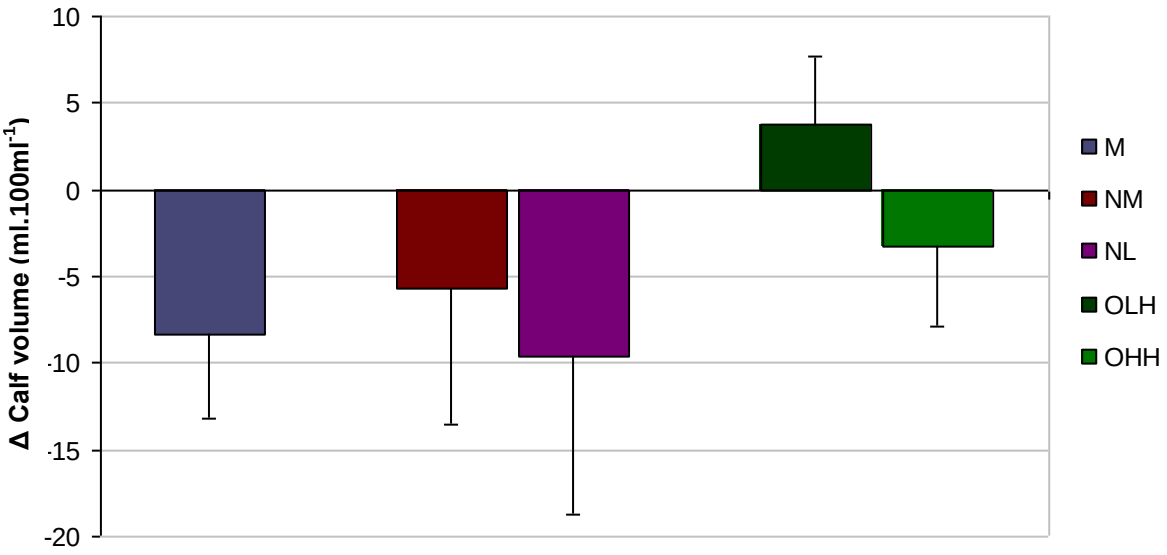


***Figure 3.12**: Calf limb volume responses to isometric leg exercise. Figure shows mean ± SEM volume changes expressed as change relative to CON value. Data are shown for separate female groups.*

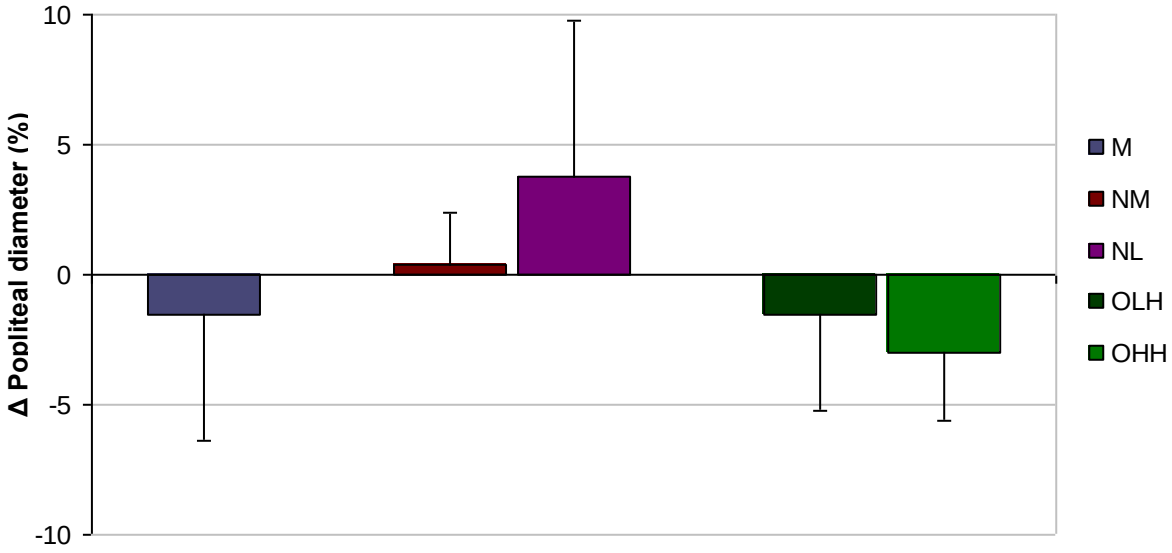


***Figure 3.13**: Popliteal vein responses to isometric leg exercise. Figure shows mean ± SEM % diameter changes expressed as change relative to CON value. Data are shown for separate female groups.*

3.3.4.2 Responses to mental stress task

Significant increases in MAP and HR were observed in all groups during the 2 minute mental stress task intervention (figures 3.14 and 3.15, $p < 0.05$). The trend appeared to be that the HR increase during mental stress was approximately double that observed during isometric exercise. A similar trend for MAP appeared for the males but not the females, whose MAP responses appeared to be very similar to those during isometric exercise. As during isometric exercise, there were no significant differences in MAP and HR response to mental stress task between the genders ($p > 0.05$) or between cycle phases ($p > 0.05$). As for ILE, calf limb volume at 3 minutes during the mental stress task distension was not significantly different to that during the control distension ($p > 0.05$, and $p > 0.05$ for MST vs. ILE). During MST, the OLH women were the only group to show a significant limb volume change ($p < 0.01$), which was an increase rather than a decrease (figure 3.16). Responses were attenuated in the luteal / high hormone phases for the women but not significantly ($p > 0.05$) and there were no significant gender differences ($p > 0.05$). Again, there was little evidence of popliteal vein diameter change in any group during mental stress task (figure 3.17, all $p > 0.05$) and although in contrast to the calf limb, responses were larger in the luteal / high hormone phases, there were no cycle phase effects ($p > 0.05$) or gender effects ($p > 0.05$).

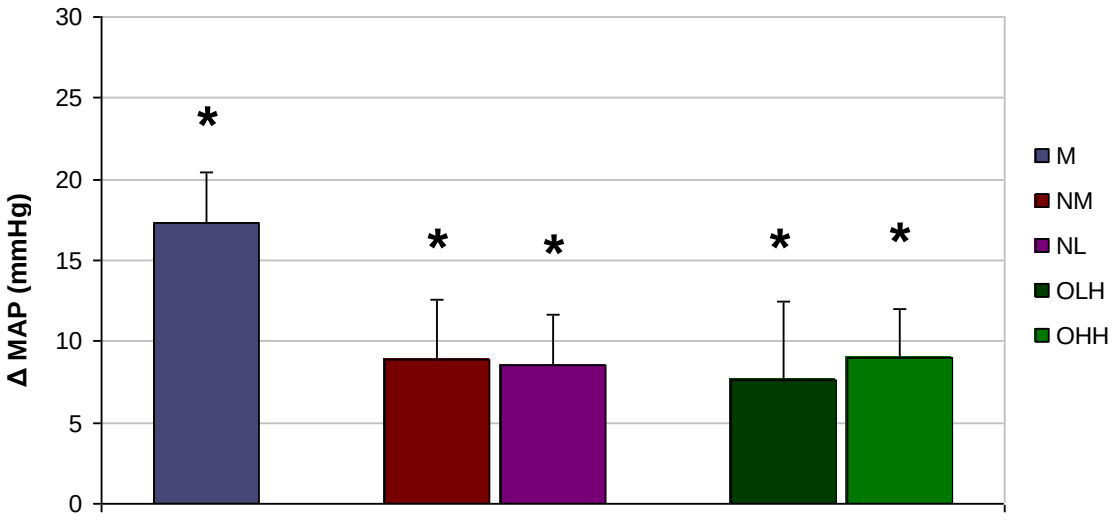


***Figure 3.14**: MAP responses to mental stress task. Figure shows mean ± SEM peak responses for males and separate female groups. * = $p < 0.05$ vs. control distension value.*

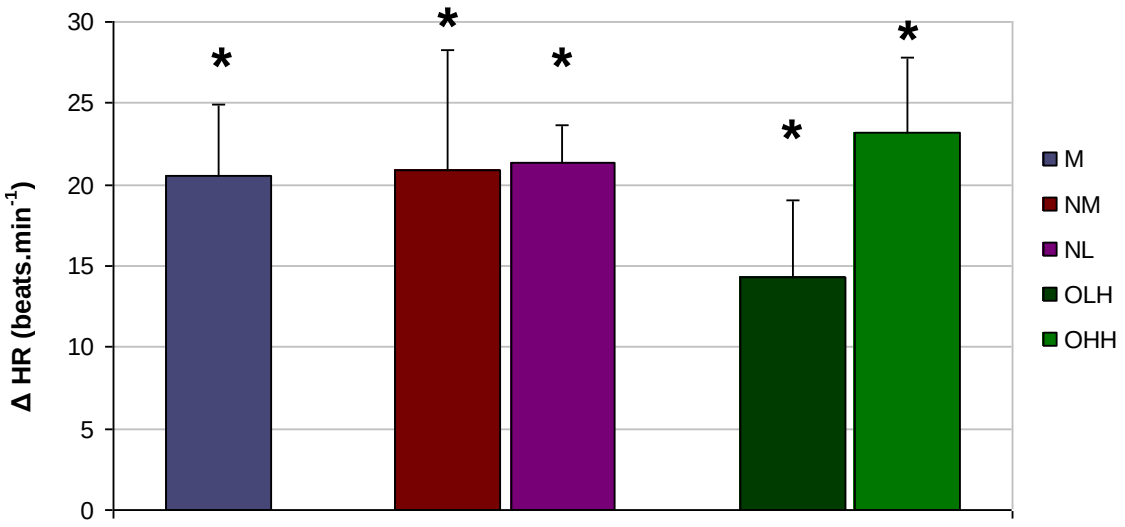


***Figure 3.15**: HR responses to MST intervention. Figure shows mean ± SEM peak responses for males and separate female groups. * = p < 0.05 vs. control distension value.*

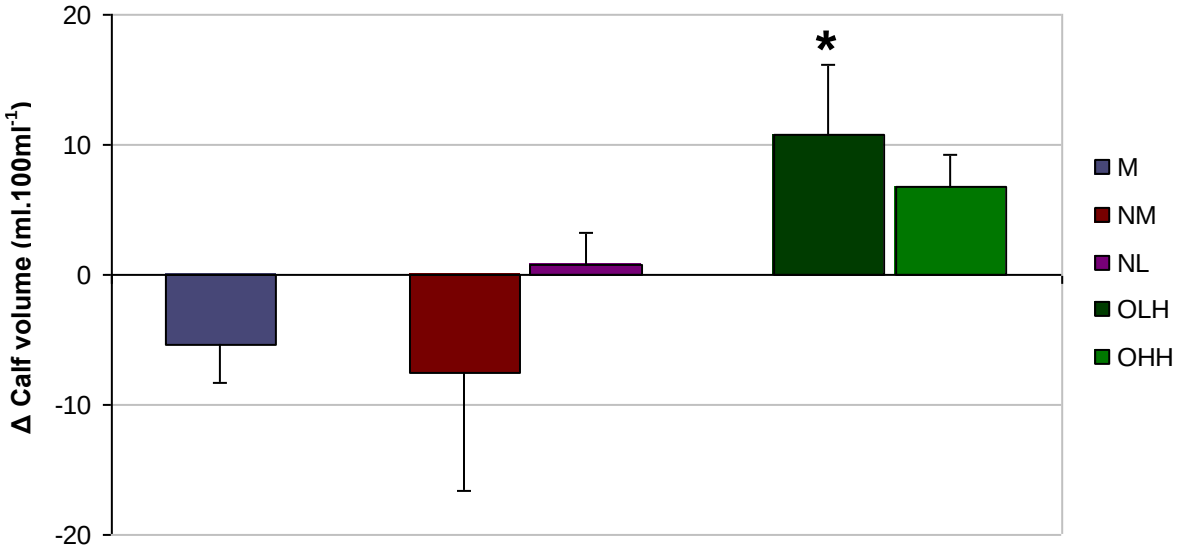


***Figure 3.16**: Calf limb volume responses to MST intervention. Figure shows mean ± SEM volume changes expressed as change relative to CON value. * = p < 0.01 for MST vs. CON.*

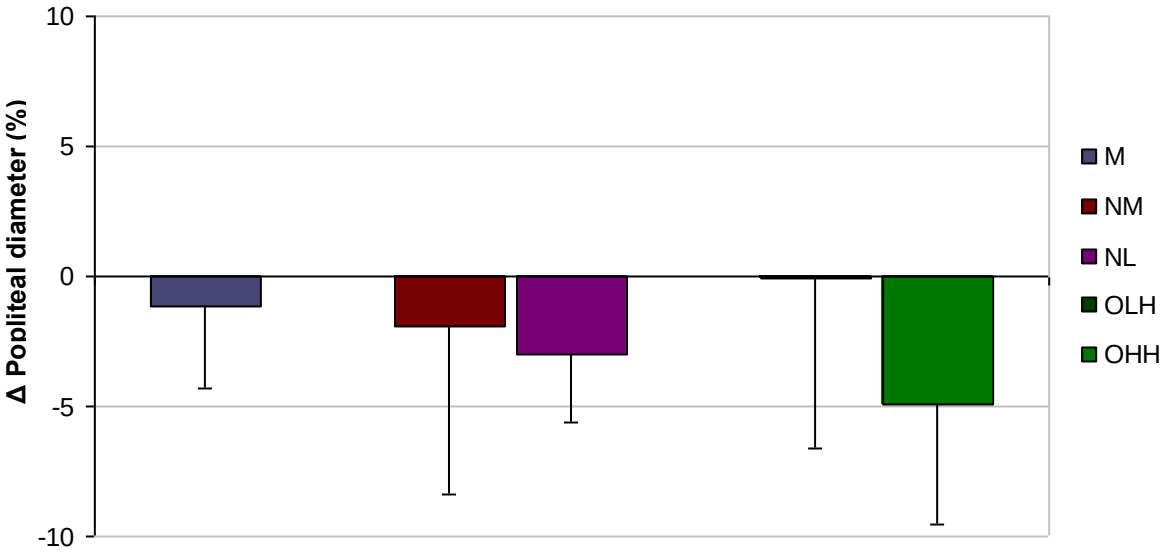


***Figure 3.17**: Popliteal vein responses to MST intervention. Figure shows mean ± SEM % diameter changes expressed as change relative to CON value. There were no significant diameter changes in any group.*

3.4 Discussion

This study tested the hypothesis that isometric exercise would induce greater leg venoconstriction than a mental stress test by examining calf volume changes (equilibration technique) and popliteal vein ultrasound diameter. Responses were compared in males and in women with low and high levels of endogenous (normal menstrual cycles) or exogenous (oral contraceptive) female hormones. In response to exercise, very modest decreases in calf venous volume were observed only in male subjects whereas females showed inconsistent changes unlikely to represent increases in venous tone and these were unaffected by menstrual cycle phase or oral contraceptive status. Mental stress likewise tended to decrease venous volume in men but not women. The only group to show differences in calf volume response to the two interventions was women taking oral contraceptives who showed no venoconstriction to exercise but a venodilation to mental stress ($p < 0.01$). The popliteal vein did not alter in diameter during either intervention or during the control distension.

3.4.1 Calf volume changes and filling during control venous distension

Filling of the calf venous vasculature during cuff inflation was similar for men and women and between women of differing hormonal status either endogenous (menstrual cycle phase) or exogenous (oral contraceptive). As in the previous chapter, there were no differences in venous capacity but this study also compared separate groups of women across the menstrual cycle with and without oral contraceptives. Arterial inflow rates were the same for

all groups and the similar capacity occurred despite men being taller and having less body fat and larger calf size than all the females. Although this finding contrasts with literature reports of gender differences, menstrual cycle phase and oral contraceptive use differences in venous distensibility (Monahan & Ray, 2003, Meendering *et al.* 2005), it is consistent with observations made in the previous chapter and may be related to the relatively high levels of fitness in the subject cohort studied. All subjects in this present study were examined for current levels of activity using the IPAQ (self-reported, short form). The results from this showed that all participants were found to be medium-high levels of activity. The similarity in resting mean arterial pressures and heart rates between groups (Table 3.2) confirms homogeneity of the groups.

The capacity and fluid filtration values presented in the results section of this chapter are approximately twice those reported in the previous chapter. This is most likely due to the positioning of the subjects for testing. In the previous study, subjects lay on a couch with their legs raised slightly above heart level, whereas in this present study, subjects lay in a semi-recumbent position. Although in this present study the subjects' calf limbs were still raised above heart level, the pelvic region was also positioned well below the level of the heart, which may have caused greater lower limb emptying at the onset, hence allowing for more filling. Although venous capacity measured 3 min after cuff inflation did not differ, there was a tendency for women taking oral contraceptives to have higher calf filtration values than women with normal menstrual cycles and not using oral contraceptives. O group values were about 40% higher than N group values in each of the cycle phases although this was not significant. In Chapter 2, the female group comprised mainly women using oral contraceptives and so the study did not compare between usage and non-usage. Female reproductive hormones have often been associated with induction of fluid filtration and tissue oedema but the evidence for this is not clear. Tollan *et al.* (1992) found increased capillary permeability for plasma proteins in the leg after six months oral contraceptive use associated with increases in plasma volume and foot swelling. In contrast, Gooding *et al.* (2005) saw no difference in calf filtration in post-menopausal women after six months hormone replacement therapy. Stachenfeld & Taylor (2007) investigated the impact of hormones on oedema formation by suppressing endogenous production with a gonadotrophin-releasing-hormone antagonist followed by controlled replacement of oestrogen and progesterone. They measured forearm Starling forces (hydrostatic and colloid oncotic pressure) and capillary filtration by plethysmography during venous occlusion periods of 7 min, reporting a lower rate of transcapillary albumin escape but no difference in fluid filtration rate. They suggested an increase in forearm blood flow via the dilator action of oestrogen could balance the reduced protein movement to leave filtration unchanged. There was no evidence of higher calf blood flow with oral contraceptive use in the present study and findings are more supportive of an

increase in fluid with oral contraceptive use.

In the present study, the popliteal vein was imaged as opposed to the long saphenous vein in Chapter 2. This vein is the main deep vein draining the lower segment of the leg and was larger in size than the long saphenous vein. In males, filled popliteal vein cross sectional area is calculated as 0.67 cm^2 which is not dissimilar to 0.83 cm^2 observed by Hitos *et al.* (2007) in young males and females in the sitting position; this would induce higher pressures in the leg than the venous occlusion used currently. Vein diameters measured when filled were also similar to those recorded by Van Diujnhioven *et al.* (2008) during venous occlusion at 60 mmHg in males aged ~ 34 years. Vein size tended to increase with leg size, being larger in men than women. However, comparing average vein diameter during the last minute of the control distension with baseline diameter shows that percent increases were similar in males (72 ± 18 %) and females (73 ± 13%) and were slightly larger than for the long saphenous vein (~ 60%). Similar relative increases in vein diameter were seen in women with and without oral contraceptives and between phases, confirming a lack of effect of female hormones on vein distensibility and diameter. Popliteal vein filling appeared to reach a plateau at a similar time to the long saphenous vein (~ 3 min; compare fig 3.6 with figure 2.10). As with the long saphenous vein, there was no evidence of hysteresis or stress relaxation in this deep vein indicating that when venous distension is prolonged beyond the time required for capacitance vessel filling, the increase in calf volume beyond this time is due to fluid filtration and / or small vein viscoelasticity.

3.4.2 Sympathoexcitation and cardiovascular responses

Heart rate rises to isometric leg exercise performed for 2 min at 40% MVC were similar for all groups (~ 15-20 $beats.min^{-1}$) with no gender, cycle phase or hormonal status differences. Exercise led to significant increases in MAP that were comparable in males and females (both N and O groups in their menstrual cycle phase). While this does not indicate a gender difference, Hogarth *et al.* (2007) found smaller increases in blood pressure to isometric handgrip in middle-aged women than men despite similar rises in MSNA. Since some of the women were post-menopausal and the cycle phase of testing was not specified in this study, it may be an effect particular to this age group. Other studies have shown that vasoconstrictor responses in the forearm to adrenergic agonists are less in women than men (Freedman *et al.* 1987, Kneale *et al.* 1997, 2000) but the similar gender rises in MAP in the current study suggest that either this was not the case for these young healthy subjects or that peripheral vasoconstriction occurred in other vascular beds to balance this out (Dishman *et al.* 2003).

In the luteal cycle phase, MAP increases to exercise were not significant for N or O females

indicating slight attenuation of peripheral vasoconstriction. However, Freedman & Girgis (2000) reported greater finger vasoconstriction to adrenergic agonists in the luteal compared to follicular cycle phase in women, and Chan *et al.* (2001) found bigger forearm vasoconstriction to noradrenaline in mid-cycle than in follicular. The attenuated MAP rise is therefore unlikely to be related to vascular reactivity in the extremities. Although it has been shown that the calf has a bigger arterial constrictor response to adrenergic activation than the forearm (Pawelczyk & Levine, 2002), there is no information about the effects of gender or hormonal status on lower limb reactivity.

Mental stress produced consistent increases in MAP of ~ 10 mmHg in all groups, and peak heart rate rises of 10-20 beats.min^{-1} were also similar in all groups. Dishman *et al.* (2003) also reported similar blood pressure and heart rate rises and calf vascular resistance decreases in men and women, independent of their level of fitness. In comparison with isometric exercise, mental stress resulted in very similar cardiovascular responses indicative of sympathoexcitation.

3.4.3 Venoconstriction to isometric exercise and mental stress

Alterations in venomotor tone during sympathoexcitation were inferred from filled calf volume changes during 2 minutes of intervention compared to the same 2 minutes during a control venous distension, during which time volume continued to increase due to fluid filtration. The control distension always carried out first to avoid persistence of any effects of the interventions although exercise and mental stress were then applied in random order. The application of three venous distensions (control, ILE, MST) each lasting 6 minutes could potentially cause disturbance to calf volume through fluid filtration but venous capacity measured during the consecutive distensions did not differ significantly in any group, and care was taken to allow time for reestablishment of calf volume baseline between distension.

Decreases in calf venous volume during isometric leg exercise or mental stress were extremely variable but where they occurred, they did so gradually to reach maximum by the end of the 2 minute intervention (see Figure 3.4). In the male group, 6 of the 7 subjects showed either a decrease or a smaller increase in volume during the ILE intervention (average approx -2%) whereas volume increased over the same time period during the control distension (average + 6%). Due to variability in response, the difference between conditions was not significant. Also in this group, 5 of the 7 subjects showed a smaller increase in volume (+1%) during mental stress than during the control distension (+6%), again not significant. Whilst these data suggest that men exhibit a degree of venoconstriction to both interventions, the changes are modest despite clear evidence, through blood pressure and heart rate rises, of sympathetic activation. For isometric exercise, compared to

the 12% reduction in calf volume observed by Seaman *et al.* (1973) during a 40% handgrip MVC performed for 2 min, the current decrease is minimal, but it is more similar to the 1% decrease in calf volume measured using impedance during 2 min of handgrip by Stewart *et al.* (2007). On this evidence, the conclusion is that calf venoconstriction during sympathoexcitation in males is modest at best.

This is borne out by the absence of any significant changes in diameter of the popliteal vein during either intervention. It had been expected that the popliteal vein imaged below the knee would show constriction during sympathoexcitation. Webb & Peplow (1968) showed that in the dog, vein segments below the knee constricted to sympathetic nerve stimulation or application of noradrenaline whereas above the knee they were unresponsive. There is a lack of clear cut histological evidence of sympathetic innervation in the human popliteal vein, and when Delaney *et al.* (2008) evoked sympathetic activation by a cold pressor test, they did not observe any shift up or down in the diameter-volume curve for the popliteal vein imaged by ultrasound, implying lack of change in venous tone. This suggests that this vessel is a passive conduit that does not respond to sympathetic activation.

The reductions in calf volume measured plethysmographically during exercise or mental stress are likely to have arisen through an increase in venomotor tone in different vessels than the large conduit popliteal vein. These could either be the more superficial veins associated with skin and subcutaneous tissue since Zelis & Mason (1969) showed these to be more reactive than deep muscle veins, or smaller venous vessels. It had been anticipated, because of the different patterns of sympathetic outflow in terms of neural activity and circulating catecholamines, that exercise would evoke arterial vasoconstriction and mental stress no change or a decrease in arterial resistance in the calf. It is not possible to comment on whether such changes actually occurred because no measurement of arterial inflow or vascular resistance was obtained in this study. This was because it proved difficult to manage logistically an additional plethysmographic measurement of blood flow since the contralateral leg was engaged in performing the isometric contraction, and it would not have been possible to use ultrasound to measure popliteal arterial flow as well as imaging the vein during the interventions. Whilst this leaves open the question of how arterial resistance actually behaved during the interventions, the fact that both exercise and mental stress tended to reduce calf volume in male subjects could be taken to indicate that venous volume changes were independent of passive blood flow-related effects.

3.4.4 Gender

The volume changes of the calf in females during either isometric exercise or mental stress were even more variable than for males. In women with normal cycles (N), the average

change in calf volume was -0.1% during isometric exercise and +5 % during mental stress compared to the control distension increase of +8-10%, but the standard deviations were more than double these values for the interventions. Although a great deal of the variability in response is likely due to movement artifact whilst performing the interventions (see Limitations below), the overall impression is that women showed even less venoconstriction than the males, particularly during mental stress. This would be consistent with the evidence that even though rises in MSNA and circulating catecholamines may be similar, women show attenuated arterial vasoconstriction during sympathoexcitation, an effect attributed to the arterial dilator effects of female hormones oestrogen and progesterone. On that basis, it would be expected that normally menstruating women show even greater attenuation of vascular reactivity in the luteal compared to menstrual cycle phase when hormone levels are considerably higher. Whereas this was true with respect to total peripheral resistance since blood pressure rises were attenuated, it was not manifest in calf volume changes which were similar between phases. Thus, hormonal effects on vascular reactivity may vary in different vascular beds.

The situation in women taking oral contraceptives was somewhat different. Average calf volume increases during isometric exercise were +14%, similar to those during control distension for this group (+14%) but greater than those in N group (-0.1%, $p<0.1>0.05$). During mental stress, calf volume in the O women increased by 22% on average, significantly more than in the N women (+5.4%, $p<0.05$). Thus, the effect of oral contraceptives was to enhance slightly the calf volume response to control venous distension (same filled venous capacity but higher filtration) and to result in further increases in venous volume during exercise and mental stress. This could be interpreted as a venodilator effect during sympathoexcitation. Alternately, venous volume could have been increased passively by arterial dilation during mental stress although this is not a consistently reported effect in the calf (Rusch *et al.* 1981). It is difficult to establish why exogenous female hormones in the form of oral contraceptives rather than endogenous hormone levels fluctuating throughout the normal menstrual cycle should modify vascular function. Chronic use of oral contraceptives has been shown to increased basal production of the endothelial dilator nitric oxide in the forearm (John *et al.* 2000) but whether this extends to the venous circulation is unknown.

The popliteal vein in women did not demonstrate any changes in tone with either intervention in agreement with a role as a passive conduit. In the women taking oral contraceptives, the discrepancy between calf volume increase (indicating venodilation) and lack of large vein response (no diameter change) to mental stress reiterates that limb volume changes must derive from smaller capacitance vessels once the large veins are filled.

3.4.5 Limitations

In addition to those limitations highlighted in Chapter 2 in relation to ambient / body temperature, hormonal status and VIA software issues, the following further limitations to this present study are acknowledged:

The obvious limitation to this present study is the small numbers in each group (n = 7), resulting in the error bars observed for each group being fairly large. A number of factors resulted in this low n, chiefly with regard to the female subjects and being able to recruit these subjects for laboratory testing within the "window of opportunity" as far as their respective menstrual cycle days were concerned and also fitting this in within the time constraints of the project itself. Although subject numbers are low within the groups, the findings presented for this Chapter do give a clear indication of trends in the whole limb and in the popliteal vein during sympathoexcitation.

As discussed in the previous Chapter, holding the ultrasound probe by hand rather than using a fixing device may present some issues with measurement accuracy in regard to vessel compression and possible movement from the point of interest. However, the hand-held method was still deemed favourable to the fixture method for this present study due to the advantages it presents in ensuring image quality (as discussed in the previous Chapter) and allowing the user to make fine adjustments in response to tissue movement, which was a particular necessity during the isometric leg exercise intervention where subjects occasionally moved slightly due to the force created against the footplate. This movement was kept to a minimum by fastening the subjects to the Biodex dynamometer with the in-built seatbelts and various Velcro fastenings situated at various points on the dynamometer.

For some subjects during isometric leg exercise, attempting to maintain the correct level of force prevented them from keeping the measurement limb still and relaxed, thus affecting plethysmograph recording, which resulted in some inaccurate data. Trying to maintain the desired 40% MVC became harder as fatigue set in and some data may be as a result of a deviation from this, contributing to variability. To counter this, data was visually inspected before analysis and it was decided that any obvious outliers would be removed from the data set. In reality, very little data were removed and the process of averaging the data over 10 s segments also served to filter and smooth outliers.

As mentioned earlier in the chapter, the positioning of the subjects in the semi-recumbent position on the Biodex dynamometer may have resulted in a larger amount of blood pooling in the pelvic region than in the previous Chapter where the subjects were position in the

supine position. This difference in position is likely to account for the observed larger values for variables such as capacity in this study compared to the last.

As in the previous Chapter, the female subjects were tested during the menstrual and luteal / high hormone phases of their respective cycles and these phases were established by self report questionnaire (Appendix C) only, which may contribute to some inaccuracy. As before, a more precise method for establishing the individuals' hormone levels would have been to use an ovulation predictor kit or to have taken blood hormone samples. However, once again, financial constraints prevented this.

3.4.6 Conclusions

Sympathoexcitation by isometric leg exercise or a mental stress test resulted in small reductions in venous vascular volume in men but not women. The popliteal vein was unresponsive to both interventions. Oral contraceptive use appeared to modify the calf volume response to mental stress indicating either venodilation or enhanced arterial vasodilation leading to a passive increase in venous volume. Because the neural and catecholamine sympathetic output is variable for these interventions a more robust autonomic activation, the cold pressor test, was used in the next chapter.

Chapter 4:

COMPARISON OF UPPER AND LOWER WHOLE LIMB VENOCONSTRICTION RESPONSES TO COLD PRESSOR TEST IN MEN AND WOMEN

4.1 Introduction

The previous chapter sought evidence of leg venoconstriction derived either from changes in calf venous volume or ultrasound observation of the popliteal vein in response to isometric exercise or a mental stress task. Both interventions raised heart rate and mean arterial pressure but they evoke different patterns of sympathoexcitation – exercise leads to increases in MSNA and vasoconstriction in the calf, while mental stress leads to increases in MSNA but not necessarily vasoconstriction in the calf, and greater levels of adrenaline than with exercise. With these contrasting effects on arterial resistance any venoconstriction that occurred would a) be independent of passive effects and b) potentially greater with exercise than with mental stress because of the different neuroendocrine responses. There were, however, only small increases in venomotor tone (assessed from calf volume) in men to these interventions and no significant change in women. The popliteal vein was unresponsive in all groups. Since autonomic activation follows different patterns during the two stressors, and it is not possible to determine which efferent stimuli leg veins might have received in greater intensity. In this present study, a further excitatory challenge to induce venoconstriction – the cold pressor test - was utilized. Gender and female hormone-associated differences in responses were again studied. Additionally, arm responses were also studied for comparison to the legs.

The cold pressor test (CPT) was one of the first procedures used to elicit a cardiovascular response under standardised laboratory conditions. It is commonly applied to examine autonomic reactivity in individuals with suspected cardiovascular dysfunction (Hilz & Dutsch, 2006, Freeman, 2006). The CPT is usually performed by immersion of either the hand or foot into an ice bath (typically ~ 4°C) or by application of a cold pack to the forehead. In the case of hand or foot immersion, measurements are usually made on the contralateral limb due to the movement involved in the act of immersion. Typically, studies have exposed their subjects to CPT for several minutes duration. The cold stimulates temperature and pain afferents from the skin, leading to activation of the central nervous system – brainstem, hypothalamus (Petrovic *et al.* 2004) and cortex (Chang *et al.* 2002). Sympathetic outflow induces increases in heart rate, mean arterial pressure and vascular resistance.

4.1.1 Blood pressure and heart rate responses to the cold pressor test

The average blood pressure and heart rate rises to a CPT in healthy subjects have been reported as 20 mmHg and 10 beats.min^{-1} respectively (Robertson, 1981, cited in Hilz & Dutsch, 2006). On the other hand, other studies have reported more modest cardiovascular responses with increases of -16 to + 13 mmHg and -8 to + 11 beats.min^{-1} respectively in healthy individuals (Fasano *et al.* 2006). Some of the variation relates to the nature of the cold stimulus and its duration of application (Mitchell *et al.* 2004), but if used under

standardised conditions, the test shows good short-term stability and little variation on test-retest (Durel *et al.* 1993, Saab *et al.* 1993, Fasano *et al.* 1996). LeBlanc *et al.* (1979) compared the time course of blood pressure and heart rate changes to a CPT and a mental stress task. Systolic pressure rises were similar and reached maximum after 1 min in both tests whereas diastolic pressure increased 15-20 mmHg within 1 min during the CPT but only 5-10 mmHg after 2 min with mental stress. By contrast, heart rate rose less with the CPT than with mental stress.

4.1.2 Sympathetic activation in response to the cold pressor test

Victor *et al.* (1987) made microneurographic measurements of muscle sympathetic nerve activity (MSNA) in the peroneal nerve in 25 subjects (21 male and 4 female) during 2 minutes of hand immersion in iced water. Total MSNA did not increase in the first 30 s of immersion but rose by 60% (from 230 ± 27 AU to 386 ± 52 AU) by the end of the first minute and by 150% (to 574 ± 73 units) during the second minute. MAP and HR both significantly increased (20 ± 2 mmHg for MAP and 7 ± 3 beats.min^{-1} for HR) and there was a significant positive correlation between MAP and total MSNA increases from rest to the second minute but no correlation between HR and MSNA increases. Others have confirmed a relatively rapid increase in MSNA (within 30 sec) during a CPT (Cui *et al.* 2002). Further evidence of a relationship between sympathetic activity and the rise in arterial pressure was shown by Dishman *et al.* (2003). They exposed 28 subjects (14 male and 14 female) to 2°C hand immersion for 2 minutes. Blood pressure (+ 24 ± 3 mmHg SBP and + 21 ± 2 mmHg DBP, both $p < 0.001$) and peroneal nerve MSNA (+ 34.6 ± 2.4 a.u., $p < 0.001$) increased significantly during CPT, and changes in MSNA were correlated significantly with changes in systolic and diastolic blood pressures.

Increases in circulating catecholamines to a CPT are also evidence of sympathetic activation. Victor *et al.* (1987) measured plasma noradrenaline in 10 of their subjects and showed a small but significant increase during the second minute of CPT (~ 25 pg.ml^{-1}) and a positive correlation between the peak changes in MSNA and plasma noradrenaline. LeBlanc *et al.* (1979) compared responses to the CPT and to mental stress (MST) and reported increases in both plasma adrenaline (+ 20 pg.ml^{-1} CPT and + 105 pg.ml^{-1} MST) and noradrenaline (+ 75 pg.ml^{-1} for both). The increase in adrenaline during CPT was significantly lower compared to mental stress but the noradrenaline increases were similar. These differences indicated greater medullary stimulation during mental stress than during CPT (Herd, 1991). Similarly, Ward *et al.* (1983) also found greater adrenaline increases in the forearm during mental stress (+ 109 pg.ml^{-1}) than during CPT (+ 38 pg.ml^{-1}) in their 8 male subjects. The increase during CPT was almost identical to that during knee exercise (+ 38 pg.ml^{-1}) but higher than during isometric handgrip exercise (+ 12 pg.ml^{-1}). All of these

increases were significantly different compared to baseline adrenaline levels ($p < 0.05$). The noradrenaline increases during CPT (+ 155 $pg.ml^{-1}$) were significantly greater than during mental stress (+ 82 $pg.ml^{-1}$), knee exercise (+ 71 $pg.ml^{-1}$) or isometric handgrip (+ 29 $pg.ml^{-1}$).

There is thus unequivocal evidence of elevated sympathetic neuroendocrine activity during the CPT which is associated with the rise in blood pressure. Despite the correlation between MSNA and noradrenaline spillover as indices of sympathetic activation (Wallin & Charkoudian, 2007), Jacob *et al.* (2000) found greater increases in noradrenaline during a CPT in the arm (+47 ± 15 %) than in the leg (+18 ± 6 %). The potential for differential vasoconstrictor effects in upper and lower limbs is apparent in measures of vascular resistance.

4.1.3 Peripheral vascular resistance responses to the cold pressor test

Sympathoexcitation with the CPT is associated with significant peripheral vasoconstriction in coronary (Momen *et al.* 2009), splanchnic (Chaudhuri *et al.* 1991) and cutaneous (Low *et al.* 1983, Feger & Braune, 2005) circulations. Reduced blood flow and increased vascular resistance are also evident in the forearm (Jacob *et al.* 2000, Thijssen *et al.* 2006) together with radial artery constriction (Perret *et al.* 1989). In the leg, however, vasoconstriction is not a consistent finding. Zbrozyna & Westwood (1983) reported that calf conductance was increased by immersion of the contralateral foot in iced water for 1 min and no change in calf vascular resistance was found by Jacob *et al.* (2000) and Dishman *et al.* (1993) despite clear evidence of sympathoexcitation (rises in MAP, HR, MSNA). On the other hand, Thijssen *et al.* (2006) saw an increase in calf vascular resistance of 130% that matched that of the forearm (211%) during a 5 minute immersion of the hand in iced water in young men.

These findings support a pattern of resistance vessel reactivity in arms and legs during reflex sympathoexcitation that emerged from studying the data on forearm and calf vascular resistance during isometric exercise and mental stress tasks. Clear cut vasoconstriction (exercise, CPT) or vasodilation (mental stress) are reported for the forearm whereas for the calf, such responses were attenuated or absent despite the similarity of MSNA traffic in the upper and lower limbs. The implication is that transduction of the efferent signals into arterial vascular responses differs between the limbs. This may have implications for capacitance vessel responses to the CPT, the evidence for which will now be reviewed.

4.1.4 Evidence for venoconstriction in response to the cold pressor test

Early studies of changes in venomotor tone in response to the CPT used the isolated vein segment or occluded limb technique. Page *et al.* (1955) measured pressure within a suitably long segment of forearm vein without communicating vessels that was temporarily isolated

from the circulation via rubber-ended clamps at each end. In 15 young healthy male subjects, the CPT increased venous pressure by 8.9 ± 4.7 mmHg without change in central venous pressure. Samueloff *et al.* (1966b) used the occluded limb technique to study pressure changes in hand, foot, forearm and calf veins during various stimuli. The venous circulation was partially arrested by cuff inflation proximal to the pressure measurement site to ~ 32 mmHg to allow filling and distension before complete arrest of the circulation was achieved (~300 mmHg) for 8 – 15 minutes. Immersion of one foot in iced water led to a rise in forearm vein pressure of 12 mmHg while radial artery pressure in the contralateral arm increased by only 1 mmHg. This was taken to indicate that the venous pressure rise was due to capacitance vessel constriction and not secondary to pre-capillary constriction. Rowell & Blackmon (1988) also studied males aged 24-32 years and observed rises in vein pressure of between 3 and 20 mmHg in the occluded forearm that peaked within 30 seconds of immersion of the contralateral hand in iced water.

Zelis & Mason (1969) used the equilibration method to study forearm venomotor tone during the application of ice to the forehead in healthy male subjects and showed that venous vascular volume decreased by 13.2%. However, in the forearm in which skin blood flow had been eliminated by adrenaline iontophoresis, venous volume was reduced by 40% compared to the untreated forearm and there was no longer any reflex venoconstriction to the CPT. Venomotor reactivity was therefore considered to be restricted to the cutaneous capacitance circulation. That superficial veins react to the CPT by constriction was confirmed by Dzeka & Arnold (2003) who observed decreases in diameter of the dorsal hand vein of 5-10% during application of ice to the forehead for 2 minute in normal healthy older individuals (62 ± 4 years). The diameter decrease was significantly greater after indomethacin was infused into the vein, leading the authors to conclude that vasodilator prostaglandins which normally attenuated venoconstriction had been eliminated.

While these studies provide definitive evidence of cold pressor-induced venoconstriction predominantly in superficial veins, they are restricted to examination of the forearm, and there has been no attempt to investigate gender differences in vein responses. Moreover, the sympathoexcitatory effects of the CPT are more stable than those of isometric exercise and mental stress since increases in neural activity and vasoconstriction are more consistently reported, albeit with some inter-individual variability (Fasano *et al.* 1996, Wirch *et al.* 2006).

4.1.5 Effects of gender and female hormones on response to the cold pressor test

With regard to blood pressure, heart rate, MSNA and forearm constrictor responses to the CPT, there are reports indicating no difference between the genders. Jones *et al.* (1996) tested young males and females during 3 min hand immersion in iced water and recorded

similar increases in systolic and diastolic blood pressures, heart rate, MSNA and plasma catecholamines in the two groups. The female group of 17 included women tested in either follicular or luteal cycle phase and 4 who were taking an oral contraceptive. Likewise, Lischauer *et al.* (1998) saw increases of ~10 mmHg in mean arterial pressure with little change in heart rate, and elevations in plasma noradrenaline of 10-15% in response to a cold fact test (ice to the forehead for 2 minute) in young men and in women with normal cycles tested both in their follicular and luteal phases. Dishman *et al.* (1993) compared young men of average fitness levels, assessed by maximal oxygen uptake, with similarly fit non-oral contraceptive using women tested in their follicular phase. The CPT (immersion of one hand into iced water for 2 min) elicited comparable increases in both genders in mean arterial pressure (~ +25 mmHg), heart rate (~ +10-20 beats.min^{-1}) and MSNA activity (~2-fold increase in bursts.min^{-1}). In addition, calf blood flow decreased to the same extent in men (-19%) and women (-22%) such that vascular resistance was unchanged. In contrast, Hogarth *et al.* (2007) compared the responses of 34 men and 34 women to CPT (4°C hand immersion) for 1 minute and found the increase in calf vascular resistance to be significantly smaller in women (+88% males vs.+46% females), whereas increases in MAP (+20% and +22%, men and women respectively), HR (+8% and +10%) and MSNA (+53% and +86%) were similar in both groups. Overall, the authors concluded that the vasoconstrictor effect of the peroneal nerve activity was attenuated in women compared to men during CPT. It should be noted that the subjects in this study were all middle-aged and almost a third of the females were post-menopausal (Hogarth *et al.* 2007).

While there appears to be little difference between the genders in response to a CPT, the same can also be said with regard to the low and high hormone phases of the menstrual cycle. Miller & Sita (1994) investigated non-OC women aged 18-39 years during their follicular and luteal cycle phases, reporting no differences in heart rate and blood pressure rises during a 2 min hand immersion CPT, confirming earlier work by Hastrup & Light (1984). Tersman *et al.* (1991), on the other hand, noted bigger rises in systolic pressure to a CPT during the luteal (+14 mmHg) compared to follicular(+9 mmHg) cycle phase in women aged 21-40 years, although this may have been confounded by inclusion of a number of women who were smokers. The lack of effect of cycle phase has also been confirmed for blood pressure, heart rate and catecholamine responses to a CPT (Litschauer *et al.* 1998). MSNA levels under resting conditions and the increase during a CPT were not different between the menstrual and early follicular cycle phases in young women (Ettinger *et al.* 1998). Other studies have found higher basal MSNA in the late luteal compared to menstrual phase in women with normal cycle (Minson *et al.* 2000a) although there was no difference in low and high hormone phases in women taking oral contraceptives (Minson *et al.* 2000b).

Overall, the evidence indicates that men and women show similar sympathoexcitation and cardiovascular response to a CPT and that, based on studies of normal menstrual cycle and oral contraceptive use, female reproductive hormones are likely to have little effect on the responses to this stressor. It is not known whether this also applies to venomotor tone.

4.1.6 Summary and Chapter aims

The cold pressor test is a sympathoexcitatory challenge that evokes similar increases in mean arterial pressure, heart rate, sympathetic neural activity and catecholamines in men and women. Forearm arterial and venous constriction are elicited but in the calf, arterial constrictor responses are not consistently found and there is no information about calf venomotor tone.

Reflex venoconstriction occurs in arms and legs (Samueloff *et al.* 1966b) whereas gender differences in vascular characteristics have most often been investigated in forearms. Forearms show less arterial adrenergic constrictor reactivity (Pawelczyk & Levine, 2002) and their venous compliance may be greater than (Halliwill *et al.* 1999), smaller than (Young *et al.* 2006) or similar to (Freeman *et al.* 2002, Risk *et al*. 2003) that of the leg. To take account of potential differences between limbs, and in view of the greater significance of leg venous function in orthostasis (Halliwill *et al.* 1998), this study will examine venoconstriction, venous capacity and compliance in both calves and forearms of men and women. It is hypothesised that:

1) **Venoconstriction to sympathetic activation by a cold pressor test will be less in women than men.**

2) **Venoconstriction may be enhanced in women exposed to high levels of exogenous female hormones via oral contraceptive use.**

3) **Changes in venomotor tone are expected to be greater in the forearm than the calf.**

4.2 Methods

4.2.1 Subjects and groups

30 subjects (10 males and 20 females) volunteered to participate in the study, which was approved by the Local Research Ethics Subcommittee, School of Sport and Exercise Sciences, University of Birmingham, UK in accordance with the declaration of Helsinki 1964. Following informed consent (Appendix A), subjects were screened by general health questionnaire (Appendix B) to ensure all participants were in good health and injury free. Exclusion criteria were: Current illness, consumption of ‘over the counter’ non-steroidal anti-

inflammatory drugs in the previous 24 hour period, cardiovascular disorders, pregnancy (either prior to or at time of study), injury to limbs (either during or at any time over 6 months prior to study) and immune, metabolic or kidney condition(s). The subjects' anthropometric characteristics are shown in table 4.1.

The female group consisted of 10 women with a normal menstrual cycle pattern who were not taking (and had not previously taken) oral contraceptives (N). Five of these women were tested in the menstrual phase (days 1 – 5) and 5 were tested in the luteal phase (days 17 – 28) of their normal cycle (day 1 was taken to be the first day of bleeding - established by questionnaire, Appendix C). The remaining 10 women were taking a "combined" oral contraceptive pill (O), which contained both a synthetic oestrogen (e.g. 30 µm ethinylestradiol) and a progestin (e.g. 150 µm levonorgestrel). As in the previous study (chapter 3), these women had been taking the combined pill for between 1 and 5 years duration (established by questionnaire) and were tested during the high hormone pill-taking phase (but between days 19 – 28). Volunteers were excluded from the study if they displayed an irregular menstrual cycle pattern i.e. markedly longer or shorter (± 3 days) than an average of 28 days or were taking a form of oral contraceptive other than a "combined" pill e.g. a progesterone only pill or the contraceptive injection.

All subjects attended one testing session. Hydration status, time of testing and the laboratory ambient temperature conditions were controlled as described previously in Chapter 2. On arrival for testing, subjects' height, weight, 4-site skinfold thicknesses and limb segment circumferential and length measurements were also made as previously described (Chapters 2 and 3).

4.2.2 General experimental setup

Subjects lay in the supine position on a laboratory couch throughout testing with all limbs supported at heart level. Whole limb measurements (see below) were made on the dominant limbs. Small inflatable cuffs (Hokanson Ltd.) of 6 cm width were placed around the ankle and wrist, a medium inflatable cuff (Hokanson Ltd.) of 13 cm was placed around the upper arm and a large inflatable cuff (Hokanson Ltd.) of 18 cm width was placed around the mid-thigh of the test limb. Mercury in silastic strain gauges (Hokanson Ltd.) - measuring 2 cm smaller than the largest limb circumference - were placed around the belly of the calf and of the forearm of the test limbs, as described in the previous chapter. Subjects were instructed to breathe at normal rhythm throughout testing and to avoid sudden gasps or deep inspirations.

***Table 4.1**: Anthropometric characteristics of subjects. Values are mean ± SD. Table shows data for male and both female groups (N and O). * = p < 0.05 vs. other 2 groups. † = p < 0.05 calf vs. forearm.*

	Males (n=10)		N (n=10)		O (n=10)	
	Mean	± SD	Mean	± SD	Mean	± SD
Age (years)	25	7	21	3	22	3
Height (cm)	178	7 *	166	8	165	6
Weight (kg)	72.4	9.5 *	63.0	11.7	62.6	8.9
Body Mass Index (kg/m^2)	23	2	23	3	23	4
Body fat (%)	12.3	3.6 *	21.6	7.8	23.5	5.6
Calf segment volume (l)	2.39	0.14 †	2.35	0.70 †	2.31	0.35 †
Forearm segment volume (l)	0.98	0.15 *	0.76	0.18	0.73	0.68

4.2.3 Measurement of variables

Beat-to-beat blood pressure, from which mean arterial pressure (MAP, calculated as diastolic pressure + 1/3 (systolic pressure – diastolic pressure) and heart rate (HR) values were derived, was detected using a Portapress photoplethysmograph incorporating height correction (Finapres medical systems, The Netherlands) placed around the left middle finger. The analogue signal from the Portapress control console was input via a Powerlab A/D converter to a PC where it was recorded at a sampling rate of 40 Hz using Chartlab software (ADInstruments Ltd.).

Blood flow, limb vascular filling, venous capacity and fluid filtration were determined using strain-gauge plethysmography. The strain gauges around the calf and forearm were connected to plethysmographs (Hokanson Inc.) and the output signals were routed to the same PC running Chartlab software via a Powerlab A/D converter. Limb volume changes were continuously recorded simultaneously with cardiovascular variables MAP and HR so that changes throughout the experiment were time-matched.

4.2.4 Resting variables

Subjects rested in the supine position for 20 minutes prior to the testing procedure. Ankle and wrist cuffs were inflated to supra-systolic pressure (> 50 mmHg above systolic pressure) to eliminate foot and hand blood flow (Williams & Lind, 1979). Following a baseline measurement period of 2 minutes during which resting MAP, HR and limb volumes were continuously recorded, the thigh and upper arm cuffs were inflated simultaneously to 50mmHg for 10 seconds to occlude the venous outflow from the limbs, thus enabling calf and forearm arterial inflow rate to be measured (Greenfield *et al.* 1963, Wilkinson & Webb 2001). Following a ~ 30 s recovery period for variables to return to baseline values, the procedure was repeated until a total of 3 calf and forearm blood flow measurements had been produced for analysis.

4.2.5 Control venous distension

A further 2 minute baseline rest period was observed following blood flow measurements. A control venous distension was then induced by simultaneous thigh and upper arm cuff inflation to 50 mmHg for 5 minutes while measurements of MAP, HR and limb volume changes were continuously recorded throughout. Values for venous capacity and fluid filtration were extracted from the filling data at specific time points as previously described and illustrated (figure 2.3) in the preceding chapters.

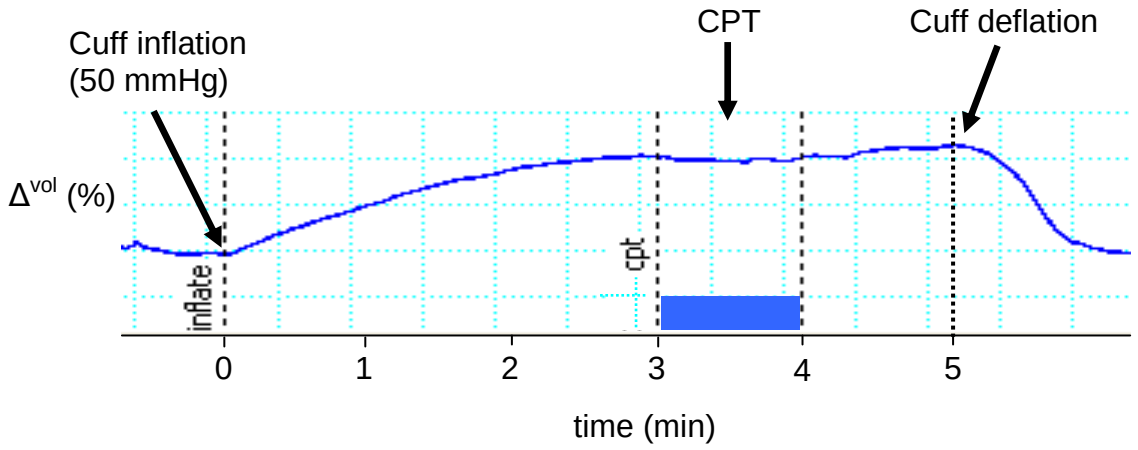


Figure 4.1*: Example of an original calf volume trace during venoconstriction distension. Figure identifies the point at which the CPT intervention (illustrated by blue shaded area) was introduced for 1 minute within the venous distension period.*

4.2.6 Sympathoexcitation by cold pressor test

A 10 minute period of rest followed the control distension. The ankle and wrist cuffs were then again inflated to supra-systolic pressure and a further 2 minutes of baseline rest data were recorded. A second venous distension was induced by simultaneous upper and lower limb cuff inflation to 50 mmHg for 5 minutes as in the control distension. To measure reflex venous constriction, sympathoexcitation was induced for 1 minute by cold pressor test (CPT) between minutes 3 and 4 of the venous distension period (figure 4.1). CPT was performed by passively lowering the subjects' left (contralateral) foot into a 4°C ice bath for 1 minute.

4.2.7 Effects of leg lowering

On a separate occasion, 4 subjects were invited back to perform two further venous distensions as described above with the exception that: upper limb volume responses were not measured and during the second intervention, although the left (contralateral) leg was passively lowered, there was no ice bath for the subject's foot to be placed into. The purpose of this protocol was to examine the effects of leg lowering *per se* to ensure that any changes observed as a direct result of the CPT intervention were not due to the partial posture change.

4.2.8 Venous compliance and hysteresis

Ankle and wrist cuffs were inflated as before following a 10 minute rest period. Venous distension was induced by a modified version of the technique used by Buckey *et al.* (1988) whereby upper limb cuffs were inflated to 20 mmHg for 2 minutes and then further inflated to 30, 40 and 50 mmHg and deflated in 10 mmHg steps at 2 minute intervals (figure 4.2). The limb volume – pressure curves generated by this procedure enabled venous compliance to

be derived from the descending arm of the relationship, and venous hysteresis, as an index of vessel wall viscoelastic creep, to be evaluated from the difference between ascending and descending curves at a pressure of 30 mmHg (Edouard *et al.* 1998).

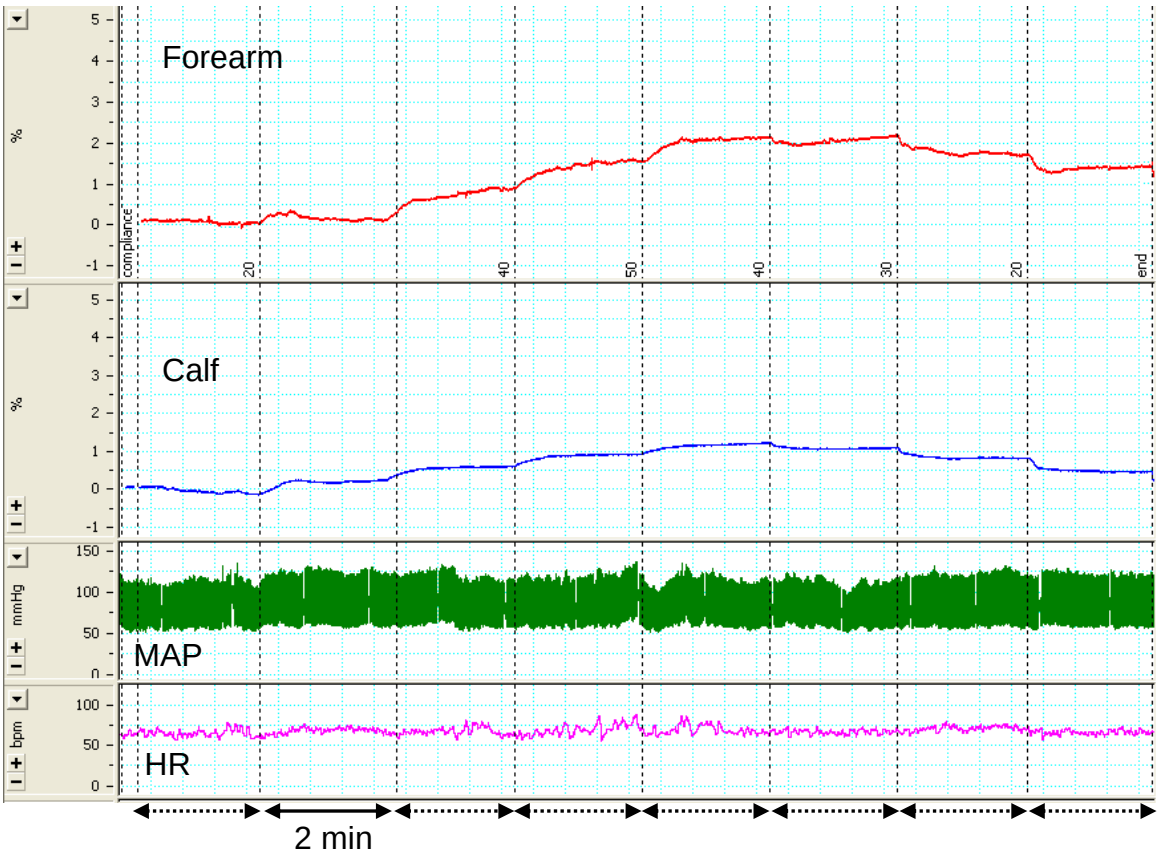


Figure 4.2*: Original chart trace segment for venous compliance and hysteresis protocol. Figure shows actual responses of variables (identified on figure) for one subject and identifies the cuff pressure (mmHg) steps within each 2 minute segment (marked by dotted lines). Calf and forearm limb volumes are observed to change linearly with the corresponding increases or decreases in cuff pressure, while MAP and HR remain unaffected.*

4.2.9 Data analyses

All measurements recorded onto the Chartlab software were initially analysed using the Microsoft Excel software package. Anthropometric data (BMI, body fat and limb segment volume) were calculated as described in chapter 2 and compared as described in chapters 2 and 3.

4.2.9.1 Sympathoexcitation by cold pressor test

Baseline values for variables, blood flows, cardiovascular variables, venous capacities and fluid filtrations were analysed as described and illustrated in Chapter 2 and figure 2.3

respectively. As described in the previous chapter, the peak change for each variable during the CPT was used for comparison with the change over the same time period during the control venous distension i.e. min 3-4. MAP and HR behaved in a similar manner to that observed during isometric exercise (figure 3.3), i.e. values increased over time and peaked towards the end of the intervention period. For detailed description of analysis methods see Chapter 3.

4.2.9.2 Effects of leg lowering

Limb volume responses to leg lowering were compared to those during venous distension without leg lowering in the same manner as for CPT vs. control venous distension.

4.2.9.3 Venous compliance and hysteresis

Venous compliance was evaluated from the relationship between limb volume and pressure during the stepped increases and decreases in cuff pressure. Compliance was calculated as the slope of the relationship between 40 and 20 mmHg on the descending (emptying) part of the pressure-volume curve via linear regression analysis. Since in this study Doppler ultrasound was not used to assess vein reactivity directly, venous visco-elastic creep was calculated as the difference between the limb volumes on the ascending and descending curves at 30 mmHg since this indicates the extent of hysteresis between filling and emptying (Edouard *et al.* 1998).

4.2.10 Statistical analyses

All statistical analyses were conducted using the Statistical Package for the Social Sciences, version 13 (SPSS, Inc., Chicago, IL). A one-sample Kolmogorov-Smirnov test was firstly performed to check for normal distribution of the data to determine the most appropriate (parametric or non-parametric) statistical analysis. The data were normally distributed and therefore the following parametric tests were applied:

Within groups, paired samples t-test was used to compare changes in variables during CPT with those during control venous distension, percentage volume changes during leg lowering with those during control distension, percentage volume changes during filling with those during emptying and calf and forearm responses.

To assess gender differences in venous function, measures were compared between men and N women by independent samples t-test (sympathoexcitation and leg lowering protocols). Previous studies have reported increased venous capacity in the luteal phase in women with normal menstrual cycles in forearm (Fawer *et al.* 1978), finger (McAusland *et al.* 1963) and calf (Meendering *et al.* 2005) but the differences were relatively small and findings

from the previous study (chapters 2 and 3) also showed very little difference in calf venous capacity between the phases. Furthermore, venous compliance has been shown to be unaffected by cycle phase (Meendering *et al.* 2005). Since changes in limb volume during the CPT in the present study were normalised to venous capacity to take account of any such variations (Stewart, 2002), and no capacity differences were observed between N women in the menstrual or luteal phase of their cycle in this study as well as the last, they were treated as a single female group. O women were all tested during their high hormone weeks and were compared against N women by separate independent samples t-test to elucidate any effects specific to long-term exposure to exogenous synthetic hormones. Data are presented as means ± S.E.M. unless otherwise stated. Differences were deemed significant if $p < 0.05$.

4.3 Results

4.3.1 Anthropometry

Male subjects were significantly taller, heavier, leaner and had significantly larger anthropometric forearm segment volumes than the females (table 4.1, $p < 0.05$). Unlike in the previous studies where calf segment volume was bigger is males than females (tables 2.2 and 3.1), the current male subjects had similar calf segment volumes to the female participants (table 4.1). Calf size was significantly larger than forearm size in all groups (table 4.1, $p < 0.05$).

4.3.2 Baseline data

Table 4.2 shows baseline values for all variables. Resting MAP was similar in all groups (M, N and O, $p > 0.05$) but HR was significantly higher in the O group compared to the M and N groups ($p = 0.01$). Resting calf and forearm blood flows were similar between groups ($p > 0.05$).

4.3.3 Control venous distension

Calf venous capacity was not significantly different between groups ($p > 0.05$, figure 4.3). Males and O females displayed significantly larger forearm venous capacity than the N females ($p = 0.01$ and $p = 0.04$ respectively, figure 4.3). Calf venous capacity was significantly smaller than forearm venous capacity in all groups ($p < 0.01$). Calf and forearm fluid filtration were similar in all groups (table 4.2, $p > 0.05$ between groups) but were lower in calf (vs. forearm) in the M and O groups.

***Table 4.2**: Resting and control values for MAP, HR and blood flows and fluid filtration of subjects. Values are mean ± SEM. Table shows data for male (M) and female groups (N and O). * = p < 0.05 O vs. other 2 groups. † = p < 0.05 calf vs. forearm.*

	Males (n=10)		N (n=10)		O (n=10)	
	Mean	± SEM	Mean	± SEM	Mean	± SEM
MAP (mmHg)	80	2	79	2	81	5
HR (beats.min^{-1})	59	2	57	3	70	4 *
Calf blood flow (ml.100ml^{-1}.min^{-1})	1.43	0.20 †	2.01	0.26	1.87	0.32
Forearm blood flow (ml.100ml^{-1}.min^{-1})	2.87	0.36	1.87	0.20	2.92	0.57
Calf fluid filtration (ml.100ml^{-1}.min^{-1}.mmHg^{-1} x 10^{-3})	1.69	0.20 †	2.52	0.60	2.00	0.36 †
Forearm fluid filtration (ml.100ml^{-1}.min^{-1}.mmHg^{-1} x10^{-3})	3.31	0.47	2.90	0.34	4.23	0.63

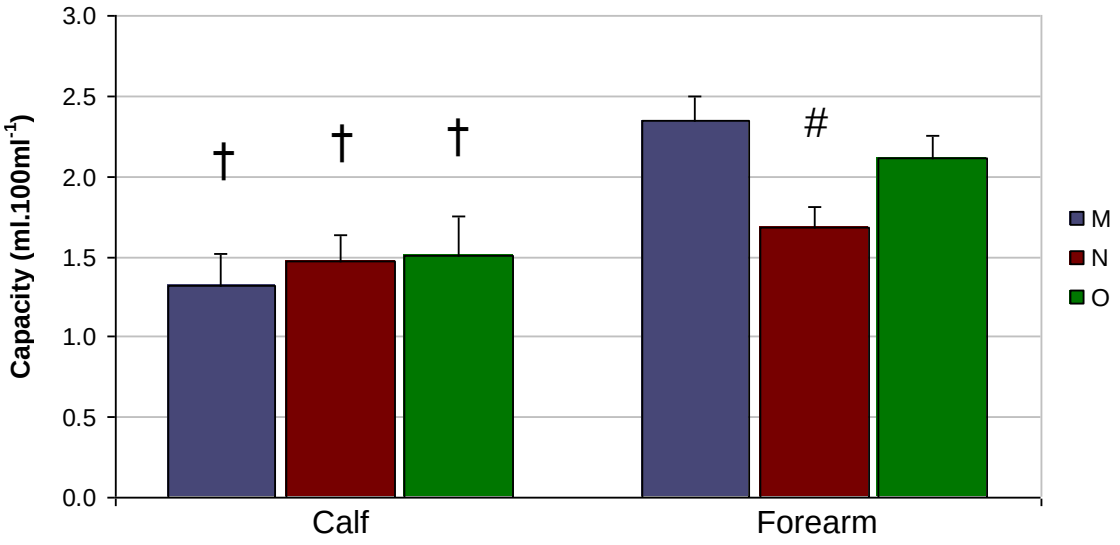


***Figure 4.3**: Calf and forearm venous capacity for male (M) and female (N and O) groups (all n = 10). Figure shows mean ± SEM for capacity (ml.100ml^{-1}) taken as the limb volume increase at 3 minutes. † = p < 0.05 calf vs. forearm. # = p < 0.05 N vs. other 2 groups for forearm.*

4.3.4 Responses to cold pressor test

Cold pressor test application induced similar significant increases in MAP and HR in all groups compared to the control distension (M = 7.7 ± 2.0 mmHg and 9.2 ± 2.5 beats.min^{-1}, N = 7.6 ± 3.2 mmHg and 7.3 ± 2.4 beats.min^{-1} and O = 5.4 ± 2.7 mmHg and 8.1 ± 1.9 beats.min^{-1} for MAP and HR respectively, all $p < 0.05$, figure 4.4). While calf limb volume significantly decreased in the M and O groups during CPT compared to the control distension (M = -5.73 ± 1.82 % and O = -4.35 ± 1.83 %, both $p < 0.05$), the decrease was not significant in the N group (-2.16 ± 2.08 %, $p > 0.05$). Males were the only group to show significant venoconstriction in the forearm (M = -7.17 ± 1.97 %, $p < 0.01$, N = -4.12 ± 2.49 %, $p > 0.05$ and O = -5.90 ± 2.81 %, $p = 0.06$). There were no significant differences in size of venoconstriction response between lower and upper limbs in any group (figure 4.5). In summary, although there were no differences between the groups, the males were the only group to show significant venoconstrictor effects for calf and forearm. The O group showed significant venoconstrictor effects for calf and nearly for forearm while the N showed no significant venoconstrictor effects at all.

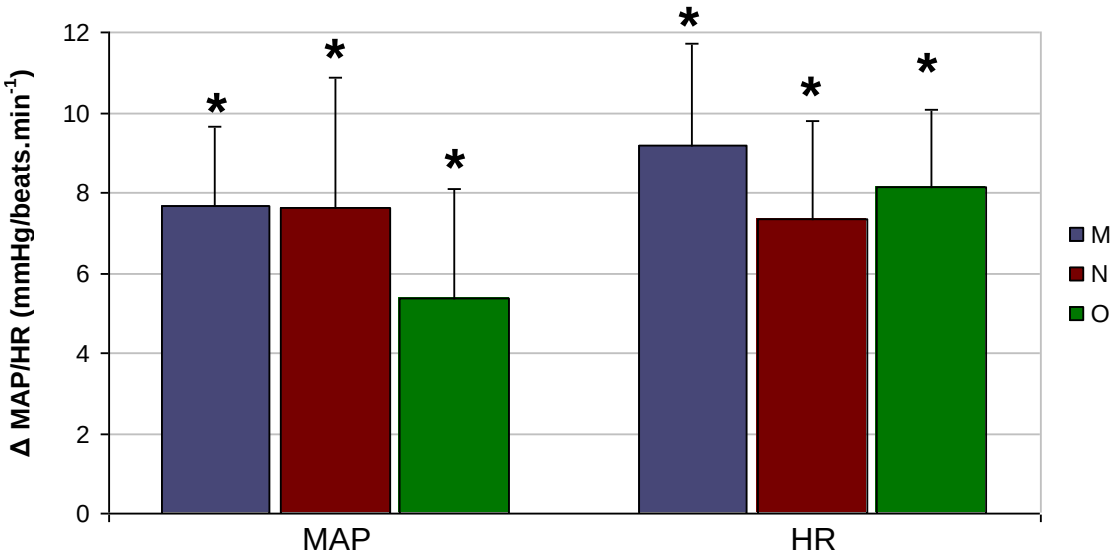


***Figure 4.4**: MAP and HR responses to cold pressor test. Figure shows mean ± SEM increases during CPT compared to the respective control distension (CON) values (represented here as 0 change). * = p < 0.05 compared to CON. There were no significant differences between groups.*

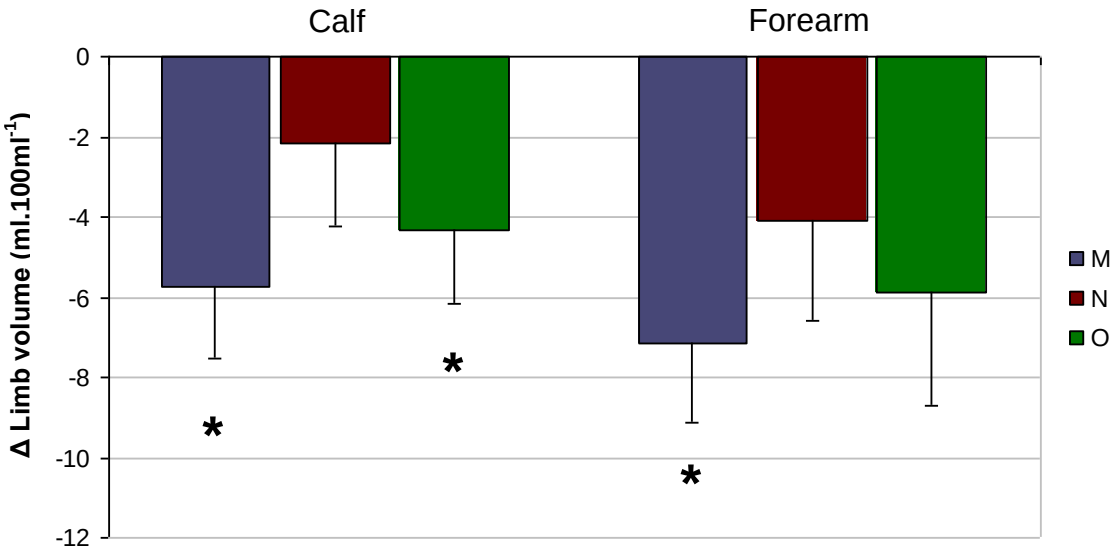


***Figure 4.5**: Limb volume responses to cold pressor test. Figure shows mean ± SEM decreases during CPT compared to the respective control distension (CON) values (represented here as 0 change). * = p < 0.05 compared to CON. There were no significant differences between groups or between limbs within groups.*

4.3.5 Effects of leg lowering

Calf volume increased 0.12 ± 0.05 ml.100ml^{-1} from the capacity value during contralateral leg lowering without immersion in iced water compared with a 0.11 ± 0.01 ml.100ml^{-1} increase from capacity during the control distension without leg lowering ($p > 0.05$).

4.3.6 Venous hysteresis and compliance

Both calf and forearm volumes at 30 mmHg were significantly different between filling and emptying phases in all groups (calves = 0.21 ± 0.04 ml.100ml^{-1}.kg^{-1}, 0.26 ± 0.03 ml.100ml^{-1}.kg^{-1} and 0.33 ± 0.06 ml.100ml^{-1}.kg^{-1}; forearms = 0.60 ± 0.13 ml.100ml^{-1}.kg^{-1} 0.47 ± 0.10 ml.100ml^{-1}.kg^{-1} and 0.69 ± 0.11 ml.100ml^{-1}.kg^{-1} for M, N and O groups respectively, all $p < 0.01$, figure 4.6). These data suggest that venous creep was greater in the forearm than in the calf for all groups. However, this only reached significance in the M and O groups ($p < 0.05$) with the N group just outside ($p = 0.07$). There were no significant differences in either calf or forearm venous creep between genders, groups or female hormone type (all $p > 0.05$).

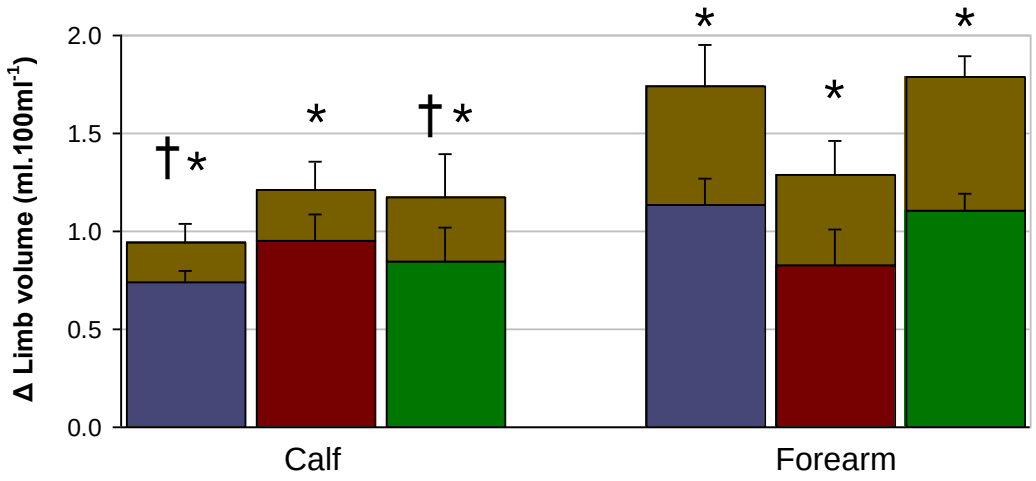


Figure 4.6*: Venous hysteresis. Figure shows the difference between filling (blue (M), red (N) and green (O) bars) and emptying (gold bars) phases mean ± SEM limb volumes at 30 mmHg.* * *= p < 0.01 filling vs. emptying.* † *= p < 0.05 calf vs. forearm. There were no significant differences between groups.*

Figure 4.7 shows venous compliance, calculated from the slope of the curve during emptying between the pressures of 40 – 20 mmHg, for all groups. Compliance was 1.5 ± 0.1 (M), 1.6 ± 0.4 (N) and 1.9 ± 0.3 (O) ml.100ml^{-1}.mmHg^{-1} x 10^{-3} for calves and 3.6 ± 0.6 (M), (N) 3.0 ± 0.4 and 3.5 ± 0.2(O) ml.100ml^{-1}.mmHg^{-1} x 10^{-3} for forearms. Venous compliance was significantly greater in the forearm than the calf in all groups (all $p < 0.02$). Overall there were no significant differences in either calf or forearm venous compliance between genders, groups or female hormone type (all $p > 0.05$).

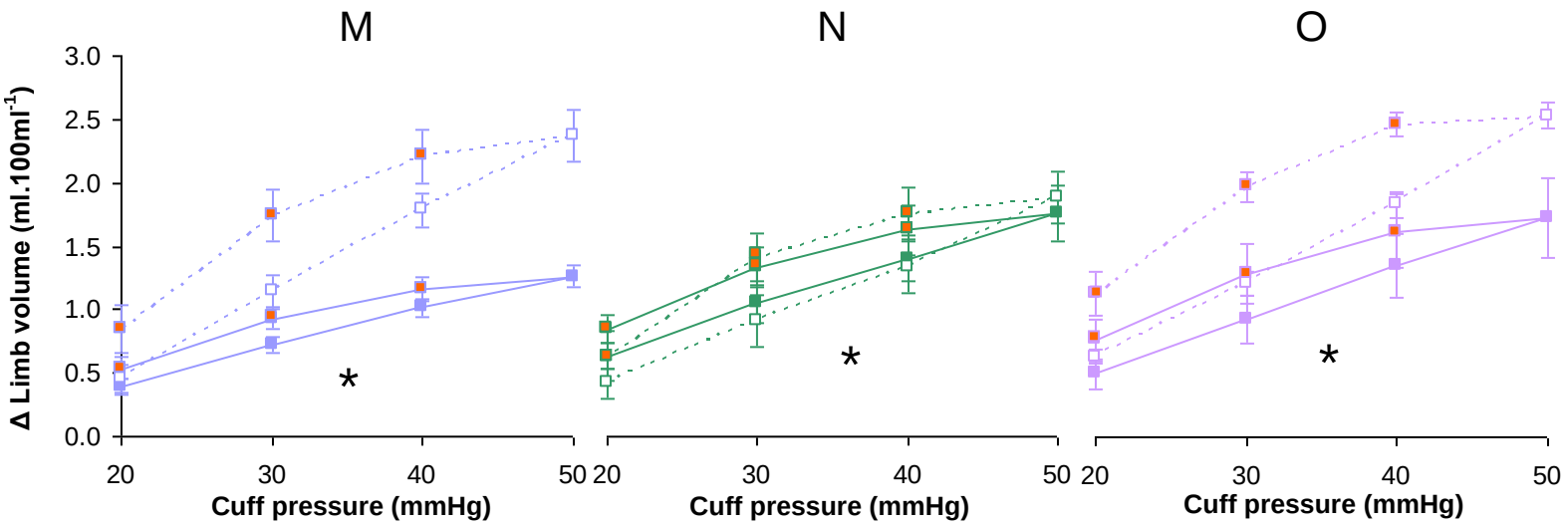


***Figure 4.7**: Venous compliance. Figure shows mean ± SEM venous pressure-volume curves for calf (solid lines and solid points) and forearm (dotted line and hollow points) for all groups. Venous compliance was derived from the slope of the descending part of the curve between 40 and 20 mmHg (points with orange fill). * = $p < 0.05$ calf vs. forearm. There were no differences between genders, groups or female hormone types (all $p > 0.05$).*

4.4 Discussion

The aim of the present study was to investigate gender differences in venous vessel reactivity by comparing men with women having normal menstrual cycles. In the lower leg and the forearm, decreases in venous vascular volume during sympathoexcitation by the cold pressor test were only significant in the men and not the women. In the women taking oral contraceptives, significant venoconstriction occurred in the legs but not in the arms but both of these leg and arm responses were still greater than those of the normally menstruating women, implying that the presence of exogenous female hormones can facilitate the response to sympathoexcitation by CPT.

4.4.1 Calf and forearm volume changes during control venous distension

As in the previous study, venous capacity showed no gender difference in contrast to studies showing smaller capacity in women than men (Hernandez & Franke, 2004, Monahan & Ray 2004, Meendering *et al.* 2005, Lindenberger & Lanne 2007). A number of factors can influence measures of venous capacity based on whole limb volume and may offer insight as to why there was no gender difference observed. Convertino *et al.* (1988) showed an inverse relationship between calf capacitance and skeletal muscle mass in men, suggesting that more muscle provided greater physical constraint for the veins. Males and females did not differ in estimated total calf volume. The subjects were young healthy exercise science students who all engaged in regular physical activity to college level or above and the relationship between calf muscle and venous capacitance is liable to be modified by training which alters both muscle mass and venous capacitance (Hernandez & Franke, 2004). In this present study, values for calf capacitance and fluid filtration were half those found in the previous chapter, as were resting values for calf blood flow. They were, however, similar to those corresponding values found in chapter 2. These findings confirm that limb positioning in relation to heart level does influence blood volume readings.

In forearms, likely to show less training-related variation, higher capacity in men than women was associated with their larger overall limb volume. On the other hand, although there are similar relative proportions of muscle versus skin and subcutaneous fat in arms and legs (Elia & Kurpad, 1993), forearm capacity overall was greater than calf capacity, in keeping with the concept of reduced physical restraint with a smaller muscle mass (Convertino *et al.* 1988) and greater vein distensibility in the arm than leg (Eiken & Kolegard, 2004). Limb size therefore appears to play a more significant role in determining venous vascular volume in the forearm than the calf. However, the O group women had bigger forearm venous capacity than the N group women, consistent with other studies demonstrating an effect of female hormones (Goodrich & Wood, 1964, Fawer *et al.* 1978, Barwin & Roddie, 1976). This could be linked to enhanced basal release of nitric oxide (John *et al.* 2000) and attenuated

myogenic constriction (Bishop & Brown, 2007) associated with prolonged oral contraceptive use.

Calf venous compliance has been shown be smaller in women than men (Monahan & Ray, 2004, Meendering *et al.* 2005) but the findings from this present study identified no gender difference in legs or arms. This may be related to levels of fitness of the subjects since Hernandez & Franke (2004) saw no difference in calf compliance between young fit women and men. Compliance was also similar in women with normal menstrual cycles and those taking oral contraceptives, in agreement with Meendering *et al.* (2005). They commented that previous studies showing increased venous distensibility under the influence of female sex hormones were based on limb volume changes during venous filling (McAusland *et al.* 1961, 1963, Goodrich & Wood, 1964, Barwin & Roddie, 1976, Fawer *et al.* 1978) and therefore subject to confounding errors due to fluid filtration and / or venous wall creep. The studies mentioned above (Monahan & Ray, 2004, Hernandez & Franke, 2004, Meendering *et al.* 2005) measured limb compliance using a method based on pressure-volume curves generated during vein emptying after a period of venous occlusion, specifically to avoid these errors (Halliwill *et al.* 1999). By constructing pressure-volume curves during both filling and emptying (Figure 4.7) it was possible to derive estimates of creep from the hysteresis, but no gender or hormonal effects were found on this parameter. There were also no gender or hormonal differences in fluid filtration in legs or arms, as previously shown (Gamble *et al.* 1998, Gooding *et al.* 2005, Stachenfeld & Taylor, 2007). In contrast, Lindenberger & Lanne (2007) partitioned out the filtration component from the calf volume response to lower body negative pressure and demonstrated higher values in women than men. This could, however, have been confounded by the effects of suction on tissue compliance and activation of cardiovascular reflexes due to splanchnic pooling. Clearly, the derivation of parameters such as capacitance and filtration based on volume changes during venous filling should be made with full appreciation of the impact that limb position, limb composition and ambient temperature can have (Jorfeldt *et al.* 2003) and for complete characterisation of venous properties (i.e. also compliance), the recording of volume during both filling and emptying is preferable.

4.4.2 Gender and cold pressor-induced venoconstriction

Limb veins constrict to sympathetic neural activity and circulating catecholamines. The cold pressor test was chosen as a reflex challenge to the sympathetic nervous system because increases in these parameters, as well as blood pressure and heart rate, are similar in men and women (Jones *et al.* 1996, Litschauer *et al.* 1998, Dishman *et al.* 2003, Hogarth *et al.* 2006), and unaffected by female sex hormones (Hastrup & Light, 1984, Ettinger *et al.* 1998, Litschauer *et al.* 1998). Furthermore, this intervention was chosen as, unlike in Chapter 3, it

allowed subjects to be positioned in the supine position without the pelvic region being situated below heart level, therefore avoiding blood pooling, which in hindsight may have occurred in the semi-recumbent position that the Biodex dynamometer offered. Indices of sympathetic activity were not directly measured but heart rate and blood pressure responses to the test were uniform across groups in support of a lack of gender or hormone-related differences. As >70% of limb volume expansion during venous occlusion is due to venous filling (Buckey *et al.* 1988), application of the cold pressor test enabled sympathetic effects on venous vessel tone to be compared between the groups assessed from the decrease in venous vascular (limb) volume.

The decreases in limb volume throughout 1 minute of cold pressor test application corresponded to those shown in the forearm by Zelis & Mason (1969) using the same equilibration technique. They represent active constriction in the more superficial venous vessels since they were abolished when skin and subcutaneous blood flow were eliminated using adrenaline iontophoresis (Zelis & Mason, 1969). During cold pressor test application, lowering of the leg per se could potentially lead to reduced venous return and reflex vasoconstriction, or invoke locally-mediated postural arterial constriction, contributing to decreases in limb volume. This is unlikely because limb volume was not influenced by this manoeuvre without cold immersion. Moreover, reduction in fluid filtration cannot account for the decrease in limb volume during cold pressor test because this parameter is not altered by sympathoexcitation during tilting (Gamble *et al.* 1997) when venous and capillary pressures are elevated to a similar extent as by the venous occlusion used in the present study.

Women with normal menstrual cycles showed similar venoconstriction as men in their forearms but only men showed significant leg venoconstriction. The literature does not appear to contain any studies measuring sympathetic neural outflow to arms and legs concurrently during a cold pressor test, but it is assumed to be comparable in both limbs, as during stressors such as head up tilt or lower body negative pressure (Wallin & Charkoudian, 2007). The difference in venoconstriction could thus be interpreted as due to a gender effect on venous vascular reactivity in the lower limbs. Arterial vasoconstriction in the leg during a cold pressor test is attenuated in women compared to men despite similar gender efferent sympathetic nerve activity (Dishman *et al.* 2003, Hogarth *et al.* 2007). In a predominantly female cohort, Jacob *et al.* (2000) measured equivalent increases in plasma catecholamines in forearms and legs during a cold pressor test associated with increases in forearm but not leg vascular resistance. Attenuation of arterial adrenergic reactivity in women has been attributed to the action of oestrogen through enhanced basal release of nitric oxide (Sudhir *et al.* 1997), augmentation of other dilator pathways (Orshal & Khalil, 2004) or inhibition of noradrenaline release (Du *et al.* 1995). Porcine veins are also relaxed by oestrogen in an

endothelial-dependent fashion (Bracamonte *et al.* 2002), and although receptors for oestrogen and progesterone are present in human veins from both genders (Mashiah *et al.* 1999), no investigations appear to have identified mechanisms of gender differences in venous vascular tone in human legs. The reason why forearm venoconstriction did not show a gender difference whereas the leg did is not clear. Limb- and gender-specific differences in flow-mediated dilation have recently been investigated, demonstrating similar dilator responses in male and female arms but a gender difference in the leg (Nishiyama *et al.* 2008). Whether this differential limb reactivity extends to the venous circulation will require further investigation.

4.4.3 Exogenous female hormones and cold pressor-induced venoconstriction

It is interesting that exposure to exogenous hormones through oral contraceptive use enhanced leg but not forearm venoconstriction. This is consistent with observations that exposure to oestrogen and progesterone in vitro (Rorie & Muldoon, 1979, Nekooeian & Pang, 2000) and *in vivo* (Varbiro *et al.* 2002) augment constriction of veins to noradrenaline. The O group women were tested during the 'luteal' days of the menstrual cycle when levels of endogenous hormones would be low (Hirshoren *et al.* 2002) but those of exogenous hormones high (Endrikat *et al.* 2002). The difference in leg venoconstriction between the two groups of women (O and N) would imply that synthetic oestrogen and progesterone modify adrenergic responses to enhance constriction in a different way to endogenous hormones (Rorie & Muldoon, 1979, Varbiro *et al.* 2002). Exogenous oestrogen may increase post-synaptic α_2 receptor density or inhibit neuronal noradrenaline reuptake (Du *et al.* 1995) and synthetic progesterone, which can have higher bioactivity than natural progesterone (Schindler *et al.* 2003), may also enhance noradrenaline release (Mercuro *et al.* 1999) and constriction (Byrne *et al.* 1988). On the other hand, chronic use of oral contraceptives increased forearm basal NO production (John *et al.* 2000), and arterial dilator effects of oestrogen in oral contraceptives may be antagonised by the progestin component (Torgrimson *et al.* 2007). The mechanisms of these antagonistic effects on vein reactivity will require further pharmacological investigation in the context that women using oral contraceptives have a higher risk of venous disease (Beebe-Dimmer *et al.* 2005).

4.4.4 Limitations

The use of ultrasound imaging in this present study would have provided a better insight into vein reactivity to the cold pressor test, particularly in the light of the significant / marked whole limb constriction that was observed. A measurement of arterial blood flow during the cold pressor test would have helped confirm whether the observed decreases in venous vascular volume were active or passive. However, the number of personnel required to include these measures made this logistically impossible for this present study – however,

the issue of individual vein responses to cold pressor test will be investigated in the next Chapter.

As in previous chapters, the female hormones issue would have been better controlled by use of ovulation predictor kits and / or blood hormone samples. The numbers of participants in the N group women may also be an issue. With n = 5 for phase groups, the data would not give enough statistical power for analysis but since there were no differences between phases in any parameter the groups were combined. Throughout the analysis process, N group data has been analyzed both as one group and as two separate groups (NM and NL) and on no occasion have the data for the two groups been statistically significant from each other. On reflection, had there been subjects readily available within the time constraints of the project then it would have been better to have larger numbers of subjects in each individual group.

4.4.5 Conclusions

Evidence of venoconstriction was present in both the forearm and calf limbs during sympathoexcitation by cold pressor test in all groups. However, this venoconstriction was only significant in both limbs for the male group and of the female groups, the N group calf response was attenuated with only the O group showed significant calf limb venoconstriction. The differences between groups with regard to gender or female hormone status (endogenous vs. exogenous) were not significant. These findings indicate limb-specific differences in venoconstrictor reactivity that can be modified by long-term synthetic female hormone exposure. Moreover, they have highlighted that there are distinctive differences between venous characteristics in the legs and arms that should be taken into account when assessing any aspect of venous function.

Chapter 5:
COMPARISON OF RESPONSES OF LONG SAPHENOUS AND POPLITEAL VEINS TO SYMPATHOEXCITATION IN MALES

5.1 Introduction

Thus far, the experimental work in this thesis has investigated changes in venomotor tone in the lower leg based on venous volume using the equilibration technique and by ultrasound imaging of the popliteal vein. The most significant evidence for venoconstriction in response to isometric exercise, mental stress or a cold pressor test was observed as decrease calf venous volume in male subjects. As explained in Chapter 1 (General Introduction), plethysmography records volume changes of all tissues in a limb and whilst venous vascular volume represents the major part of this (Buckey *et al.* 1988, Cirovic *et al.* 2006), contributions from fluid filtration and visco-elastic creep are involved especially when venous distension is elicited, either by cuff inflation or upright tilt (Halliwill *et al.* 1999). Among the capacitance vessels, it is not clear which exhibit the venoconstriction that is detected by plethysmography.

5.1.1 Innervation and reactivity of the long saphenous and popliteal veins

As also stated in the General Introduction, the venous system of the lower leg comprises deep veins lying within the fascial envelope of the leg, including inter- and intra-muscular veins, and superficial veins in the subcutaneous tissue, with perforating veins that join the two systems (Ludbrook, 1962). During rhythmic calf exercise, pressure in both the deep and superficial systems rises during contraction and falls on relaxation, with superficial pressure always remaining higher so that blood drains into the deep veins where valves help to maintain forward flow for venous return. Since the early work of Donegan (1921), the majority of evidence from animal studies supports the notion that it is the superficial veins that are more responsive to sympathetic activation. Anatomical demonstration of adrenergic nerves using catecholamine histochemistry showed sparse supply to the intrasmuscular veins in cat gastrocnemius muscle (Fuxe & Sedvall, 1965) whereas cutaneous and superficial veins are more densely innervated (Shepherd & Vanhoutte, 1975). The most prominent superficial vein of the human lower leg is the long saphenous vein (see Figure 1.3) and because of its widespread use in surgery as a graft for arterial bypass, it has been the subject of most investigation. Histochemical methods for detection of catecholamines show a plexus of sympathetic perivascular nerve fibres on human saphenous vein (Loesch & Dashwood, 2009) and stimulation of these nerves evokes venoconstriction via complex co-transmission and neuromodulation involving noradrenaline, neuropeptide Y and ATP (Fabi *et al.* 1996, Loesch & Dashwood, 2009).

In contrast, there is a lack of information about the innervation of the human popliteal vein, the main conduit vessel carrying blood from below to above the knee, and whether it responds to sympathetic activation. In Chapter 3, the popliteal vein did not react to sympathoexcitation by either isometric leg exercise or mental stress task and did not

increase in diameter after reaching capacity at 3 minutes, implying that it does not contribute to venoconstriction. Studies have investigated the popliteal vein by using Doppler ultrasound imaging. Hertzberg *et al.* (1997) measured popliteal vein diameters below the knee in 975 legs in vivo and found that vessel diameter was greatest at the level of the common femoral vein (10.57 ± 2.88 mm) and became progressively smaller as it travelled down to the level of the mid superficial femoral vein segment (6.41 ± 1.72 mm). Mean popliteal vein diameter was reported as 6.80 ± 2.11 mm. Neglen & Raju (1995) measured popliteal vein diameter during graded head up tilt and estimated hydrostatic pressure at the imaging site in order to construct diameter-pressure plots and calculate popliteal compliance. Although they found reasonable agreement between vein compliance and that of the calf based on plethysmographic venous volume, their sample population involved both healthy and post-thrombotic limbs and data was not separated out for these groups. De Groot et *al.* (2005) also described a method to measure popliteal vein compliance from cross-sectional area – pressure plots during thigh cuff inflations to set pressures and reported significant correlation (R^2=0.39) with plethysmographically-derived calf compliance in normal and spinal-cord injured subjects. Young *et al.* (2006) subsequently modelled popliteal vein compliance from cross-sectional area – pressure plots during emptying in healthy young men and women. They also administered a cold pressor test and sublingual nitroglycerin to induce constriction and dilation respectively and neither treatment had any effect on popliteal vein size, supporting the notion that it acts primarily as a passive conduit and makes little contribution to calf volume changes during a cold pressor test.

The involvement of the long saphenous vein during sympathoexcitation by cold pressor test has not been investigated to date. Evidence that, compared to deep vessels, this vein is capable of reflex changes in venomotor tone comes from a study of whole body heat stress (Abraham *et al.* 1994), where the CSA of the long saphenous vein doubled while that of the femoral vein remained unchanged. Early work by Page *et al.* (1955), Samueloff *et al.* (1966a) and Zelis & Mason (1969) all demonstrated venoconstriction in the forearm following reflex sympathoexcitation by various manoeuvres and in the only study to compare venomotor tone in upper and lower limbs, Samueloff *et al.* (1966b) reported that constriction to a deep breath was greatest in hand and foot veins, intermediate in a forearm vein and least in the long saphenous vein. This might imply that despite the presence of innervation, the superficial long saphenous vein does not greatly respond to sympathetic activation. However, there was no information provided in this study about the systemic cardiovascular changes so that it is not possible to estimate whether the degree of sympathoexcitation was comparable under all conditions. The only direct evidence for differential venoconstriction in superficial and deep veins remains that from Zelis & Mason (1969) based on their use of adrenaline iontophoresis of the whole forearm to eliminate skin and subcutaneous blood flow. In this situation,

decreases in forearm venous volume to a deep breath, leg exercise or a cold pressor test were abolished, but again, no data on blood pressure and heart rate changes to the interventions or any other indices of sympathetic activation were given to verify that cardiovascular status was similar between conditions.

5.1.2 Ultrasound imaging of the superficial and deep lower limb venous vessels

Doppler ultrasound imaging allows direct measurement of vein diameter changes under different conditions e.g. bed-rest, applied pressure, tilt, standing and has been used to view many of the vessels of the lower leg (Louisy *et al.* 1997, Haenen *et al.* 1999, Eiken & Kolegard 2004, Chauveau *et al.* 2006, Cirovic *et al.* 2006, Arbeille *et al.* 2008). A number of studies (Woodman *et al.* 2001, Newey & Nassiri, 2002, Sidhu *et al.* 2002) have recently used on-line, i.e. “live”, edge-detection software to continuously measure brachial artery diameter change during flow-mediated dilation technique. Briefly, a segment of vessel, in this case – brachial artery, is imaged in long-section by Doppler ultrasound, while the software continuously (~25 Hz) detects the vessel walls via 2 artificial neural networks, which have been “trained” to track vessel wall movement and from this, the software can calculate vessel diameter within the image region (Newey & Nassiri, 2002). This approach was used in Chapter 2 to monitor long saphenous vein diameter changes during filling and in Chapter 3, to measure popliteal diameter during isometric exercise and mental stress. Although it offers an ideal tool with which to compare reactivity of deep and superficial veins to autonomic challenge, no studies appear to have directly compared deep and superficial vessels *in vivo* during sympathoexcitation.

5.1.3 Summary and Chapter aims

On the basis of data presented in Chapter 3, the popliteal vein does not make any contribution to the reduced calf venous volume during reflex sympathetic activation by isometric exercise or mental stress. It is not known whether this vessel responds to a cold pressor test or to what extent constriction of the long saphenous vein is involved. In light of the established increases in venomotor tone in response to the cold pressure test in the calves of men as demonstrated in Chapter 4, the aim of this study is to use Doppler ultrasound imaging in conjunction with on-line edge-detection software to continuously monitor and compare reactivity of superficial (long saphenous) and deep (popliteal) veins of the lower limb during sympathoexcitation via cold pressor test. It is hypothesized that:

The long saphenous vein of the calf will constrict during CPT stimulation but the popliteal vein will not.

5.2 Methods

5.2.1 Subjects

10 young healthy males volunteered to participate in two venous distension protocols to investigate differences in deep vein (popliteal, POP) and superficial vein (long saphenous, LS) filling characteristics and reactivity to sympathoexcitation by cold pressor test (CPT). All procedures were approved by the Local Research Ethics Subcommittee, School of Sport and Exercise Sciences, University of Birmingham, UK in accordance with the declaration of Helsinki 1964. Following informed consent (Appendix A), all subjects were screened by general health questionnaire (Appendix B) in order to ensure all subjects were in good health and injury free. Exclusion criteria were: Current illness, consumption of 'over the counter' anti-inflammatory drugs in the previous 24 hour period, cardiovascular disorders, injury to limbs (either during or at any time over 6 months prior to study) and immune, metabolic or kidney condition(s). The subjects' anthropometric characteristics are shown in table 5.1.

Table 5.1*: Anthropometric characteristics of subjects. Values are mean ± SD.*

	(n = 10)	
	Mean	± SD
Age (years)	26	4
Height (cm)	177	5
Weight (kg)	72.4	7.4
Body Mass Index (kg/m^2)	23	2
Calf limb segment volume (l)	2.65	0.27

5.2.2 Other considerations

Subjects were fasted before the testing session. The modified euhydration protocol described in the previous chapters was used to ensure subjects were sufficiently hydrated. The study was conducted in the Vascular laboratory at the School of Sport and Exercise Sciences, University of Birmingham, UK. The laboratory is situated along a quiet corridor and silence was requested from passers by so that experimental subjects were not distracted. The environmental temperature within the laboratory was maintained at 21 °C to keep room temperature fluctuations and its associated effects on calf volume measurements by strain gauge plethysmography to a minimum. Lighting inside the laboratory was dimmed to ensure subjects remained relaxed.

The two protocols (POP and LS) were held on separate days and were performed in a counter-balanced order by the subjects so that half performed the POP protocol on the first

day and the LS on the second day, and vice versa. On both days, subjects completed a control venous distension and a venous distension with a CPT intervention. All procedures were the same in both protocols, the only difference between them being that a different vein was measured. All subjects were tested in the afternoon to avoid any differences due to circadian effects on metabolic rate or catecholamine levels (Aschoff & Heise, 1972, Kleitman & Ramsaroop, 1948, Wenger *et al.* 1976, Sowers, 1981, Stephenson & Kolka, 1985).

5.2.3 General experimental setup

Subjects lay in the supine position with lower limbs raised slightly above heart level in order to aid venous emptying (see figure 2.1). All whole limb and direct vein measurements were made on the dominant calf. A small inflatable cuff (Hokanson Ltd.) of 6 cm width was placed around the ankle and a large inflatable cuff (Moor Instruments Ltd.) of 13 cm width was placed around the mid-thigh of the test limb. A mercury in silastic strain gauge (measuring 2 cm smaller than the largest circumference of the calf, Hokanson Ltd.) was placed around the belly of the calf. Subjects were instructed to breathe at normal rhythm throughout testing and to avoid sudden gasps or deep inspirations.

5.2.4 Measurement of variables

Beat-to-beat blood pressure, from which mean arterial pressure (MAP, calculated as diastolic blood pressure + 1/3 (systolic blood pressure - diastolic blood pressure) and heart rate (HR) values were derived, was detected using a Portapress photoplethysmograph incorporating height correction (Finapres Medical systems, Netherlands) placed around the right middle finger. The analogue signal from the Portapress control console was input to a Powerlab A/D converter and displayed on a PC where it was recorded at a rate of 40 Hz using Chartlab software (ADInstruments Ltd.).

Blood-flow, whole-limb vascular filling, venous vascular capacity and filtration were determined using strain-gauge plethysmography. The mercury in silastic strain gauges placed around the calf and forearm were connected to a plethysmograph (Hokanson Inc.) and the output signals were connected to the same PC running Chartlab software via a Powerlab A/D converter. Whole limb volume changes were continuously recorded simultaneously with cardiovascular variable changes so that changes throughout the experiment were time-matched.

Venous filling and filled vein diameter were all detected using Doppler ultrasound imaging. Popliteal and long saphenous vein diameters were imaged in longitudinal section with a linear 3 - 12 MHz probe positioned by hand 5 - 7 cm below the line of the popliteal fossa over the respective vein (figure 1.3). The transducer head (probe) was manipulated, ensuring to

not press into the leg tissue, in order to obtain an optimum image of the vessel borders. The ultrasound image signal was relayed to a second PC running Vascular Image Analysis (VIA®) software (Newey & Nassiri, 2002), which, as described previously, uses two artificial neural networks to detect anterior and posterior walls of the vessel and produce a digital display tracking vessel diameter at a rate of ~ 25 Hz.

5.2.5 Blood flow and control distension measurements

Subjects rested in the supine position for 20 minutes prior to the testing procedure. The ankle cuff was inflated to supra-systolic pressure (> 50 mmHg above systolic pressure) to eliminate foot blood flow (Williams and Lind, 1979). Following a baseline measurement period of 2 minutes where resting MAP, HR and limb volume was recorded, calf arterial inflow rate was measured as described in the previous chapters (Greenfield *et al.* 1963, Wilkinson & Webb 2001). Briefly, the thigh cuff was inflated to 50mmHg for 10 seconds and the rate of increase in calf limb volume was measured by strain gauge plethysmography. Following a ~ 30 s recovery period for variables to return to baseline values, the procedure was repeated until a total of 3 calf blood flow measurements had been produced for analysis.

5.2.6 Control venous distensions

The ankle cuff was again inflated to supra-systolic pressure to eliminate foot blood flow and a 2 minute baseline rest period was observed. Venous distension was then induced by thigh cuff inflation to 50 mmHg for 5 minutes; in this experiment by means of a Moor Instruments Ltd. DRT4 automatic cuff inflator. Measurements of MAP, HR and limb volume changes were continuously recorded throughout. Values for venous capacity, fluid filtration and filled vein diameter were extracted from the whole limb and direct vein filling data at specific time points as in the previous Chapters.

5.2.7 Venoconstriction to cold pressor test

After a 10 minute period of rest following the control distension, the ankle cuff was again inflated to supra-systolic pressure and a further 2 minutes of baseline rest was observed. A second venous distension was induced by thigh cuff inflation to 50 mmHg for 5 minutes as in the control distension. To measure reflex venous constriction, sympathoexcitation was induced by (left) hand immersion in iced water (4°C CPT) for one minute between minutes 3 and 4 of the distension protocol (see figure 4.1 for protocol illustration). Hand immersion was used in order to eliminate any movement artifact caused by the leg lowering required for foot immersion, which was used in the previous study.

5.2.8 Data analyses

All measurements recorded onto the Chartlab and VIA software were initially analysed using

the Microsoft Excel software package. Calf blood flow (rate of arterial inflow) was calculated from the rising slope of the plethysmograph trace ($ml.100ml^{-1}.min^{-1}$) as the mean of the three 10 s cuff inflations performed prior to the control distensions. Baseline values for all variables (MAP, HR and limb volumes) were averaged over the 2 minute rest period prior to venous distension. Venous capacity was reported as the limb volume increase at 3 minutes (Gamble *et al.* 1993, Lanne & Olsen, 1997). This also represented the "baseline" volume from which changes during the intervention period (minutes 3 – 4) were evaluated. Fluid filtration was taken as the volume increase from capacity to the volume at the end of the distension period (figure 2.5). Filled vein diameter was taken as the value at 3 minutes.

To compare the two venous distensions (CON and CPT) in each protocol (POP and LS), the recorded signal for all variables were averaged over 10 s segments. The patterns of response for both cardiovascular and limb volume variables were identical in this study to the patterns observed in the previous study and therefore, analyses between the two conditions (CON and CPT) were conducted in the same manner: MAP and HR remained similar to baseline levels during the control distension but increased, peaked and remained at their highest levels until the end of the intervention period during CPT. To assess the influence of CPT on the cardiovascular variables, peak MAP and HR during both distensions (CON and CPT) in each protocol were compared to their respective values at the end of minute 3 (as in the previous chapters). Limb volume continued to increase during the control distension (figure 2.5) whereas it decreased on application of the CPT (figure 4.1). To evaluate this, the change in limb volume during minutes 3 – 4 for both distensions was normalised to the respective capacity at 3 minutes (e.g. change in volume = (volume at 3.5 minutes) – (volume at 3 minutes)). The peak change from the capacity value for both distensions was taken and converted to a percentage change, thus providing a single value for limb volume change, which was used to describe the magnitude of the venoconstrictor response to CPT (Zelis & Mason, 1969). For an illustration of the process for analysing limb volume described above, see figure 3.4 in Chapter 3.

Direct vein diameter changes were analysed in the same manner as limb volume changes: The change in vein diameter during minutes 3 – 4 for both distensions was normalised to the respective filled vein diameter at 3 minutes. The peak change from the filled vein diameter value for both distensions was then converted to a percentage change, providing a single value for vein diameter change.

5.2.9 Statistical analyses

All statistical analyses were conducted using the Statistical Package for the Social Sciences, version 13 (SPSS, Inc., Chicago, IL). A one-sample Kolmogorov-Smirnov test was firstly

performed to check for normal distribution of the data to determine the most appropriate (parametric or non-parametric) statistical analysis. A paired samples t-test was used to compare changes in variables during CPT with those during control distension and also changes in variables during the same condition (e.g. CON) in the different protocols (i.e. POP vs. LS). Data are presented as means ± S.E.M. unless otherwise stated. Differences were deemed significant if $p < 0.05$.

5.3 Results

5.3.1 Resting and control values for variables

Resting MAP and HR were similar prior to the respective POP and LS protocols (table 5.2, $p > 0.05$) and there were no significant differences in resting calf blood flow between the two protocols.

Table 5.2*: Resting and control distension values for variables. Figure shows mean ± SEM for variables during POP and LS protocols.* ******* *= p < 0.01 POP vs. LS.*

	POP		LS	
	Mean	± SEM	Mean	± SEM
MAP (mmHg)	76	3	71	3
HR (beats.min^{-1})	58	3	60	4
Calf blood flow (ml.100ml^{-1}.min^{-1})	2.54	0.21	2.85	0.22
Filled vein diameter (mm)	10.52	0.93 *	3.10	0.27
Fluid filtration (ml.100ml^{-1}.min^{-1}.mmHg^{-1} x 10^{-3})	1.81	0.29	1.86	0.36

5.3.2 Venous distension

5.3.2.1 Time course patterns of responses to venous distension

In agreement with the findings from chapters 2 and 3, calf limb volume, long saphenous vein diameter and popliteal vein diameter all increased markedly during venous distension (figure 5.1). Both veins became full (point of inflection) at approximately 60 s earlier than in the previous studies but appeared to follow a similar pattern to the previous studies in that the popliteal vein filled more rapidly to reach this point of “fullness” first (Pop = 1 min vs. 2 min in Chapter 3 and LS = 2 min vs. 3 min in Chapter 2, figure 5.1b & c). These findings are consistent with previously published data that deep veins fill more rapidly than superficial veins (Buckey *et al.* 1988).

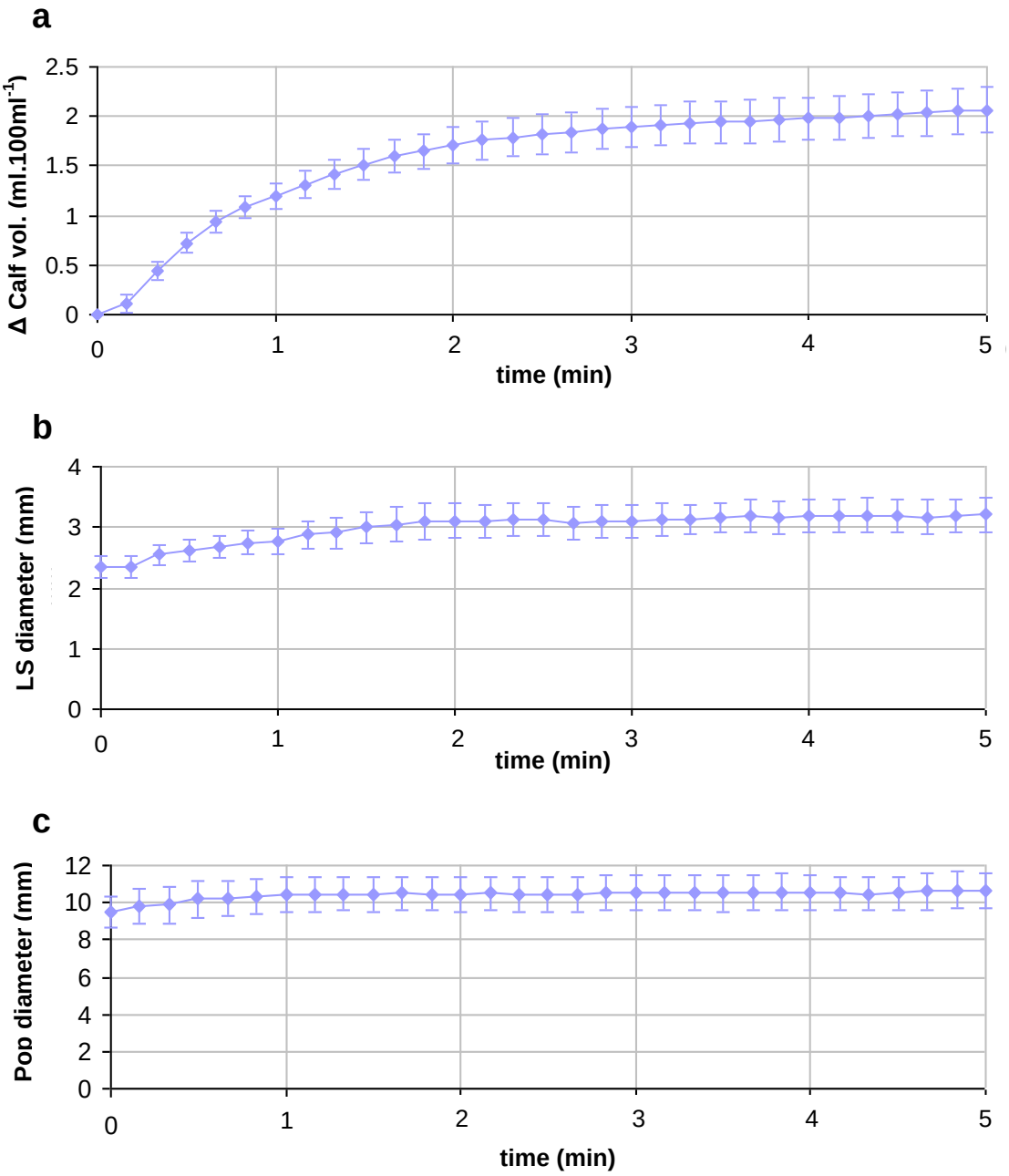


***Figure 5.1**: Calf limb (a), long saphenous vein (b) and popliteal vein (c) filling. Figure shows mean ± SEM limb volume and vein diameter changes during venous distension.*

In comparison to the filling data in chapters 2 and 3, the calf limb response (figure 5.1a) was similar to that in chapter 2 (figure 2.8), which were approximately half that observed in chapter 3 (figure 3.5) in terms of overall volume increase ($p < 0.05$). Long saphenous vein filling was similar to that observed in Chapter 2 in that both increased in diameter by approximately 1 mm over the course of the distension (figures 2.10 and 5.1b) but the baseline and filled diameters were in the region of 1 mm and 0.5 mm larger in this present

study than in Chapter 2. Similarly, filled popliteal vein diameter was larger in this present study compared with that in Chapter 3 (~ 1 mm larger, figure 3.6) but there was a difference of in the region of 3 mm in baseline diameters. However, popliteal filling was slightly smaller in this present study compared to that observed in Chapter 3. These differences perhaps highlight the influence of leg positioning during venous volume studies. As in all studies, MAP and HR remained unaltered from their respective baseline values throughout venous distension.

5.3.2.2 Control venous distensions

Capacity (figure 5.2) and fluid filtration (table 5.2) were almost identical during the two control distensions (both $p > 0.05$). Filled popliteal vein diameter (t = 3 minutes) was significantly larger than filled long saphenous vein diameter taken at the same time point (table 5.2, $p < 0.01$).

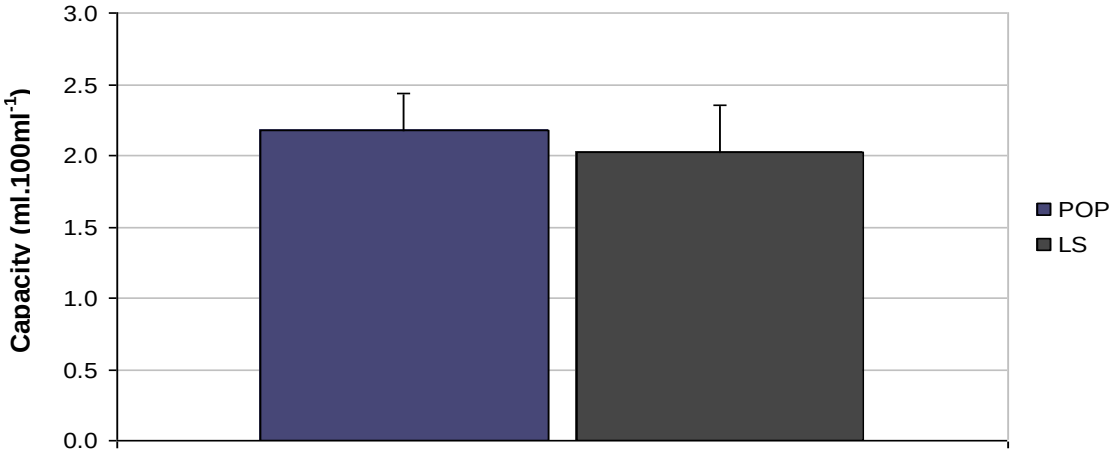


Figure 5.2*: Venous capacity. Figure shows mean ± SEM venous capacity (ml.100ml^{-1}) for POP and LS protocols.*

5.3.3 Venoconstriction

MAP and HR significantly increased during CPT compared to the control distension in both the POP and LS protocols (POP = 18 ± 3 mmHg and 11 ± 3 beats.min^{-1} and LS = 22 ± 3 mmHg and 14 ± 2 beats.min^{-1}, all $p < 0.01$, figure 5.3). These increases were not significantly different between the two protocols ($p > 0.05$). Calf limb volume significantly decreased by almost exactly the same amount during CPT in the two protocols compared to the control distension (POP = -16.0 % and LS = -15.8 %, both $p < 0.05$, figure 5.6, $p > 0.05$). Popliteal vein diameter was not different between the control distension and the CPT distension (figure 5.4) illustrating that it does not constrict during sympathoexcitation by CPT. In contrast, long saphenous vein diameter reduced markedly at the onset of the CPT intervention (figure 5.5) and was significantly constricted through the remainder of the CPT and up to 30 s into the final minute after the intervention had ceased. The peak difference between CPT and CON

distensions was -13.46 ± 3.46 % ($p < 0.01$, figure 5.6), providing direct evidence that venoconstriction had occurred. Paired t-test confirmed that popliteal and long saphenous vein reactivity to CPT was significantly different (figure 5.6, $p < 0.01$).

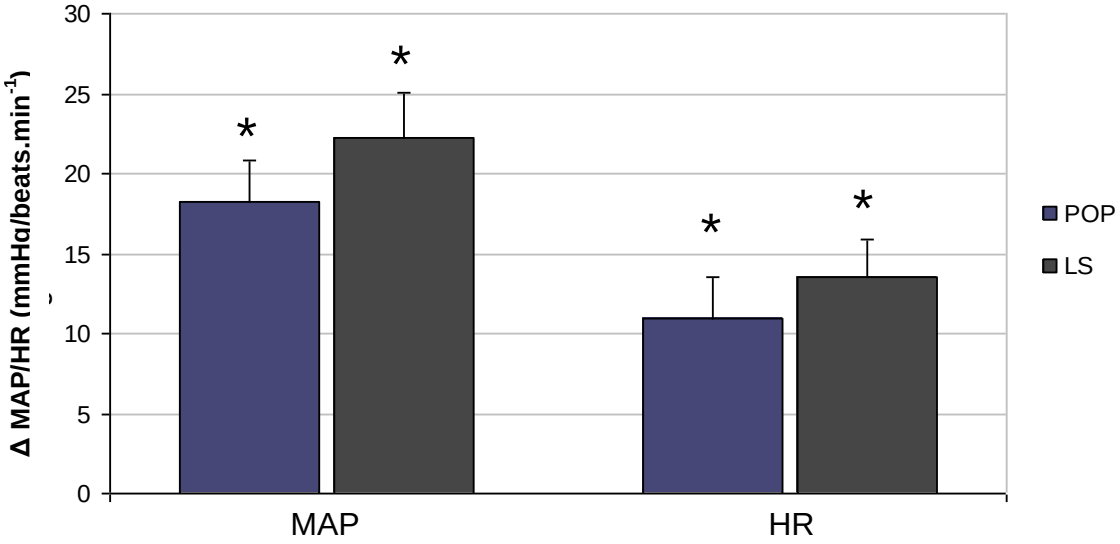


***Figure 5.3**: MAP and HR responses to cold pressor test. Figure shows mean ± SEM increases compared to control distension values during both protocols. * = $p < 0.05$ compared to CON.*

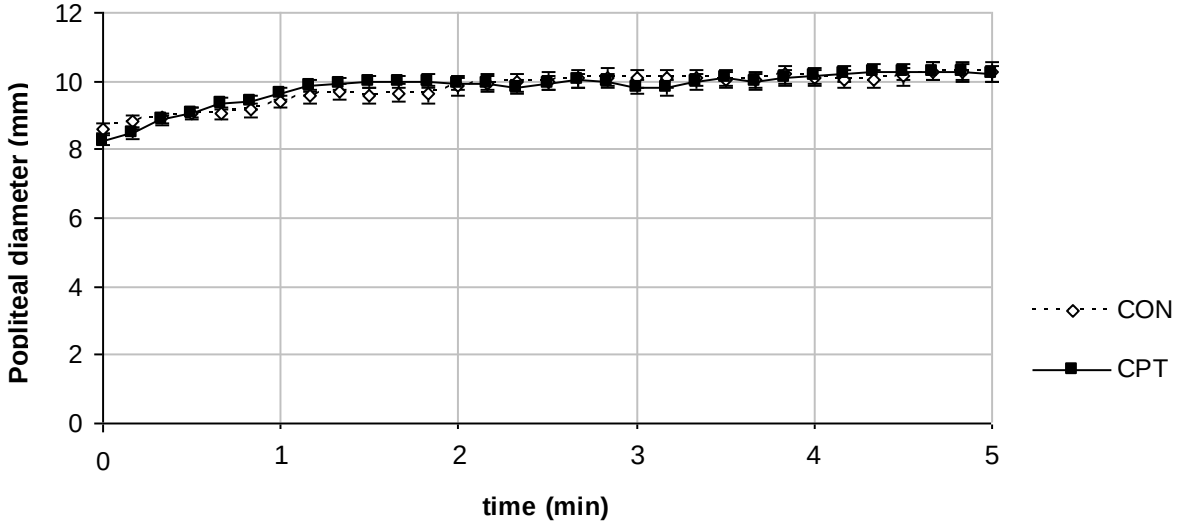


***Figure 5.4**: Popliteal vein filling and reactivity during control and cold pressor test distensions. Figure shows mean ± SEM diameter changes and clearly illustrates that the popliteal vein does not react to CPT.*

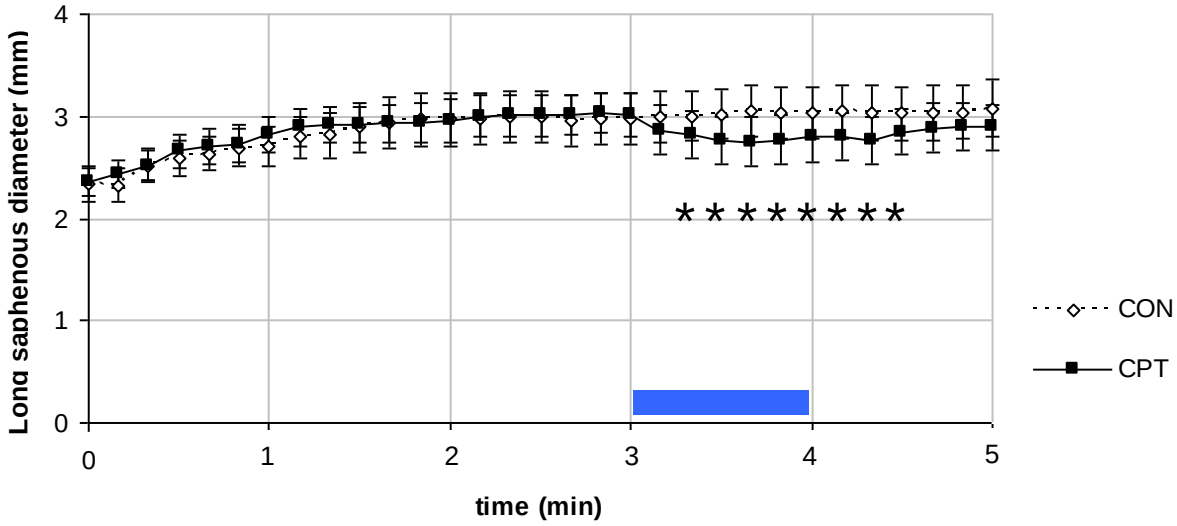


***Figure 5.5**: Long saphenous vein filling and reactivity during control and cold pressor test distensions. Figure shows mean ± SEM diameter changes and clearly illustrates the marked decrease in vein diameter at the onset and throughout application of the CPT (identified by the blue shaded area).* ⁎ *= p < 0.05 compared to control value (CON).*

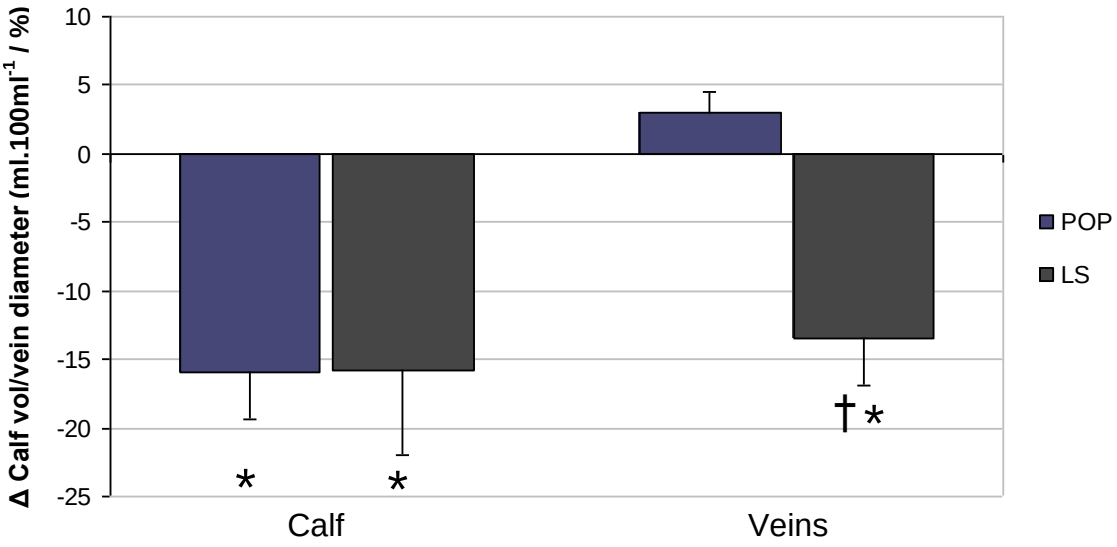


***Figure 5.6**: Calf limb and vein responses to cold pressor test. Figure shows mean ± SEM changes compared to control distension values during both protocols.* ⁎ *= p < 0.05 compared to CON. † = p < 0.01 LS vs. POP.*

5.4 Discussion

The aim of the present study was to use Doppler ultrasound imaging in conjunction with on-line edge-detection software to continuously monitor and compare reactivity of superficial (long saphenous) and deep (popliteal) veins of the lower limb during sympathoexcitation via cold pressor test. In support of the study hypothesis, the main finding is that the long saphenous (superficial) vein actively constricts during CPT, while the popliteal (deep) vein shows no active contribution to a reduction in total venous capacity in the lower limb during the same stimulus.

5.4.1 Responses to control venous distension

Both veins imaged (long saphenous and popliteal) increased in size rapidly at the onset of cuff inflation to 50 mmHg. The findings were in agreement with previous chapters in so far as that the veins became “full” at different time points into the distension procedure. The popliteal vein filled more rapidly and reached its capacity at 1-1.5 minutes into the distension procedure, while the long saphenous vein took longer and became full at around 2-2.5 minutes. These findings are consistent with previously published data that deep veins fill more rapidly than superficial veins (Buckey *et al.* 1988). However, there is somewhat of a discrepancy between the findings of this present study and the findings in previous chapters, where the responses took approximately 30 seconds longer in each case. Apart from the possibility these differences were due to the different cohort of subjects used, it is possible that the different cuff inflator that was used in this study (DRT4 in present study vs. Hokansen in previous studies), which had a slightly different inflation rate than that used in the other studies, may have caused the limb to fill more rapidly.

Baseline diameter, although extremely hard to measure at rest due to the very small size and the purported irregular cross sectional profile due to the low blood volume, was larger in this present study than in the previous studies. It has already been discussed in Chapter 3 that the positioning of the subjects may have caused the veins to be more “emptied” than in this present study, which may also largely account for the discrepancy in popliteal vein size and filling (filling from baseline level was approximately 1 mm greater in Chapter 3) but does not account for the difference between this study and that in Chapter 2, as the subjects were tested in the same position. Since calf limb segment volume was 2.65 ± 0.27 l in this study and 2.7± 0.5 l in the males in Chapter 2, this difference in vein diameter cannot be explained by larger segment volume and so the already mentioned very small size and irregular profile, which make the vessel difficult to image and measure accurately in combination with the possibility that the subject cohort simply produced a larger group mean would seem to be the most likely explanation for the variation in baseline vein size between these Chapters.

In line with the direct vein measurements, whole limb measurements of resting blood flow, capacity and filtration although slightly higher in this present study compared to the findings in the previous Chapter were not significantly different ($p > 0.05$). Neither were they dissimilar to those findings in Chapter 2. As mentioned above, calf limb segment volume was similar between Chapters 2, 4 and 5 but all of these values were approximately half those found in Chapter 3. The differences between the Chapter 3 findings and those in the other Chapters have largely been related to body position during the experiments, which was different in Chapter 3 and may have promoted greater emptying of the leg veins. However, all calf limb segment measurements were performed in the same position in all of the studies (in the supine position) and the fact that the Chapter 3 subjects displayed a much larger calf segment volume must account for some of the discrepancies observed.

5.4.2 Venoconstriction to cold pressor test

The calf limb responded to CPT stimuli by showing an approximately 15% reduction in limb volume (measured by plethysmography) during the intervention in both LS and POP protocols. These responses were approximately twice that observed for the males in the previous Chapter when a CPT intervention was also used. As in previous chapters, mean arterial pressure (MAP) and heart rate (HR) were used as indices of sympathoexcitation as they have previously been found to accompany more direct measures of sympathoexcitation i.e. increases in noradrenaline (LeBlanc *et al.* 1979, Ward *et al.* 1983, Victor *et al.* 1987, Jacob *et al.* 2000) and MSNA (Victor *et al.* 1987, Dishman *et al.* 2003, Hogarth *et al.* 2007). Resting MAPs were slightly lower in comparison to the previous Chapter (76 ± 3 and 71± 3 vs. 80 ± 2 mmHg) but HRs were almost identical (58 ± 3 and 60 ± 4 vs. 59 ± 2 beats.min^{-1}). During CPT, MAP responses (like the calf responses) were approximately twice those observed in the previous study and HR responses were similar but still slightly higher in the present study, which may indicate a greater sympathoexcitatory affect. The water was the same temperature (4°C) and was cooled in the same way in both experiments and was also checked using the same thermometer immediately prior to the protocol being performed. The CPT intervention was performed by immersing the subject's hand in cold water in the present study, whereas in the previous study it was the subject's foot that was immersed. The hand, in particular - the finger tips, contains the densest area of nerve endings in the body with nerve receptors in abundance (Vazquez *et al.* 2003) and it is possible that a lesser sympathoexcitatory effect is elicited from foot immersion CPT than from hand immersion due to the presence of fewer nerve endings. However despite this, Mark (1987) had shown that leg MSNA responses to CPT were greater following contralateral foot immersion than following hand immersion and therefore in light of this the differences between the findings in this Chapter and those in the last are a little puzzling. None-the-less both still illustrate significant venoconstriction via CPT in males and the only way of knowing which protocol

had elicited the greatest leg MSNA response would have been to make direct microneurographic measurements during the experiments, which was not possible at the time.

Within this present study, although slightly higher MAP and HR responses were observed during the LS protocol, there were no significant differences between these responses during the two different protocols (LS and POP). The reason why MAP and HR responses were slightly higher during the LS protocol cannot be explained since the trials were completed in a randomised order so that half of the subjects completed the POP protocol first and vice versa.

Zelis & Mason (1969) previously used iontophoresis to imply that the superficial veins of the forearm displayed marked venoconstriction during sympathoexcitation by CPT, while the deep veins were unresponsive to the same stimulus. The findings of Zelis & Mason (1969) and those of Eiken & Kolegard (2004) for the lower limb during pressure stimuli, along with histological evidence for more sparsely innervated deep veins (Fuxe & Sedvall, 1965, Ehinger *et al.* 1967, Burnstock *et al.* 1970), provided the basis for the hypothesis for this present study that only significant venoconstriction would be observed in the (superficial) long saphenous vein when compared to the (deep) popliteal vein. As found in Chapter 3, the popliteal vein in this present study provided no evidence of deep vein reactivity to sympathetic stimulus and these findings are strengthened in the light that the sympathoexcitation was evidently greater in this study. In contrast, the long saphenous vein displayed clear venoconstriction (13.5% reduction in diameter) to the same stimulus. That this degree of venoconstriction is sufficient on its own to maintain venous return during orthostatic challenge is unlikely but what these findings do provide is evidence for the greater relative contribution of the superficial veins of the lower limb in countering venous pooling compared to the more sparsely innervated deep capacitance vessels. However, the significant contribution of skeletal muscle pump in aiding the deep venous vasculature in maintaining venous return during orthostasis cannot be overlooked.

In the previous Chapter, the issue was raised that without making direct measurements of the venous vessel responses or measurements of blood flow, one could not be sure whether the “venoconstriction” observed was genuine venoconstriction or resulting from arterial constriction. Certainly arterial constriction is a contributing factor in the observed whole limb constriction since the arteries are also innervated by sympathetic nerves, but the direct measurement of two major leg veins also confirms that significant constriction of the superficial leg veins also contributes to the observed whole limb constriction. The differing responses of the two veins, despite the similar sympathoexcitatory challenge, suggests that

changes in whole limb volume when measuring sympathoexcitation by plethysmography will reflect constriction in the more superficial but not the deep veins.

5.4.3 Limitations

As in the studies in the previous two chapters, indices of sympathoexcitation were not directly measured, i.e. MSNA and noradrenaline responses, and these measurements would have enhanced the findings in this present study.

The difference in filling rates between this Chapter and the previous Chapters has been attributed in part to the different thigh cuff inflator used in this present study. Had an arterial blood flow rate been taken during the distension procedure then this could have been confirmed or discredited. The DRT4 cuff inflator that was used in this study allows the user to run a baseline period before automatically inflating the thigh cuff to a predetermined pressure and therefore makes it easier to run an experiment with low numbers of personnel. It may have been more appropriate to use the same cuff inflator throughout all of the experiments, however at the time of running this study, the Hokansen pump that had been used in all other experiments had a pressure fault and had gone for repair.

One of the overall aims of this project was to identify gender and hormonal differences during sympathoexcitation. This present study was conducted using only male subjects on the basis that in the previous experiments, only the males had demonstrated significant or a marked tendency towards venoconstriction and that male subjects were the most likely to produce venoconstrictor responses again rather than females. In the light of such positive results from these experiments in support of the study hypothesis, it is regrettable that a female cohort of subjects was not also studied in order to better answer the gender and female hormone status questions.

5.4.4 Conclusions

This study directly and continuously observed and compared measurements of superficial and deep vein reactivity during sympathoexcitation by means of Doppler ultrasound imaging and on-line edge-detection software. The main finding was that the popliteal (deep) vein did not actively contribute to a reduction in total venous capacity in the lower limb during sympathoexcitation, whereas the long saphenous (superficial) vein actively constricted. These differences could largely be due to differences in sympathetic nerve innervation in that the long saphenous vein is more greatly innervated than the popliteal vein.

Chapter 6:
GENERAL CONCLUSIONS

The veins of the human lower leg are distended due to gravitational blood pooling when in the upright posture, and venous return under this condition is aided during dynamic exercise by the calf muscle pump. Although it is generally thought that these capacitance vessels are relatively weakly innervated compared to arteries and respond little to adrenergic stimulation, an increase in leg venomotor tone would assist in countering pooling and help to maintain a pressure gradient for forward flow. Previous studies of venoconstriction have been carried out mainly in the forearm, demonstrating significant responses to interventions known to evoke increase in sympathetic autonomic activity such as a deep breath, Valsalva's manoeuvre, upright tilt, lower body negative pressure, exercise, mental stress and cold stimulus. There are very few investigations of gender differences in venous reactivity despite evidence that women are more orthostatically intolerant than men and experience a higher incidence of venous disease, both conditions in which attenuated venoconstriction could play a role. The main aim of this thesis was thus to investigate the reactivity of veins in the lower leg in men and women to three specific sympathoexcitatory interventions.

6.1 Methodological considerations

Measurements of changes in venous tone in humans have been carried out using a variety of methods (see Chapter 1, page 26). In the limbs, vein function can be studied in single superficial vessels, by extrapolation from changes in venous vascular volume and in single deeper vessels using ultrasound. In the present work, the equilibration technique was used whereby limb volume was measured plethysmographically during venous distension by an occluding cuff held at constant pressure, and any changes in volume taken to due to altered venous tone. In addition, ultrasound imaging was used to investigate the responses of superficial and deep large veins of the lower leg. There are several issues to be considered when measuring limb volume as a surrogate for venous vascular volume because vein filling can be influenced by the rate of arterial inflow and experimental conditions such as limb position relative to heart level and ambient temperature.

In Chapter 2, ultrasound imaging was employed to investigate arterial flow to the lower leg (popliteal artery) during a period of venous occlusion (5 min, thigh cuff inflated to 50 mmHg). It was confirmed that flow velocity was reduced without any change in artery diameter so that estimated volume flow was decreased ~ 35-40%. When vascular resistance was calculated using mean arterial pressure and taking into account the back pressure applied by the thigh cuff, there was no significant change, indicating that the reduction in flow was due to the lower arterio-venous pressure difference rather than any active change in arterial tone. This finding confirmed steady state conditions of arterial inflow during venous distension and justified the use of the equilibration technique for assessment of venomotor tone.

Laboratory temperature was carefully controlled in all studies in this thesis, but there were differences in venous filling observed that can be related to leg position. When calf blood flow and venous capacity in males are compared across the four different studies (Chapters 2,3,4 and 5), it is evident that they were higher in Chapter 3 (3.85 ±0.74 $ml.100ml^{-1}.min^{-1}$ and 4.4 ± 0.3 $ml.100ml^{-1}$ respectively) and lower in Chapter 4 (1.43 ± 0.20 $ml.100ml^{-1}.min^{-1}$ and 2.1 ± 0.3 $ml.100ml^{-1}$). The main difference between these studies was body posture. In Chapter 3, the subjects were semi-recumbent in a Biodex isokinetic dynamometer with their calves at heart level but slightly higher than the hips, allowing for better drainage, whereas in Chapter 4, subjects were lying flat with the calves and hips at heart level. While these postures were consistent within each study, the absolute values of venous capacity representing the baseline from which decreases due to venoconstriction were measured clearly differed. For this reason, changes in limb volume resulting from the sympathoexcitatory interventions were normalized to capacity to enable comparison between studies. A similar approach was adopted by Stewart (2002).

Another confounding factor when measuring limb volume during periods of venous occlusion lasting several minutes is fluid filtration which occurs because of the raised capillary hydrostatic pressure. Relative to venous capacity measured after 3 minutes of occlusion, filtration over the subsequent 2 minutes accounted for a further 7-8% in calf volume in the men studied in Chapters 3 and 5 even though absolute values of capacity and filtration were lower in the second of these studies. This consistent effect enabled decreases in volume during sympathoexcitation to be determined relative to capacity without undue influence from interstitial fluid accumulation.

There is, however, yet another potential factor in the volume increase of a limb during venous distension, namely viscoelastic venous creep. This property derives from the biomechanical characteristics of connective tissue elements in the vein wall that undergo delayed relaxation when under pressure. It has been demonstrated in venous vessels in rat skeletal muscle (Skalak & Schmid-Schonbein, 1986) and inferred from the hysteresis in the pressure-volume curve of a limb during venous filling and emptying (Halliwill *et al.* 1999), but the exact contribution of creep to venous volume during distension is unclear. Ultrasound imaging was used in Chapter 2, 3 and 5 to capture vein diameter during distensions lasting several minutes and any changes in diameter once filling reached maximum would have indicated creep. For both the long saphenous vein (Chapters 2 and 5) and popliteal vein (Chapters 3 and 5), maximum diameter was achieved within 2-3 minutes of thigh cuff inflation and there was no evidence of substantial increase thereafter. With no creep occurring in these large veins, there remains the potential for smaller venous vessels to contribute a creep component to limb volume, but this cannot be determined with the current

methodology because ultrasound vessel diameter measurement becomes increasingly tricky as the vessels become smaller in size and may prove to be extremely unreliable in veins smaller than 3-4 mm in diameter (personal observations).

Ultrasound vein imaging has, however, been used to asses what proportion of the calf volume response during venous distension is due to filling of the larger veins. Cirovic *et al.* (2006) summed the cross-sectional area of all visible large veins at mid-calf level during venous distension induced by leg dependency. This manoeuvre was estimated to increase venous pressure at the calf by ~ 65 mmHg and vein CSA accounted for only 20% of the increase in calf CSA measured by strain gauge, the remainder assumed due to distension of small venous vessels. Buckey *et al.* (1988), on the other hand, measured 70% of the calf volume increase during thigh cuff inflation as due to distension of large veins in the calf, based on MRI imaging. In the present work, only one main vein in the lower leg was imaged at a time whilst calf volume increase was registered during distension at 50 mmHg, but as Figures 6.1 and 6.2 show, over time there was very good agreement between the increase in diameter of the long saphenous and popliteal veins respectively and the increment in calf volume in male subjects (r^2 = 0.97 and 0.97 for long saphenous and popliteal veins respectively vs. calf).

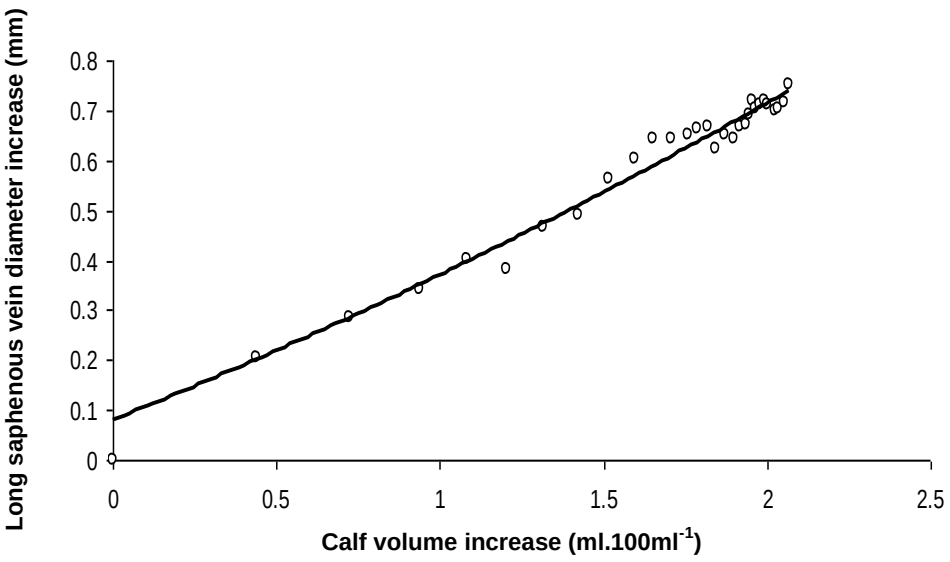


Figure 6.1*: Relationship between calf volume increase and long saphenous vein diameter increase (r^2 = 0.97).*

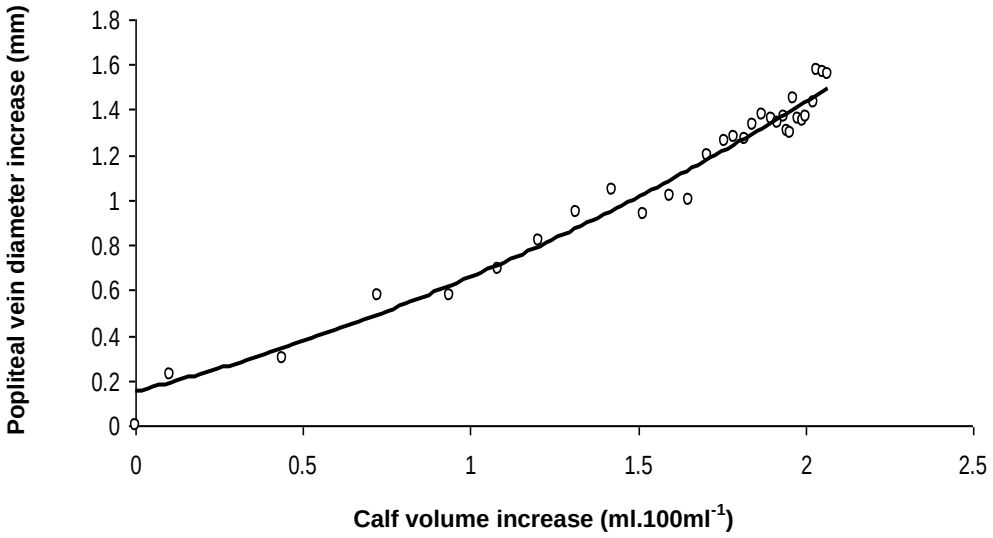


Figure 6.2*: Relationship between calf volume increase and popliteal vein diameter increase (r^2 = 0.97).*

6.2 Venomotor responses to sympathoexcitation in male subjects

Venoconstriction was assessed by the equilibration technique in the calf in response to isometric exercise and mental stress (Chapter 3) and a cold pressor test (Chapters 4 and 5). Decreases in calf venous volume were relatively modest to the first two interventions despite clear evidence of sympathoexcitation from the rises in heart rate and mean arterial pressure and a longer duration stimulus (2 minutes) compared to the cold stimulus (1 minute). There were differences in the responses seen to a cold pressor test in the two studies in which this was employed. A pronounced venoconstrictor effect was seen in Chapter 5 (calf volume decrease of ~ 17%) compared to that in Chapter 4 (calf volume decrease of ~ 6%). This difference is not immediately easy to explain as the protocols and measurements were made in similar fashion in both studies. However, blood pressure and heart rate responses to the CPT were larger in Chapter 5 where the hand was immersed in iced water than in Chapter 4 where the foot was immersed. The sympathetic neural response to a CPT is related to the size of the stimulus in that greater nerve activity was recorded with two hands immersed than with one (Mark, 1987; Seals, 1990) and there is a greater abundance of nerve receptors in the hand than the foot (Vazquez *et al.* 2003).

With regard to the question of differing responses in different vascular beds, a comparison of arm vs. leg responses to CPT was made in Chapter 4 where the findings showed that the decreases in limb volume throughout one minute of cold pressor test application were significant in both the lower (Chapters 4 and 5) and upper limbs (Chapter 4) in men. These findings corresponded to those shown in the forearm by Zelis & Mason (1969) using the same equilibration technique. On comparison of deep and superficial venous beds in the

lower limb, there was an absence of any significant changes in diameter of the popliteal vein during any intervention (CPT, ILE & MST) throughout the experimental works, suggesting that this vessel is a passive conduit that does not respond to sympathetic activation. While conversely, the long saphenous vein displayed clear venoconstriction (13.5% diameter reduction) to the cold pressor test. These findings provide direct evidence that the reductions in calf volume measured by plethysmography during sympathoexcitation arise through an increase in venomotor tone in different vessels than the large conduit popliteal vein (or other deep venous vessels) such as the long saphenous vein and other small venous vessels.

6.3 Venomotor responses to sympathoexcitation in female subjects

The findings from Chapters 2, 3 and 4 showed that there were no gender differences when comparing males and menstrual phase females in responses to venous distension or venous distension in conjunction with sympathetic stimuli. The results from all of these studies clearly demonstrate only anatomical differences between the genders, which do not appear to impact upon venous characteristics.

Calf venous capacity and fluid filtration measured by plethysmography were not different between men and women (menstrual phase) during any study. No relationship was found between whole limb venous capacity and calf size ($p > 0.05$) suggesting that anatomical size is not a factor even though men and women had different sized calves in Chapters 2 and 3. Other studies that have reported differences in capacity have examined forearms (see introductions in previous Chapters) and in Chapter 4 there was a difference in forearm capacity between men and women. Compared to the calf, the forearm has less muscle mass (Chapter 4) and so this perhaps made vascular volume more ‘accessible’, i.e. it was easier to detect the changes in volumes by plethysmography. However, ‘accessibility’ of the specific lower limb veins was not issue when using ultrasound and also this technique clearly showed slight differences in filled venous diameter between the genders, as for the whole limb measurement, there were no significant gender differences observed in the lower limb in regard to venous characteristics. However, these findings may suggest the case for using a more direct method of venous measurement (such as ultrasound) in conjunction with plethysmography to measure venous vascular responses in the lower limb, particularly in cases where the vessels may be deemed ‘inaccessible’ due to large segment size. Additionally, all of the subjects examined in these studies were young fit sports science students most of whom were regularly undertaking either recreational or competitive level physical activities. Calf venous volume is higher in athletes than non-athletes (Boutcher & Boutcher, 2005) and in those with higher fitness levels (Hernandez & Franke, 2004, 2005) and is decreased by inactivity (Bleeker *et al.* 2004). Therefore, it is possible that differences between the genders are less evident in fitter individuals.

The calf volume changes in females during isometric leg exercise and mental stress task were more variable than for males and the overall impression was that women showed less venoconstriction than the males, particularly during mental stress. As with the males, women showed much greater lower limb venoconstrictor responses to CPT. These findings in relation to CPT responses would be consistent with the evidence that even though rises in MSNA and circulating catecholamines may be similar, women show attenuated arterial vasoconstriction during sympathoexcitation, an effect attributed to the arterial dilator effects of female hormones oestrogen and progesterone. As for men, there was no evidence of change in popliteal vein tone in women during isometric leg exercise and mental stress task in agreement with a role as a passive conduit.

Venoconstrictor reactivity to CPT of the venous vasculature in the forearms (Chapter 4) did not differ between genders or with exogenous female hormone use. In Chapter 4, lower leg venoconstriction was attenuated in women with normal menstrual cycles compared to men but not in women using oral contraceptives. The reason why forearm venoconstriction did not show a gender difference whereas the leg did is not clear and no other investigations appear to have identified mechanisms of gender differences in venous vascular tone in human legs.

The long saphenous vein tended to be bigger when filled in men than in women (Chapter 2, $p > 0.05$). While the popliteal vein also tended to be bigger in men than in women (Chapter 3, $p > 0.05$). Statistical analysis also revealed that popliteal vein size was correlated with calf size ($p < 0.05$) but long saphenous vein size had no relationship with calf size ($p > 0.05$). This latter finding is presumably in keeping with the notion that the deep veins, particularly the popliteal vein, are the main drainage point for the lower leg and therefore must reflect the size, and therefore overall blood volume, of the limb.

The long saphenous vein tended to be bigger in the luteal than menstrual phase (Chapter 2, $p = 0.06$) but there was no difference in popliteal vein size between the menstrual and luteal / high hormone phase of the respective normal cycle (N) or oral contraceptive using (O) groups ($p = 0.61$). During sympathoexcitation, the only difference in responses between the phases in the N group women was a smaller MAP increase to isometric leg exercise during the luteal phase than in the menstrual phase.

Oral contraceptive use appears to enhance the leg response to CPT in women (Chapter 4). These findings are consistent with observations that exposure to oestrogen and progesterone in vitro (Rorie & Muldoon, 1979, Nekooeian & Pang, 2000) and in vivo (Varbiro *et al.* 2002) augment constriction of veins to noradrenaline. The reason why exogenous

hormones (OCs) would enhance this affect may be due to an increased basal production of the endothelial dilator nitric oxide associated with long-term oral contraceptive use (John *et al.* 2000). All female subjects who were using oral contraceptives in these studies had typically been doing so consistently for between 3 and 5 years, which could be considered relatively long-term use and therefore this explanation is entirely feasible. Additionally, it is also possible that exogenous oestrogen may increase post-synaptic α_2 receptor density or inhibit neuronal noradrenaline reuptake (Du *et al.* 1995) and synthetic progesterone may also enhance noradrenaline release (Mercuro *et al.* 1999) and constriction (Byrne *et al.* 1988).

As a final point, in the women taking oral contraceptives, the discrepancy between calf volume increase (indicating venodilation) and lack of large vein response (no diameter change) to mental stress reiterates that limb volume changes must derive from smaller capacitance vessels once the large veins are filled.

6.4 Limitations

The individual Chapters of this thesis presented the specific limitations for each study. However the key limitations of the experimental works carried out for this Thesis as a whole are that:

1. Direct indices of sympathoexcitation, MSNA and noradrenaline responses, were not directly measured and, had they been included, may have enhanced the findings of the studies in relation to being able to quantify the level of sympathoexcitation within the lower limb to each intervention.

2. Arterial measurements during sympathoexcitation would have given a clearer indication of changes in arterial flow.

3. Female hormone levels in relation to menstrual cycle phase were established by self report only. This issue would have been better controlled by use of ovulation predictor kits and / or blood hormone samples.

4. The numbers of subjects in the groups in Chapter 3 may have led to a lot of variation in responses and a lack of clarity with regard to the whole limb findings.

6.5 Significance of findings

Moving from the supine to the upright posture (orthostasis) presents a significant challenge to the human body. During this action, approximately 500 - 700 ml of blood is translocated from the thoracic compartment into the highly compliant capacitance vessels (veins) of the

lower limb causing a transient decrease in blood pressure in the vessels above the lower limb (Levick, 2003). Therefore maintaining the end diastolic volume in orthostatic hypotension is crucial (Rothe, 2006). Sympathetically-mediated reflex constriction of the veins in the lower body has been suggested as one compensatory adjustment to orthostasis (Smit *et al.* 1999) as it redistributes the blood volume and reduces the contained blood volume and the rate of venous pooling in the lower limb (Samueloff *et al.* 1966a, Rothe, 2002). Attenuated venoconstriction has been implicated in conditions of pathological orthostatic intolerance (Streeten & Scullard, 1996; Manyari *et al.* 1996; Streeten, 1999). However, quantifiable proof of active venoconstriction in humans is sparse and the majority of work in this field has been done on animals (Rothe, 2006, Hainsworth & Drinkhill, 2006).

The studies within this thesis have looked directly at lower limb responses to venoconstriction in humans and have investigated potential differences in the responses of vein vs. whole limb, differing types of stimuli (greater neural stimulus (mental stress) vs. lesser neural stimulus (isometric exercise)), gender and female hormones, upper limb vs. lower limb and deep vs. superficial vein responses. Since the sympathoexcitation invoked by the interventions used in this thesis are non-baroreceptor mediated, their effects on the venous vasculature cannot be directly extrapolated to the situation of changes in posture and orthostatic responses. Nonetheless, the data do demonstrate lower limb venous reactivity to adrenergic stimulation – venoconstriction – which has been suggested as a compensatory adjustment to orthostasis.

With regard to gender and female hormone differences, women have been shown to be less orthostatically tolerant than men (White *et al.* 1996; Convertino, 1998; Shoemaker *et al.* 2001). One reason proposed for this gender difference is that women experience enhanced lower body blood pooling because of greater capacitance and / or compliance of the leg veins (Monahan and Ray, 2004). However, venous capacitance and compliance were both found to be similar between males and females in these present studies and have been found to be similar or lower in women than men by others (Monahan & Ray, 2004; Hernandez & Franke, 2004; Meendering *et al.* 2005) and therefore the evidence does not support this proposal. It therefore seems unlikely that differences in limb venous pooling contribute to orthostatic intolerance in women. Since increases in sympathetic nerve outflow and total peripheral resistance during upright tilting are equivalent in women and men (Fu *et al.* 2005), it may be that the differences during orthostasis are due to the transduction of these adrenergic signals at the venous vessel wall being modified by the presence of female reproductive hormones. Oestrogen and progesterone have relaxant effects on venous vessels (Nekooeian & Pang, 2000; Herkert *et al.* 2000) but enhance the constriction to noradrenaline (Rorie & Muldoon, 1979; Herkert *et al.* 2000; Varbiro *et al.* 2002). In these

present studies, males displayed greater venoconstriction than females during all three stimuli used (ILE & MST (Chapter 3) and CPT (Chapter 4)). Oral contraceptive use appeared to modify the calf volume response to mental stress (Chapter 3) indicating either venodilation or enhanced arterial vasodilation leading to a passive increase in venous volume and during cold pressor test intervention (Chapter 4), the responses of the women taking oral contraceptives were also modified compared to those with normal menstrual cycles but in this case to enhance venoconstriction. It is clear from these experiments that oral contraceptive use modifies the venous responses to sympathetic stimuli in females. However it should be noted that these modified responses were only slight and that overall there were no significant gender or female hormone differences found for lower (or upper, Chapter 4) limb reactivity during any of the experiments (all $p > 0.05$) and therefore clearly more work needs to be carried out in this area to establish the exact reasons for female orthostatic intolerance.

The respective data for vein filling of the long saphenous and popliteal veins (Chapters 2, 3 and 5) provide further evidence in support of the literature (Buckey *et al.* 1988) that the deep capacitance vessels of the lower limb fill to capacity before the smaller venous vessels on the periphery. Chapters 3 and 5 also provided direct evidence that the popliteal vein did not actively contribute to a reduction in total venous capacity in the lower limb during sympathoexcitation by any of the stimuli used, whereas the long saphenous vein actively constricted. These findings for the lower limb are akin to those reported by Zelis & Mason (1969) for the forearm, whereby they implied via iontophoresis that the superficial forearm vessels provide the major active component in forearm venoconstriction during cold pressor test stimulus. Since supine humans have irregular bursts of MSNA of ~ 1Hz and during orthostasis this may increase to ~ 2Hz (Graham *et al.* 2004), venoconstriction would therefore reduce lower limb capacitance by ~ 100ml during standing (Hainsworth & Drinkhill, 2006) and so could make a contribution to maintaining cardiac output.

There is also the issue of establishing the extents of both active and passive venoconstriction and their relative contributions during orthostasis (Chapter 1, page 29). There is little doubt that constriction was observed in the whole limb and in the long saphenous vein during sympathoexcitation via cold pressor test. However, active and passive components of venoconstriction are difficult to separate since sympathetic activity not only constricts veins but arteriols also and so some of the observed whole limb “venoconstriction” that was observed in Chapters 4 & 5 may have been resultant from a passive reduction in arterial inflow. This is also the conclusion that Hainsworth & Drinkhill (2006) came to. Active venoconstriction occurs at low levels of activity (Noble *et al.* 1997), which would suggest that in supine humans, 50% of the sympathetic “drive” would already have been engaged

(Graham *et al.* 2004) before movement into orthostasis took place and therefore it would be unlikely that active venoconstriction of the superficial venous vessels alone would be sufficient on its own to maintain end diastolic volume. As it appears that the majority of blood pooling occurs in the larger capacitance vessels of the lower limb due to both their size and that they fill more rapidly than their superficial counterparts (Chapters 2, 3 & 5) and also that they do not appear to actively venoconstrict during sympathoexcitation (Chapters 3 & 5), then it would seem that the deep lower limb veins such as the popliteal vein may be more reliant on skeletal muscle pump or a passive reduction in venous flow (caused by sympathetic activation of arteriols) to assist blood flow and counter venous pooling during orthostasis and it is this that may be of more importance than venoconstriction during orthostasis.

6.6 Future directions

The findings from these studies have shown that venoconstriction occurs in the superficial lower limb veins during sympathoexcitation but not in the deep veins. With regard to orthostasis, whether venoconstriction of the lower limb superficial veins alone is sufficient to maintain venous return during orthostatic challenge is unlikely but may warrant further investigation in humans to categorise the precise contribution. Furthermore, the question of active vs. passive venoconstriction and the respective contributions of arterial and venous vessels during sympathoexcitation and orthostasis also need to be addressed. There was no evidence in these studies of significant gender or female cycle phase differences during measures of venous reactivity to venous distension or sympathoexcitation. However, use of exogenous female hormones in the form of the oral contraceptive pill clearly modified responses to sympathoexcitation and requires further investigation, particularly in the area of long-term use and its implications.

APPENDICES

Appendix A:
INFORMED CONSENT FORM

School of Sport and Exercise Sciences

Consent Form

Investigation:

Investigators:

Subject:

Name ----------

Address ----------

DOB ----------

I have read the attached Information sheet and discussed the investigation with..... who has explained the procedures to my satisfaction. I am willing to undergo the investigation but understand that I am free to withdraw at any time without having to give an explanation and that doing so will not affect any treatment or care I may receive.

Signed

Witnessed ----------

Date ----------

Appendix B:
GENERAL HEALTH QUESTIONNAIRE

The University of Birmingham

School of Sport and Exercise Sciences

General Health Questionnaire

Name: .....
Address: .....
.....
Phone: .....

Name of the responsible investigator for the study:

.....

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1.	You are.....	Male	Female
2.	What is your exact date of birth? Day..... Month.....Year..19..... So your age is..... Years		
3.	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
4.	Are you currently taking any medication?	YES	NO
5.	Are you currently taking the contraceptive pill?	YES	NO
6.	Have you taken any anti-inflamatory drugs such as ibuprofen in the last 24 hours?	YES	NO
7.	Have you had a cold or feverish illness in the last month?	YES	NO
8.	Has your doctor ever said you have “heart trouble”?	YES	NO
9.	Has your doctor ever said you have high blood pressure?	YES	NO
10.	Have you ever taken medication for blood pressure or your heart?	YES	NO

11.	Has your doctor ever told you that you have circulation problems in your limbs?	YES	NO
12.	Has your doctor ever told you that you have any vascular disease or venous disorders such as venous congestion or varicose veins?	YES	NO
13.	Has your doctor (or anyone else) said that you have a raised blood cholesterol?	YES	NO
14.	If you are female, are you experiencing amenorrhea or irregular menstrual cycles?	YES	NO
15.	If you are female, to your knowledge, are you pregnant?	YES	NO
16.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
17.	Do you suffer from asthma?	YES	NO
18.	Do you have any joint or bone problems or have you injured any of your limbs in the past 6 months?	YES	NO
19.	Has your doctor ever said you have diabetes?	YES	NO
20.	Have you ever had viral hepatitis?	YES	NO
21.	Do you know of any reason, not mentioned above, why you should not participate in this study?	YES	NO
22.	Do you exercise regularly? If so, how many hours per week? ……..	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signed: .....

Date: .....

Appendix C:
MENSTRUAL CYCLE QUESTIONNAIRE

The University of Birmingham
School of Sport and Exercise Sciences
Subject Questionnaire

Name: **Age:** **Date:**

We would be grateful if you could answer the following questions. (All information is confidential and will be held in a secure sight).

1. **Do you have regular periods?** Y/N

2. **When was the first day of your current cycle?**..... (date)
   (Day 1 = First day of bleeding)

3. **What is the usual duration of your <u>full</u> cycle?**..... (days)

4. **Do you take oral contraceptives?** Y/N
   If yes,
   i. Which type?
   ii. How long have you used them for?
   iii. At what time of the day do you take them?

Thank you for completing this questionnaire. All information given is completely confidential.

Appendix D:

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (IPAQ, short version)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

REFERENCES

Abe T, Kearns CF & Fukunaga T (2003) Sex differences in whole body skeletal muscle mass measured by magnetic resonance imaging and its distribution in young Japanese adults. *British Journal of Sports Medicine* 37(5):436-40

Abraham P, Leftheriotis G, Desvaux B, Saumet M & Saumet JL (1994) Venous return in lower limb during heat stress. *American Journal of Physiology* 267(4 Pt 2):H1337-40

Aellig WH (1994) Clinical pharmacology, physiology and pathophysiology of superficial veins--2. *British journal of clinical pharmacology* 38 (4): 289-305

Aharinejad S, Nedwed S, Michlits W, Dunn R, Abrahem D & Verndakis A (2001) Valvular density alone cannot account forsites of chronic venous insufficiency and ulceration in the lower extremity. *Microcirculation* 8: 347-54

Ahluwalia & Vallance (1997) Evidence for functional responses to sensory nerve stimulation of rat small mesenteric veins. *Journal of Pharmacology and Experimental Therapeutics* 281 (1): 9-14

Alexander R S (1963) *The peripheral venous system*. In W F Hamilton and P Dows (Eds) Handbook of Physiology, II. Circulation Washington DC: American Physiology Society: 1075-98

Amenta F, Cavallotti C, De Rossi M & Vatrella F (1983) Beta-adrenoceptors in the rat kidney. Immunohistochemical study. *Naunyn-Schmiedebergs Archives of Pharmacology* 324(2): 94-8

Andersen EB, Boesen F, Henriksen O & Sonne M (1986) Blood flow in skeletal muscle of tetraplegic man during postural changes *Clinical Science (London)* 70: 321-5

Ando S, Imaizumi T, Harada S, Hirooka Y & Takeshita (1992) Atrial natriuretic peptide increases human capillary filtration and venous distensibility. *Journal of Hypertension* 10 (5):451-7

Arangino S, Cagnacci A, Angiolucci M, Longu G, Melis G & Volpe A (1998) Effect of desogestrel-containing oral contraceptives on vascular reactivity and catecholamine levels. *Contraception* 58 (5): 289: 93

Arbeille P, Kerbeci P, Mattar L, Schoemaker JK & Hughson R (2008) Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. *American Journal of Physiology Heart & Circulatory physiology* 295 (5): H1846-54

Babaei H & Azarmi Y (2008) 17beta-estradiol inhibits calcium-dependent and -independent contractions in isolated human saphenous vein. *Steroids* 73 (8): 844-50

Banks M, Wei CM, Kim CH, Burnett JC Jr. & Miller VM (1996) Mechanism of relaxations to C-type natriuretic peptide in veins. *American Journal of Physiology* 271 (5 Pt 2): H1907-11
.

Barcroft (1960) The mechanism of the vasodilatation in the forearm muscle during stress (mental arithmetic). *Clinical Science* 19: 577-86

Barwin & Roddie, (1976) Venous distensibility during pregnancy determined by graded venous congestion. *American Journal of Obstetrics and Gynecology* 125 (7): 921-3

Beebe-Dimmer JL, Pfeifer JR, Engle JS & Schottenfeld D (2005) The epidemiology of chronic venous insufficiency and varicose veins. *Annals of Epidemiology* 15 (3): 175-84

Belin de Chantemèle E, Pascaud L, Custaud MA, Capri A, Louisy F, Ferretti G, Gharib C, Arbeille P. (2004) Calf venous volume during stand-test after a 90-day bed-rest study with or without exercise countermeasure. *Journal of Physiology* 561 (Pt 2): 611-22

Bell M & White M (2005) Cardiovascular responses to external compression of human calf muscle vary during graded metaboreflex stimulation. *Experimental Physiology* 90 (3): 383-91

Bellard et al. (2003) Haemodynamic response to peripheral venous congestion in patients with unexplained recurrent syncope. *Clinical Science (London)* 105 (3): 331-7

Bengtsson (1978) Estrogen induced inhibition of 3H-noradrenaline release in the uterus and portal vein of the rat. *Acta Physiologica Scandinavica* 104 (3): 287-98

Berczi et al. (1992) Pressure-induced activation of membrane K+ current in rat saphenous artery. *Hypertension* 19 (6 pt 2): 725-9

Berczi et al. (2005) Non-invasive assessment of human large vein diameter, capacity, distensibility and ellipticity in situ: dependence on anatomical location, age, body position and pressure. *European Journal of Applied Physiology* 95 (4): 283-9

Bevan, (1975) Evidence for alpha-adrenergic receptors on intimal endothelium. *Blood vessels* 12 (5): 307-10

Bevegard BS & Shepherd JT (1965) Effect of local exercise of forearm muscles on forearm capacitance vessels. *Journal of Applied Physiology* 20 (5): 968-74

Bevegard BS & Shepherd JT (1966) Reaction in man of resistance and capacity vessels in forearm and hand to leg exercise. *Journal of Applied Physiology* 21 (1): 123-32

Birch DJ, Turmaine M, Boulos PB & Burnstock G (2008) Sympathetic innervation of human mesenteric artery and vein. *Journal of Vascular Research* 45 (4): 323-32

Bishop GD, Oldfield MA & Brown MD (2005) Vasoconstriction in response to venous distension in the legs of women and men. *Journal of Physiology* 565P, PC8 (abstract)

Bishop GD & Brown MD (2007) Cutaneous postural vasoconstriction is modified by exogenous but not endogenous female hormones in young women. *Clinical Autonomic Research* 17(2):85-92

Bjornberg J & Maspers M (1991) Comparative studies of the venous resistance and capacitance functions and their sympathetic regulation in skeletal muscle. *Acta Physiologica Scandinavica* 142 (3): 329-337

Blackman DJ, Morris-Thurgood JA, Atherton JJ, Ellis GR, Anderson RA, Cockcroft JR & Frenneaux MP (2000) Endothelium-derived nitric oxide contributes to the regulation of venous tone in humans. *Circulation* 101(2):165-70

Bleeker JJ (1959) Experience with promarit as a hemostatic. *Medicine & Journalsim* 56 (8): 521-28

Boegehold MA (1996) Shear-dependent release of venular nitric oxide: effect on arteriolar tone in rat striated muscle. *American Journal of Physiology* 271 (2 Pt 2): H387-95

Boutcher YN & Boutcher SH (2005) Limb vasodilatory capacity and venous capacitance of trained runners and untrained males. *European Journal of Applied Physiology* 95 (1): 83-7

Bowyer L, Brown MA & Jones M (2001) Vascular reactivity in men and women of reproductive age. *American Journal of Obstetrics & Gynecology* 185 (1): 88-96

Braam B, Koomans HA, Rabelink TJ & Berckmans R (1989) Pitfalls of venous occlusion method for determination of capillary pressure in humans. *Journal of Applied Physiology* 66: 997-1002

Bracamonte MP, Jayachandran M, Rud KS & Miller, VM (2002) Acute effects of 17beta -estradiol on femoral veins from adult gonadally intact and ovariectomized female pigs. *American Journal of Physiology Heart & Circulatory Physiology* 283 (6): H2389-96

Brown E, Greenfield ADM, Goei JS & Plassaras G (1966) Filling and emptying of the low pressure blood vessels of the human forearm. *Journal of Applied Physiology* 21: 573-82

Browse NL, Shepherd JT, Donald DE (1966) Differences in response of veins and resistance vessels in limbs to same stimulus. *American Journal of Physiology* 211: 1241-7

Browse NL & Hardwick PJ (1969) The deep-breath-venoconstriction reflex. *Clinical Science* 37 (1): 125-135

Buckey JC, Peshock RM & Blomqvist CG (1988) Deep venous contribution to hydrostatic blood volume change in the human leg. *American Journal of Cardiology* 62 (7): 449-53

Burnstock G, Gannon B, & Iwayama, T (1970) Sympathetic innervation of vascular smooth muscle in normal and hypertensive animals. *Circulation Research* 27: 23

Butt C, Pathmadeva C & Spencer C (1999) Forearm and calf blood flow in response to cortical arousal in normal male and female subjects. *Clinical Autonomic Research* 9 (2): 103-107

Byrne KB, Stanton BJ, Coupar IM & Burcher E (1988) Effect of levonorgestrel and ethinyloestradiol on vasoconstriction in rat isolated vasculature. *Journal of Pharmacy & Pharmacology* 40 (8): 562-6

Caggiati A, Phillips M, Lametschwandtner A & Allegra C (2006) Valves in small veins and venules. *European Journal of Vascular & Endovascular Surgery* 32 (4): 447-452

Callister R, Suwarno NO & Seals DR (1992) Sympathetic activity is influenced by task difficulty and stress perception during mental challenge in humans. *Journal of Physiology* 454: 373-87

Callow ID, Campisi P, Lambert ML, Feng Q & Arnold JM (1998) Enhanced in vivo alpha1- and alpha2-adrenoceptor-mediated venoconstriction with indomethacin in humans. *American Journal of Physiology* 275 (3 Pt 2): H837-43

Carter JR, Ray CA & Cooke WH (2002) Vestibulosympathetic reflex during mental stress. *Journal of Applied Physiology* 93 (4): 1260-4

Carter JR, Cooke WH & Ray CA (2005) Forearm neurovascular responses during mental stress and vestibular activation. *American Journal of Physiology Heart & Circulatory Physiology* 288 (2): H904-7

Carter JR & Lawrence JE (2007) Effects of the menstrual cycle on sympathetic neural responses to mental stress in humans. *Journal of Physiology* 585 (2): 635-41

Carter JR & Ray CA (2009) Sympathetic neural responses to mental stress: responders, nonresponders and sex differences *American Journal of Physiology Heart & Circulatory Physiology* 296 (3): H847-53

Cavezzi A, Labropoulos N, Partsch H, Ricci S, Caggiati A, Myers K, Nicolaides A & Smith PC (2006) Duplex ultrasound investigation of the veins in chronic venous disease of the lower limbs--UIP consensus document. Part II. Anatomy. *European Journal of Vascular & Endovascular Surgery* 31 (3): 288-99

Ceballos C, Ribes C, Amado JA, Perez J, Garcia Unzueta MT & de B Jr. (2000) Venous endothelial function in postmenopausal women who are receiving long-term estrogen and progestagen therapy. *Fertility & Sterility* 74 (2): 268-73

Chan NN, MacAllister RJ, Colhoun HM, Vallance P & Hingorani AD (2001) Changes in endothelium-dependent vasodilatation and alpha-adrenergic responses in resistance vessels during the menstrual cycle in healthy women. *Journal of Clinical Endocrinology & Metabaloism* 86 (6): 2499-504

Chan YC, Leung FP, Yao X, Lau CW, Vanhoutte PM, Huang Y. (2005) Raloxifene relaxes rat pulmonary arteries and veins: roles of gender, endothelium, and antagonism of Ca2+ influx. *Journal of Pharmacology & Experimental Therapeutics* 12(3):1266-71

Chang PF, Arendt-Nielsen L & Chen AC (2002) Dynamic changes and spatial correlation of EEG activities during cold pressor test in man. *Brain Research Bulletin* 57(5):667-75

Chaudhuri KR, Thomaides T, Hernandez P, Alam M & Mathias CJ (1991) Noninvasive quantification of superior mesenteric artery blood flow during sympathoneural activation in normal subjects. *Clinical Autonomic Research* 1 (1): 37-42

Chauveau M, Bassez S, Tra S & Scherrer B (2006) Area-pressure relationship of lower limb main veins in man. *Vasa* 35 (2): 59-66

Chen HI & Ta C (1994) The sympathetic efferent innervation of the cutaneous and muscle veins in cats. A comparative study using retrograde localization with horseradish peroxidase. *Journal of Autonomic Nervous System* 46 (3):189-97

Christ F, Gamble J, Baschnegger H & Gartside IB (1997) Relationship between venous pressure and tissue volume during venous congestion plethysmography in man. *Journal of Physiology* 503 (2): 463-7

Christensen NJ & Galbo H (1983) Sympathetic nervous activity during exercise. *Annual Review of Physiology* 45: 139-153

Christou DD, Jones PP, Jordan J, Diedrich A, Robertson D & Seals DR (2005) Women have lower tonic autonomic support of arterial blood pressure and less effective baroreflex buffering than men. *Circulation* 111 (4): 494-8

Chu ZM & Beilin LJ (1997) Effects of 17 beta-oestradiol on vascular responses in the in situ blood-perfused mesentery of Wistar-Kyoto rats. *Clinical & Experimental Pharmacology & Physiology* 24 (6): 430-2

Cirovic S, Walsh C, Fraser WD & Gulino A (2006) Venous filling and elastance in the calf positioned above and below heart level. *Aviation Space Environment Medicine* 77 (10): 1009-14

Claydon VE, Younis NR & Hainsworth R (2006) Phase of the menstrual cycle does not affect orthostatic tolerance in healthy women. *Clinical Autonomic Research* 16 (2): 98-104

Clifford PS, Hamann JJ, Valic Z & Buckwalter JB (2005) Counterpoint: The muscle pump is not an important determinant of muscle blood flow during exercise. *Journal of Applied Physiology* 99 (1): 372-374

Clements IP, Strelow DA, Becker GP, Vlietstra RE & Brown ML (1981) Radionuclide evaluation of peripheral circulatory dynamics: new clinical application of blood pool scintigraphy for measuring limb venous volume, capacity, and blood flow. *American Heart Journal* 102 (6 part 1): 980-3

Coffman JD & Lempert JA (1975) Venous flow velocity, venous volume and arterial blood flow. *Circulation* 52: 141-145

Coleridge-Smith P, Labropoulos N, Partsch H, Myers K, Nicolaides A & Cavezzi A (2006) Duplex ultrasound investigation of the veins in chronic venous disease of the lower limbs--UIP consensus document. Part I. Basic principles. *European Journal of Vascular & Endovascular Surgery* 31 (1): 83-92

Convertino VA (1998) Gender differences in autonomic functions associated with blood pressure regulation. *American Journal of Physiology Regulatory, Integrative & Comparative Physiology* 275 (6): R1909-20

Crandall CG, Shibasaki M & Yen TC (2002) Evidence that the human cutaneous venoarteriolar response is not mediated by adrenergic mechanisms. *Journal of Physiology* 538: 599-605

Cui J, Wilson TE & Crandall CG (2002) Baroreflex modulation of muscle sympathetic nerve activity during cold pressor test in humans. *American Journal of Physiology Heart & Circulatory Physiology* 282 (5): H1717-23

Danielsson G, Eklof B & Kistner RL (2005) Association of venous volume and diameter of incompetent perforator veins in the lower limb--implications for perforator vein surgery. *European Journal of Vascular and Endovascular Surgery* 30 (6): 670-3

Davies TS, Frenneaux MP, Campbell RI & White MJ (2007) Human arterial responses to isometric exercise: the role of the muscle metaboreflex. *Clinical Science (London)* 112 (8): 441-7

de Boer D, Ring C & Carroll D (2006) Time course and mechanisms of hemoconcentration in response to mental stress. *Biological Psychology* 72 (3): 318-24

de Graaff JC, Ubbink DT, Lagarde SM & Jacobs MJ (2003) Postural changes in capillary pressure in the hallux of healthy volunteers. *Journal of Applied Physiology* 95 (6): 2223-8

de Groot E, Jukema JW, Montauban van Swijndregt AD, Zwinderman AH, Ackerstaff RG, van der Steen AF, Bom N, Lie KI & Bruschke AV (1998) B-mode ultrasound assessment of pravastatin treatment effect on carotid and femoral artery walls and its correlations with coronary arteriographic

findings: a report of the Regression Growth Evaluation Statin Study (REGRESS). *Journal of the American College of Cardiology* 31 (7): 1561-7

de Groot E, Hovingh GK, Zwinderman AH, Wiegman A, Smit AJ & Kastelein JJ (2005) Data density curves of B-mode ultrasound arterial wall thickness measurements in unaffected control and at-risk populations. *International Angiology* 24 (4): 359-65

Delaney EP, Young CN, Disabatino A, Stillabower ME & Farquhar WB. (2008) Limb venous tone and responsiveness in hypertensive humans. *Journal of Applied Physiology* 105 (3): 894-901

Delis KT, Nicolaides AN & Stansby G (2000) Effect of posture on popliteal artery hemodynamics. *Archives of Surgery* 135 (3): 265-9

Delius W (1971) Variability in measurement of venous tone reactions with the occluded limb technique. *Scandinavian Journal of Clinical Labaoratory Investigation* 27 (3): 207-12

Dhawan V, Brookes ZL & Kaufman S (2005) Repeated pregnancies (multiparity) increases venous tone and reduces compliance *American Journal of Physiology Regulatory, Integrative & Comparative Physiology* 289 (1): R23-8

Dietz NM, Rivera JM, Eggener SE, Fix RT, Warner DO & Joyner MJ (1994) Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. *Journal of Physiology* 480 (Pt 2): 361-8

Dietz NM, Engelke KA, Samuel TT, Fix RT & Joyner MJ (1997) Evidence for nitric oxide-mediated sympathetic forearm vasodiolatation in humans. *Journal of Physiology* 498 (Pt 2): 531-40

Dishman RK, Jackson EM & Nakamura Y (2002) Influence of fitness and gender on blood pressure responses during active or passive stress. *Psychophysiology* 39 (5): 568-76

Dishman RK, Nakamura Y, Jackson EM & Ray CA (2003) Blood pressure and muscle sympathetic nerve activity during cold pressor stress: fitness and gender. *Psychophysiology* 40 (3): 370-80

Donadio V, Kallio M, Karlsson T, Nordin M & Wallin BG (2002) Inhibition of human muscle sympathetic activity by sensory stimulation. *Journal of Physiology* 544 (1): 285-92

Donegan JF (1921) The physiology of the veins. *Journal of Physiology* 55 (3-4): 226-45

Doorenbos CJ, Blauw GJ & van BP (1991) Arterial and venous effects of atrial natriuretic peptide in the human forearm. *American Journal of Hypertension* 4 (4 Pt 1): 333-40

Dornyei G, Monos E, Kaley G & Koller A (1996) Myogenic responses of isolated rat skeletal muscle venules: modulation by norepinephrine and endothelium. *American Journal of Physiology* 271 (1 Pt 2): H267-72

Dornyei G, Kaley G & Koller A (1997) Release of nitric oxide and prostaglandin H2 to acetylcholine in skeletal muscle venules. *American Journal of Physiology* 272 (6 Pt 2): H2541-6

Du XJ, Riemersma RA & Dart AM (1995) Cardiovascular protection by oestrogen is partly mediated through modulation of autonomic nervous function. *Cardiovascular Research* 30 (2): 161-5

Ducharme DW, Weeks JR & Montgomery RG (1968) Studies on the mechanism of the hypertensive effect of prostaglandin F2-alpha. *Journal of Pharmacology & Experimental Therapeutics* 160 (1): 1-10

Duggan JJ, Love VL & Lyons RH (1953) A study of reflex venomotor reactions in man. *Circulation* 7 (6): 869-73

Durel LA, Kus LA, Anderson NB, McNeilly M, Llabre MM, Spitzer S, Saab PG, Efland J, Williams R & Schneiderman N (1993) Patterns and stability of cardiovascular responses to variations of the cold pressor test. *Psychophysiology* 30 (1): 39-46

Durnin JV & Womersley J (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition* 32: 77-97

Dyer NH & Dawson AM (1970) Diagnosis of Crohn's disease. A continuing source of error. *British Medical Journal* 1 (5698): 735-7

Dyer NH, Child JA, Mollin DL & Dawson AM (1972) Anaemia in Crohn's disease *QJ Med* 41 (164): 419-36

Dzeka TN, Kuzminski P & Arnold JM (2000) Venous responsiveness to norepinephrine in healthy subjects: effects of single doses of 325 mg aspirin. *Clinical Pharmacology & Therapeutics* 67 (3): 299-304

Dzeka TN & Arnold JM (2003) Prostaglandin modulation of venoconstriction to physiological stress in normals and heart failure patients. *American Journal of Physiology Heart & Circulatory Physiology* 284 (3):H790-7

Dzeka TN, Townley R & Arnold JM (2003) Effects of enalaprilat on venoconstriction to norepinephrine: role of prostaglandins. *Cardiovascular Research* 59 (1): 250-6

Edouard DA, Pannier BM, London GM, Cuche JL & Safar ME (1998) Venous and arterial behavior during normal pregnancy. *American Journal of Physiology* 274 (5 Pt 2): H1605-12

Eiken O & Kolegard R (2004) Comparison of vascular distensibility in the upper and lower extremity. *Acta Physiologica Scandinavica* 181 (3): 281-7

Eklund B & Kaijser L (1974) Forearm blood flow after isometric contraction at different loads in relation to potentially vasodilating substances. *Scandinavian Journal of Clinical Laboratory Investigation* 34 (1): 23-9

Eklund B, Kaijser L & Knutsson E (1974) Blood flow in resting (contralateral) arm and leg during isometric contraction. *Journal of Physiology* 240 (1): 111-24

Eklund B & Kaijser L (1976) Effect of regional alpha- and beta-adrenergic blockade on blood flow in the resting forearm during contralateral isometric handgrip. *Journal of Physiology* 262 (1): 39-50

Eklund B & Kaijser L (1978) Blood flow in the resting forearm during prolonged contralateral isometric handgrip at maximal effort. *Journal of Physiology* 277: 359-66

Elliott KJ, Cable NT & Reilly T (2005) Does oral contraceptive use affect maximum force production in women? *British Journal of Sports Medicine* 39 (1):15-9

Endrikat J, Klipping C, Cronin M, Gerlinger C, Ruebig A, Schmidt W & Dusterberg B (2002) An open label, comparative study of the effects of a dose-reduced oral contraceptive containing 20 microg ethinyl estradiol and 100 microg levonorgestrel on hemostatic, lipids, and carbohydrate metabolism variables. *Contraception* 65 (3): 215-21

Epstein SE, Beiser GD, Stampfer M & Braunwald E (1968) Role of the venous system in baroreceptor-mediated reflexes in man. *Journal of Clinical Investigation* 47 (1): 139-52

Eston R, Evans R & Fu F (1994) Estimation of body composition in Chinese and British men by ultrasonographic assessment of segmental adipose tissue volume. *British Journal of Sports Medicine* 28: 9-13

Ettinger SM, Silber DH, Collins BG, Gray KS, Sutliff G, Whisler SK, McClain JM, Smith MB, Yang QX & Sinoway LI (1990) Influences of gender on sympathetic nerve responses to static exercise. *Journal of Applied Physiology* 80 (1): 245-51

Ettinger SM, Silber DH, Gray KS, Smith MB, Yang QX, Kunselman AR & Sinoway LI (1998) Effects of the ovarian cycle on sympathetic neural outflow during static exercise. *Journal of Applied Physiology* 85 (6): 2075-81

Fabi F, Argiolas L, Chiavarelli M & del BP (1996) Nitric oxide-dependent and -independent modulation of sympathetic vasoconstriction in the human saphenous vein. *European Journal of Pharmacology* 309 (1): 41-50

Fabi F, Di MG, Musumeci F & del BP (2004) Endothelium-dependent noradrenergic hyperresponsiveness induced by thapsigargin in human saphenous veins: role of thromboxane and calcium. *European Journal of Pharmacology* 484 (2-3): 277-85

Farag NH, Vincent AS, McKey BS, Al'Absi M, Whitsett TL & Lovallo WR (2006) Sex differences in the hemodynamic responses to mental stress: Effect of caffeine consumption. *Psychophysiology* 43 (4): 337-43

Fasano ML, Sand T, Brubakk AO, Kruszewski P, Bordini C & Sjaastad O (1996) Reproducibility of the cold pressor test: studies in normal subjects. *Clinical Autonomic Research* 6 (5): 249-53

Fawer R, Dettling A, Weihs D, Welti H & Schelling JL (1978) Effect of the menstrual cycle, oral contraception and pregnancy on forearm blood flow, venous distensibility and clotting factors. *European Journal of Clinical Pharmacology* 13 (4): 251-7

Feger J & Braune S (2005) Measurement of skin vasoconstrictor response in healthy subjects. *Autonomic Neuroscience* 120 (1-2): 88-96

Ferguson RA & Brown MD (1997) Arterial blood pressure and forearm vascular conductance responses to sustained and rhythmic isometric exercise and arterial occlusion in trained rock climbers and untrained sedentary subjects *European Journal of Applied Physiology& Occupational Physiology* 76 (2): 174-80

Fisher JP & White MJ (2003) The time course and direction of lower limb vascular conductance changes during voluntary and electrically evoked isometric exercise of the contralateral calf muscle in man. *Journal of Physiology* 546 (1): 315-23

Fisher JP & White MJ (2004) Muscle afferent contributions to the cardiovascular response to isometric exercise. *Experimental Physiology* 89 (6): 639-46

Fisher JP, Sander M, MacDonald I & White MJ (2005) Decreased muscle sympathetic nerve activity does not explain increased vascular conductance during contralateral isometric exercise in humans. *Experimental Physiology* 90 (3): 377-82

Franke WD, Johnson CP, Steinkamp JA, Wang R & Halliwill JR (2003) Cardiovascular and autonomic responses to lower body negative pressure: do not explain gender differences in orthostatic tolerance. *Clinical Autonomic Research* 13 (1): 36-44

Freedman RR & Girgis R (2000) Effects of menstrual cycle and race on peripheral vascular alpha-adrenergic responsiveness. *Hypertension* 35 (3): 795-9

Freeman R, Lirofonis V, Farquhar WB & Risk M (2002) Limb venous compliance in patients with idiopathic orthostatic intolerance and postural tachycardia. *Journal of Applied Physiology* 93 (2): 636-44

Frey MA & Hoffler GW (1988) Association of sex and age with responses to lower-body negative pressure. *Journal of Applied Physiology* 65 (4): 1752-6

Freyschuss U, Hjemdahl P, Juhlin-Dannfelt A & Linde B (1988) Cardiovascular and sympathoadrenal responses to mental stress: influence of beta-blockade. *American Journal of Physiology* 255 (6 Pt 2): H1443-51

Fronek A, Criqui MH, Denenberg J & Langer RD (2001) Common femoral vein dimensions and hemodynamics including Valsalva response as a function of sex, age, and ethnicity in a population study. *Journal of Vascular Surgery* 33 (5): 1050-6

Fu Q, Witkowski S & Levine BD (2004) Vasoconstrictor reserve and sympathetic neural control of orthostasis. *Circulation* 110 (18): 2931-7

Fu Q, Witkowski S, Okazaki K & Levine BD (2005) Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress. *American Journal of Physiology Regulatory, Integrative & Comparative Physiology* 289 (1): R109-16

Fu Q, Okazaki K, Shibata S, Shook RP, VanGunday TB, Galbreath MM, Reelick MF & Levine BD (2009) Menstrual cycle effects on sympathetic neural responses to upright tilt. *Journal of Physiology* 587 (9): 2019-31

Fuller NJ, Laskey MA & Elia M (2008) Assessment of the composition of major body regions by dual-energy X-ray absorptiometry (DEXA), with special reference to limb muscle mass. *Clinical Physiology and functional Imaging* 12 (3): 253-66

Fung YC (1993) *Biomechanics – mechanical properties of living tissue*. 2nd ed. New York, Springer-Verlag

Furness JB & Marshall JM. (1974) Correlation of the directly observed responses of mesenteric vessles of the rat to nerve stimulation and noradrenaline with the distribution of adrenergic nerves. *Journal of Physiology* 239 (1): 75-88

Fuxe K & Sedvall G (1965) The distribution of adrenergic nerve fibres to the blood vessels in skeletal muscle. *Acta Physiologica Scandinavica* 64: 75-86

Gamble J, Gartside IB & Christ F (1993) A reassessment of mercury in silastic strain gauge plethysmography for microvascular permeability assessment in man. *Journal of Physiology* 464: 407-22

Gamble J, Christ F & Gartside IB (1997) The effect of passive tilting on microvascular parameters in the human calf: a strain gauge plethysmography study. *Journal of Physiology* 498 (2): 541-52

Gamble J, Christ F & Gartside IB (1998) Human calf precapillary resistance decreases in response to small cumulative increases in venous congestion pressure. *Journal of Physiology* 507 (Part 2): 611-7

Gamble J, Christ F & Gartside IB (1998) Human calf precapillary resistance decreases in response to small cumulative increases in venous congestion pressure. *Journal of Physiology* 507 (Part 2): 611-7

Gamble J, Joyner MJ, Dietz NM & Shepherd JT (2005) A restrospective perspective. *Journal of Applied Physiology* 98 (2): 762-3

Gauer OH & Thron HL (1962) Properties of veins in vivo: integrated effects of their smooth muscle. *Physiological Reviews Supplemental* 5: 283-308

Geelen G, Laitinen T, Hartikainen J, Lansimies E, Bergstrom K & Niskanen L (2002) Gender influence on vasoactive hormones at rest and during a 70 degrees head-up tilt in healthy humans. *Journal of Applied Physiology* 92 (4): 1401-8

Gelman, S (2008) Venous function and central venous pressure: a physiologic story. *Anesthesiology* 108 (4): 735-48

Gerova M & Gero J (1975) The ability of collecting veins to sustain sympathetic constriction. *Physiologia Bohemoslovaca* 24 (3): 193-8

Giessler C, Wangemann T, Silber RE, Dhein S & Brodde OE (2002) Noradrenaline-induced contraction of human saphenous vein and human internal mammary artery: involvement of different alpha-adrenoceptor subtypes. *Naunyn-Schmiedebergs Archives of Pharmacology* 366 (2): 104-9

Gisclard V, Flavahan NA & Vanhoutte PM (1987) Alpha adrenergic responses of blood vessels of rabbits after ovariectomy and administration of 17 beta-estradiol. *Journal of Pharmacology & Experimental Therapeutics* 240 (2): 466-70

Gooding KM, MacLeod KM, Spyer G, Ewings P, Tooke JE & Shore AC (2005) Impact of hormone replacement therapy on microvascular function in healthy and Type 2 diabetic postmenopausal women. *Diabetic Medicine* 22 (5): 536-42

Goodrich SM & Wood JE (1964) Peripheral venous distensibility and velocity of venous blood flow during pregnancy or during oral contraceptive therapy. *American Journal of Obstetrics & Gynecology* 90: 740-44

Goodrich SM & Wood JE (1966) The effect of estradiol-17-beta on peripheral venous distensibility and velocity of venous blood flow. *American Journal of Obstetrics & Gynecology* 96 (3): 407-12

Greenfield AD, Whitney RJ & Mowbray JF (963) Methods for the investigation of peripheral blood flow. *British Medical Bulletin* 19: 101-9

Groban L, Cowley AW Jr. & Ebert TJ (1990) Atrial natriuretic peptide augments forearm capillary filtration in humans. *American Journal of Physiology* 259 (1 Pt 2): H258-63

Guyton AC & Richardson TQ (1961) Effect of hematocrit on venous return. *Circulation Research* 9: 157-64

Haenen JH, van L H, Janssen MC, Wollersheim H, van't hof MA, van Asten WN, Skotnicki SH & Thien T (1999) Venous duplex scanning of the leg: range, variability and reproducibility. *Clinical Science (London)* 96 (3): 271-7

Hainsworth R (1986) Vascular capacitance: its control and importance. *Reviews of Physiology, Biochemistry & Pharmacology* 105: 101-73

Hainsworth R (1990) Non-invasive investigations of cardiovascular reflexes in humans. *Clinical Science (London)* 78 (5): 437-43

Hainsworth R & Drinkhill MJ (2006) Counterpoint: active venoconstriction is not important in maintaining or raising end-diastolic volume and stroke volume during exercise and orthostasis. *Journal of Applied Physiology* 101 (4): 1264-5

Halliwill JR, Lawler LA, Eickhoff TJ. Dietz NM, Nauss LA & Joyner MJ (1997) Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *Journal of Physiology* 504 (Pt 1): 211-20

Halliwill JR, Lawler LA, Eickhoff TJ, Joyner MJ & Mulvagh SL (1998) Reflex responses to regional venous pooling during lower body negative pressure in humans. *Journal of Applied Physiology* 84 (2): 454-8

Halliwill JR, Minson CT & Joyner MJ (1999) Measurement of limb venous compliance in humans: technical considerations and physiological findings. *Journal of Applied Physiology* 87 (4): 1555-63

Hamer M, Boutcher YN, Park Y & Boutcher SH (2006) Reproducibility of skeletal muscle vasodilatation responses to Stroop mental challenge over repeated sessions *Biological Psychology* 73 (2): 186-9

Hargreaves AD & Muir AL (1992) Haemodynamic responses to glyceryl trinitrate: influence of rate and duration of delivery. *European Heart Journal* 13 (7): 960-5

Hasegawa GR (1983) Ventricular preexcitation. *Archives of Internal Medicine* 143 (11): 2215-8

Hastrup JL & Light KC (1984) Sex differences in cardiovascular stress responses: modulation as a function of menstrual cycle phases *Journal of Psychosomatic Research* 28 (6): 475-83

Heidbreder E, Ziegler A, Heidland A, Kirsten R & Gruninger W (1982) Circulatory changes during mental stress in tetraplegic and paraplegic man. *Klinische Wochenschrift* 60 (15):795-801

Heitz NA, Eisenman PA, Beck CL & Walker JA (1999) Hormonal Changes Throughout the Menstrual Cycle and Increased Anterior Cruciate Ligament Laxity in Females. *Journal of Athletic Training* 34 (2): 144-9

Henriksen O. (1991) Sympathetic reflex control of blood flow in human peripheral tissues. *Acta Physiologica Scandinavica Supplemental* 603: 33-9

Henriksen O, Amtorp O, Faris I & Agerskov K (1983) Evidence for a local sympathetic venoarteriolar "reflex" in the dog hindleg. *Circulation Research* 52 (5): 534-42

Herbst WM, Eberle KP, Ozen Y & Hornstein OP (1992) The innervation of the great saphenous vein: an immunohistochemical study with special regard to regulatory peptides. *Vasa* 21 (3): 253-7

Herd JA (1991) Cardiovascular response to stress. *Physiological Reviews* 71 (1): 305-30

Herkert O, Kuhl H, Busse R & Schini-Kerth VB (2000) The progestin levonorgestrel induces endothelium-independent relaxation of rabbit jugular vein via inhibition of calcium entry and protein kinase C: role of cyclic AMP. *British Journal of Pharmacology* 130 (8): 1911-8

Hernandez JP & Franke WD (2004) Age- and fitness-related differences in limb venous compliance do not affect tolerance to maximal lower body negative pressure in men and women. *Journal of Applied Physiology* 97: 925-9

Hernandez JP, Karandikar A & Franke WD (2005) Effects of age and fitness on tolerance to lower body negative pressure. *Journal of.Gerontology Series A Biological Sciences & Medical Sciences* 60 (6): 782-6

Hertzberg BS, Kliewer MA, DeLong DM, Lalouche KJ, Paulson EK, Frederick MG & Carroll BA (1997) Sonographic assessment of lower limb vein diameters: implications for the diagnosis and characterization of deep venous thrombosis. *American Journal of Roentgenology* 168 (5):1253-7

Hiatt WL, Huang SY, Regensteiner JG, Micco AJ, Ishimoto G, Manco-Johnson M, Drose J, Reeves JT (1989) Venous occlusion plethysmography reduces arterial diameter and flow velocity. *Journal of Applied Physiology* 66: 2239-44

Hilz MJ & Dutsch M (2006) Quantitative studies of autonomic function. *Muscle & Nerve* 33 (1): 6-20

Hirshoren N, Tzoran I, Makrienko I, Edoute, Y, Plawner MM, Itskovitz-Eldor J & Jacob G (2002) Menstrual cycle effects on the neurohumoral and autonomic nervous systems regulating the cardiovascular system. *Journal of Clinical Endocrinology & Metabolism* 87 (4): 1569-75

Hisdal J, Toska K, Flatebo T, Waaler B & Walloe L (2004) Regulation of arterial blood pressure in humans during isometric muscle contraction and lower body negative pressure. *European Journal of Applied Physiology* 91 (2-3): 336-41

Hitos K, Cannon M, Cannon S, Garth S & Fletcher JP (2007) Effect of leg exercises on popliteal venous blood flow during prolonged immobility of seated subjects: implications for prevention of travel-related deep vein thrombosis. *Journal of Thrombosis & Haemostasis* 5 (9): 1890-5

Hogarth AJ, Mackintosh AF & Mary DA (2007) Gender-related differences in the sympathetic vasoconstrictor drive of normal subjects *Clinical Science (London)* 112 (6): 353-61

Holland CK, Brown JM, Scoutt LM & Taylor KJW (1998) Lower extremity volumetric arterial blood flow in normal subjects. *Ultrasound in Medicine & Biology* 24: 1079-86

Huang A & Kaley G (2004) Gender-specific regulation of cardiovascular function: estrogen as key player. *Microcirculation* 11: 9-38

Hughes A, Thom S, Goldberg P, Martin G & Sever P (1988) Direct effect of alpha-human atrial natriuretic peptide on human vasculature in vivo and in vitro. *Clinical Science (London)*. 74 (2): 207-11

Hughes J & Vane JR (1970) Relaxations of the isolated portal vein of the rabbit induced by nicotine and electrical stimulation. *British Journal of Pharmacology* 39 (3): 476-89

Ireland MA, Davies P & Littler WA (1983) Some aspects of the arterial response to venous occlusion in man. *Clinical Science* 65: 1-8

Jacob G, Costa F, Shannon J, Robertson D & Biaggioni I (2000) Dissociation between neural and vascular responses to sympathetic stimulation: contribution of local adrenergic receptor function. *Hypertension* 35 (1 Pt 1): 76-81

Janse de Jonge XA, Boot CR, Thom JM, Ruell PA & Thompson MW (2001) The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans. *Journal of Physiology* 530 (1): 161-6

Jeanneret C, Labs KH, Aschwanden M, Bollinger A, Hoffmann U & Jager K (1999) Physiological reflux and venous diameter change in the proximal lower limb veins during a standardised Valsalva manoeuvre. *European Journal of Vascular & Endovascular Surgery* 17 (5): 398-403

Jeanneret C, Jager KA, Zaugg CE & Hoffmann U (2007) Venous reflux and venous distensibility in varicose and healthy veins *European Journal of Vascular & Endovascular Surgery* 34 (2): 236-42

Jespersen J, Petersen KR & Skouby SO (1990) Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid. *American Journal of Obstetetrics & Gynecology* 163 (1 Pt 2): 396-403

John S, Jacobi J, Schlaich MP, Delles C & Schmieder RE (2000) Effects of oral contraceptives on vascular endothelium in premenopausal women. *American Journal of Obstetetrics & Gynecology* 183 (1): 28-33

Jones PP, Spraul M, Matt KS, Seals DR, Skinner JS & Ravussin E (1996) Gender does not influence sympathetic neural reactivity to stress in healthy humans American *Journal of Physiology* 270 (1 part 2): H350-7

Jorfeldt L, Vedung T, Forsstrom E & Henriksson J (2003) Influence of leg position and environmental temperature on segmental volume expansion during venous occlusion plethysmography. *Clinical Science (London)* 104 (6): 599-605

Journo HJ, Chanudet XA, Pannier BM, Laroque PL, London GM & Safar ME (1992) Hysteresis of the venous pressure-volume relationship in the forearm of borderline hypertensive subjects. *Clinical Science (London)* 82 (3): 329-34

Joyner MJ, Dietz NM, & Shepherd JT (2001) From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *Journal of Applied Physiology* 91 (6): 2431-41

Joyner MJ & Halliwill JR (2000) Sympathetic vasodilatation in human limbs. *Journal of Physiology* 526 (3): 471-80

Kamiya A, Michikami D, Fu Q, Niimi Y & Iwase S (2000) Muscle sympathetic nerve activity (MSNA) during high-intensity, isometric leg exercise in humans. *Environmental Medicine* 44 (1): 49-52

Kerslake DM (1949) The effect of the application of an arterial occlusion cuff to the wrist on the blood flow in the human forearm. *Journal of Physiology* 108 (4): 451-7

Kim MB & Sarelius IH (2003) Distributions of wall shear stress in venular convergences of mouse cremaster muscle. *Microcirculation* 10 (2): 167-78

Kleitman N & Ramsaroop A (1948) Periodicity in body temperature and heart rate. *Endocrinology* 43 (1): 1-20

Kneale BJ, Chowienczyk PJ, Cockcroft JR, Coltart DJ & Ritter JM (1997) Vasoconstrictor sensitivity to noradrenaline and NG-monomethyl-L-arginine in men and women. *Clinical Science (London)* 93 (6): 513-8

Kneale BJ, Chowienczyk PJ, Brett SE, Coltart DJ & Ritter JM. 2000 Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *Journal of the American College of Cardiology* 36 (4): 1233-8

Kooijman M, Thijssen DH, de Groot PC, Bleeker MW, van Kuppevelt HJ, Green DJ, Rongen GA, Smits P & Hopman MT (2008) Flow-mediated dilatation in the superficial femoral artery is nitric oxide mediated in humans. *Journal of Physiology* 586 (4):1137-45

Kopin IJ, Lake RC & Ziegler M (1978) Plasma levels of norepinephrine. *Annals of Internal Medicine* 88 (5): 671-80

Kresse H, Filipovic I, Iserloh A & Buddecke E (1970) Comparative studies on the chemistry and the metabolism of arterial and venous tissue. *Angiologica* 7 (6): 321-32

Kroger K, Ose C, Rudofsky G, Roesener J, Weiland D & Hirche H (2003) Peripheral veins: influence of gender, body mass index, age and varicose veins on cross-sectional area. *Vascular Medicine* 8 (4): 249-55

Kugler C, Strunk M & Rudofsky G (2001) Venous pressure dynamics of the healthy human leg. Role of muscle activity, joint mobility and anthropometric factors. *Journal of Vascular Research* 38 (1): 20-9

Kuhl (1999) Vascular effects of gestagens – biochemistry versus epidemiology. *Zentralbl Gynakol* 121: 67-78

Kuipers NT, Sauder CL, Carter JR & Ray CA (2008) Neurovascular responses to mental stress in the supine and upright postures. *Journal of Applied Physiology* 104 (4): 1129-36

Lake CR, Ziegler MG & Kopin IJ (1976) Use of plasma norepinephrine for evaluation of sympathetic neuronal function in man. *Life Science* 18 (11): 1315-25

Lanne T & Olsen H (1997) Decreased capacitance response with age in lower limbs of humans--a potential error in the study of cardiovascular reflexes in ageing. *Acta Physiologica Scandinavica*161 (4): 503-7

Laughlin MH (1987) Skeletal muscle blood flow capacity: role of muscle pump in exercise hyperemia. *American Journal of Physiology* 253 (5 Pt 2): H993-1004

Laughlin MH & Schrage WG (1999) Effects of muscle contraction on skeletal muscle blood flow: when is there a muscle pump? *Medicine in Science &.Sports & Exercise* 31 (7): 1027-35

LeBlanc J, Cote J, Jobin M & Labrie A (1979) Plasma catecholamines and cardiovascular responses to cold and mental activity. *Journal of Applied Physiology* 47 (6): 1207-11

Lenders J, Houben H, van V R, Willemsen J & Thien T (1988) Reproducibility of haemodynamic and plasma catecholamine responses to isometric exercise and mental arithmetic in normo- and hypertensive subjects. *Clinical Science (London)* 75 (6): 615-9

Leuenberger UA, Mostoufi-Moab S. Herr M, Gray K, Kunselman A & Sinoway LI (2003) Control of skin sympathetic nerve activity during intermittent static handgrip exercise. *Circulation* 108 (19): 2329-35

Levick JR (2003) *An Introduction to Cardiovascular Physiology.* Hodder Arnold (4th Edition)

Levy BI, Valladares WR, Ghaem A & Martineaud JP (1979) Comparison of plethysmographic methods with pulsed Doppler blood flowmetry. *American Journal of Physiology* 236 (6): H899-903

Lewin MR, Stein J, Wang R, Lee MM, Kernberg M, Boukhman M, Hahn IH & Lewiss RE (2007) Humming is as effective as Valsalva's maneuver and Trendelenburg's position for ultrasonographic visualization of the jugular venous system and common femoral veins. *Annals of Emergency Medicine* 50 (1): 73-7

Lewis DH and Mellander S (1962) Competetive effects of sympathetic control and tissue metabolites on resistance capillary filtration in skeletal muscle. *Acta Physiologica Scandinavica* 56: 162

Linde B, Hjemdahl P, Freyschuss U & Juhlin-Dannfelt A (1989) Adipose tissue and skeletal muscle blood flow during mental stress. *American Journal of Physiology* 256 (1 Pt 1): E12-18

Lindenberger M & Lanne T (2007) Sex-related effects on venous compliance and capillary filtration in the lower limb. *American Journal of Physiology Regulatory, Integratory & Comparative Physiology* 292 (2): R852-9.

Lindqvist M, Davidsson S, Hjemdahl P & Melcher A (1996) Sustained forearm vasodilation in humans during mental stress is not neurogenically mediated *Acta Physiologica Scandinavica* 158 (1): 7-14

Litschauer B, Zauchner S, Huemer KH & Kafka-Lützow A (1998) Cardiovascular, endocrine, and receptor measures as related to sex and menstrual cycle phase. *Psychosomatic Medicine* 60 (2): 219-26

Loesch A & Dashwood MR (2009) On the sympathetic innervation of the human greater saphenous vein: relevance to clinical practice. *Current Vascular Pharmacology* 7 (1): 58-67

Lott MEJ, Hogeman C, Herr M, Bhagat M, Kunselman A & Sinoway Li (2009a) Vasoconstrictor responses in the upper and lower limbs to increases in transmural pressure. *Journal of Applied Physiology* 106: 302-10

Lott MEJ, Hogeman C, Herr M, Bhagat M & Sinoway LI (2009b) Sex differences in limb vasoconstriction responses to increases in transmural pressures. *American Journal of Physiology* 296: H186-194

Low PA, Neumann C, Dyck PJ, Fealey RD & Tuck RR (1983) Evaluation of skin vasomotor reflexes by using laser Doppler velocimetry. *Mayo Clinic Proceedings* 58 (9): 583-92

Ludbrook J (1962) Functional aspects of the veins of the leg. *American Heart Journal* 64: 706-13

Ludbrook J (966) Collateral artery resistance in the human lower limb *Journal of Surgical Research* 6 (10): 423-34

Mah IR, Tooke JE, Shore AC (1995) Capillary pressure during and after incremental venous pressure elevation in man. *Journal of Physiology* 485.1: 213-9

Manyari DE, Malkinson TJ, Robinson V, Smith ER & Cooper KE (1988) Acute changes in forearm venous volume and tone using radionuclide plethysmography. *American Journal of Physiology* 255 (4 Pt 2): H947-52

Manyari DE, Rose S, Tyberg JV & Sheldon RS (1996) Abnormal reflex venous function in patients with neuromediated syncope. *Journal of American College of Cardiology* 27 (7): 1730-5

Marin ML, Gordon RE, Veith FJ, Panetta TF, Sales CM & Wengerter KR (1994) Human greater saphenous vein: histologic and ultrastructural variation. *Cardiovascular Surgery* 2 (1): 56-62

Mark AL, Schmid PG, Eckstein JW & Wendling MG (1971) Venous responses to prostaglandin F2 alpha. *American Journal of Physiology* 220 (1): 222-6

Mark AL, Victor RG, Nerhed C & Wallin BG (1985) Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans *Circulation Research* 57 (3): 461-9

Mark AL (1987) Microneurography: A technique for study of the sympathetic nervous system in human hypertension *Journal of Cardiovascular Pharmacology* 10 (Suppliment 5): S56-9

Maros T (1981) Data regarding the typology and functional significance of the venous valves. *Morphology & Embryology* 27: 195-214

Marshall JM (1982) The influence of the sympathetic nervous system on individual vessels of the microcirculation of skeletal muscle of the rat. *Journal of Physiology* 332: 169-86

Marshall JM, Stone A & Johns EJ (1991) Analysis of the responses evoked in the cutaneous circulation of one hand by heating the contralateral hand. *Journal of Autonomic Nervous System* 32 (2): 91-9

Martin DS, Appelt C, Rodrigo MC & Egland MC (1996) Acute stress increases venomotor tone in conscious rats. *American Journal of Physiology* 271(4 Pt 2): H1375-83

Martin DS, Egland MC, Barnes LU & Vogel EM (2006) Adrenergic nerves mediate the venoconstrictor response to PVN stimulation. *Brain Research* 1076 (1): 93-100

Mashiah A, Berman V, Thole HH, Rose SS, Pasik S, Schwarz H & Ben-Hur H (1999) Estrogen and progesterone receptors in normal and varicose saphenous veins. *Cardiovascular Surgery* 7 (3): 327-31

Mason DT & Braunwald E (1964) Studies on digitalis. X. effects of oubanin on forearm vascular resistance and venous tone in normal subjects and in patients in heart failure. *Journal of Clinical Investigation* 43: 532-43

Mathias CJ (2002) To stand on one's own legs. *Clinical Medicine* 2 (3): 237-45

McAusland SD (1961) A new treatment of leg ulcers. Practitioner 186: 227-37

McCausland AM, Holmes F, Trotter AD Jr (1963) Venous distensibility during the menstrual cycle. *American Journal of Obstetrics & Gynecology* 86: 640-5

McGee & Gould (2003) Preventing complications of central venous catherization. *The New England journal of medicine* 348: 1123-33

Meendering JR, Torgrimson BN, Houghton BL, Halliwill JR & Minson CT (2005) Effects of menstrual cycle and oral contraceptive use on calf venous compliance. *American Journal of Physiology Heart & Circulatory Physiology* 288 (1): H103-10

Mercuro G, Pitzalis L, Podda A, Zoncu S, Pilia I, Melis GB & Cherchi A (1999) Effects of acute administration of natural progesterone on peripheral vascular responsiveness in healthy postmenopausal women. *American Journal of Cardiology* 84 (2): 214-8

Miller SB & Sita A (1994) Parental history of hypertension, menstrual cycle phase, and cardiovascular response to stress. *Psychosomatic Medicine* 56 (1): 61-9

Miller JD, Pegelow DF, Jacques AJ & Dempsey JA (2005) Effects of augmented respiratory muscle pressure production on locomotor limb venous return during calf contraction exercise. *Journal of Applied Physiology* 99 (5):1802-15

Minson CT, Halliwill JR, Young TM & Joyner MJ (2000) Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation* 101 (8): 862-8

Minson CT, Halliwill JR, Young TM & Joyner MJ (2000) Sympathetic activity and baroreflex sensitivity in young women taking oral contraceptives. *Circulation* 102 (13): 1473-6

Mitchell JH & Wildenthal K (1974) Static (isometric) exercise and the heart: physiological and clinical considerations. *Annual Review of Medicine* 25: 369-81

Mitchell LA, MacDonald RA & Brodie EE (2004) Temperature and the cold pressor test. *Journal of Pain* 5 (4): 233-7

Molderings GJ, Likungu J, Hentrich F & Gothert M (1988) Facilitatory presynaptic angiotensin receptors on the sympathetic nerves of the human saphenous vein and pulmonary artery. Potential involvement in beta-adrenoceptor-mediated facilitation of noradrenaline release. *Naunyn-Schmiedeberg's Archives of Pharmacology* 338 (3): 228-33

Momen A, Mascarenhas V, Gahremanpour A, Gao Z, Moradkhan R, Kunselman A, Boehmer JP, Sinoway LI & Leuenberger UA (2009) Coronary blood flow responses to physiological stress in humans. *American Journal of Physiology Heart & Circulatory Physiology* 296 (3): H854-61

Monahan KD & Ray CA (2004) Gender affects calf venous compliance at rest and during baroreceptor unloading in humans. *American Journal of Physiology Heart & Circulatory Physiology* 286 (3): H895-901

Monos E, Berczi V & Nadasy G (1995) Local control of veins: biomechanical, metabolic, and humoral aspects. *Physiological Reviews* 75 (3): 611-66

Monos E, Contney SJ, Dornyei G, Cowley AW Jr. & Stekiel WJ (1993) Hyperpolarization of in situ rat saphenous vein in response to axial stretch. *American Journal of Physiology* 265 (3 Pt 2): H857-61

Monos E, Lorant M, Dornyei G, Berczi V & Nadasy G (2003) Long-term adaptation mechanisms in extremity veins supporting orthostatic tolerance. *News Physiology Science Online* 18: 210-14

Mortensen JD, Talbot S & Burkart JA (1990) Cross-sectional internal diameters of human cervical and femoral blood vessels: relationship to subject's sex, age, body size. *The Anatomical Record* 226 (1): 115-24

Murakami Y, Yokotani K, Okuma Y & Osumi Y (1996) Nitric oxide mediates central activation of sympathetic outflow induced by interleukin-1 beta in rats. *European Journal of Pharmacology* 317 (1): 61-6

Muntinga JH & Visser KR (1992) Estimation of blood pressure-related parameters by electrical impedance measurement. *Journal of Applied Physiology* 73 (5):1946-57

Neglen P & Raju S (1995) The pressure/volume relationship of the calf: a measurement of vein compliance? *Journal of Cardiovascular Surgery (Torino)* 36 (3): 219-24

Neglen P & Raju S (2000) Ambulatory venous pressure revisited. *Journal of Vascular Surgery* 31 (6): 1206-13

Neglen P & Raju S (2000) Differences in pressures of the popliteal, long saphenous, and dorsal foot veins. *Journal of Vascular Surgery* 32 (5): 894-901

Nekooeian AA & Pang CC (2000) Effects of estrogen on venous function in rats with chronic heart failure. *American Journal of Physiology Heart & Circulatory Physiology* 278 (6): H1941-7

Newey VR & Nassiri DK (2002) Online artery diameter measurement in ultrasound images using artificial neural networks. *Ultrasound in Medicine & Biology* 28 (2): 209-16

Ng AV, Callister R, Johnson DG & Seals DR (1994) Sympathetic neural reactivity to stress does not increase with age in healthy humans. *American Journal of Physiology* 267 (1 Pt 2): H344-53

Nishiyama SK, Wray DW & Richardson RS (2008) Aging affects vascular structure and function in a limb-specific manner. *Journal of Applied Physiology* 105 (5): 1661-70

Okazaki K, Fu Q, Martini ER, Shook R, Conner C, Zhang R, Crandall CG & Levine BD (2005) Vasoconstriction during venous congestion: effects of venoarteriolar response, myogenic reflexes, and hemodynamics of changing perfusion pressure. *American Journal of Physiology* 289: 1354-13

Oldfield MA & Brown MD (2006) Evaluation of the time course of vascular responses to venous congestion in the human lower limb. *Journal of Vascular Research* 43 (2): 166-74

Olsen H & Lanne T (1998) Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. *American Journal of Physiology* 275 (3 pt2): H878-86

Ooue A, Ichinose-Kuwahara T, Shamsuddin AK, Inoue Y, Nishiyasu T, Koga S & Kondo N (2007) Changes in blood flow in a conduit artery and superficial vein of the upper arm during passive heating in humans. *European Journal of Applied Physiology* 101 (1): 97-103

Ooue A, Ichinose TK, Inoue Y, Nishiyasu T, Koga S & Kondo N (2008) Changes in blood flow in conduit artery and veins of the upper arm during leg exercise in humans. *European Journal of Applied Physiology* 103 (3): 367-73

Orshal JM & Khalil RA (2004) Gender, sex hormones, and vascular tone. *American Journal of Physiology Regulatory, Integrative & Comparative Physiology* 286 (2): R233-49

Page EB, Hickam JB, Sieker HO, McIntosh HD & Pryor WW (1955) Reflex venomotor activity in normal persons and in patients with postural hypotension. *Circulation* 11 (2): 262-70

Palmer GJ, Ziegler MG & Lake CR (1978) Response of norepinephrine and blood pressure to stress increases with age. *Journals of Gerontology Series A: Biological Sciences & Medical Sciences* 33 (4): 482-7

Pang CC (2001) Autonomic control of the venous system in health and disease: effects of drugs. *Pharmacology & Therapeutics* 90 (2-3): 179-230

Pang CC (2000) Measurement of body venous tone. *Journal of Pharmacology & Toxicology Methods* 44 (2): 341-60

Pawelczyk JA & Levine BD (2002) Heterogeneous responses of human limbs to infused adrenergic agonists: a gravitational effect? *Journal of Applied Physiology* 92 (5): 2105-13

Perret F, Mooser V, Waeber B, Yanik T, Jean-Jacques M, Mooser E, Nussberger J & Brunner HR (1989) Effect of cold pressor test on the internal diameter of the radial artery. *American Journal of Hypertension* 2 (9): 727-8

Perrot-Applanat M (1996) Estrogen receptors in the cardiovascular system. Steroids 61 (4): 212-15

Peters JK, Lister G, Nadel ER & Mack GW (1997) Venous and arterial reflex responses to positive-pressure breathing and lower body negative pressure. *Journal of Applied Physiology* 82(6):1889-96

Petrofsky JS, LeDonne DM, Rinehart JS & Lind AR (1976) Isometric strength and endurance during the menstrual cycle. *European Journal of Applied Physiology Occupation Physiology* 35 (1): 1-10

Petrovic P, Petersson KM, Hansson P & Ingvar M (2004) Brainstem involvement in the initial response to pain. *Neuroimage* 22 (2): 995-1005

Phillips MN, Jones GT, van Rij AM & Zhang M (2004) Micro-venous valves in the superficial veins of the human lower limb. *Clinical Anatomy* 17 (1): 55-60

Planken RN, Keuter XH, Hoeks AP, Kooman JP, van der Sande FM, Kessels AG, Leiner T & Tordoir JH (2006) Diameter measurements of the forearm cephalic vein prior to vascular access creation in end-stage renal disease patients: graduated pressure cuff versus tourniquet vessel dilatation. *Nephrology Dialysis Transplantation* 21 (3): 802-6

Pollack AA & Wood EH (1949) Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. *Journal of Applied Physiology* 1 (9): 649-62

Prather JW, Taylor AE & Guyton AC (1969) Effect of blood volume, mean circulatory pressure, and stress relaxation on cardiac output. *American Journal of Physiology* 216 (3): 467-72

Prince S, Sullivan R and Hackel A (1976) Percutaneous catheterization of the internal jugular vein in infants and children *Anesthesiology* 44 (2): 170

Protter AA, Wallace AM, Ferraris VA & Weishaar RE (1996) Relaxant effect of human brain natriuretic peptide on human artery and vein tissue. *American Journal of Hypertension* 9 (5): 432-6

Racchi H, Irarrazabal MJ, Howard M, Moran S, Zalaquett R & Huidobro-Toro JP (1999) Adenosine 5'-triphosphate and neuropeptide Y are co-transmitters in conjunction with noradrenaline in the human saphenous vein. *British Journal of Pharmacology* 126 (5): 1175-85

Racz K, Tiszai-Szucs T, Gal J & Kertai DM (2009) Preoperative revascularization in high-risk patients undergoing vascular surgery. *Orv Hetil* 150 (8): 341-52

Raju S, Neglen P, Doolittle J & Meydrech EF (1999) Axillary vein transfer in trabeculated postthrombotic veins. *Journal of Vascular Surgery* 29 (6): 1050-62

Ray CA & Mark AL (1993) Augmentation of muscle sympathetic nerve activity during fatiguing isometric leg exercise. *Journal of Applied Physiology* 75 (1): 228-32

Ray CA & Wilson TE (2004) Comparison of skin sympathetic nerve responses to isometric arm and leg exercise. *Journal of Applied Physiology* 97 (1): 160-4

Reed KL & Anderson CF (2000) Changes in umbilical venous velocities with physiologic perturbations. *American Journal of Obstetrics & Gynecology* 182 (4): 835-8

Risk MR, Lirofonis V, Armentano RL & Freeman R (2003) A biphasic model of limb venous compliance: a comparison with linear and exponential models. *Journal of Applied Physiology* 95 (3): 1207-15

Robertson D, Shand DG, Hollifield JW, Nies AS, Frolich JC & Oates JA (1979) Alterations in the responses of the sympathetic nervous system and renin in borderline hypertension. *Hypertension* 1 (2): 118-24

Robertson D (2008) The pathophysiology and diagnosis of orthostatic hypotension. *Clinical Autonomic Research* 18 (Suppl 1): 2-7

Rorie DK & Muldoon SM (1979) Increased reactivity of isolated rabbit saphenous vein after treatment with estrogen and progesterone. *Blood Vessels* 16 (5): 252-8

Rothe CF (2006) Active venoconstriction is important in maintaining or raising end-diastolic volume and stroke volume during exercise and orthostasis. *Journal of Applied Physiology* 101 (4): 1264-5

Rothe C (1983) Reflex control of veins and vascular capacitance. *Physiological Reviews* 63 (4): 1281-1342

Rowell, Loring B. (1993) *Human cardiovascular control* New York ; Oxford : Oxford University Press.

Rowell LB (2006) Comments on Point: Counterpoint series “Active venoconstriction is / is not important in maintaining or raising end-diastolic volume and stroke volume during exercise and orthostasis. *Journal of Applied Physiology* 101 (4): 1267-8

Rowell LB & Blackmon JR (1988) Venomotor responses during central and local hypoxia. *American Journal of Physiology* 255 (4 Pt 2): H760-4

Rudner XL, Berkowitz DE, Booth JV, Funk BL, Cozart KL, D'Amico EB, El-Moalem H, Page SO, Richardson CD, Winters B, Marucci L & Schwinn DA (1999) Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. *Circulation* 100 (23): 2336-43

Rusch NJ, Shepherd JT, Webb RC & Vanhoutte PM (1981) Different behavior of the resistance vessels of the human calf and forearm during contralateral isometric exercise, mental stress, and abnormal respiratory movements. *Circulation Research* 48 (6 Pt 2): I118-30

Saab PG, Llabre MM, Hurwitz BE, Schneiderman N, Wohlgemuth W, Durel LA, Massie C & Nagel J (1993) The cold pressor test: vascular and myocardial response patterns and their stability. *Psychophysiology* 30 (4): 366-73

Samueloff SL, Browse NL & Shepherd JT (1966) Response of capacity vessels in human limbs to head-up tilt and suction on lower body. *Journal of Applied Physiology* 21 (1): 47-54

Samueloff SL, Bevegard BS & Shepherd JT (1966) Temporary arrest of circulation to a limb for the study of venomotor reactions in man. *Journal of Applied Physiology* 21 (1): 341-6

Santos AC, Alves MJ, Rondon MU, Barretto AC, Middlekauff HR & Negrão CE (2005) Sympathetic activation restrains endothelium-mediated muscle vasodilatation in heart failure patients. *American Journal of Physiology Heart & Circulatory Physiology* 289 (2): H593-9

Sarrel PM (1999) The differential effects of oestrogens and progestins on vascular tone. *Human Reproduction Update* 5 (3): 205-9;

Sato N (1988) Cyclic changes in sex steroids, prostaglandins and oxytocin receptors of normal fallopian tube throughout the menstrual cycle. *Nippon Sanka Fujinka Gakkai Zasshi* 40 (9): 1432-8

Sato N & Miyake S (2004) Cardiovascular reactivity to mental stress: relationship with menstrual cycle and gender. *Journal of Physiology Anthropology Applied Human Science* 23 (6): 215-23

Schank BJ, Acree LS, Longfors J & Gardner AW (2007) Differences in vascular reactivity between men and women. *Angiology* 57(6):702-8.

Schindler AE (2003) Differential effects of progestins on hemostasis. *Maturitas* 46 (Suppl 1): S31-7

Schmitt M, Blackman DJ, Middleton GW, Cockcroft JR & Frenneaux MP (2002) Assessment of venous capacitance. Radionuclide plethysmography: methodology and research applications. *British Journal of Clinical Pharmacology* 54 (6): 565-76

Schmitt M, CockCroft JR & Frenneux MP (2003) Modulation of the natriuretic peptide system in heart failure: from bench to bedside? *Clinical Science* 105: 141-60

Seals DR & Victor RG (1991) Regulation of muscle sympathetic nerve activity during exercise in humans. *Exercise & Sport Science Reviews* 19: 313-49

Seaman RG, Wiley RL, Zechman FW & Goldey JA (1973) Venous reactivity during static exercise (handgrip) in man. *Journal of Applied Physiology* 35 (6): 858-60

Seddon MD, Chowienczyk PJ, Brett SE, Casadei B & Shah AM (2008) Neuronal nitric oxide synthase regulates basal microvascular tone in humans in vivo. *Circulation* 117 (15): 1991-6

Sejresen P, Henriksen O, Paaske W & Nielsen S (1981) Duration of increase in vascular volume during venous stasis. *Acta Physiologica Scandinavica* 111: 293-8

Sharpey-Schafer EP (1963) Venous tone: effects of reflex changes, humoral agents and exercise. *British Medical Bulletin* 19: 145-8

Sharpey-Schafer E, Semple S, Halls R, & Howarth S (1965) Venous constriction after exercise: its relation to acid-base changes in venous blood *Clinical Science* 29: 397

Shepherd JT & Vanhoutte PM (1975) *Veins and their control.* Saunders, London: 181-189

Shepherd JT, Rusch NJ, & Vanhoutte PM (1983) Effect of cold on the blood vessel wall. *General Pharmacology* 14 (1): 61-4

Sheriff D (2005) Point: The muscle pump raises muscle blood flow during locomotion. *Journal of Applied Physiology* 99 (1): 371-2

Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS, & Sinoway LI (2001) Gender affects sympathetic and hemodynamic response to postural stress. *American Journal of Physiology Heart & Circulatory Physiology* 281 (5): H2028-35

Sidhu JS, Newey VR, Nassiri DK & Kaski JC (2002) A rapid and reproducible on line automated technique to determine endothelial function. *Heart* 88 (3): 289-92

Sjoberg T, Norgren L, Andersson KE & Steen S (1989) Comparative effects of the alpha-adrenoceptor agonists noradrenaline, phenylephrine and clonidine in the human saphenous vein in vivo and in vitro. *Acta Physiologica Scandinavica* 136 (3): 463-71

Skagen K, Jensen K, Henriksen O & Knudsen L (1982) Sympathetic reflex control of subcutaneous blood flow in tetraplegic man during postural changes Clinical Science (London) 62: 605-9

Skalak TC & Schmid-Schönbein GW (1986) Viscoelastic properties of microvessels in rat spinotrapezius muscle. *Journal of Biomechanical Engineering* 108: 193-200

Smit AA, Halliwill JR, Low PA & Wieling W (1999) Pathophysiological basis of orthostatic hypotension in autonomic failure. *Journal of Physiology* 519 (Pt 1): 1-10

Smith SA, Mitchell JH & Garry MG. (2006) The mammalian exercise pressor reflex in health and disease. *Experimental Physiology* 91 (1): 89-102

Sowers JR (1981) Dopaminergic control of circadian norepinephrine levels in patients with essential hypertension. *Journal of Clinical Endocrinology & Metabolism* 53 (6): 1133-7

Stachenfeld NS, Silva C, Keefe DL, Kokoszka CA & Nadel ER (1999) Effects of oral contraceptives on body fluid regulation. *Journal of Applied Physiology* 87 (3): 1016-25

Stachenfeld NS & Taylor HS (2007) Exogenous oestradiol and progesterone administration does not cause oedema in healthy young women. *Clinical Endocrinology (Oxford)* 66 (3): 410-18

Steen S, Sjoberg T, Skarby T, Norgren L & Andersson KE (1984) The postjunctional alpha-adrenoceptors of the human saphenous vein. *Acta Pharmacologica Toxicologia (Copenhagen)* 55 (5): 351-7

Steen S, Castenfors J, Sjoberg T, Skarby T, Andersson KE & Norgren L (1986) Effects of alpha-adrenoceptor subtype-selective antagonists on the human saphenous vein in vivo. *Acta Physiologica Scandinavica*126 (1): 15-19

Stephenson LA & Kolka MA (1985) Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating *American Journal of Physiology* 249 (2 Pt 2): R186-91

Stewart JM (2003) Microvascular filtration is increased in postural tachycardia syndrome. *Circulation* 107: 2816-22

Stewart JM, McLeod KJ, Sanyal S, Herzberg G, & Montgomery LD (2004) Relation of postural vasovagal syncope to splanchnic hypervolemia in adolescents. *Circulation* 110 (17): 2575-81

Stewart JM (2002) Pooling in chronic orthostatic intolerance: arterial vasoconstrictive but not venous compliance defects. *Circulation* 105 (19): 2274-81

Stewart JM & Weldon A (2000) Vascular perturbations in the chronic orthostatic intolerance of the postural orthostatic tachycardia syndrome. *Journal of Applied Physiology* 89 (4): 1505-12

Stewart JM & Weldon A (2001) The relation between lower limb pooling and blood flow during orthostasis in the postural orthostatic tachycardia syndrome of adolescents. *Journal of Pediatrics* 138 (4): 512-19

Stewart et al. Stewart JM, Montgomery LD, Glover JL & Medow MS. (2007) Changes in regional blood volume and blood flow during static handgrip. *American Journal of Physiology Heart & Circulatory Physiology* 292 (1): H215-23

Stooker W, Gok M, Sipkema P, Niessen HW, Baidoshvili A, Westerhof N, Jansen EK, Wildevuur CR & Eijsman L (2003) Pressure-diameter relationship in the human greater saphenous vein. *Annals of Thoracic Surgery* 76 (5): 1533-8

Stratton JR, Halter JB, Hallstrom AP, Caldwell JH & Ritchie JL (1983) Comparative plasma catecholamine and hemodynamic responses to handgrip, cold pressor and supine bicycle exercise testing in normal subjects. *Journal of the American College of Cardiology* 2 (1): 93-104

Streeten DH (1990) Pathogenesis of hyperadrenergic orthostatic hypotension. Evidence of disordered venous innervation exclusively in the lower limbs. *Journal of Clinical Investigation* 86 (5): 1582-8

Streeten DH & Scullard TF (1996) Excessive gravitational blood pooling caused by impaired venous tone is the predominant non-cardiac mechanism of orthostatic intolerance. *Clinical Science (London)* 90 (4):277-85

Streeten DH (1999) Orthostatic intolerance. A historical introduction to the pathophysiological mechanisms. *American Journal of Medical Science* 317 (2): 78-87

Streeten DH (2001) Role of impaired lower-limb venous innervation in the pathogenesis of the chronic fatigue syndrome. *American Journal of Medical Science* 321 (3): 163-7

Sudhir K, Jennings GL, Funder JW & Komaseroff PA. (1996) Estrogen enhances basal nitric oxide release in the forearm vasculature in perimenopausal women. *Hypertension* 28: 330-4

Sumner A, Zelis R, Bennett M & Gascho JA (1989) Effect of the venodilated state on sympathetic-induced venoconstriction in normal subjects. *American Journal of Cardiology* 63 (13): 973-6

Suzuki T, Nakamura Y, Moriya T & Sasano H (2003) Effects of steroid hormones on vascular functions. *Microscience Research & Technology* 60 (1): 76-84

Svejcar J, Prerovsky I, Linhart J & Kruml J (1962) Content of collagen, elastin, and water in walls of the internal saphenous vein in man. *Circulation Research* 11: 296-300

Taneja I, Moran C, Medow MS, Glover JL, Montgomery LD & Stewart JM (2007) Differential effects of lower body negative pressure and upright tilt on splanchnic blood volume. *American Journal of Physiology Heart & Circulatory Physiology* 292 (3): H1420-6

Tank J, Diedrich A, Szczech E, Luft FC & Jordan J (2005) Baroreflex regulation of heart rate and sympathetic vasomotor tone in women and men. *Hypertension* 45 (6): 1159-64

Tersman Z, Collins A & Eneroth P (1991) Cardiovascular responses to psychological and physiological stressors during the menstrual cycle. *Psychosomatic Medicine* 53 (2): 185-97

Theisen D, Vanlandewijck Y, Sturbois X & Francaux M (2000) Blood distribution adaptations in paraplegics during posture changes: peripheral and central reflex responses. *European Journal of Applied Physiology* 81: 463-9

Thijssen DH, de Groot P, Kooijman M, Smits P & Hopman MT (2006) Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *American Journal of Physiology Heart & Circulatory Physiology* 291 (6): H3122-9

Thomson HL, Atherton JJ, Khafagi FA & Frenneaux MP (1996) Failure of reflex venoconstriction during exercise in patients with vasovagal syncope. *Circulation* 93 (5): 953-9

Todo Y, Tanimoto M, Yamamoto T & Iwasaki T (1986) Radionuclide assessment of peripheral hemodynamics: a new technique for measurement of forearm blood volume and flow. *Journal of Nuclear Medicine* 27 (2): 192-7

Tollan A, Kvenild K, Strand H, Oian P & Maltau JM (1992) Increased capillary permeability for plasma proteins in oral contraceptive users. *Contraception* 45 (5): 473-81

Tombaugh TN (2006) A comprehensive review of the Paced Auditory Serial Addition Test (PASAT). *Archives of Clinical Neuropsychology* 21 (1): 53-76

Torgrimson BN, Meendering JR, Kaplan PF & Minson CT (2007) Endothelial function across an oral contraceptive cycle in women using levonorgestrel and ethinyl estradiol. *American Journal of Physiology Heart & Circulatory Physiology* 292 (6): H2874-80

Tripathi A, Shi X, Wenger CB & Nadel ER (1984) Effect of temperature and baroreceptor stimulation on reflex venomotor responses. *Journal of Applied Physiology* 57 (5): 1384-92

Tschakovsky M, Shoemaker JK & Hughson RL (1995) Beat-to-beat forearm blood flow with Doppler ultrasound and strain-gauge plethysmography. *Journal of Applied Physiology* 79: 713-719

Tschakovsky ME & Sheriff DD (2004) Immediate exercise hyperemia: contributions of the muscle pump vs. rapid vasodilation. *Journal of Applied Physiology* 97 (2): 739-47

Tyberg JV (2002) How changes in venous capacitance modulate cardiac output. *Pflugers Archives* 445 (1): 10-17

van Duijnhoven NT, Bleeker MW, de Groot PC, Thijssen DH, Felsenberg D, Rittweger J & Hopman MT (2008) The effect of bed rest and an exercise countermeasure on leg venous function. *European Journal of Applied Physiology* 104 (6): 991-8

Vanhoutte PM & Shepherd JT (1985) Adrenergic pharmacology of human and canine peripheral veins. *Fed Proc* 44 (2): 337-40

Vanhoutte P & Leusen I (1969) The reactivity of isolated venous preparations to electrical stimulation. *Pflugers Archives* 306 (4): 341-53

Vanhoutte PM & Janssens WJ (1978) Local control of venous function. *Microvascular Research* 16 (2): 196-214

Varbiro S, Vajo Z, Nadasy GL, Monos E, Acs N, Lorant M, Felicetta JV & Szekacs B (2002) Sex hormone replacement therapy reverses altered venous contractility in rats after pharmacological ovariectomy. *Menopause* 9 (2): 122-6

Vazquez ED, Lopez-Costa JJ, Caballero AB, Otano Sahores AL and Pecci Saavedra J (2003) Sensitive nerve receptors beneath the extensor apparatus of the hand fingers. *Journal of Orthopaedic Traumatology* 4: 35-8

Veldhuijzen van Zanten JJ, Thrall G, Wasche D, Carroll D & Ring C (2005) The influence of hydration status on stress-induced hemoconcentration *Psychophysiology* 42 (1): 98-107

Victor RG, Leimbach WN Jr., Seals DR, Wallin BG & Mark AL (1987) Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension* 9 (5): 429-36

Vissing SF & Nielsen SL (1988) Regional blood volume in man determined by radiolabelled erythrocytes. *Clinical Physiology* 8: 303-8

Vissing SF, Scherrer U & Victor RG (1991) Stimulation of skin sympathetic nerve discharge by central command. Differential control of sympathetic outflow to skin and skeletal muscle during static exercise. *Circulation Research* 69 (1): 228-38

Von Kugelgen A (1955) Relation of the circular musculature and internal pressure of the large veins in humans. *Zeitschrift fuer Zellforschung und Mikroskopische Anatomie* 43 (2): 168-83

Wallin BG, Morlin C & Hjemdahl P (1987) Muscle sympathetic activity and venous plasma noradrenaline concentrations during static exercise in normotensive and hypertensive subjects. *Acta Physiologica Scandinavica* 129: 489-97

Wallin BG, Victor RG & Mark AL (1989) Sympathetic outflow to resting muscles during static handgrip and postcontraction muscle ischemia. *American Journal of Physiology* 256: H105-10

Wallin BG & Charkoudian N (2007) Sympathetic neural control of integrated cardiovascular function: insights from measurement of human sympathetic nerve activity. *Muscle & Nerve* 36: 595-614

Wasmund WL, Westerholm EC, Watenpaugh DE, Wasmund SL & Smith ML (2002) Interactive effects of mental and physical stress on cardiovascular control. *Journal of Applied Physiology* 92: 1828-34

Webb-Peploe MM & Shepherd JT (1968) Veins and their control. *The New England Journal of Medicine* 278: 317-22

Webb DJ, Collier JG, Seidelin PH & Struthers AD (1988) Regulation of regional vascular tone: the role of angiotensin conversion in human forearm resistance vessels. *Journal of Hypertension Supplemental* 6: S57-9

Webb DJ & Haynes WG (1993) Venoconstriction to endothelin-1 in humans is attenuated by local generation of prostacyclin but not nitric oxide. *Journal of Cardiovascular Pharmacology* 22 Suppl 8: S317-20

Weinstein JS, Grossman W, Weintraub RM, Thurer RL, Johnson RG & Morgan KG (1989) Differences in alpha-adrenergic responsiveness between human internal mammary arteries and saphenous veins. *Circulation* 79: 1264-70

Weiss LW & Clark FC (1985) Ultrasonic protocols for separately measuring subcutaneous fat and skeletal muscle thickness in the calf area. *Physical Therapy* 65: 477-81

Wenger CB, Roberts, MF, Stolwijk JA & Nadel ER (1976) Nocturnal lowering of thresholds for sweating and vasodilation. *Journal of Applied Physiology* 41 (1): 15-19

Wenger CB & Roberts MF (1980) Control of forearm venous volume during exercise and body heating. *Journal of Applied Physiology* 48 (1): 114-19

Wesly RL, Vaishnav RN, Fuchs JC, Patel DJ & Greenfield JC Jr. (1975) Static linear and nonlinear elastic properties of normal and arterialized venous tissue in dog and man. *Circulation Research* 37 (4): 509-20

Whitney RJ (1953) The measurement of volume changes in human limbs. *Journal of Physiology* 121 (1): 1-27

Wilkinson IB & Webb DJ (2001) Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *British Journal of Clinical Pharmacology* 52 (6): 631-46

Williams CA & Lind AR (1979) Measurement of forearm blood flow by venous occlusion plethysmography: influence of hand blood flow during sustained and intermittent isometric exercise. *European Journal of Applied Physiology Occupational Physiology* 42 (3): 141-9

Williamson JW, Fadel PJ & Mitchell JH (2006) New insights into central cardiovascular control during exercise in humans: a central command update. *Experimental Physiology* 91 (1): 51-8

Wilson TE, Dyckman DJ, Ray CA (2006) Determinants of skin sympathetic nerve responses to isometric exercise. *Journal of Applied Physiology* 100 (3):1043-8

Wirch JL, Wolfe LA, Weissgerber TL & Davies GA (2006) Cold pressor test protocol to evaluate cardiac autonomic function. *Applied Physiology Nutrition & Metabolism* 31 (3): 235-43

Wood JE & Eckstein JW (1958) A tandem foream plethysmograph for study of acute responses of the peripheral veins of man: the effect of environmental and local temperature change, and the effect of pooling blood in the extremities. *Journal of Clinical Investigation* 37 (1): 41-50

Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA & Green D (2001) Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *Journal of Applied Physiology* 91 (2): 929-37

Wu JS, Yang YC, Lu FH, Wu CH & Chang CJ. (2008) Population-based study on the prevalence and correlates of orthostatic hypotension/hypertension and orthostatic dizziness. *Hypertension Research* 31 (5): 897-904

Wieling W & Schatz IJ (1996) The consensus statement on the definition of orthostatic hypotension: a revisit after 13 years. *Journal of Hypertension* 27 (5): 935-8

Yamazaki F, Okuno C, Nagamatsu S & Sone R (2002) Effects of whole-body and local thermal stress on hydrostatic volume changes in the human calf. *European Journal of Applied Physiology* 88 (1-2): 61-6

Young CN, Stillabower ME, DiSabatino A & Farquhar WB (2006) Venous smooth muscle tone and responsiveness in older adults. *Journal of Applied Physiology* 101(5):1362-7

Zbrozyna AW & Westwood DM (1988) Habituation of vasodilatation in the calf elicited by repeated sensory stimulation in man. *European Journal of Applied Physiology Occupational Physiology* 58 (3): 284-90

Zelis R & Mason DT (1969) Comparison of the reflex reactivity of skin and muscle veins in the human forearm. *Journal of Clinical Investigation* 48 (10): 1870-7

Zitnik RS & Lorenz RR (1969) Sensitivity of methods for determination of active changes in venous wall tension. *American Journal of Cardiology* 24: 220

PRINTED ABSTRACTS

Brown MD, Phillips C, Witham K & Lawler R (2009) Lower leg artery and vein responses to venous distension and head up tilt in healthy men and women. *Proceedings of the Physiological Society* 14: PC30

Phillips CJ, Horsburgh J, Balanos G & Brown MD (2008) Heterogenous venoconstriction in human legs. *FASEB Journal* 22: 1211.3

Brown MD, Phillips CJ, Clabburn B, Smith A, Freeman G & Saunders P (2007) Ultrasound imaging of the popliteal vein during sympathetic activation in men. *Microcirculation* 14 (6): 644

Phillips CJ, Clabburn B, Freeman G, Saunders P, Smith A & Brown MD (2007) The effects of endogenous and exogenous female hormones on vein responses to sympathoexcitation. *Proceedings of the Physiological Society, Proc Life Science:* PC24

Phillips CJ, Bishop G, Balanos GM & Brown MD (2006) Venoconstriction in young healthy women is attenuated compared to men. *Journal of Vascular Research* 43 (Supplement 1): 90

Bishop GD, Phillips CJ, Balanos GM & Brown MD (2006) Single leg vein properties measured by ultrasound during the menstrual cycle and oral contraceptive use in women. *Proceedings of the Physiological Society* 3: PC193