

The effect of OBesity on Venous Impedance
and Outflow measured by UltraSound

“The OBVIOUS Study”

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ABSTRACT

Obesity has been identified epidemiologically as a risk factor for development of chronic venous disease. To examine whether abdominal adiposity obstructs venous outflow from the legs, 26 females aged 34-49 years with no clinical venous disease and body mass index (BMI) between 20.9 – 46.7 kg/m² were studied. A novel measurement of the extent of abdominal fat along the legs when seated correlated well with BMI and other measures of central fat (sagittal-abdominal diameter, ultrasound fat thickness, % truncal fat by DEXA scan). On sitting, inguinal tissue pressure recorded by needle manometry increased more in obese (BMI > 30) than normal weight (BMI 20-25) subjects (8.2 vs 1.5 mmHg, p<0.01) as did the femoral vein cross-sectional area (129 vs 60%, p<0.05). Both measures correlated with increasing abdominal fat but were not associated with each other. In the lower leg, saphenous vein distensibility and compliance correlated positively with abdominal fat and BMI, but there was no such association for the popliteal vein. Female sex hormones, physical activity levels and insulin status did not affect venous haemodynamics. Thus, increased abdominal fat can potentially hinder venous return when sitting, leading to distension and changes in vein biomechanics, which could overtime contribute to venous disease.

ABBREVIATIONS

ANP	Atrial Naturetic Peptide
BMI	Body Mass Index
CEAP	Clinical classification of chronic lower extremity venous disease
CPT	Cold Pressor Test
CSA	Cross Sectional Area
CT	Computed Tomography
CVI	Chronic Venous Insufficiency
DEXA	Dual Energy X-ray Absorptiometry
EDTA	Ethylene-diamine-tetra-acetic acid
EVS	Edinburgh Vein Study
FT	Foundation Trust
FV	Femoral Vein (previously called the Superficial Femoral Vein)
GSV	Great Saphenous Vein
HOMA	Homeostasis Model Assessment
HRT	Hormone Replacement Therapy
IAP	Intra Abdominal Pressure
IR	Insulin Resistance
IVC	Inferior Vena Cava
Kf	Capillary Filtration
MAP	Mean Arterial Pressure
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
mmHG	millimetres of mercury

n	number
NHS	National Health Service
p	Level of significance (0.05)
PAD	Peripheral Arterial Disease
PPG	Photoplethysmography
Pvest	Calf Venous Pressure
Pvi	Isovolumetric Venous Pressure
ROI	Region of Interest
SAD	Sagittal Abdominal Diameter
SA/SP	Sacro Abdominal/Sacro Patella Index
SD	Standard Deviation
SFJ	Sapheno Femoral Junction
SGP	Strain Gauge Plethysmography
SMC	Smooth Muscle Cells
SPJ	Sapheno Popliteal Junction
SSV	Short Saphenous Vein
USS	Ultrasound
UHNS	Universty Hospital Birmingham
VIA	Vascular Imaging Analysis
VV	Varicose Veins
WHO	World Health Organisation
W-H	Waist-Hip ratio
ρ	Rho, Spearman's Rank correlation coefficient
%	Percentage

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CHAPTER 1:

BACKGROUND AND LITERATURE REVIEW

Obesity has been identified as a risk factor associated with the development of chronic venous disease. Epidemiological studies indicate a higher occurrence of varicose veins, chronic venous insufficiency or both in people who are overweight or obese as defined by their body mass index, especially women. The pathogenesis of venous disease involves valvular incompetence although it is not known if this is the primary defect or whether it occurs secondary to venous dilatation in response to venous hypertension. It has been suggested that obesity could be linked to venous disease by abdominal adiposity presenting a mechanical impeding pressure on peripheral venous return, promoting hypertension (Beebe-Dimmer et al. 2005), or through raised levels of inflammatory mediators such as cytokines and adipokines (Allison et al. 2010).

The majority of studies investigating the association between obesity and venous disease involve populations that already show clinical manifestations of the condition, and characterise obesity through body mass index, and venous dysfunction by visual examination and ultrasound assessment of reflux and/or obstruction. Once the veins have become diseased, they show increased distensibility because of the pathological changes in their wall composition (van Rij et al 2008). Such case-control studies of obesity and chronic venous disease are therefore limited in that this design does not establish a temporal sequence between exposure and disease. Moreover, while body mass index gives a ‘whole body’ view of obesity, it does not specifically define the abdominal obesity that might contribute to impairment of venous return.

The work presented in this thesis aims to address these issues by examining the relationship of body composition and abdominal obesity to measures of leg vein function in a cohort of

individuals without clinical venous disease. By comparing venous capacity and compliance, as physiological parameters that can signify subtle pre-disease alterations in vein wall biomechanical properties, with abdominal fat across a range of otherwise healthy people, the potential for obesity-induced mechanical impediment to venous return may be determined. Furthermore, the relevance for venous function of confounding factors such as levels of physical activity and female hormone status will be controlled for and evaluated.

1.1 PREVALENCE AND INCIDENCE OF CHRONIC VENOUS DISEASE

Venous disease of the lower extremities is the commonest reported vascular condition and is a major source of the morbidity in the Western world. The spectrum of the disease ranges from uncomplicated varicose veins (VV) through to chronic venous insufficiency (CVI) which is characterised by oedema, venous eczema, haemosiderin deposits, lipodermatosclerosis and eventually ulceration. Several large population studies over the last decade report the incidence of chronic venous disease, reviewed by Beebe-Dimer et al. (2005). VV estimates range from 2 – 56% in males and <1 - 73.2% in females (Carpentier et al. 2004, Stanhope, 1975, Lake et al. 1942) whilst CVI has been estimated at 9% in males and 7% in females in the Edinburgh Vein Study (Lee et al. 2003). The San Diego study (Criqui et al. 2003, Criqui et al. 2007) estimated that 27.9% of their target population had functional venous disease (i.e. VV and CVI). The incidence of venous disease given by the Framingham study was 1.9% in men and 2.6% in woman per annum (Brand et al. 1988). The variation in these figures is partly due to differences in definition of venous disease and since several studies only report the incidence based on symptomatic reporting by patients, it is possible that this excludes large numbers of sufferers. The numbers are thus likely to underestimate the true situation. Leg ulceration, of which the majority (~80%) is attributable to venous disease (Naik et al.

2000), affects 1% of the population in their lifetime (Callam et al. 1987) and this presents a huge burden to NHS resources since 2% of the present NHS budget goes on the treatment of venous ulceration (Laing, 1992).

CVI itself can be a debilitating illness with loss of self-esteem and self-confidence. Sufferers can become dependent on medical care and lose independence, and loss of time at work, trips to the general practitioner, travel to and from medical appointments, can all be considered as a consequence of the illness. Sam et al. (2004, 2006) demonstrated clearly a marked improvement in the quality of life scores people recorded following treatment for VV yet there is very little health promotion carried out in this area where preventative measures may offer major functional, quality of life and financial benefits. It is thus clear that this is an area of major health concern which can be severely detrimental to people's physical and psychological well being.

Although venous disease prevalence increases with age in all the studies reported, it is not just a disease of the elderly. Callam et al. (1987) in their examination of 600 ulcerated legs found that 20% of ulcers had started before the age of 40, and the incidence of CVI in the EVS in the 35-44 year old age category was 2.53% in men and 3.76% in woman. This raises the question of what factors predispose and / or initiate venous disease in the population at a time when individuals may otherwise be relatively healthy.

1.2 CLASSIFICATION OF CHRONIC VENOUS DISEASE

In the literature, chronic venous disease is classically divided into two different categories, varicose veins and chronic venous insufficiency. VV are defined by the WHO (Prerovsky) as "saccular dilatations of the veins which are often tortuous", while the Basle study (Widmer, 1978) identifies three different components:- dilated saphenous veins (trunk veins), dilated

superficial tributaries (reticular veins) and dilated venules (hyphenwebs). The definition of CVI encompasses multiple presentations of similar pathologies characterised by the failure of the lower limbs to remove sufficient blood via the venous system leading to symptomatic presentation (Pappas et al. 2005). The condition ranges in presentation from symptoms of aching and swelling in the leg, through varicose veins to skin changes of venous eczema, haemosiderin deposits and lipodermatosclerosis, eventually leading to ulceration. Venous disease is now categorized using a universally accepted structure that recognizes that the two diseases are part of the same spectrum. The Clinical, Etiologic, Anatomic, Pathophysiologic (CEAP) classification system was devised by Moneta & Porter (1995) and endorsed by the American Venous Forum and the Joint Council of the Society for Vascular Surgery and the North American-International Society for Cardiovascular Surgery (Moneta et al 1995, Padberg 2005). The system has four areas of classification for labelling the disease listed in Table 1.1. The majority of clinicians categorise using the version in table 1.2

Mark	Definition
C	Clinical signs (grade ₀₋₆), supplemented by (s) for symptomatic and (a) for asymptomatic presentation
E	Etiologic Classification (Congenital, Primary, Secondary)
A	Anatomic Distribution (Superficial, Deep, or Perforator, alone or in combination)
P	Pathophysiologic Dysfunction (Reflux or Obstruction, alone or in combination)

Table 1.1. CEAP Clinical classification of chronic lower extremity venous disease (Moneta & Porter 1995).

Class	Clinical signs
0	No visible or palpable signs of venous disease
1	Teleangiectases, reticular veins, malleolar flare
2	Varicose veins
3	Edema without skin changes
4	Skin changes ascribed to venous disease (pigmentation, venous eczema, lipodermatosclerosis)
5	Skin changes (as defined above) in conjunction with healed ulceration
6	Skin changes (as defined above) in conjunction with active ulceration

Table 1.2. Breakdown of CEAP classification into the 7 categories of clinical signs used for practical purposes (Moneta & Porter 1995).

1.3 LOWER LIMB VENOUS ANATOMY AND FUNCTION

Before considering in detail the pathological changes associated with chronic venous disease, a brief description of the venous anatomy of the lower leg will be given (Figure 1.1). This is classified into the deep and superficial systems, the deep system comprising the venae comitantes of the crural vessels and their confluence into the popliteal vein and its continuation into the femoral vein (FV) and iliac veins draining into the inferior vena cava (IVC). The superficial system has two main vessels that drain the skin and subcutaneous tissue. The great saphenous vein (GSV) is a confluence of the veins on the medial side of the foot that passes anterior to the medial malleolus and runs on the antero-medial aspect of the whole limb draining into the FV at the sapheno-femoral junction (SFJ). It is enveloped along its entire length in a fascial sheath/compartiment supplied by tributaries which lie outside this. This may explain why greater tortuosity is initially seen in primary VV in the tributaries and less in the main GSV trunk (Caggiati, 1999). The Short Saphenous Vein (SSV) commences at the outer border of the foot and runs posterior to the lateral malleolus. It pierces the fascia and

drain into the popliteal vein between the two head of the gastrocnemius muscle at the sapheno-popliteal junction (SPJ) (Browse et al 1999).

The deep and superficial systems are connected together by perforator veins that drain the superficial system into the deep system at various variable points (Delis, 2005). The drainage of all the systems is kept flowing by the presence of multiple valve in the veins that prevent backflow. There is substantial variation in the course and tributaries of these systems with other named veins in existence (Delis et al 2004).

The venous drainage leaves both legs under the inguinal ligament in the femoral canal and passes along the back of the abdominal cavity in the retro-peritoneum coming together to form the Inferior Vena Cava. It is joined by the circulation returning from the liver, kidneys and abdominal organs before piercing the diaphragm at the level of the tenth vertebra. Here it is subjected to the pressure variations that affect all structures in the abdominal cavity and retro-peritoneum (e.g. straining, lifting, coughing etc.) Once entering the chest the venous return is affected by the pressure in the right atrium and the intra-thoracic pressure variations. Venous disease is more common in the left than the right leg and this may reflect the fact that the left Common Iliac Vein must pass below the Right Common Iliac Artery to reach the inferior cava causing impedance to flow. If this becomes of clinical significance it is termed May-Turner Syndrome.

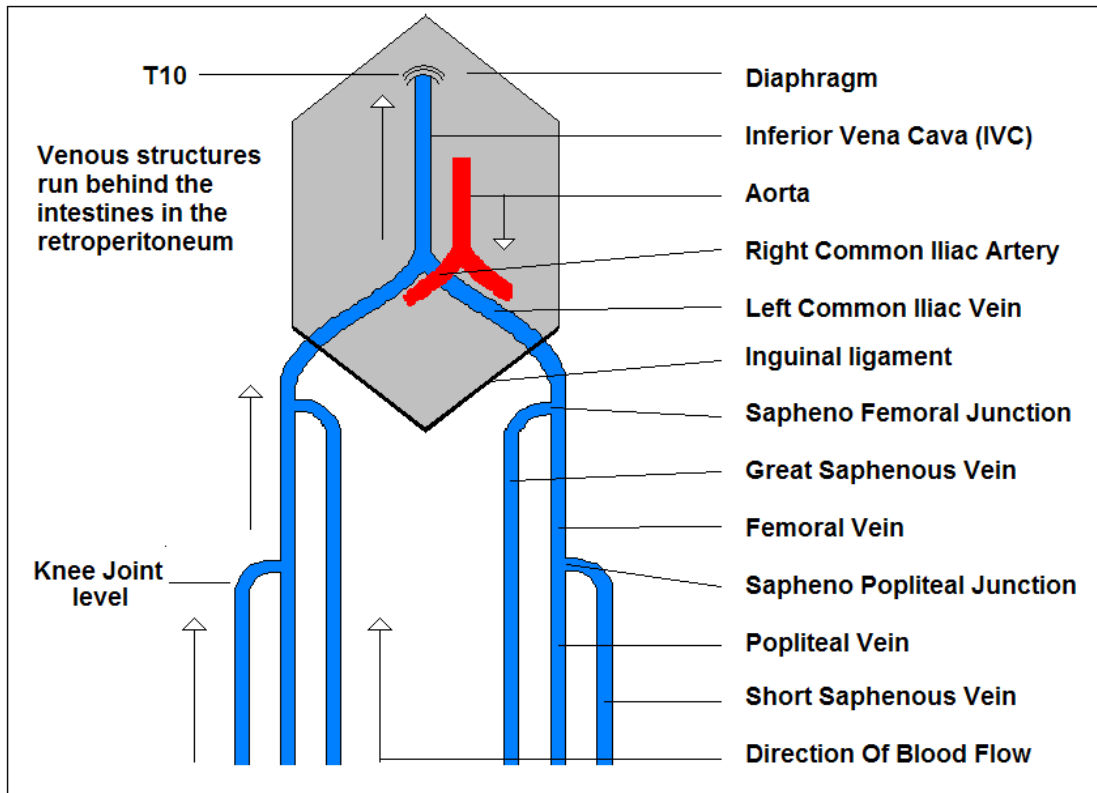


Figure 1.1. The lower limb venous drainage and its passage through the retroperitoneum of the abdominal cavity.

With the body in a supine position, lower limb venous pressure is low (~ 5-10 mmHg) and venous return is achieved by the pressure gradient to the right atrium. In the upright position, hydrostatic load increases venous pressure in the foot to approximately 100 mmHg, and venous return is aided by the presence of valves to prevent backflow, the calf muscle pump, and the respiratory pump. The calf muscle pump involves the deep veins of the calf (supplied by blood from the superficial compartment through perforator veins) running through the muscle bellies of gastrocnemius and soleus (see Figure 1.2). When these muscles contract they exert a pressure of anywhere between 200 and 300 mmHg expelling more than 60% of the venous blood contained within them into the large popliteal vein, equating to approximately 30-40ml per contraction. This substantially lowers the pressure in the deep and

superficial venous systems until refill occurs by arterial inflow (Raju et al 1993). After 10 contractions of muscle this takes approximately 25 seconds (Browse et al 1999; see Figure 1.3). Reflux in the deep system is prevented by valves in the tibial, popliteal and femoral veins and no reflux occurs into the superficial system as valves prevent flow outward in the perforator veins.

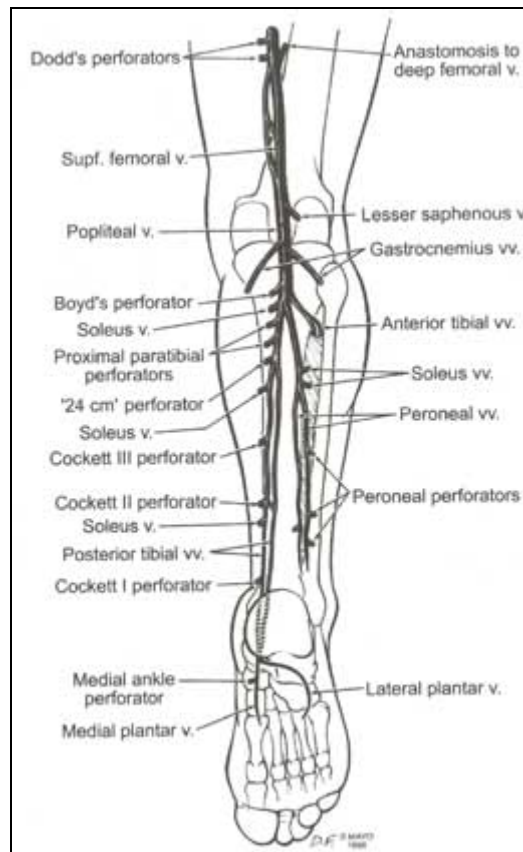


Figure 1.2. The calf muscle pump showing the arrangement of veins in the calf interwoven between muscle layers that act to squeeze the veins on contraction (Gloviczki et al 2001¹⁷⁶).

The respiratory pump acts through decreased intra-thoracic pressure on inspiration which draws blood up from the IVC into the chest, driving it on expiration into the right atrium as pressure increases in expiration. The pressure gradient between limbs and heart is also aided by the increasing diameter of the veins approaching the heart, through La Place's Law. Since

the equilibrium relationship between transmural pressure difference (dP), wall tension (T), and radius of curvature \mathbb{R} in a concave surface for a cylinder is defined as $dP = T/R$, this equates to blood moving from small caliber higher pressure chambers to larger lower pressure chambers (Daintree et al 1975).

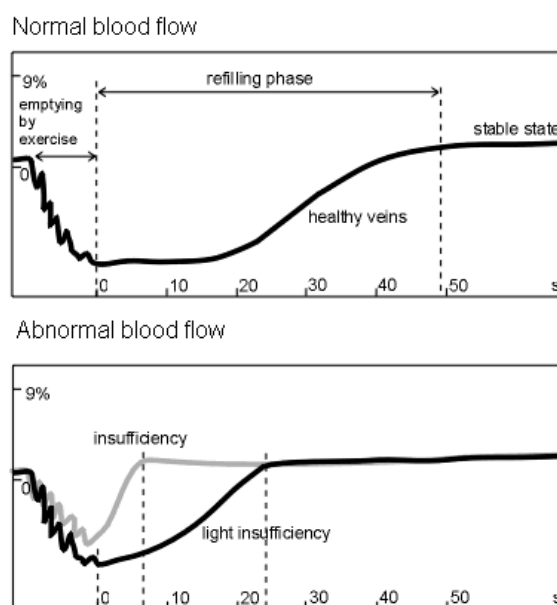


Figure 1.3. The effect of calf contraction on the blood volume of the lower limb as measured by photoplethysmography. The time it takes to return to normal should be greater than 25 seconds (Browse et al. 1999).

1.4 PATHOLOGY OF CHRONIC VENOUS DISEASE

Failure of the structural integrity of the venous system in lower limbs leads to varicose veins (VV) and chronic venous insufficiency (CVI). The two key changes that occur leading to the formation of venous reflux and varicosities are 1) weakening and dilation of the vein wall and 2) incompetence of the venous valves (Figure 1.4).

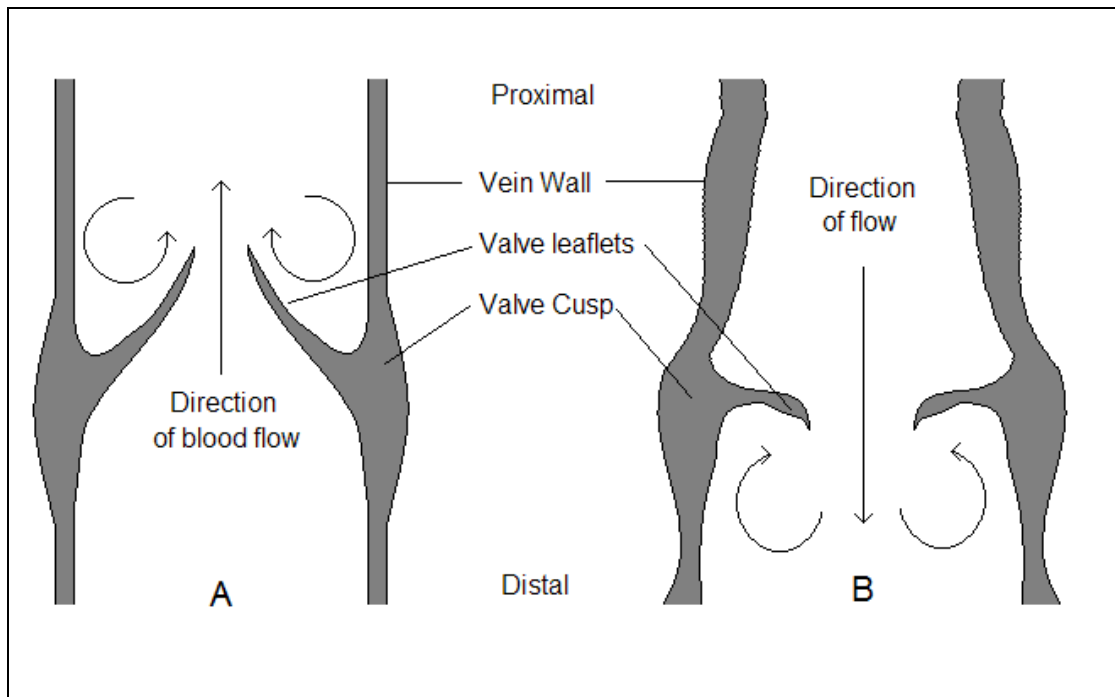


Figure 1.4. A) Normal healthy venous valves with the leaflets preventing back flow. B) Diseased venous valve with disruption of the vein wall and loss of integrity of the valve. Failure to control blood flow is seen with reflux through the separated leaflets.

Compared to the normal GSV, varicose veins show thickening of the wall intima and media and a transverse greater diameter, changes in smooth muscle composition, endothelial disruption, and fibrosis. Studies have shown hypertrophic changes in the smooth muscle cells (SMC) in the media of vein walls with remodeling of the alignment and structure of the muscles cells (Sansilvestri-Morel et al. 2001, Elsharawya et al. 2007). Reports have described increased, decreased and no change in the volume of smooth muscle cells. Overall the literature tends to support a higher content of SMC, which suggests that the pathological abnormalities in varicose veins are not due to deficiency of smooth muscle but to the inability

of the SMC to produce the necessary venous tone (Elsharawya et al. 2007). There is generalized thickening of the wall and hypertrophy of the sub-endothelial tissues can cause protrusions of the wall into the vessel lumen. There is elongation of the intima, thrown into excessive folds and invagination with discontinuity of the endothelial sheet and desquamation in many places. Wrinkling and invagination of the endothelial layer, not seen in normal veins, is found. These invaginations were noted to fold over on themselves trapping blood cells and then blebbing off into the circulation leaving exposed areas of intima (Wali et al. 2002).

The composition of the vein wall in terms of the connective tissue elements has been investigated and the relationship between elastin and collagen plays an important role in determining its distensibility properties and hence its ability to withstand pressure. Both are altered in venous disease. Elastin production has been shown to initially increase in the early stages (Naoum et al. 2007, Kirsch et al. 2000, Elsharawya et al. 2007) but with progression, the fibres become reduced in number and fragmented with the loss of the normal latticework in the intima and media. Venturi et al. (1996) found a strong association between the loss of elastin and the development of varicose veins. Evidence indicates that people with varicose veins have a higher turnover of elastin in tissue remodeling (Buján et al. 2003), and a study by Zsotér & Cronin (1966) showed that patients who had varicose veins in the lower limb also demonstrated increased distensibility of the forearm veins, implying a generalized defect. Reduction in elastin was noted in elderly populations, as expected, leading to reduced distensibility with time (Naoum et al. 2007). Measurement of collagen in the vein wall during venous disease has yielded conflicting results with reports of higher concentrations (Sansilvestri-Morel et al. 2001, Gandhi et al. 1993, Venturi et al. 1996), lower concentrations or no difference. However, more recent studies have accepted that the collagen contents is increased contributing to vein wall fibrosis and SMC separation (Venturi et al. 1996), but

have focused on the balance of the different types of collagen fibres. Within an overall increase in collagen production, Sansilvestri-Morel et al. (2001) noted a marked reduction in the production of collagen III fibres (involved in elasticity) compared to an increase in collagen I (structural strength). This imbalance of production occurred in the face of normal mRNA expression. Further studies by this group have found that not only is the saphenous vein affected by this imbalance but also the skin, suggesting that the connective tissue problem is at least part inherited (Sansilvestri-Morel et al. 2001, 2002, 2005, 2007).

Valve deficiency in chronic venous disease is also evident as separation and shortening of the valve leaflets with depression of the valves commissures (Edwards et al. 1940, Rokitansansky 1852). Valve cusp perforation, tearing and elongation have been described with disappearance of valves altogether (Takase et al. 2004, 2004). These changes are associated with inflammation as observed by Ono et al. (1998) who took histological samples of veins at human surgery and found monocyte infiltration. The San Diego group (Takase et al. 2004) speculated that the changes seen may be due to induced apoptosis of the cells in the valve leaflets but were not able to support this hypothesis biochemically.

1.5 PATHOGENESIS OF CHRONIC VENOUS DISEASE AND LINK TO VENOUS HYPERTENSION

A key question concerning the initiation of venous disease is which of these events – valve dysfunction or vein wall weakening - occurs first and how the two are related. On the one hand, it is considered that increased venous diameter due to structural wall changes and dilation could cause dysfunction of the valve leaflets. The disturbance in the wall ratio of elastin and collagen and the smooth muscle and endothelial changes described above alter the mechanical properties of the vein, resulting in reduced distensibility and dilatation, and

expose the valves to raised pressures that could precipitate their incompetence (Bergan et al. 2008). This theory is based on three observations made by King(1950):- 1) that initial varicosities communicate with a normal venous system, as clearly seen on duplex in patients attending vascular clinic who have no saphenous reflux but varicose veins in the lower limb; 2) that vein wall dilations occur distal to valves therefore making the backwash reflux of blood through the valves an unlikely theory (also suggested by Cotton in 1961) and 3) that histological examinations of the vein wall indicates that valves increase to a certain degree with venous dilation but then cease to operate beyond a maximal diameter.

On the other hand, valve dysfunction as the primary defect would permit backflow and raised venous pressure, which could lead to dilation and weakening of the vein wall. The role of valves in the development of venous disease is shown in congenital valve aplasia where the total absence of valves, albeit a rare situation, leads to development of severe varicosities and secondary complications. It was previously suggested that the absence of valves in the ileo-femoral vein segments (see Figure 1.1) increased hydrostatic pressure on the sapheno-femoral junction (SFJ) and that this led to descending valvular failure (Browse et al. 1999). Post-mortem analysis has shown that up to 40% of the population may be affected. A similar situation could also arise following disruption to, rather than absence of, valves in the ileo-femoral segments. However, no causative link has been proven and there is strong evidence that reflux in the GSV can be present without SFJ incompetence (Labropoulos et al. 1999, Abu-Own et al. 1994). The number of valves in the GSV has been noted to be significantly lower in varicosed veins although this is not necessarily related to junctional reflux. These points make a descending valvular incompetence theory less likely.

Whatever the exact sequence of the events that result in chronic venous disease, it is evident that venous hypertension, either through vein wall dilation or because of valve dysfunction, is

a major contributing factor. The inflammatory changes in valve leaflets that are characteristic of the disease process can be mimicked by chronic pressure elevation in animal models (Bergan et al. 2008). In venous hypertension induced by arterio-venous fistulae, Takase et al. (2004) showed that the changes seen in diseased valves could be replicated after only short periods of hypertension with vein wall dilatation, reflux, and increases in inflammatory markers accompanied by leukocyte infiltration in the valve leaflets.

There are a number of physical and haemodynamic characteristics of veins in humans that could predispose to venous hypertension and the sequelae that define chronic venous disease. These characteristics can be modified by many different factors including body composition (height, weight), obesity, raised arterial blood pressure, altered calf muscle pump function, levels of physical activity, outflow obstruction, attenuation of venous smooth muscle tone, and female reproductive hormone status. In order to evaluate the literature that relates to the involvement of these factors in venous disease, it is necessary to first define the parameters that describe these physical and haemodynamic aspects and describe how they are evaluated.

1.6 DEFINING LOWER LIMB VENOUS CHARACTERISTICS

The physical, mechanical and physiological characteristics of veins have been described by Rothe in two excellent reviews (Rothe, 1983 & 1986). When the veins are emptied (supine/head-down Trendelenberg position) they adopt an elliptical shape supported by the surrounding tissues (Clarke et al 1989, Zamboni et al. 1997). Their pressure is low and there is little resistance to flow. On assumption of the upright posture, blood pools under hydrostatic pressure in the leg veins which fill into their circular shape (Figure 1.5). The relationship between venous volume contained and pressure is important because it enables several characteristics of the veins to be defined. Over at least part of the physiological

operating range, the incremental change in volume for a small incremental change in transmural pressure tends to be constant; i.e. the pressure-volume relationship may be considered linear. At abnormally high pressures, the veins are stiff and there is little change in volume as the pressure is increased.

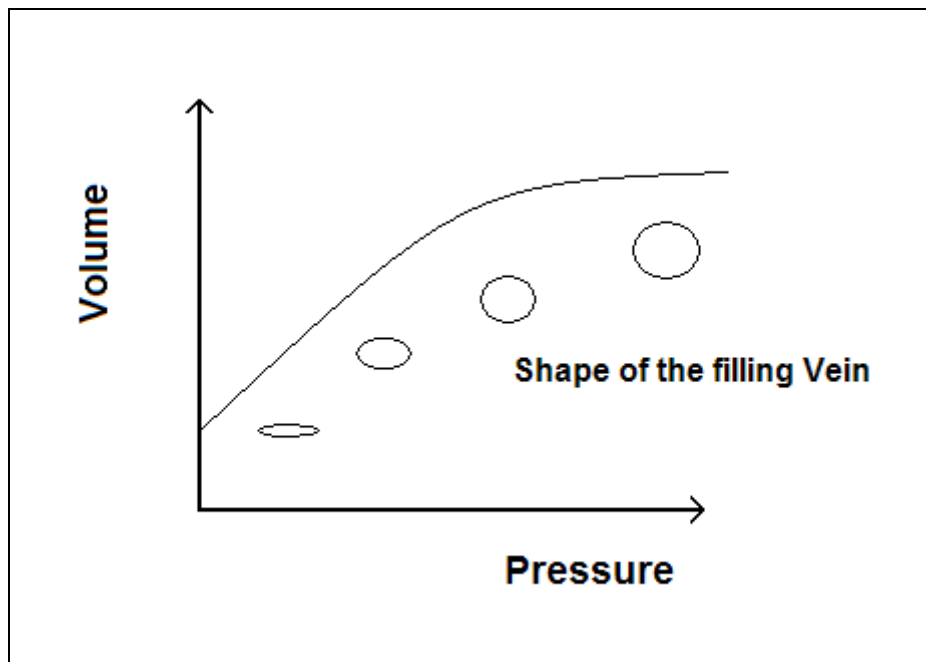


Figure 1.5. The volume pressure curve for veins depicting the predicted shape that veins take at different volumes and pressures.

Venous capacitance defines contained volume of the veins at a given pressure over the normal physiological range and can be altered by changes in the venous tone (i.e. the contractile status of venous smooth muscle), allowing the pressure-volume curve to be shifted down by venoconstriction and up by venodilation (Figure 1.6). The linear part of the normal pressure-volume relationship intercepts the volume axis at a positive value, representing what is called the unstressed volume. This is the volume contained at zero transmural pressure that does not

contribute to vein distension. When fully filled, volume contained can be defined as maximum venous capacity.

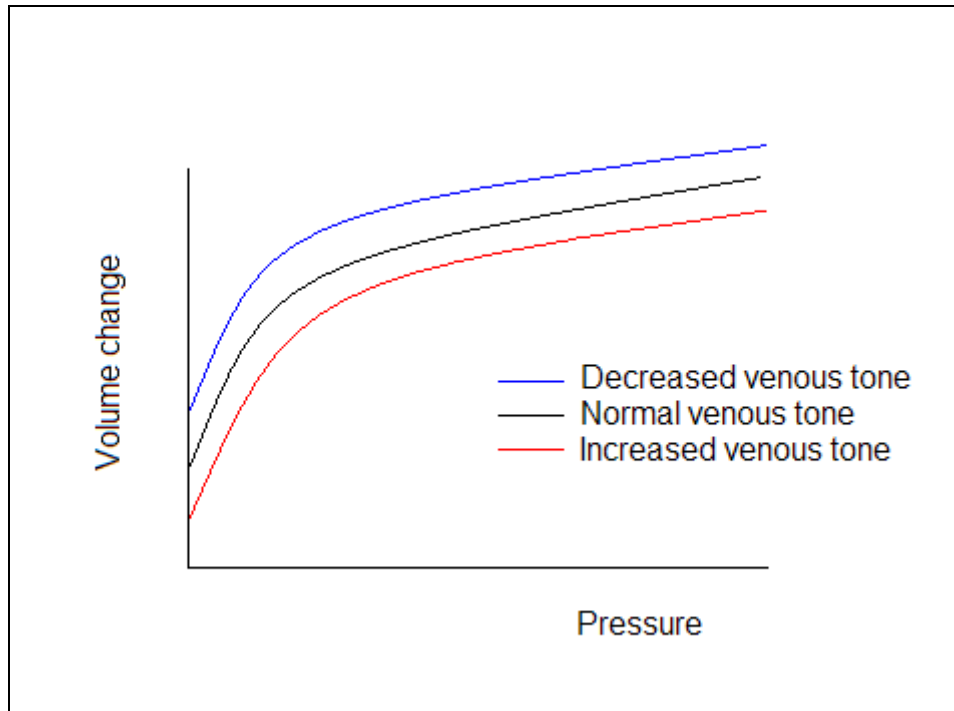


Figure 1.6 Pressure-Volume curves with normal, increased and decreased venous tones. The graph shows how it is possible for venous and or whole limb compliance to remain the same as venous tone is altered.

In order to describe the venous pressure-volume relationship insofar as it is determined by the properties of the vein wall, two other terms – distensibility and compliance – are often used. Distensibility is a qualitative term implying that when a force is applied to a material, it changes its dimensions. Vascular distensibility has been defined as $\Delta V/(V^{\circ}/\Delta P)$, the fractional change in volume per unit change in pressure where V° is the total vascular volume at the point on the pressure-volume curve under consideration. In practice, venous distensibility

should be calculated, for the reasons as explained above, over a linear portion at a defined range of pressures. Venous compliance is the ratio of the change in volume (ΔV) to the concomitant small change in the transmural distending pressure (ΔP): $C = \Delta V / \Delta P$. It is the slope of the pressure-volume relationship at a specified pressure or volume. Because of the overall nonlinear distensibility characteristics of blood vessels, the location on the vascular pressure- volume curve should be specified in terms of the pressure or total contained volume. It should be noted that, as shown in Figure 1.6, venous capacitance may be reduced or increased by venoconstriction or venodilation respectively without a change in the slope of the pressure-volume relationship, i.e. without change in compliance. Venous smooth muscle tone can be increased by autonomic sympathetic neural and hormonal activity even though the density of adrenergic innervation is much less than in arteries, especially in the smaller veins (Rothe, 1983). It is also sensitive to a wide range of local and circulating vasoactive substances (see Appendix A). Passive tone is dependent on the elastic properties of the vein wall. This property cannot be changed and is the major source of venous tone at rest (Browse et al. 1999).

Moreover, there is another property of the vein wall that impacts upon filling and has implications for estimations of distensibility and / or compliance. This is hysteresis which refers to the considerable discordance between venous volumes measured during a rise in venous pressure versus a fall in venous pressure. It is due to the presence of the elastin and collagen components of the vein wall, which impart viscoelasticity and allow for the property of 'creep' or what is called 'stress relaxation' or 'delayed compliance'. The shape of the hysteresis curve during vein filling and emptying is depicted in Figure 1.7, illustrating that contained volume at a given venous pressure in the filling phase of venous engorgement is less than for the same venous pressure during venous emptying i.e. the effect lags behind the

cause. Initially the response as described is overcoming the effect of the elastin fibres in the intima. This is the period of rapid expansion of volume with minimal increase in venous pressure. Once filling has occurred the resulting stretch is dependent on forces acting on the structural strength supplied by the collagen fibres. This is why the pressure in the vein rises rapidly for minimal increase in volume.

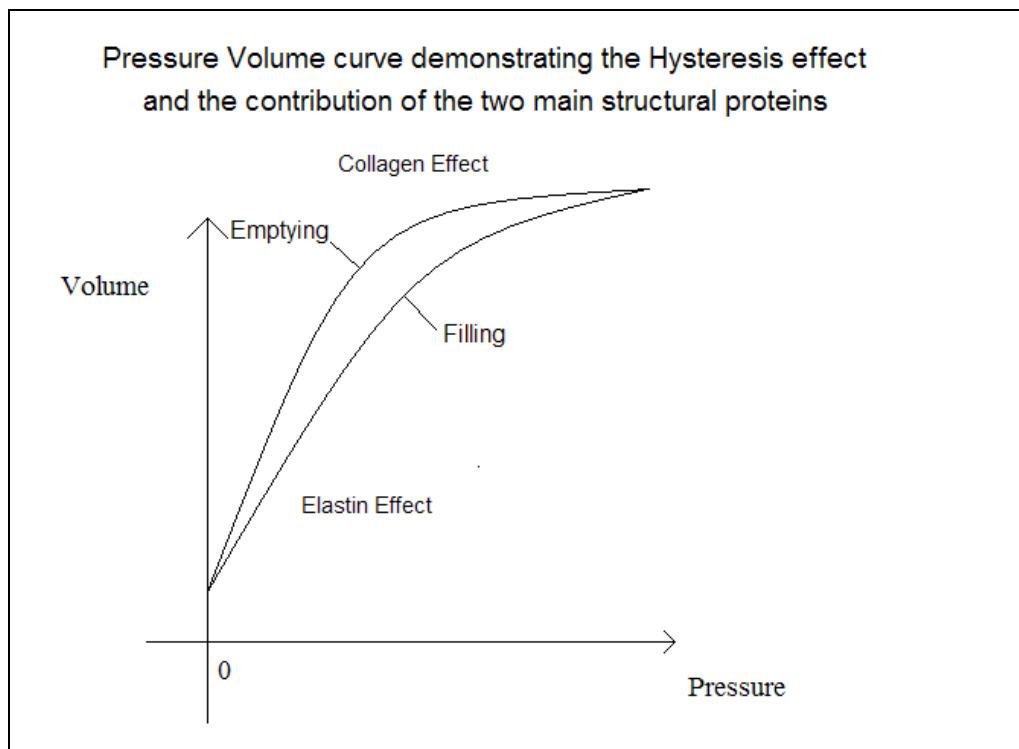


Figure 1.7. Diagrammatic representation of venous filling against pressure and hysteresis in a normal vein showing the different structural proteins effects on filling and emptying.

During filling, the early part of the curve can be modified by venous smooth muscle tone, whereas differences in vein wall composition - quantity of smooth muscle, elastin and collagen - modify the hysteresis curve in the upper pressure range. Because of 'creep' or 'stress relaxation', it is considered that a more accurate assessment of the passive elastic recoil

properties of the vein wall due to elastin and collagen is achieved by recording a pressure-volume curve during emptying. In any case, alterations in vein wall composition as a consequence of venous disease will result in changes in the shape of pressure-volume relationship characteristic of increased distensibility and capacity, and reduced compliance.

1.7 METHODS OF ASSESSMENT OF LOWER LIMB VENOUS FUNCTION

There are three main methods available for examining leg vein properties described by Pang (2000). Superficial veins in the foot can be studied for their vasoreactivity by measurement of diameter changes using the linear differential transformer technique as described by Aellig (1994) in response to local infusion, injection or oral administration of agonists and antagonists. For example, Streeten (2001) used this method to show supersensitivity to noradrenaline in the foot veins of patients with orthostatic hypotension. Since this method is limited to smaller superficial vessels, it may not provide information about the larger and / or deeper leg veins which play a greater role in determining pressure within the system. For this, venous occlusion combined with limb plethysmography or USS are more suitable methods.

A widely used approach to study leg venous function is to measure limb volume changes as a surrogate for venous vascular volume during filling either by postural manoeuvres or by application of a venous occlusion cuff. From red blood cell scintigraphy, it has been estimated that up to 65% of total blood volume in the filled limb resides in the venous circulation (Gelman, 2008), and pressure-volume curves representative of venous vascular volume can be constructed from plethysmographic recordings of limb volume during graded pressure increases applied by a proximal cuff. A typical trace obtained from such a filling procedure is illustrated in Figure 1.8. The shape of the curve is typical of that shown for individual veins in Figures 1.4 and 1.6, displaying a biphasic volume-pressure curve with a

steep initial slope representing rapid filling and a more gradual slope that levels off representing fluid filtration out of the microcirculation into the tissues (Risk et al. 2003). The inflection point between the steep and gradual part of the curve represents filled venous capacity and thereafter limb expansion can be attributed to venous wall “creep” during maintenance of high venous pressure as well as interstitial fluid accumulation (Knaapen et al. 2005).

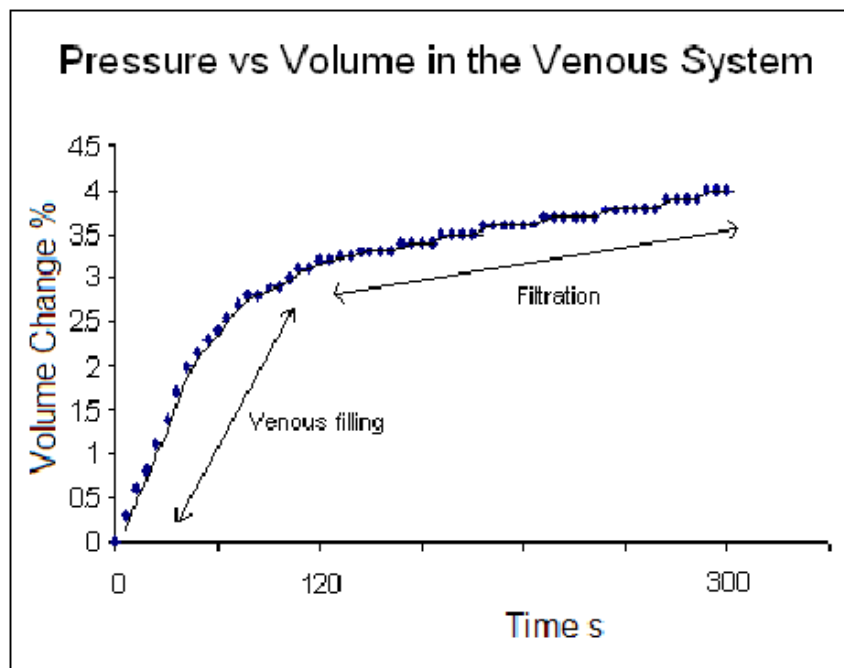


Figure 1.8. The effect of increasing pressure on limb volume demonstrating the venous filling and fluid filtration phases. Note that the venous filling and capillary filtration stages can be considered linear.

Using limb plethysmography to record volume changes, different venous occlusion protocols are employed to determine varying aspects of both arterial and venous haemodynamics. Brief occlusions to 40-50 mmHg (5-10 sec) allow the first few seconds of limb expansion as the

capacitance vessels fill to be measured, equating to the rate of arterial inflow as described by Joyner et al. (2001). A longer period of occlusion to record a complete filling curve (e.g. 5 min) will provide measures of rate of arterial inflow (initial slope), venous capacity (volume at inflection point) and fluid filtration (slope of gradual increase). Distensibility may also be derived during filling. From the filled state, a controlled rate of deflation of the occluding cuff (e.g. 1 mmHg/s) can be used to generate volume-pressure curves from which venous compliance (elastic recoil) is calculated. Distensibility and compliance are both measures of the volume-pressure relationship. They are different due to the effects of hysteresis. Distensibility is difficult to calculate as it is dependent on venous filling which cannot easily be controlled. Deflation of the limb can be controlled and compliance can be calculated using the technique described by Halliwill et al. (1999) (see methods section 2.2.3D).

Although many venous occlusion plethysmography protocols employ a step increase in cuff pressure, an alternative approach is to apply incremental small pressure steps (8-10 mmHg), each lasting ~ 5 min (Gamble et al. 1993). The rationale for this is that it avoids activation of the venoarteriolar response, a pre-capillary constriction triggered by venous distension that prolongs the time taken for filling by attenuating arterial inflow rate (Henriksen, 1991). The relationship between initial limb volume expansion at each step and pressure can be extrapolated to zero pressure to provide an estimate of ambient resting venous pressure, while the relationship between fluid filtration at each step and pressure represents limb filtration capacity and, when extrapolated to zero volume change, the isovolumetric pressure (i.e. pressure at which the Starling hydrostatic and oncotic pressures governing filtration are in balance).

Plethysmography at the calf combined with various venous distension protocols described above is frequently used to provide information about leg venous function involving all

classes of vessels. The recorded volume changes also include soft tissues (skin, adipose tissue) and these elements' contribution must be acknowledged. Doppler ultrasound imaging techniques on the other hand can be used to study the properties of specific leg veins, deep or superficial, providing that they are of a size to be visualized with good resolution as described in the consensus document by Cavezzi et al. (2006). This approach has been used not only to detect the presence of venous disease (Hertzberg et al. 1997, Danielsson et al. 2005, Jeanneret et al. 2007) but also to compare the anatomy of different leg veins in relation to body size, age, gender (Jeanneret et al. 1999, Fronck et al. 2003, Kröger et al. 2003, Bérczi et al. 2005, Haenen et al. 1999), to establish the relationship between vein cross-sectional area and pressure during cuff inflation (Planken et al. 2006) or tilting (Chauveau et al. 2006), to evaluate vein distensibility (Eiken et al. 2004) or compliance (Neglén et al. 1995, De Groot et al. 2005, Young et al. 2008, Delaney et al. 2008) and to study modifications to venous function during exercise (Ooué et al. 2008), inactivity (Belin de Chantemele et al. 2004, Arbeille et al. 2008) and heat stress (Abraham et al. 1994, Ooué et al. 2007).

In summary, the physical and functional properties of lower leg veins – size, filled capacity, distensibility, compliance and hysteresis – can be determined by use of a thigh cuff for venous occlusion together with measurement of calf volume by plethysmography or individual vein imaging by ultrasound. With plethysmography, the influence of soft tissues, venous 'creep' and fluid filtration must be acknowledged and taken into account. With ultrasound, the limits of image resolution that determine the smallest vessels that can be accurately visualized are an important consideration.

1.8 FACTORS AFFECTING LOWER LIMB VENOUS FUNCTION

Exposure to venous hypertension is implicated as a major feature in the pathogenesis of venous disease whether the initial defect is valve dysfunction or alterations in vein wall properties. Hydrostatic pressure in the leg during quiet standing is approximately 80-100 mmHg at the ankle depending on height and is equally high in superficial and deep veins (Recek, 2006). On walking, pressure decreases in both venous systems by about 50 mmHg (Pollack et al. 1949; Stick et al 1992) due to the calf muscle pump, but as pressure in the femoral vein does not change, this gradient will favour retrograde flow (Recek, 2006). Venous hypertension during walking is defined as the absence of the usual drop in pressure in lower leg and foot veins due to valvular incompetence, outflow obstruction or limited joint mobility.

There are a number of important factors that can modulate leg venous function, increase the likelihood of high venous pressure and hence predispose to the development of venous disease over time. Some of these impose hydrostatic load, e.g. body size, mechanical outflow obstruction, impaired calf muscle pump function, whereas others affect vein wall composition and hence distensibility and / or compliance more directly, e.g. aging, hormonal and metabolic status. The evidence for a relationship between any of these factors and development of venous disease will now be discussed.

1.8.1 Body Size, Composition and Occupation

A) Height

Increasing orthostatic pressure in the upright position is thought to increase the risk of developing venous disease. In healthy young subjects, foot venous pressure measured directly during quiet standing and the decrease in pressure on walking were greater with increasing

height (Kügler et al. 2001). Despite this association, the Edinburgh study showed only a significant risk in females with an increase in height (Lee et al. 2003) as did Beaglehole et al. (1975) and Sisto et al. (1995). Abramson et al. (1981) looked at height in the Jerusalem study but they found it was not significant when other factors were controlled. There is a lack of any data in the literature that reviews the effect of height directly on venous functional measures such as distensibility, capacitance or compliance. Fronek et al. (2001) commented upon a strong correlation between height and venous disease, but as height corresponds with other indices of body composition (e.g. weight, calf size), there were felt to be multiple interactions with other factors.

B) Weight

In healthy individuals, Kügler et al (2001) demonstrated a greater venous pressure in the foot during standing, an increase in FV cross sectional area (CSA) and GSV CSA with an increase in body weight associated with greater height and calf muscle CSA, but not with thigh circumference. Time to minimal pressure during walking was also longer in those of greater weight. Weight was seen as an independent variable in the size of the FV and GSV CSA in a study by Fronek et al. (2001). Iannuzzi et al. (2002) suggested that body weight in post menopausal women plays a specific and independent role in the development of varicose veins. The EVS by Lee et al. 2003 multi-adjusted for all factors and showed a significant risk of truncal varices in women with increasing weight but a reduction in risk in males. This may suggest that different environmental factors affect men and women, such as body position at their occupation. Weight is not only related to body size and frame but also to levels of body fat. Body mass index (BMI) incorporates both height and weight and is thus a commonly-used measure to indicate obesity in relation to body proportions.

C) Body Mass Index (BMI)

Within a population, BMI, measured in Kg/m^2 , is defined as normal when it falls between 20-25, overweight between 25-30 and obese when over 30. Association between BMI and the occurrence of varicose veins has been demonstrated in several population-based studies and smaller studies (Brand et al. 1988, Danielsson et al. 2002, Ciardullo et al. 2000), and appears to hold predominantly for women rather than men (Lee et al. 2003, Beaglehole et al. 1975, Seidell et al. 1986). In post-menopausal women, clinical evidence of VV was associated with obesity even after adjustment for levels of oestradiol as found by Ianuzzi et al. (2002). However, two studies have demonstrated no correlation between obesity and varicose veins formation in women (Hirai et al. 1990, Komsuoğlu et al. 1994). This may represent a genetic difference in the population but also life style factors may play a role. Danielsson et al. (2002) reported from a study of 99 males and 173 females aged 14-90 years, overweight ($\text{BMI} > 25 \text{ kg/m}^2$) individuals were more likely to have skin changes and ulceration, and there was a significant association between clinical severity of venous disease and BMI. Associations have also been noted in the severity of the presentation of venous disease in CVI with obesity and these correlate with increasing symptomatology (Danielsson et al. 2002). There is, however, no consistent link demonstrated between obesity and CVI (Benigni et al. 2006), and, as previously described by Padberg et al. (2003), obese patients can exhibit the symptoms of CVI without detectable venous dysfunction. This may be due to the fact that the overweight are also less physically active, a condition that can impact on calf muscle pump function (see section 1.8.1 E).

It is possible that obesity *per se* results in adverse changes in structural and functional vein wall properties under the influence of the associated haemodynamic and hormonal alterations that ensue. Venous compliance of the forearm was lower in obese compared to lean subjects,

with a smaller reduction in central blood volume on standing (Stepniakowski et al. 1995). In contrast, the greater increase in leg vein size on standing with higher BMI, independent of age, implies greater venous distensibility (Kröger et al. 2003). It is not clear, therefore, to what extent BMI impacts on vein function through mechanical hydrostatic factors or through specific changes that alter wall composition. It must also be taken into account that BMI may not always be the best option for studying the effects of obesity in a population as it does not give information on the distribution of body fat. Potentially, variations in disposition of abdominal rather than peripheral adiposity could present mechanical obstruction to the venous outflow from the lower legs and contribute to venous hypertension and disease.

Venous return on its way back to the heart from the legs passes through the retroperitoneum of the abdomen (Figure 1.1) where it is subjected to changes in intra abdominal pressures. Multiple studies have shown that an increase in obesity raises the intra-abdominal pressure (Cobb et al. 2005, Sugerman et al. 1997, Sugerman, 2001). Arfvidsson et al. (2005) demonstrated that the pressure in the femoral vein was directly correlated with the intra abdominal pressure in the morbidly obese suggesting that compression in the abdominal compartment is acting to inhibit venous return from the limbs. Sugerman et al. (2001) showed an improvement in venous symptomatology following weight loss following gastric surgery for obesity. Arfvidsson et al. 2005 reinforced this by showing a decrease in venous reflux post gastric banding procedures. It is not clear, however, how much of this is due to decreased intra-abdominal pressure or general body weight loss.

Adipose distribution in the region of the hips and thighs may also present mechanical hindrance to venous return. For example, the effects of artificial compression on the lower limb were investigated by Pottier et al. (1969) who showed that compression of the posterior thigh when seated increases lower limb swelling, suggestive of raised venous pressure and

fluid filtration. Epidemiological evidence from the Netherlands suggests that the prevalence of varicose veins is higher in women who store the majority of their adipose tissue around the hip and thigh region, termed gynoid adiposity (Seidell et al. 1986). L'Hermitte et al. (2003) suggested that the symptoms of venous and lymphatic congestion seen in women were independently related to their gynoid adiposity, shown by a negative correlation with waist-hip ratio rather than with abdominal adiposity.

D) Occupation

Two studies which clearly discuss the impact of occupation on the prevalence of venous disease are the EVS by Fowkes et al. (2001) and Tomei et al. (1999) from Rome. Both involved targeted questionnaires to ascertain the level of activity undertaken at work combined with a clinical examination to evaluate the prevalence of disease. Both studies showed the effects of standing at work as the major risk factor for developing venous disease, the Italian study suggesting that people who spend greater than 50% of time standing are at greatest risk. Prolonged sitting has been shown to cause distension of leg veins and venous stasis by Delis et al. (2004) investigating the causes of deep venous thrombosis. Ashby et al. (1995) measured femoral vein flow velocity and demonstrated that blood moves slowest out of the limbs in the seated position. No literature has been found by the author to ascertain if occupation has any demonstrable effect on venous compliance. Epidemiological studies have linked venous disease to occupation through standing, but how the occupation relates to the presence of abdominal and / or gynoid adiposity has not been established and this may well play a major part in the overall effect of occupation.

E) Calf Muscle Pump Function

As the calf muscle pump is integral to the venous return from the lower limbs and acts to counter raised pressure in the upright posture (see section 1.3), any impairment will

predispose to venous hypertension and chronic venous disease. In healthy subjects, higher venous pressures were recorded in the foot during quiet standing in those with larger (>36 cm) than smaller (< 36 cm) calf cross sectional area (CSA), and pressure decreases on walking were bigger (Kügler et al. 2001). This implies that muscle mass is an important factor for appropriate muscle pump action. To determine efficiency of the pump, non-invasive protocols have been developed which test the ability of calf contractions to empty the lower leg venous circulation (Fronek et al. 2000, Haenen et al. 2000). In general these measure venous volume indirectly by strain gauge or photoplethysmography, red cell scintigraphy, or use ultrasound to measure venous flow velocity, and examine changes during standardized calf exercise (dorsi- and / or plantar-flexion) or calf compression as demonstrated by Van Den Broek et al. (1991).

Calf muscle pump dysfunction is well established in patients with chronic venous disease (Christopoulos et al. 1989, Yang et al. 1999) but it is not clear how much of this is due to reduced size and strength of calf muscle itself or to defective venous valves, because calf size is seldom assessed. Moloney et al. (2007) performed MRI imaging to calculate calf muscle volume in older patients with chronic venous disease and ulceration and compared this with their muscle pump function. They found no correlation between calf size and peak popliteal vein velocity during voluntary calf contractions, but because the patients already had severe disease (CEAP 6), it is impossible to ascertain cause and effect from this work. Qiao et al. (2005) examined calf muscle biopsies from patients with VV and CVI as well as controls, and reported morphological abnormalities characteristic of denervation and reinnervation in the patients that correlated with poorer calf muscle pump function (assessed by lowering of foot venous pressure on walking). Again, this association does not help to explain whether it is reduced muscle size that promotes pump dysfunction and chronic venous disease, or whether

the disease state compromises pump function and impairs patient mobility, leading to inactivity, calf muscle atrophy and worsening of pump function.

There is evidence from studies in healthy populations without recorded venous disease that calf size impacts upon venous function. Convertino et al. (1988) determined the compliance of the calf in young males by relating strain gauge volume changes during venous occlusion to the applied cuff pressure (i.e. during filling). They showed an inverse relationship between this value and calf CSA and volume, estimated from CT scanning, and suggested that increased support from the greater muscle mass reduced the ability of the veins to distend in response to hydrostatic pressure, hence lowering compliance. Based on a multivariate analysis of factors such as muscle size, strength and peak oxygen consumption in trained and untrained individuals, they discussed the likelihood that physical training, which can cause muscle hypertrophy, would decrease calf compliance. This is at odds with data from studies that have measured calf compliance during venous emptying after training and shown significant increases in the trained state (Hernandez et al. 2004, Monahan et al. 2001). The discrepancy may be related to the mode of testing compliance in that pressure-volume relationships during filling will differ from those during emptying because of hysteresis (Convertino et al. 1988, see Section 1.6), but studies demonstrating an increase in filling venous capacity after training (Kenney et al. 1987, Boutcher & Boutcher 2005) have not concurrently measured calf muscle size to assess its impact.

Those patients with chronic venous disease who have undergone superficial venous treatment have been reported to show improvement in the efficiency of the calf muscle pump by Struckmann (1987) and abolition of deep venous reflux by Mackenzie et al. (2004). This suggests that reduced venous stasis of blood in the limb has a positive effect on the functioning of the venous system.

1.8.2 Age, Gender, Female Sex Hormones and Pregnancy

A) Age

The prevalence of venous disease has been shown to increase with age (Beaglehole et al. 1975, Criqui et al. 2003). In the EVS the 55-64 year old age groups of men and women showed greater than 40% prevalence for truncal disease (Lee et al. 2003, Evans et al. 1999). It is, however, difficult to distinguish effects specific to the ageing process because of its association with lower levels of physical activity, weight gain etc. The study by Kröger et al (2003) stated that the cross sectional area of the lower leg veins measured by ultrasound imaging did not change with age but was positively associated with BMI especially in the standing position. Fronck et al. (2001) however noted a decrease in the FV CSA and a marked decrease in femoral vein flow velocity with age. Monahan et al. (2004) showed that the capacitance of the lower limb was less in young females than young males when measured by strain gauge plethysmography and that it reduced in both groups with age. This would fit with the decrease noted above by Fronck et al. (2001) in CSA of the FV. The effect of ageing on venous compliance in the upper and lower limbs was examined by Young et al. (2006) using mercury in silastic strain gauge plethysmography. The group found that venous compliance decreases with age in both males and females. This is supported by previous studies (Young et al 2006, Monahan et al 2004, Olsen et al 1998, Monahan et al 2004, Hernandez et al 2004). This has however been challenged in the literature by Lindenberger et al. (2007) who suggested no decrease in venous compliance with increasing age in women. Gascho et al. (1989) demonstrated a reduced dilatory response to nitroglycerin in upper limb veins in an elderly population, accompanied by reduced distensibility. This was in 40 males and 50 females ranging from 21 to 78 years in age. Overall the evidence points to a reduction with age in the size of the lower limb veins which have become less reactive through exposure to

lifestyle factors. This is supported by the epidemiological data although the confounding factors and inherent bias in each study makes multivariate analysis complicated and unreliable.

B) Gender

There is a general preconception that women suffer more from varicose veins than men. Beaglehole et al. (1975), Abramson et al. (1981) and Sisto et al. (1995) all reported that the condition is more common in females than males, although these studies often rely on self-reported data. The EVS (Lee et al. 2003) challenged the conception that females suffer more from varicose veins because a higher prevalence was found in males on clinical examination. This is supported by Scott et al. (1995) who found male gender to be a significant risk factor for CVI of the lower limbs, although the authors conceded that this may in part be related to concurrent arterial disease.

Kröger et al. (2003) and Fronck et al. (2001) found as expected (due to smaller body size) that the CSA of the FV and GSV was smaller in females than males and that the increase in absolute volume when moving from lying to standing was also less. Fronck et al. (2001) demonstrated no change in flow in FV when moved to standing positions which equated to a higher flow velocity in females in their study. The studies by Meendering et al. (2005), Monahan et al. (2004) and Lindenberger et al. (2007) all demonstrated that the compliance of the lower limb was less in females than males. Lindenberger et al. (2007) subsequently demonstrated that the venous capacitance of the calf was less in females but their fluid filtration was equal to males.

C) Female Sex Hormones and Oral Contraceptive Usage.

Females have been shown to have a reduced venous capacitance in line with less tissue mass compared to males (Muntinga et al. 1997, Monahan et al. 2001) and a smaller venous

diameter as measured by Kröger et al. (2003). Meendering et al. (2005) and Monahan et al. (2004) both demonstrated that the compliance of the lower limb was less in females than males. The epidemiological evidence that females may or may not have a higher incidence of varicose veins has lead to the investigation of three areas of female health that have been suggested to be causative factors.

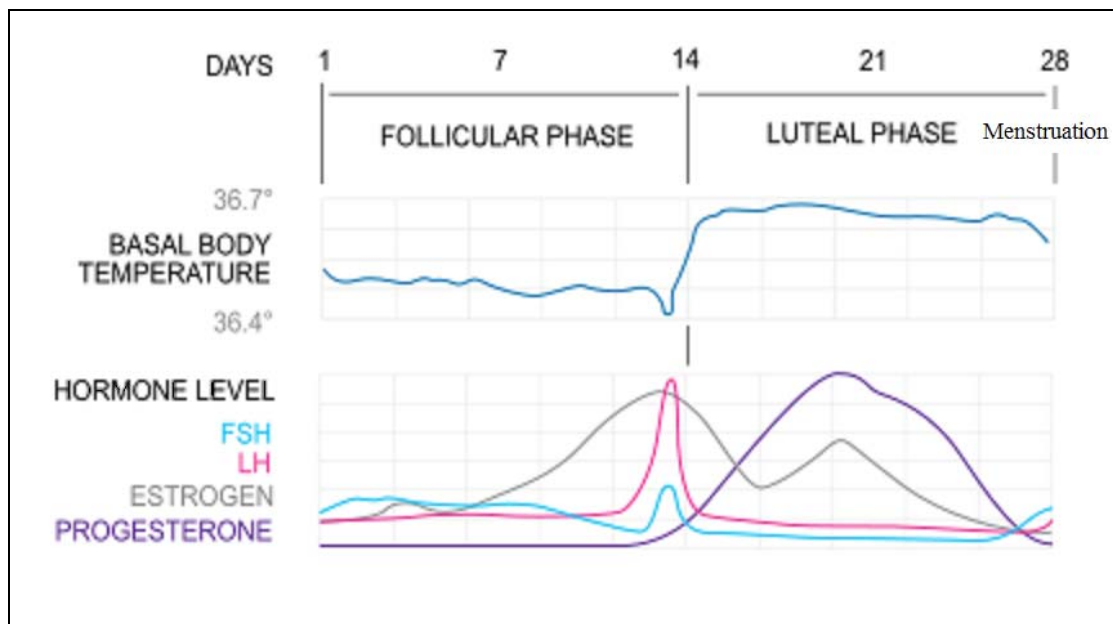


Figure 1.9. The menstrual cycle showing the levels of females sex hormones and their relation to one another over a 28 day period.

Female Sex Hormones

Existing evidence shows that healthy venous vascular smooth muscle has functional oestrogen and progesterone receptors (Karas et al. 1995, Karas et al. 1994, Haas et al. 2007). Perrot-Applanat et al. (1995) demonstrated the presence of progesterone receptors in the smooth muscle layer in 90% of surgically excised varicose veins but oestrogen receptors was

only found in 17% of their samples. This is in contrast to Knaapen et al. (2005) who demonstrated smooth muscle hypertrophy directly mediated by oestrogen receptors in excised varicose vein samples. Haas et al (2007) demonstrated that 17β -estradiol alone did not affect venous contractions in vitro but enhance contractions of endothelin-1. This effect of enhanced venoconstriction in the presence of 17β -estradiol was opposite to the effect in the arterial system where 17β -estradiol demonstrated arterial vasoconstriction. The studies above all look at either cellular biochemical activity of in vitro reactions. What evidence is there in vivo?

Walters et al. (1977) demonstrated that the distensibility of the dorsal hand vein during filling was increased in the first half of the menstrual cycle (follicular phase, see Figure 1.9) but reduced during menstruation. This is in contrast to Fawer et al. (1978) who showed increase venous distensibility in the luteal phase of the cycle. Goodrich et al. (1966) both demonstrated an increase in venous distensibility during venous filling with administration of 17β -estradiol measured by calf strain gauge plethysmography in healthy young subjects. The same relationship was noted by Ciardullo et al. (2000) in post menopausal ladies with high estradiol levels using the same technique.

Distensibility of the veins is complicated by the rate of limb blood flow. Keates et al. (1969) in normal ovulating women demonstrated increased blood flow in the limb in the follicular phase of the menstrual cycle by strain gauge plethysmography. In post menopausal women Vehkavaara et al. (2000) & Volterrani et al. 1995 have both demonstrated increase in forearm blood flow with administration of exogenous 17β -estradiol. It is therefore more appropriate to measure compliance of the venous system in order to ascertain the effect of hormone levels. Meendering et al. (2005) could demonstrate no difference in venous tone or compliance throughout the menstrual cycle on the calf or during the use of oral contraceptive using whole limb plethysmography and quadratic interpretation of deflation protocols for definition of

compliance. Mercurio et al. (1997), using the same investigation but not stipulating how compliance when using transdermal oestrogen It also showed an increase in blood flow, decreased vascular resistance and increased venous compliance.

Oral Contraceptive Pill

The prevalence of varicose veins in patients who have taken oral contraception (OCP) was found to be lower but not significantly so than those who had not in the EVS (Lee et al. 2003). Giribela et al. (2007) reported no significant effect on the venous endothelial derived venous dilatation in dorsal hand vein during OCP use although a (non-significant) reduction in venous dilatation post use of OCP was reported . Goodrich et al. (1966) demonstrated an increase venous distensibility in the lower limb with OCP usage. Gosling et al. (1977) reported that the blood flow was reduced by the use of the OCP but that venous distensibility was not affected. This is surprising as oestrogen has been shown to inhibit vasoconstriction in the presence of norepinephrine (Danielsson et al. 2002). Meendering et al. (2005) could find no increase in the the venous compliance of the lower limb with use of the OCP.

Hormone Replacement Therapy

In post menopausal women, Ciardullo et al. (2000) found the incidence of varicose veins to be higher in those with increased oestradiol levels, but the Edinburgh study showed a decrease incidence with the use of HRT (Fowkes et al. 2001). The San Diego Study (Criqui et al. 2007) had a greater risk of venous disease in females who had undergone oophorectomy but did not comment on the use of HRT. They state that other hormonal factors are at risk in females but do not comment on which these are. Nonetheless, this data indicates that high levels of female hormones, particularly oestrogen, may be beneficial in protecting against venous disease. Ceballos et al. (2000) reported that the use of HRT improves veno-dilatation in dorsal hand veins but that this effect is quickly lost following withdrawal. This finding is supported by

Varbiro et al. (2002) who demonstrated that the decreased distensibility of mesenteric rat veins post oophorectomy could be reversed with supplemental oestradiol.

Multiple techniques have been used to assess the effects of sex hormones and the variation causes confusion. Distensibility is dependent on filling and oestrogen has a clear affect to increase limb blood flow which will affect the rate of filling of the venous system whilst oestrogen also has been demonstrated to inhibit the effects of adrenergic constrictors and this may affect venous distensibility but should not affect compliance. Meendering et al. (2005) used filled systems and measured deflation which should exclude the problems with distensibility. The papers that this group published show no effect of the menstrual cycle or OCP usage on whole limb/calf compliance. One of the major problems is that the impact of oestrogen/OCP/HRT has been measured on the whole limb/calf to establish distensibility or compliance and that capillary filtration has a role to play when this measurement technique is used. Direct visualisation of venous properties is being performed and this may exclude this problem.

Overall the effect of exogenous administration of oestrogen/OCP/HRT appears to be an increase in arterial inflow and an increase in venous distensibility but no effect on venous compliance. This would suggest that arterial inflow is the major contributor to venous distensibility. The menstrual cycle has been suggested to increase blood flow in the follicular as well as the luteal phases although the published data is contradictory. There is no clear evidence as to the effect of the balance between the sex hormones on the venous wall function or the balance of structural elements. It is safe to state that oestrogen and progesterone receptors are present but their effect is unclear. The long term effects of raised oestrogen or progesterone levels is equally unclear as any structural changes would presumably be related to prolonged periods of low or high hormones. Establishment of the effect of long term

exposure to low or high levels of female sex hormones may help to answer some of these questions.

D) Pregnancy

Varicose veins are associated with pregnancy by the medical world but also in the mind of the general public. The Edinburgh and San Diego studies (Fowkes et al. 2001, Criqui et al. 2007) showed an increased but not significantly higher prevalence of varicose veins with previous parity. Jukkola et al. (2006) found in a Finnish population that an association existed between parity and varicose veins, but this was only a significant difference between nulliparous women and those who had 3 or more pregnancies. The fact that the number of pregnancies rather than weight gained in pregnancy is the determinant factor again suggests another mode of cause other than mechanical impedance. A 2005 study by Dhawan et al. in rats showed a reduced compliance and increased venous tone in rat splanchnic veins which were multiparous over nulliparous, suggesting that the effects of pregnancy last after the event.

The major sex hormone present during pregnancy is progesterone and receptors for this hormone have been demonstrated in saphenous veins (Perrot-Appianat et al. 1995). Sparey et al. (1999) investigated the changes that occur in the lower limb veins throughout pregnancy and showed a marked increase in venous diameter with pregnancy up to the point of parturition. Normal diameter was restored at 6 weeks post delivery. They found no development of new venous reflux in their population but that people with reflux disease deteriorated. Venous distensibility follows a similar pattern with an increase up to the point of term, as demonstrated by Barwin et al. (1976) in the calf and forearm and by Fawer et al. (1978) in the forearm. This global effect as opposed to a local effect due to a gravid uterus is reinforced as the changes are visible in the first trimester of pregnancy, before any gravid effect would be of consequence. The upper limb changes were not however demonstrated in

study by Edouard et al. (1998) although lower limb changes were noticed. No studies could be identified that have investigated venous parameters both pre and post pregnancy in the same population.

The evidence would suggest that the effect of hormones clinically can be overcome by removal of the stimulus, either the presence of the foetus or the hormones associated with pregnancy. However increasing numbers of pregnancies is associated with an increased risk of venous disease and therefore the changes that occur during and because of pregnancy must be assumed to all have a contributing element that has the long term consequence of venous damage.

1.8.3 Life Style Factors

A) Diet and bowel habit

Studies have looked at the prevalence of varicose veins and CVI and the intake of dietary fibre, transit times and straining at stool. Lee et al. (2003) showed an increase risk of VV prevalence in men who strained at stool, whilst Beaglehole et al. (1975) reported an increase in a South Pacific population with the intake of refined sugars. Constipation and dietary fibre were not risk factors, however, in the San Diego study Criqui et al. (2007). Venous compliance or distensibility has not been assessed against these variables.

B) Insulin and Diabetes

All insulin resistance states are associated with significant macro- and microvascular defects Evcimen et al. (2007). These are well documented for the arterial circulation and include the loss of insulin-mediated decreases in large artery stiffness (Yki-Jarvinen et al. 2007, Cameron et al. 2007), generalised endothelial dysfunction, reduced insulin-mediated vasodilation of pre-capillary vessels, and impaired capillary perfusion (Kim et al. 2006, Serné et al. 2007). In

skeletal muscle, these disrupt glucose dispersal and metabolism, while other organs e.g. skin, eye, kidney, suffer microvascular and end-organ damage. These defects may cause or be caused by sympathetic adrenergic overdrive and hypertension (Serné et al 2007, Grassi et al 2007), which together inevitably increase the vascular damage and cardiovascular risk.

Far less attention has been paid to the venous circulation in insulin-resistance states despite the fact that lower limb ulceration affects 15% of people with diabetes (Brem et al. 2007). Venous hypertension is a key factor in the development of ulcers by causing microvessel inflammation, oedema, skin hardening and impaired oxygen delivery. Skin pre-capillary arterial vessels lose their ability to buffer blood pressure in diabetics (Fegan et al. 2003), leading to arteriovenous shunting (Fagrell et al. 1999) and raised venous pressure (Purewal et al. 1995). There is also, however, evidence that insulin has direct effects on venous vessel tone that are altered in insulin-resistant states and could contribute to development of venous hypertension. Insulin causes venodilation (Feldman et al. 1993) and can attenuate adrenergic venoconstriction (Sung et al. 1998). Vein dilator sensitivity to insulin, tested in the dorsal hand vein, was decreased by hypertension (Feldman et al. 1993), obesity (Feldman et al. 1995) and high cholesterol (Sung et al. 1998). Moreover, in a rat model of metabolic syndrome, endothelial dilator modulation of venous tone by nitric oxide was depressed (Song et al. 2006). All these changes reduce the ability to counter constrictor tone and encourage raised venous pressure. This is of particular importance in light of the hyperadrenergic conditions in insulin-resistance states. Epidemiological data from Sisto et al. (1995) in Finland, however, suggested a lower incidence of varicose veins in their population with established diabetes but the study does not give figures for the number of volunteers with diabetes from the population. The San Diego and EVS do not comment on the levels of diabetes in their populations. The author was unable to obtain any data from the literature on

the relationship between glucose, insulin or insulin resistance and the properties of the lower limb venous system.

C) Hypertension, Renin and Atrial Natriuretic Peptide

The heart requires a continuous flow of blood to maintain a sufficient arterial pressure to perfuse the organs. The venous system acts as a reservoir which can be used to increase or decrease blood flowing into the heart as required. The ability of the veins to distend and contract when required is paramount for this. If the system is too compliant, then the condition of orthostatic intolerance can develop whereby insufficient blood flow from the lower limbs reaches the heart. However if the system is less compliant, it may increase venous return and increase cardiac output by overfilling the right side of the heart (Safar et al. 1987). Arterial hypertension has been investigated along with multiple endogenous and exogenous vasoactive substances to determine their effect on the venous system (Appendix A). Criqui et al. (2007) showed that in the presence of hypertension there was a slight reduction in the incidence of mild varicose disease. This was not the case in severe venous disease where hypertension was more prevalent than the normal population. Brand et al. (1998) in the Framingham study reported an increase in the incidence of varicose veins in the presence of hypertension but unfortunately the Edinburgh study does not comment in its published results. Delaney et al. (2008) looked at the effect of hypertension in the young on the capacitance and compliance of the upper and lower limb veins and reported that capacitance is reduced (possibly due to raised smooth muscle tone). This study was unable to demonstrate any effect of hypertension on venous compliance. Takeshita et al. (1979) demonstrated a decrease in the venous distensibility in forearm veins in young borderline hypertensive males which was felt to be due to non-adrenergic mechanisms and possibly due to structural changes. Stepniakowski & Egan (1995) noted a similar effect of decreased venous distensibility but

also recognized the effect of obesity and insulin sensitivity in the process with possible additive affect. The study by Simon et al. (1979) from Minnesota on human forearm veins demonstrated a similar effect but also demonstrated an increase in the level of capillary filtration during occlusion plethysmography suggesting an increase in capillary permeability in hypertensive adults. This is possibly an adaptive change to higher arterial and venous pressure. All these results were corroborated by Fitzpatrick et al. (1986) who also showed that reduced distensibility could be linked to a decrease in renin release and a lower increase in heart stroke volume in hypertensive patients when the limb is occluded, blunting the physiological response to renin release.

The effect of Atrial Natriuretic Peptide (ANP) on the venous system has been investigated by Schmitt et al. (2003) who showed a venodilatory effect and reduced resting venous tone with even small doses of ANP on human forearm veins, whereas Doorenbos et al. (1991) showed no alteration in the compliance of the venous system. ANP is thought to have an antagonistic affect on Angiotensin 2 and to act by possibly inhibiting noradrenaline release at the pre-synaptic membrane. This may explain why it has the action of venous dilatation at rest. ANP's maximal venous dilatory response was noted in the presence of high levels of vasoconstrictors akin to those present in sufferers from congestive cardiac failure (Doorenbos et al. 1991). Ando et al. (1992) reported an increase in capillary filtration with ANP in the forearm. This is not the same effect in the leg as ANP has been reported to decrease lower limb filtration by Watenpaugh et al. (1995). This may perform a protective function to help maintain circulating volume and blood return to the heart. The author was unable to locate any data on the effect of ANP on the compliance of the lower limb venous system. ANP at levels found in the body in the resting state appears to have little effect on the overall venous function. The affects of vasoconstriction are only visible in times of severe cardiac distress.

D) Smoking

Gourgou et al. (2002) carried out a case-control study of 1806 patients with CVI against 1806 controls. The population was gathered from primary care and the results showed a significant association between smoking and the likelihood of developing CVI. The odds ratio was 1.7 if 10-19 cigarettes were smoked per day rising to 2.4 for greater than 20. The San Diego study (Criqui et al. 2007) showed that smoking in the male gender increased the severity of the CVI but no effect was noted in females. The mechanism of action is not clearly understood. Higman et al. (1993, 1996) reported that the response of veins to endothelium-derived relaxing factor in normal and varicose veins was inhibited in heavy smokers. The group also noted a decrease in the nitrous oxide release (a potent venodilator produced in the endothelium of the vein) when smokers' veins were studied. This would suggest that smoking damages the endothelium and therefore reduce endothelium-dependent venous dilatation. No data could be found in the literature by the author that described the effects of smoking on the properties of the lower limb venous system.

E) Family History

Major published studies have reported an increase in the prevalence of varicose veins and chronic venous disease in people who have a family history of the disease (Criqui et al. 2007, Fowkes et al. 2001), but such investigations have suffered from the limitations of self reporting. The relationship was evaluated by Ahti et al. (2009) in a longitudinal Finnish study showing a positive correlation but this was not as strong as they had expected. Where two parents are affected by venous disease, Cornu-Bernand et al. (1994) demonstrated there is a 90% chance of developing varicose veins but only a 20% chance when neither parent was affected. Brinsuk et al. (2003) reported a comparison on the compliance and capacitance of the venous system in monozygotic and dizygotic twins. They found a stronger correlation in

compliance and capacitance between the monozygotic twins than dizygotic twins. The level of evidence for the effect of family history on the venous properties is limited except for that described. Family history clearly has a role to play in the development of venous disease but the studies examining family history have concentrated more on it as a risk factor and not looked at the physiological or genetic predisposition present required for the development of venous disease.

1.9 CONCLUSIONS

The development of venous hypertension is a risk factor for the development of chronic venous disease, and various anthropometric and life style factors can modify the functioning of lower leg veins in such a way as to predispose to this condition. These include height, weight, BMI, age, gender, female hormonal status, pregnancy, calf muscle pump function, levels of physical activity, occupation, diet, bowel habits, insulin status, arterial hypertension, smoking and family history. Their effects in terms of promoting the venous stasis and valve dysfunction that presage CVD are expressed either through adverse changes in venous haemodynamics or by impairment of venous tone and disruption of vein wall structure.

The greater incidence of venous disease in people with high BMI is, on the one hand, related to elevated venous pressure associated with height, supported by the relationship between disease and occupational time spent in the standing posture. On the other hand, venous disease in individuals who are overweight or obese has led to the concept that abdominal adiposity can impede peripheral venous return from the legs by mechanical hindrance, in accord with the disease prevalence in pregnant women. Such a mechanism would be most evident when in the seated position, causing leg vein distension and venous stasis. The conventional means for classification of obesity - BMI - does not adequately describe the

distribution of body fat in the abdominal region, and specific measures of abdominal obesity such as sagittal abdominal diameter and waist-hip ratio are made in the supine and standing positions respectively. To date, only one study (Van Rij et al. 2008) has reported a higher foot venous pressure on sitting in obese than normal weight individuals based on BMI values, but all were patients with symptomatic venous disease and abdominal adiposity was not defined. There are no studies that have examined explicitly the relationship between the presence of abdominal fat and peripheral venous function in otherwise healthy individuals to see if mechanical hindrance occurs and whether it is associated with measurable alterations in leg vein physiology consistent with repeated exposure to impeded flow. Demonstration of any such relationship would help to establish how obesity over time can present a risk for future development of venous disease.

In view of the multiple factors that can impact on leg vein function, it is also necessary to take into account those that are themselves linked to obesity. For example, levels of physical activity or inactivity are related to the overweight condition and can lead to alterations in calf muscle pump function that either impair venous return or increase distensibility. The female reproductive hormones oestrogen and progesterone are venodilators and have been implicated in modifying venous tone and distensibility; oestrogen may also alter vein wall properties through effects on collagen metabolism. Hormonal changes during the normal menstrual cycle, oral contraceptive use and pregnancy (which itself presents weight loading and abdominal obstruction) may therefore play a role in the development of venous disease. Metabolic status and its effect on the venous system remains a field, which has not been fully explored. The factors implicated in the development of arterial disease (hypertension, renin, insulin levels, insulin resistance) have been shown to inhibit the venodilation and constriction

which are required to control venous return and this inability to react may lead to a loss of the natural mechanisms designed to prevent the formation of venous hypertension and disease.

1.10 THESIS AIMS, HYPOTHESES AND OVERALL STUDY DESIGN

This study aimed specifically to examine people without chronic venous disease to see whether leg vein physiology is altered by the presence of abdominal fat in a way that would indicate potential risk for future disease development. It is known that varicose and diseased veins already display dilatation, reduced distensibility and lower compliance due to the loss of elastic wall elements. If signs of similar preliminary changes were to be seen in otherwise healthy but obese individuals during sitting, when mechanical impediment to peripheral venous return is most likely, this would suggest a mechanism that, over time, could progress to overt disease.

Accordingly, the following hypotheses will be tested.

1. Increased abdominal obesity in a healthy middle-aged population causes obstruction to peripheral venous return in the sitting position.

This will be tested by correlation of anthropometric indices of abdominal fat (sagittal abdominal diameter, waist-hip ratio, DEXA scan, ultrasound fat thickness and a new measure of seated abdominal fat overhang) with ultrasound measurements of femoral vein size and flow velocity in supine and sitting positions. It is hypothesised that greater amounts of abdominal fat will be associated with bigger vein size and decreased flow velocity due to distension of the vein by impedance to outflow from the legs.

In order to obtain a quantitative estimate of the degree of mechanical hindrance due to abdominal fat when sitting, a needle manometry technique will be used to record inguinal

tissue pressure. It is hypothesised that bigger amounts of abdominal fat will be associated with higher inguinal pressures and greater obstruction to venous return. A further indication of the extent of outflow obstruction will be gained from durometry of the thigh, a measure of tissue hardness. It is hypothesised that obesity will be associated with greater tissue hardness in the seated position because of compression of the venous microcirculation and fluid filtration into the tissues giving rise to turgor.

2. Increased gynoid (hip, thigh) adiposity in a healthy middle-aged population contributes to obstruction of venous return in the sitting position by compression.

This will be tested by correlation of femoral vein size in supine and sitting positions with anthropometric measures of leg size and composition (circumference, skinfold thicknesses, fat-free cross-sectional area). It is hypothesised that greater vein distension will be observed when seated in individuals with greater amounts of peripheral limb fat.

3. By causing impediment to peripheral venous return, increasing abdominal obesity in a healthy middle-aged population leads to venous hypertension which begins the process of damage to the lower leg vein walls.

This will be tested by correlation of anthropometric indices of abdominal fat with measurements of venous capacity and compliance in the lower leg. In much of the previous work examining lower leg venous physiology, strain gauge plethysmography of the whole limb has been used as a surrogate for venous volume during occlusion protocols to induce vein filling and emptying. This method is not specific to the veins because of fluid filtration into soft tissues during periods of venous occlusion. Direct ultrasound imaging of individual lower leg veins would enable more accurate examination of the

properties of the vein wall. Therefore popliteal and saphenous vein capacity and compliance will be measured using ultrasound in tandem with strain gauge plethysmography for calf venous capacity and compliance. It is hypothesised that, in relation to abdominal obesity, venous capacity will be increased due to loss of elasticity while venous compliance will be reduced.

As indicated above, it is evident from the literature that there are other key factors that impact on either venous haemodynamics or vein wall properties. To take these confounding influences into account, additional data will be obtained for correlation with femoral vein haemodynamics and calf vein capacity and compliance as follows:-

- Increasing exposure to female sex hormones and long-term oral contraceptive use are associated with higher venous distensibility and hence blood levels of oestrogen and progesterone will be measured and self-reported oral contraceptive use recorded.
- To gain insight into the weight burden experienced by individuals over time, a self-reported body weight history will be taken and the number of full-term pregnancies noted.
- Physical activity can increase venous capacity and current levels will be evaluated by a standardised activity recall questionnaire.
- Since insulin is both an arterial and venous dilator agent, raised pressure in the microcirculation combined with an inability to respond with vasomotor tone could exacerbate venous distensibility. The metabolic status of the study population will therefore be estimated as the HOMA2 index based on fasting blood levels of insulin and glucose.

CHAPTER 2:

METHODS

2.1 SUBJECT POPULATION

The study population was healthy male and female (non pregnant) volunteers recruited by advertisements placed in the local press, hospital intranet, local slimming clubs and university newspapers. As participants without overt venous disease were required, the age range of 30-50 years was selected and a duplex ultrasound scan of the femoral vein (FV), the popliteal vein (PV), the great saphenous vein (GSV), the sapheno-femoral junction, the short saphenous vein (SSV) and the sapheno-popliteal junction (SPJ) was carried out to rule out the presence of any evidence of old or new deep vein thrombosis or deep or superficial clinical reflux (defined as greater than 0.5 seconds). This was performed at a screening visit to the Vascular Department, Selly Oak Hospital (Appendix B), where volunteers also underwent an ankle brachial pressure index (ABPI) measure to exclude peripheral arterial disease (PAD). Systolic pressures at the ankle of the dominant leg and the brachial artery were measured using a hand-held Doppler device and standard blood pressure cuff inflated to suprasystolic pressure around the calf and upper arm respectively whilst subjects lay supine. Volunteers were excluded from the study if the ratio of these values lay outside normal limits (0.9-1.1) as defined by Al-Qais et al. (2009).

At the screening visit, a medical history was taken to determine that volunteers fitted the exclusion criteria: deep venous thrombosis (DVT), post thrombotic syndrome, Type 1 diabetes mellitus, pregnancy, heart failure, exercise-limiting arthritis, liver failure, or clinical use of steroids. Those participating would later have blood samples analysed for fasting glucose level to determine any glucose intolerance or Type II diabetes

Subjects of all body size were recruited to include normal weight, overweight, obese and very obese individuals. A questionnaire soliciting recall of weight history from the age of twenty to the time of study (see Appendix C) was completed by all participating subjects. Men and premenopausal women were recruited for the study and women completed a reproductive history questionnaire to assess their habitual menstrual cycle, oral contraceptive use and past pregnancies (see Appendix D). This questionnaire was used to determine the time of all testing procedures for women so that they coincided with the menstrual phase of their cycle, when endogenous and exogenous female sex hormones would be at their lowest levels (Hirshoren et al. 2002). Volunteers were requested to complete the Stanford Seven Day Physical Activity Recall Questionnaire in order to assess their daily energy expenditure (Washburn et al. 2003). (Appendix E)

Volunteers who satisfied inclusion and exclusion criteria for the study received a verbal and written explanation (see Information sheet, Appendix F) of the study aims, and procedures and informed consent was obtained. The study conformed to the Declaration of Helsinki and was approved by the Black Country Research Ethics Committee and the local research ethics committee of the School of Sport and Exercise Sciences.

The aim of the study was to look for associations between the presence of abdominal adiposity and leg vein haemodynamics and a population with a range of body sizes was therefore sought. It was decided to use the BMI categories of normal, overweight and obese for group comparisons since BMI is a universal measure of obesity and would allow for comparison with other published data. Abdominal obesity has been described and will be measured as such in the study but as this variable does not have a recognised scale of categories, correlational analysis with vein function is more appropriate.

Initial power calculations were performed to estimate the number of subjects required for group comparisons of different obesity levels based on BMI, using published data for one of the primary outcome measures, superficial femoral venous flow velocity. Median supine femoral flow velocity at rest in normal healthy individuals has been measured at 13 cm/s with an interquartile range of 9-18 cm/s and standard deviation of 6.7 cm/s (Fromy et al. 1997). A total of 30 participants in each of the three BMI groups would give 80% power to detect a difference of 5.5 cm/s between groups for parametric data. For non-parametric data, 30 in each group would give 80% power to detect a difference of 6 cm/s in between groups. In the sitting position, a median velocity of 3 cm/s, interquartile range 2.7-5 cm/s, standard deviation 1.5 cm/s (Delis et al. 2004), were used to calculate that 30 participants in each group would give 80% power to detect a difference of 1.25 cm/s between groups for parametric data, and 1.35 cm/s between groups for non-parametric data. Calculations were conducted by Dr Peter Nightingale, Wolfson Building, University Hospital Birmingham, Edgbaston, Birmingham, UK. In practice it proved difficult to recruit sufficient numbers of subjects within each BMI class, with consequences for statistical analysis and interpretation that will be considered in the Discussion.

2.2 MEASUREMENTS

Participants were tested on one day at two different locations for a range of measures relating to body composition, and lower limb venous and vascular function. The protocols for testing at these sessions will be described later. Throughout each testing station the mean arterial blood pressure and pulse of the volunteers was checked repeatedly to look for fluctuations, which may affect the blood flow into the limb. The following sections describe procedures for the individual measurements that were made.

2.2.1 Body Composition

A) Anthropometric Measures

BMI and Historical BMI

Subjects' weight (Seca scales) and height (stadiometer) were recorded to calculate body mass index as Kg/m^2 . The volunteers completed a weight history recall questionnaire. They were required to recall their weight at 5 year intervals since the age of twenty and this was then averaged. Using their present measured height their historic BMI was derived from these two figures. (See Appendix C)

Waist-Hip Ratio

Whilst standing, waist (3cm superior to the iliac wing) and hip (at the level of the greater trochanter) circumferences were taken with a hand-held tape measure. The waist-hip ratio was calculated by dividing waist circumference by hip circumference.

Limb Size

Circumferences were also measured at both wrists and ankles. Thigh and calf circumferences were measured 15 cm proximal to the medial femoral condyle and 15cm proximal to the medial malleolus respectively with subjects standing. Each subject's dominant leg was determined by asking which leg would be used to kick a football.

Abdominal Adiposity

Abdominal adiposity was estimated by sagittal-abdominal diameter (SAD) measured using Holtain calipers. With subjects supine, the caliper base was inserted under the small of the back and the caliper arm lowered to rest on the abdominal wall at the level of the umbilicus. The recorded distance in centimetres (SAD) correlates significantly with estimates of the volume of visceral fat (Després, 2007). A key aim of the study was to investigate the impact of abdominal obesity on leg venous function. A novel index to quantify the degree of

impingement of the abdominal wall over the inguinal region was calculated in volunteers in the seated position with their legs straight out in front of them and their back and thighs supported at right angles Figure 2.1. The distance from the sacrum to the most anterior part of the abdominal wall (SA) was measured by hand-held tape measure and divided by the sacrum patella (SP) distance. The index SA/SP was designed as a measure of the obstructive effect of fat in the abdominal/inguinal region when in the seated position.

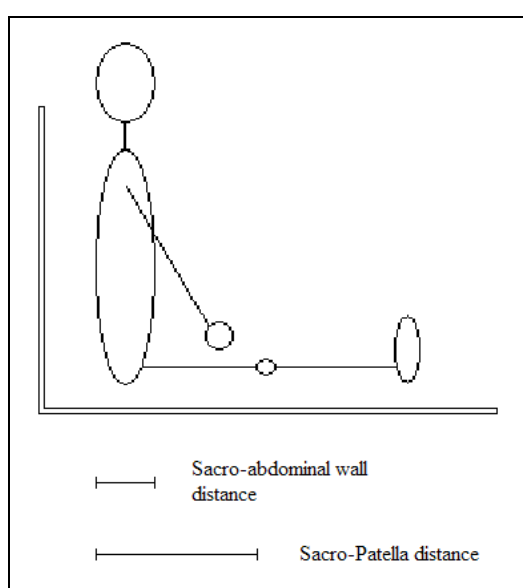


Figure 2.1. Diagram of the measurements made to calculate the SA/SP ratio.

Body Composition by DEXA Scan

Body composition was also determined by Dual Energy X-ray Absorptiometry (DEXA) scan. This is a low energy X-ray giving a radiation dose of 0.15mSv (approximately 0.7% of background one year radiation exposure). The principle of the technique is that two different wave lengths of x-rays are passed into the body and the absorption of the waves is known in different materials. By subtracting out the known absorption patterns of bone, fat or lean tissue it is possible to accurately estimate the percentage of each type of tissue in different

body compartments. The scan takes approximately 7 minutes and is not painful or invasive. The information obtained using previously recorded height and weight allowed for estimation of truncal and limb proportions of fat, lean tissue and bone content as percentages of each individual body section. This percentage truncal adiposity was used for analysis. This technique has been validated by Haderslev et al. (2005) and is effective for measuring soft tissue in obese subjects.

B) Measures of Abdominal and Lower Body Composition by Ultrasound and Skinfold Calipers

Ultrasound Tissue Depth

With subjects lying supine and rested for 10 min, skin and subcutaneous tissue depth were imaged at five sites in B mode ultrasound mode with a Philips EnVisor HD© Ultrasound machine, 3-12 MHz linear probe. Using frozen static images, the in-built automatic calipers were used to measure in centimetres the distance:- 1) at the abdomen from the skin surface to the anterior rectus sheath at a level 3cm below the umbilicus; 2) from the skin surface to the level of deep fascia overlying the muscle groups at 15 cm up from the medial femoral condyle on each thighs; 3) from the skin surface to the level of deep fascia overlying the muscle at 15cm above the medial malleolus in each calf. In all cases care was taken to make sure that there was a gel offset for the probe so that it did not press on the tissues, that the long saphenous vein did not obscure the image, and that the underlying structure was muscle not bone. Readings were taken by one observer in duplicate, and averaged to reduce sampling error.

Skinfold Thickness

Harpender calipers were used at the same sites as described above to record skinfold thickness in centimetres. A pinch-fold of skin was taken between thumb and forefinger and the calipers applied perpendicular to this and allowed to close. All readings were taken by one observer in duplicate and averaged to reduce sampling error.

C) Tissue Hardness Measured by Durometry

Tissue Hardness

To investigate the effect of body composition on skin tissue hardness or 'turgor', a Rex Gauge Durometer© was used (Figure 2.2). This device is used in industry to assess the hardness of materials in production. It has been used to aid the identification of serious skin disease such as systemic sclerosis, Raynaud's and fibrosing syndromes, and durometer measures have been correlated against ultrasound skin thickness measures (Falanga et al. 1993). In venous disease, the technique has been correlated against the venous skin severity score and found to have a direct linear relationship (Romanelli et al. 1995).

With subjects in the supine position with knees bent to 20° and legs externally rotated at the hips allowing the lower limbs to fall laterally, the probe was placed in the positions described in the above sections (abdomen, both thighs and calves) and the sites marked on the skin surface to enable repeated measurement (avoiding underlying bone). The dominant limb was the limb chosen for measurement. The durometer was placed on the skin surface at 90° to the skin and the under its own weight (400g) allowed to rest and give an analogue reading of hardness scaled 0-100 (Figure 2.3). No pressure was applied to the probe and the highest reading was recorded. Skin creep has been noted in previous studies suggesting that interstitial fluid is moved out of tissue by probe pressure. Repeat measures at each site were

separated by 20 seconds to allow the tissue to return to its resting state. Subjects were then moved into the seated position as described for the measurement of SA/SP index (see Figure 2.1) for 5 minutes and repeat measurements were made at thighs and calves (no abdominal reading was possible). All readings were taken twice and averaged by one observer to reduce sampling error for further analysis.



Figure 2.2. A 1600 Rex Gauge Durometer©

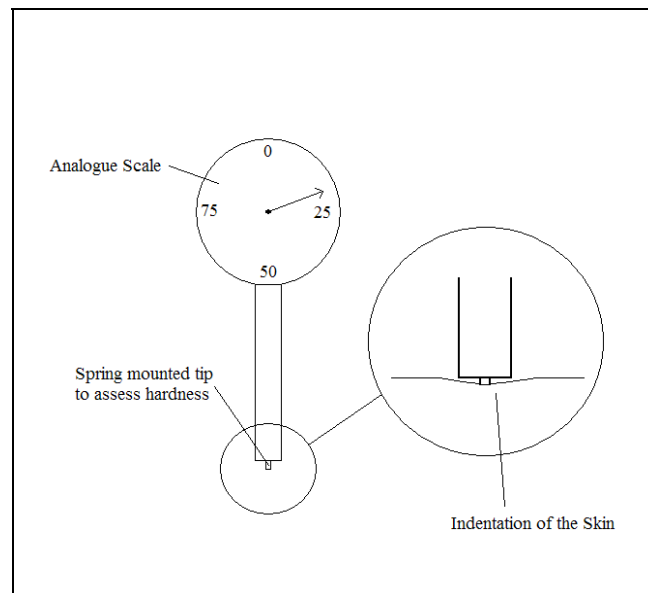


Figure 2.3. Representation of the Durometer probe and the indentation of the spring mounted probe on the skin.

D) Effect of Body Composition on Inguinal Tissue Pressure

Subjects lay supine on a mechanical bed and rested for 20 minutes. A pressure transducer line was prepared and attached to a Hewlett Packard ProPac© recorder. An EnVisor HD ultrasound machine in B mode was used to image the SFV, the GSV and the SFJ. In the groin skin crease in line with the SFJ, 10mls of 1% lignocaine was introduced and given 5 minutes to take effect. Under direct ultrasound guidance a 14 gauge Optiva® 2 cannula was passed through the skin to rest medial to the SFV and superior to the SFJ. This was aspirated to check it was not in a venous structure (Figure 2.4). The metal trochar was removed to leave the plastic cannula. This was attached to the prepared transducer line and flushed for 1 second with saline 0.9%. The transducer was placed at the level of the mid thigh and the system was zeroed. The subjects rested for two minutes and on each of the next 2 minutes a baseline pressure reading was taken and recorded in mmHg. The mechanical bed was then used to move the subject from the supine to seated position, with trunk at 90° to legs straight out, requiring the individual to make no effort. Once in position, the line was flushed again for 1 second to check it was not kinked and readings were taken at the end of each subsequent minute for a further 5 min to record tissue pressure in mmHg. Subjects were then returned to the supine position using the mechanical bed and the line was flushed and readings taken at the end of a further two minutes of supine rest (total of 9 readings). The process was repeated four more times, giving 5 sets of readings of resting, maximum seated and difference in moving from supine to seated pressure (mmHg) in the inguinal region. Pressure was applied to the region upon removal of the cannula to arrest any bleeding post-procedure. The procedure was carried out by one observer trained in vascular surgery and duplex ultrasound imaging of the groin.

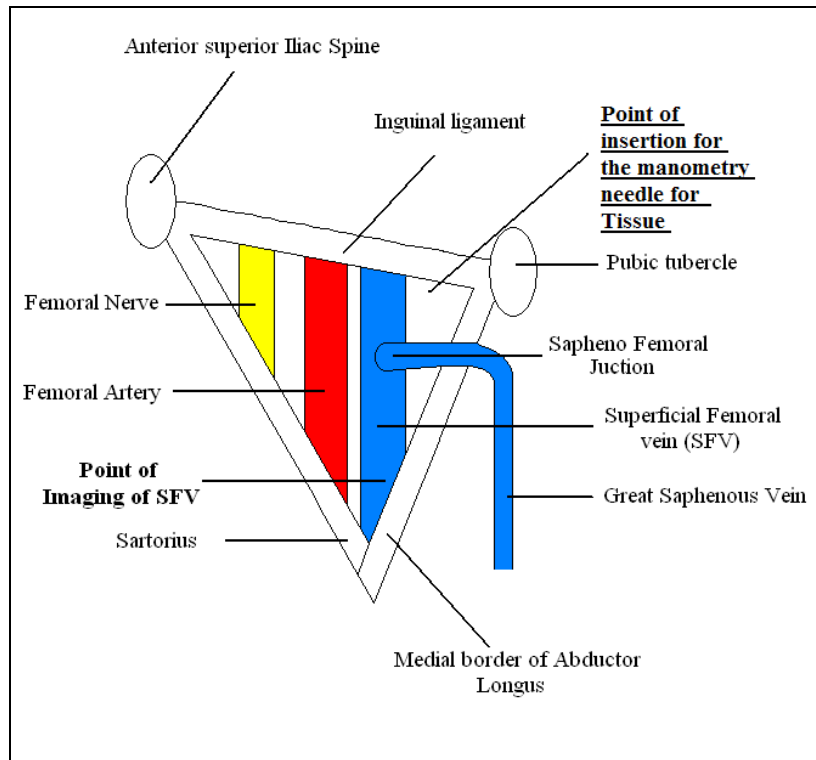


Figure 2.4. Positioning of the plastic cannula for the measurement of inguinal tissue pressure.

In order to evaluate the reproducibility of tissue pressure measurements with this method, the typical within-subject error was calculated from the readings for the 5 repeat trials for each subject as the group mean standard deviation expressed as a % of the group mean, i.e. coefficient of variation (Hopkins, 2000). This was done separately for the supine and seated repeated readings. The minute values (2 supine and 5 seated) were averaged for the normal BMI group and displayed graphically as pressure against time (see Results figure 3.5). This demonstrated a step increase in pressure over all volunteers from supine to the seated position. For each individual, the change in pressure on sitting was calculated as the difference between the mean supine and the mean seated values after averaging their data for all 5 trials. These figures were used for further analysis.

2.2.2 Measures of Venous Function

A) Clinical Venous Status

Subjects were classified for any visible signs of lower extremity venous dysfunction or disease by the Clinical Etiologic Anatomic Pathophysiologic (CEAP) scoring system as described by Porter & Moneta, (1995) see Table 1.1 & 1.2.

B) Venous Refill Time

Photoplethysmography (PPG) is a non-invasive test that measures the time taken for the venous system to refill following activation of the veno-muscular pump. This technique has been shown to correlate well with ambulatory venous pressure by Abramowitz et al. (1979) and has been used successfully by Fronck et al. (2000) to study venous valvular dysfunction. More recently the technique has been questioned, but Sam et al. (2006) showed that it is reproducible for superficial venous disease. It involves the use of infrared light passing into the tissue. The different absorption spectrum of tissue with blood in and that without allows for the time taken for the leg to refill with blood after emptying to be calculated. (Figure 2.5)

With volunteers in a seated position with feet flat on the floor, a PPG probe connected to a Huntleigh Vascular Assist Device© was attached at 10cm above the medial malleolus on the dominant limb. A base line reading was achieved and the patients were asked to perform ten dorsiflexions of the foot with the heel placed on the ground, followed by 45 seconds of rest. The time taken for the PPG reading to return to base line was taken. Readings greater than 25seconds were taken as normal, readings 15-25 seconds as signs of venous impairment and < 15 seconds are taken as diagnostic of venous insufficiency (Figure 1.3). This test was used as a screening tool and was not used for further analysis.

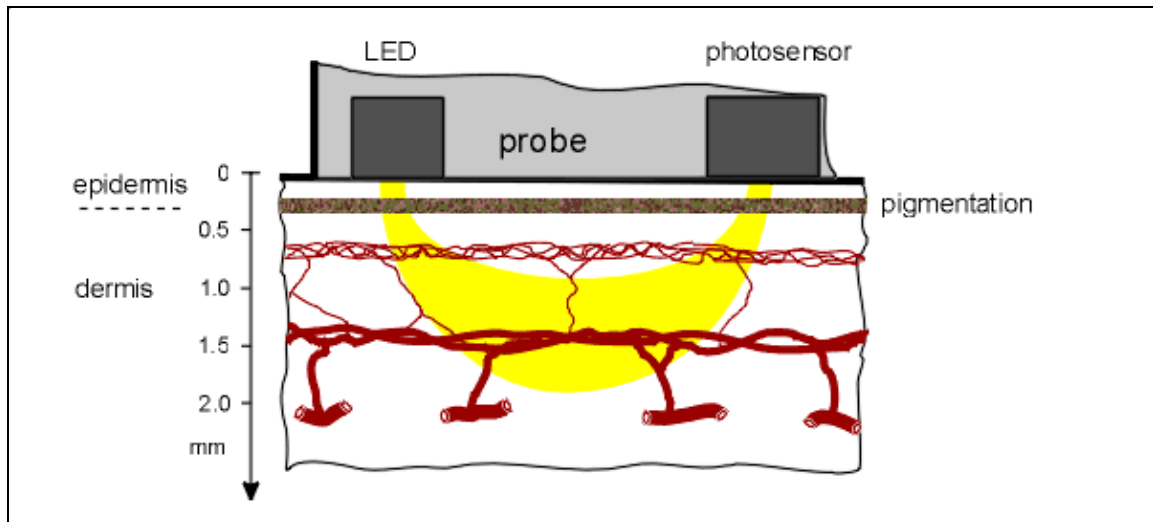


Figure 2.5. Photoplethysmograph sensor. LED emitting infrared light with photosensor to detect changes in spectral signal

C) Diameter, Velocity and Flow in the Femoral Vein

The study employed ultrasound technology to image the femoral vein and measure vein diameters using the Envisor HD© in-built calipers. The measurement of venous flow velocity was carried out using pulse wave mode using duplex ultrasound which is based on the Doppler effect (Figure 2.6 & 2.7). The sound reflected from a moving structure shows variation in frequency called the Doppler effect. The shift in frequency can be converted into an audible frequency which forms the principle of the Doppler probe. The probe produces a high frequency sound wave normally 4 or 8MHz that is passed through the tissue, the frequency of the returning waves being detected by the probe. When measuring flow of blood in a vessel it is possible to calculate the speed of movement by the formula:

$$\text{Frequency Shift} = \frac{2F_i \times V \times \cos\Theta}{c}$$

(As c , the speed of sound in tissues, and F_i the incident frequency of sound, are constant and if Θ , the Doppler angle is kept constant, the frequency shift depends directly on the blood flow velocity V) (Armstrong & Wastie, 1998).

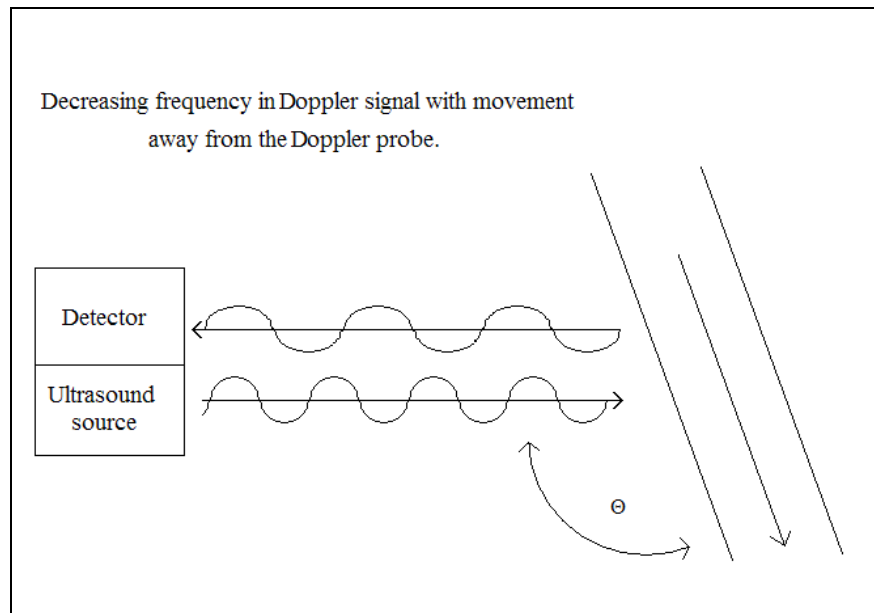


Figure 2.6. The effect of fluid moving away from the Doppler probe on the frequency of the returning ultrasonic signal.

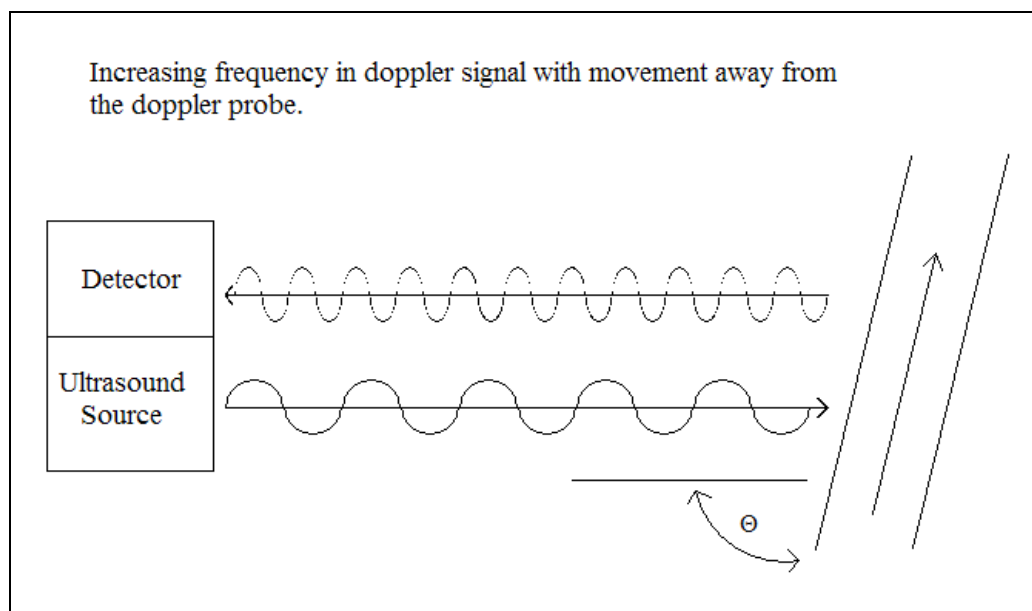


Figure 2.7. The effect of fluid moving towards the Doppler probe on the frequency of the returning ultrasonic signal.

Diameter and flow velocity were measured in the Femoral Vein (FV) of subjects' dominant leg in the supine and seated position. The FV was imaged at the lower end of the femoral triangle where it lies next to and not behind the superficial femoral artery (Figure 2.8). This position was marked and, in B mode ultrasound longitudinal plane, the intra luminal diameter (cm) of the FV was measured with the in-built caliper, readings being taken twice to reduce sampling error. The mode was then changed to spectral analysis and a standard 3-lead ECG signal was input to the Envisor HD© to produce a cardiac trace. The mean systolic velocity, time averaged velocity mean, peak systolic velocity and the diastolic velocity were recorded from the spectral trace all in cm/s. These supine resting readings were averaged over 15 cardiac cycles as denoted by the ECG trace and repeated twice to compensate for any variations in cardiac and respiratory cycles. Subjects were advised to maintain normal respiratory rhythm and avoid gasping or taking deep breaths during recording. The calculation of flow was performed by using the mean of the two readings of FV diameter and the mean of the two readings of the FV time averaged mean velocity. The cross sectional area (CSA) of the FV was calculated and multiplied by the FV time average mean velocity to calculate the total FV flow. This was the result used for further analysis.

A cuff attached to a Hokanson Rapid Inflation system was then placed around the dominant calf with the foot supported to remove pressure from the calf tissue. The cuff was inflated to 80 mmHg for approximately 5 sec. to squeeze the calf and, in spectral mode, the peak systolic flow velocity in the FV was recorded. This was repeated three times after a 45 second pause to allow for venous refilling in between inflations, and the average of the three readings was taken to represent the peak FV flow velocity. Peak FV flow was then calculated by multiplying FV CSA and FV peak flow velocity.

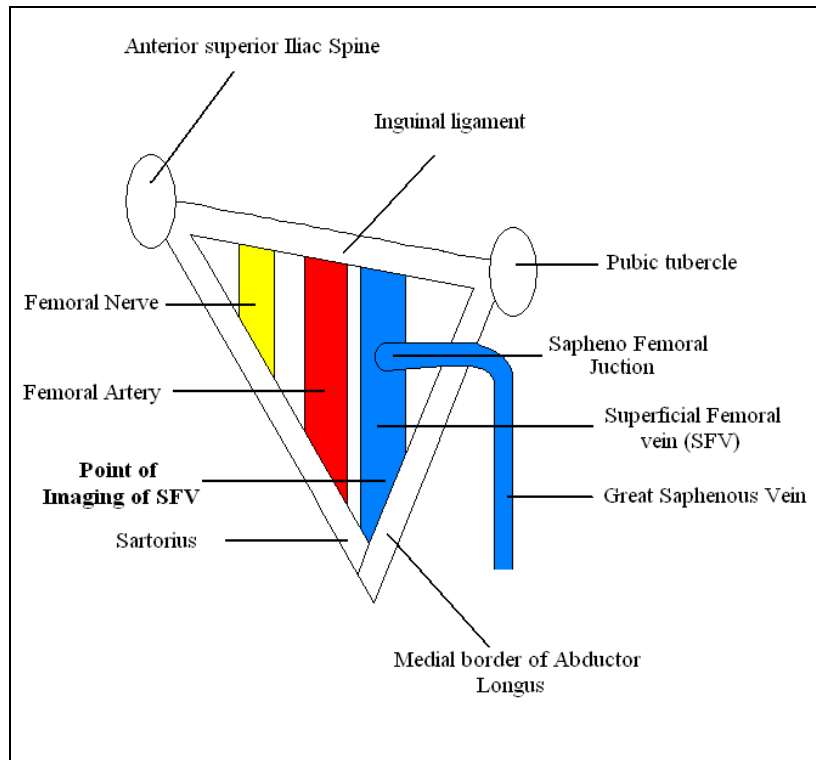


Figure 2.8. The position of the major blood vessels and nerves in the Femoral Triangle for measurement of Femoral Vein cross-sectional area and Femoral Vein flow velocity.

The same sequence of measurements and calculations as described above of FV diameter, FV flow velocity, FV flow, peak FV flow velocity and peak femoral vein flow during calf compression was repeated with the subjects placed in the seated position with the trunk and legs at 90° with legs out stretched. (Figure 2.9)

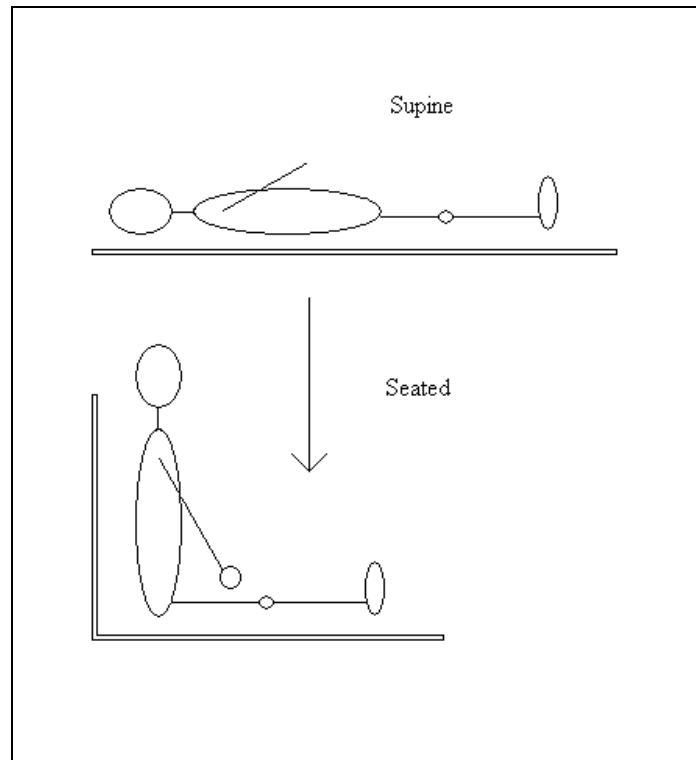


Figure 2.9. Change in position for the measurement of Femoral Vein flow velocity.

2.2.3 Venous Occlusion Plethysmography

Venous occlusion plethysmography applied to the lower leg can provide information on 1) rate of arterial inflow, 2) venous vascular volume or capacity, taken as the magnitude of increase in limb volume after rapid filling is complete, 3) microvascular fluid filtration, as the rate of slow volume increase once filling is complete and (Wilkinson & Webb, 2001) 4) venous compliance, taken from the volume-pressure relationship of the calf during emptying once the occluding cuff is released as described by Halliwill et al. (1999) & Meendering et al. (2005) further explained in section 2.2.3D.

The underlying principle of venous occlusion plethysmography (VOP) is simple: when venous drainage is interrupted, arterial inflow is unaltered. Blood can enter but not leave the limb past the inflated cuff. Volume rises in a linear fashion until the potential space in the

venous volume has been filled. The increase can be measured as percentage change in limb volume and be used to calculate the blood flow into the limb. Although this technique was developed for the forearm it has been used successfully on the lower limb. (Hewlett and van Zwaluwenberg, 1909, Wilkins & Bradley, 1946, Wilkinson & Webb, 2001)

A) Calf Blood Flow

Subjects rested supine on an examination couch, with feet and calves elevated to aid drainage (i.e. above or at the level of the heart) (Figure 2.10) Both feet were secured using an evacuated bean bag pad.

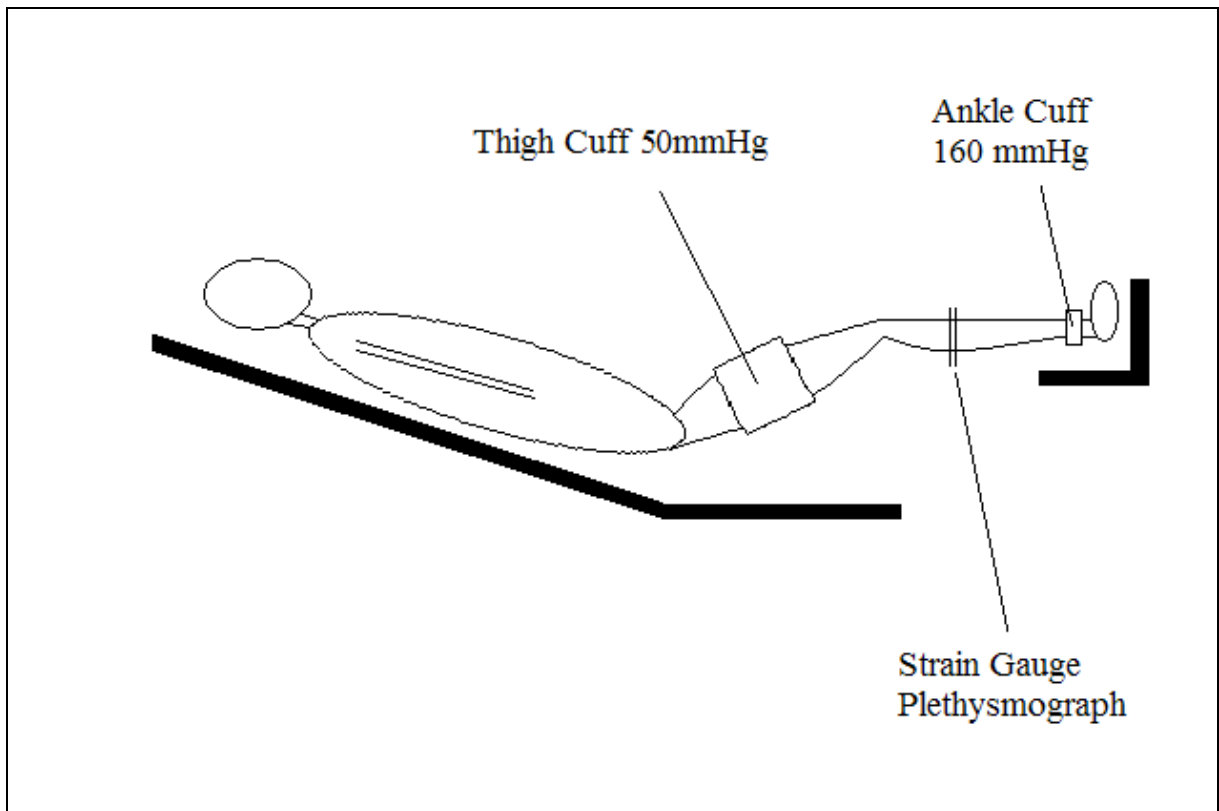


Figure 2.10 Positioning for lower limb occlusion plethysmography with the limbs elevated.

Ankle and thigh cuffs are shown in place.

Cuffs were applied to both ankles to exclude pedal circulation (inflated to 160mmHg prior to all venous occlusions). Cuffs were applied around both thighs and attached to a Hokanson Rapid Inflation system that provided simultaneous inflation to 50 mmHg. Bilateral calf maximal circumferences were measured and strain gauges of 2 cm smaller were selected and fixed around the both calf maximal circumferences and connected to Hokanson Plethysmographs from which the outputs were routed via a Powerlab module (AD Instruments) to a computer running Chart v5.2.2 for Windows© (Figure 2.11).

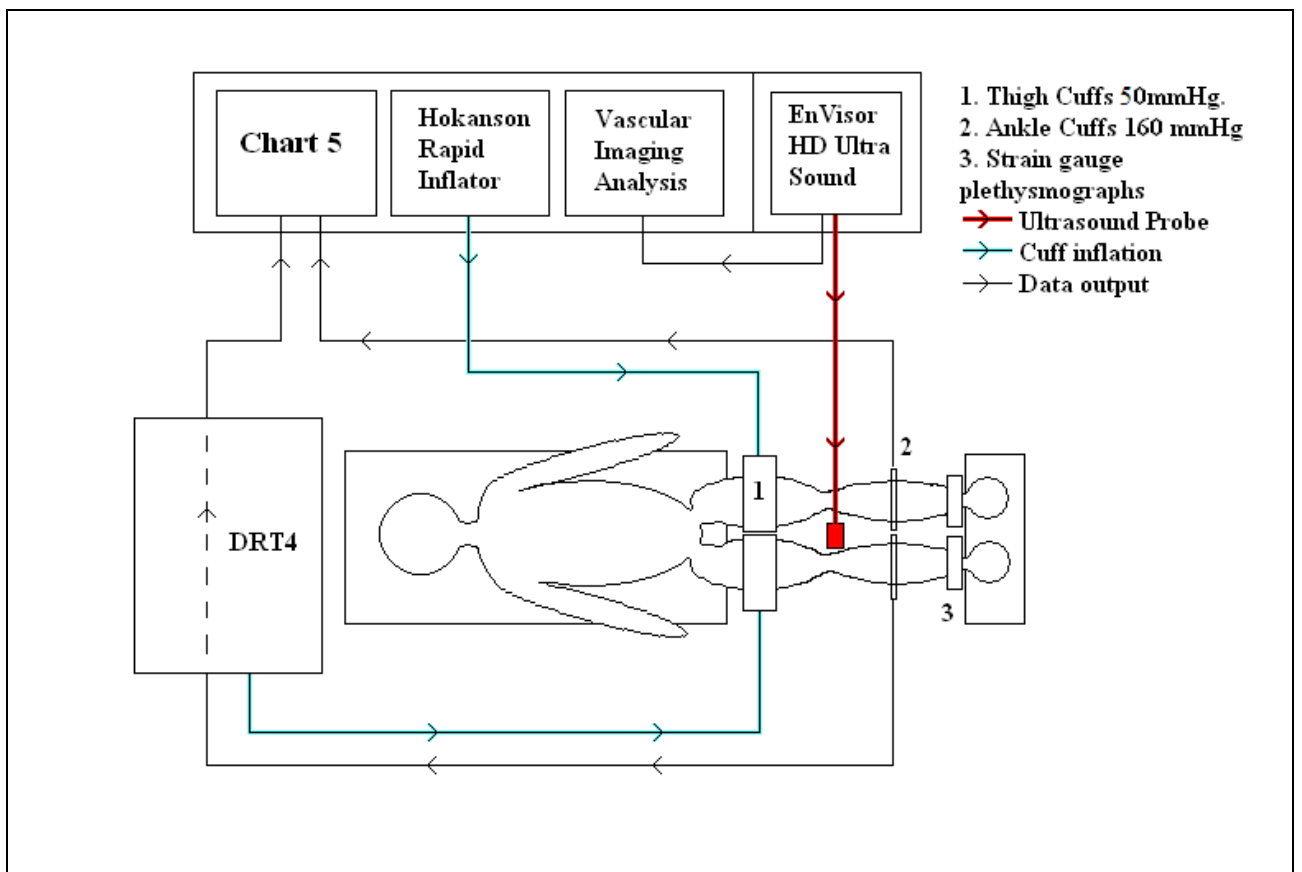


Figure 2.11. Equipment and volunteer placement for the evaluation of whole limb and Great Saphenous Vein distension and controlled deflation during lower limb occlusion plethysmography.

The limbs were supported by elasticated straps at knee level to reduce the requirement for muscle activity to support the limbs. Resting blood flow measurements were obtained by inflating the thigh cuffs to 50 mmHg for 10 seconds three separate times. Arterial inflow was measured as the rate of increase in limb volume against time (ml/100ml/min) (figure 2.12). The average of the three readings was used for analysis as this is standard practice given the reproducibility of calf plethysmographic flow values (8 – 24%, Thijssen et al 2005).

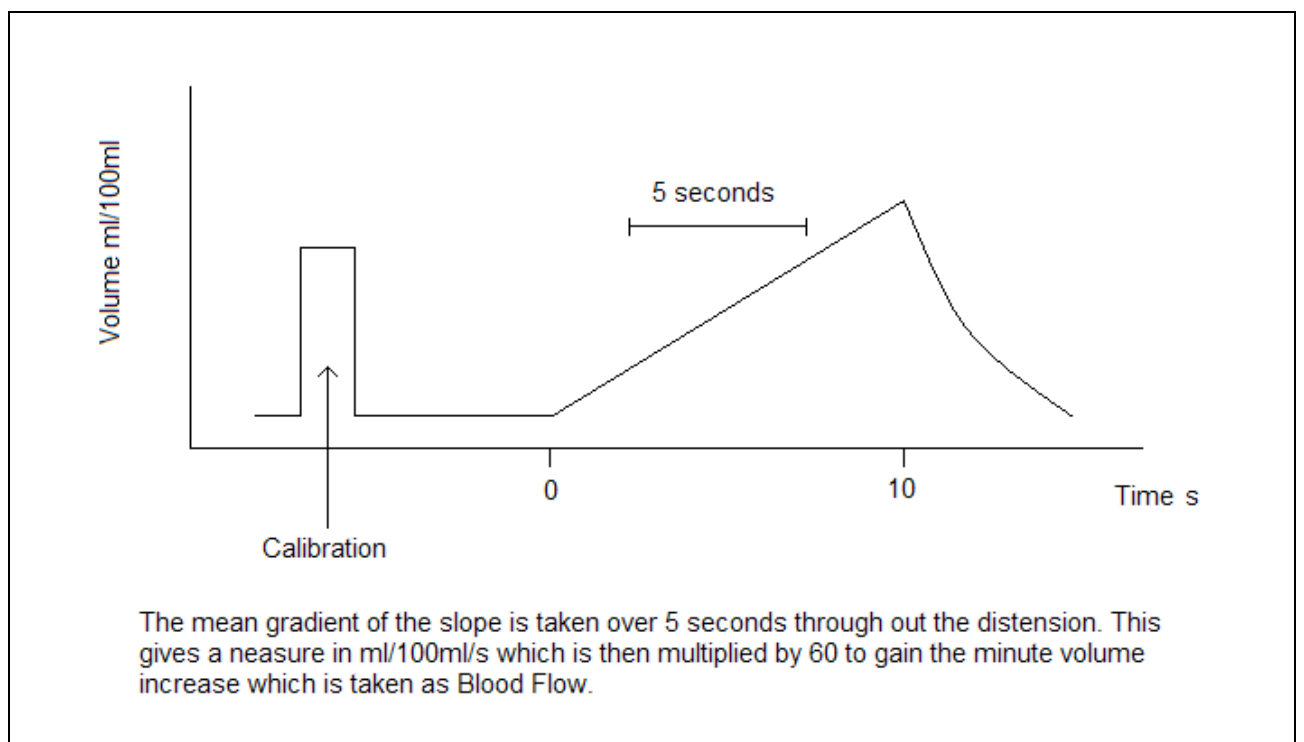


Figure 2.12. Example of a blood flow trace produced with 10seconds of occlusion plethysmography.

B) Venous Capacity

Calf venous capacity is determined from the increase in limb volume during venous occlusion that is maintained for several minutes, long enough to allow for vascular filling but excluding fluid filtration (Lindenberger & Länne, 2007) Although the rapid vascular filling phase when venous outflow is occluded will occur at different rates depending on arterial inflow and the time it takes for pressures to equilibrate throughout the venous circulation, it is accepted that filling is mostly completed within 2 min. Beyond this time, the increase in limb volume includes a component due to fluid filtration out into the tissues as venous valves become incompetent above a back pressure of 40 mmHg (Brown et al. 1966) and capillary hydrostatic pressure equates to occluding cuff pressure (Mahy et al. 1995). In the same situation as described above (Figure 2.10), the cuff around the dominant thigh only was inflated by the pressure system of a DRT4 laser Doppler flux monitor (Moor Instruments) which allowed for both rapid inflation and a controlled rate of deflation. Inflation was to a pressure of 50 mmHg over five seconds and was maintained for 5 min (300 sec). Deflation was set to occur at a rate of 1 mmHg per second to enable calculation of the whole limb compliance by the method described Halliwell et al. (1999) and Meendering et al. (2005) (see later 2.2.3D). The signal from the strain gauge around the dominant calf was recorded both to the computer running Chart via the Powerlab©, and to the DRT4© running Moorsoft© analysis software, for the determination of whole limb venous capacity and calculation of microvascular fluid filtration as described below.

During venous distension, the change in limb volume recorded by the strain gauge was averaged in 10 sec. bins using the chart software. The volume increase of the limb follows the course of a rapid phase (venous filling) and slow phase (fluid filtration) (Figure 2.13).

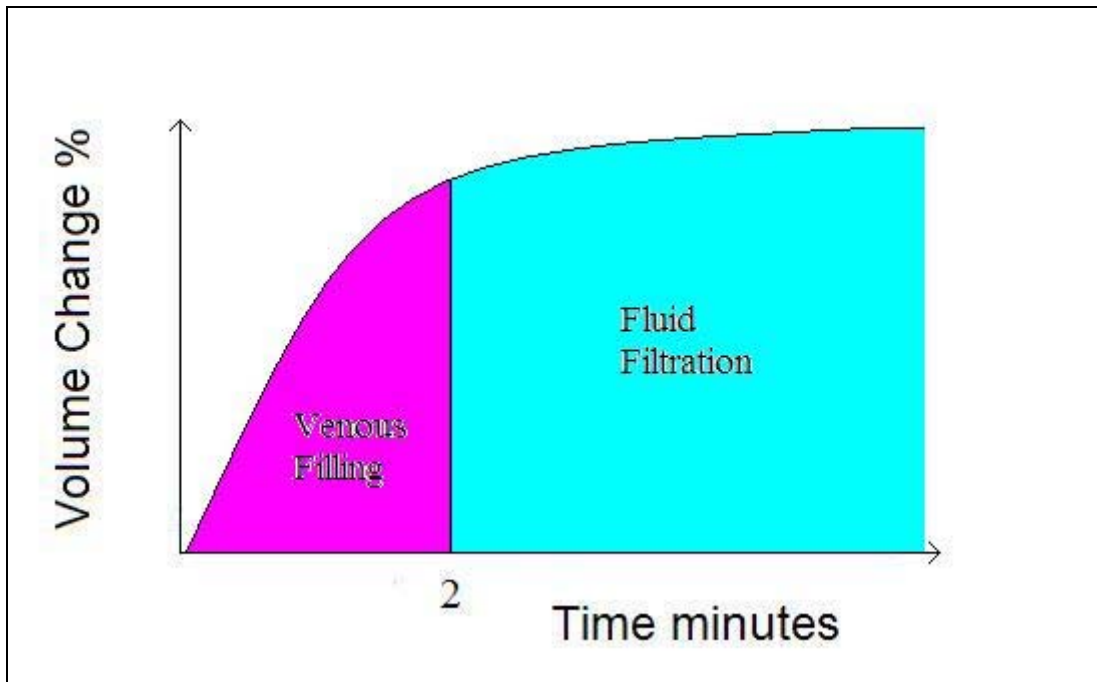


Figure 2.13. Occlusion Plethysmography causes swelling of the limb in two phases. Initial venous filling takes approximately 2 minutes. Fluid filtration occurs once the venous capacity has been exhausted and fluid is forced to move out of the venous system into the tissues.

Venous capacity (ml/100ml) was taken as the volume increase at two minutes after onset of cuff inflation. This coincides with the inflection of the curve from rapid to slow change and allows sufficient time for filling of the venous vascular volume as described by Gamble et al. (1993) and Olsen & Länne, (1998). The maximal limb capacity was also recorded at the end of the five minute inflation period. These were the two results used for analysis.

C) Microvascular Filtration Capacity

Limb volume changes were recorded during cuff inflation to 50 mmHg for 5 min on AD Instruments Chart v5.2.2 for Windows© as described above. In order to determine the capillary filtration (Kf) that occurred during the occlusion period the trace was analysed by taking 10 second averages throughout the distension. Having allowed for venous filling to

occur over the first two minutes of the distension (Figure 2.13), the following 120 seconds or 12 average readings were analysed. The difference between the volume at 4 minutes and 2 minutes was calculated and divided by 2 to give a minute value. This value was then divided by the venous occlusion pressure (50 mmHg) to give the rate of volume change per unit of pressure for this distension or the capillary filtration rate (Kf).

There are, however, problems that have been identified with this method. Single large (>25 mmHg) steps in cuff pressure have been shown to decrease arterial inflow to the limbs over a period of a cuff inflation as shown by Gamble et al. (1998), by activation of a veno-arteriolar local response thought to be stimulated by stretch receptors in the distended veins and vessels, even at sub-diastolic pressures (Henriksen et al. 1991). Small stepwise increases in venous occlusion pressure have been used to avoid this venoarteriolar response (Christ et al. 2000, Gamble et al. 2002) (Figure 2.14). In this study, in order to avoid activation of the veno-arteriolar response and potential consequences for estimation of filtration, a stepwise incremental venous occlusion protocol was used, increasing the occlusion pressure by 10 mmHg at a time. For each step a period of venous filling occurred followed by a period of capillary filtration recorded by strain gauge plethysmography.

For this protocol, an electromagnetic strain gauge around the calf was used instead of the mercury-in-silastic gauge and the thigh cuff inflation was driven by the in-built software of a Filtrass machine. The use of this type of strain gauge has been validated against the mercury-in-silastic gauge by Christ et al. (2000) and shown to yield comparable values. A protocol of six four minute inflations in 10 mmHg steps up to 60 mmHg was initiated taking a total of 24 minutes (Figure 2.14).

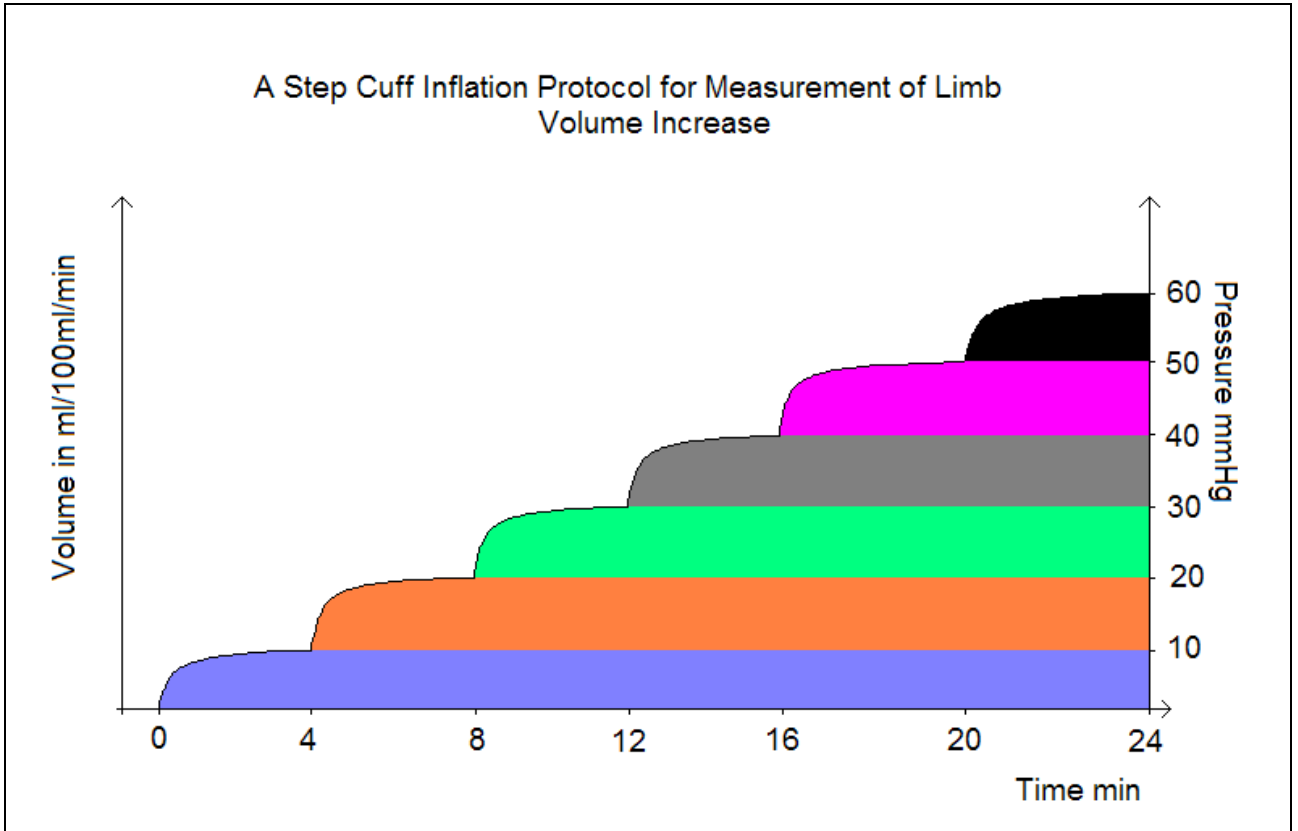


Figure 2.14. A multi step cuff inflation protocol of venous occlusion plethysmography using the Filtrass© Machine.

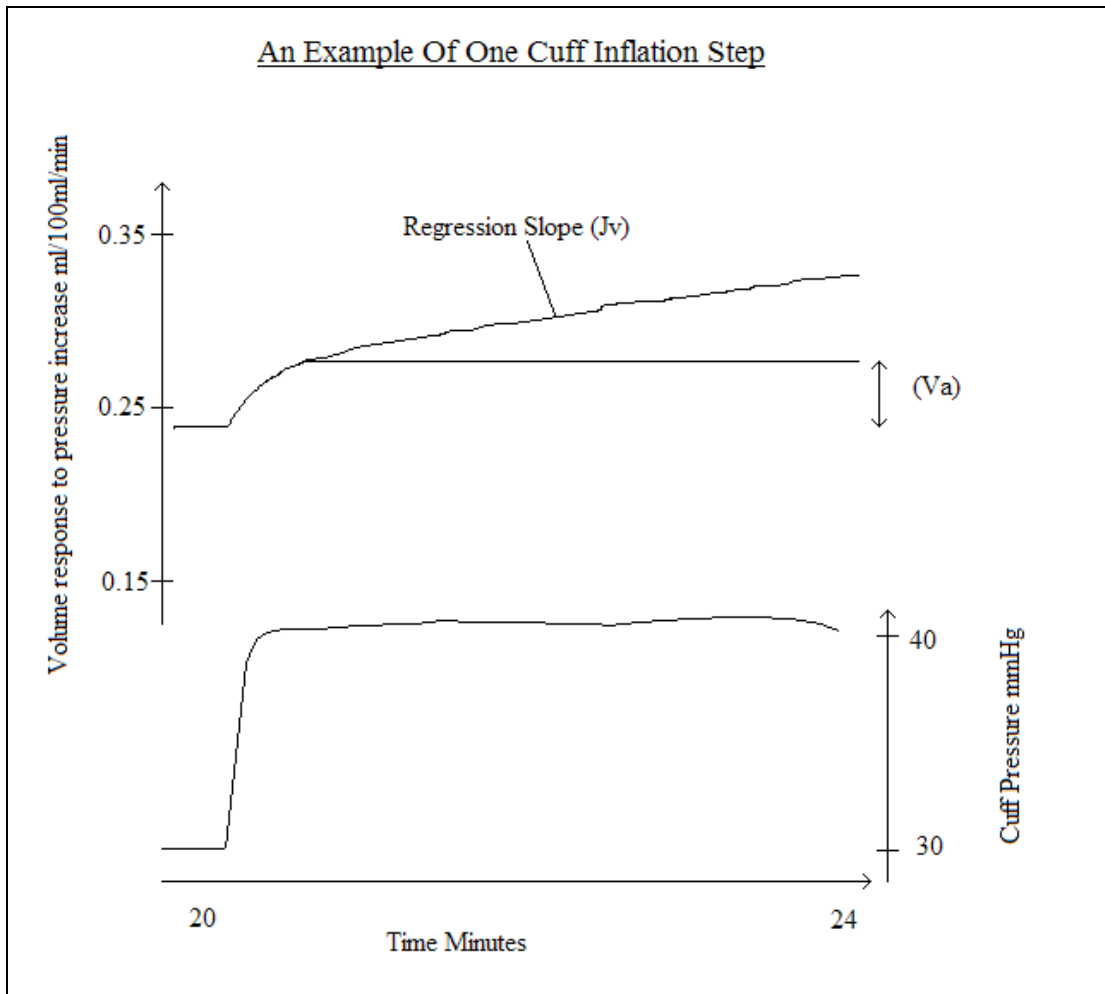


Figure 2.15. An example of the limb volume response to one of the small pressure increments of 10 mmHg.

The filtration slope at each increment of pressure or J_v (Figure 2.15) is plotted against thigh cuff pressure. The derivation of this slope provides the microvascular fluid filtration value, K_f using this technique. The variables of P_{vi} (mmHg) and P_{vest} were calculated by the filtrass inbuilt software using the same regression slope (Figure 2.16).

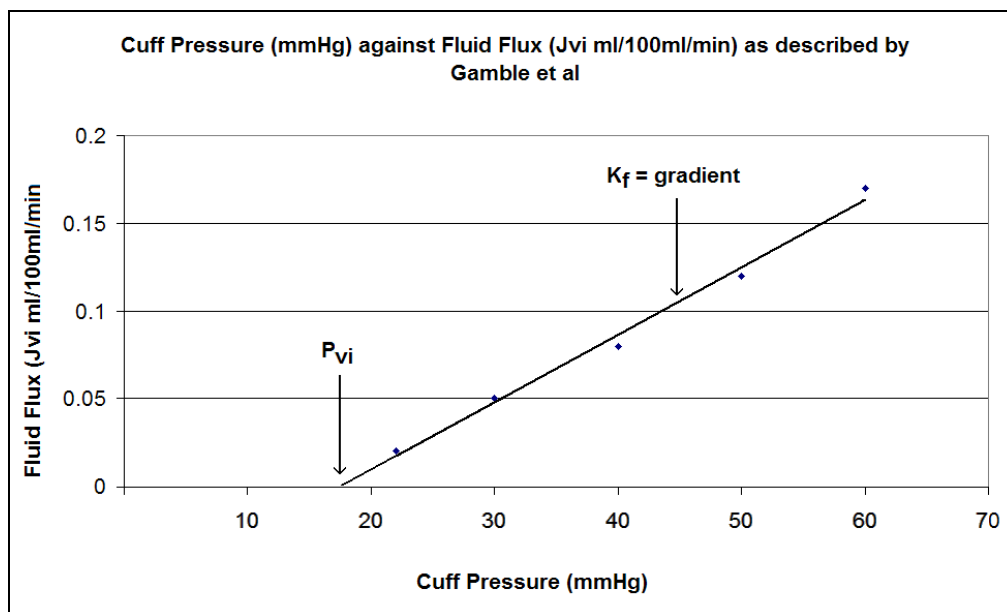


Figure 2.16. Relationship of J_v to Cuff pressure P_v . Microvascular filtration is the slope of the linear regression. P_{vi} is the venous pressure required for fluid filtration to occur.

Reading	Explanation
P_{vest}	The venous pressure in the limb which has been shown to be equivalent to the pressure in the limb cuff
V_a	Once the cuff is inflated there is a rapid period of venous filling due to arterial inflow. This is measured in ml/100ml/min.
J_v	Once venous filling is complete for each pressure step, filtration continues to occur. The gradient of this slope is defined as J_v in ml/100ml/min
K_f	Regression analysis of J_v in ml/100ml/min against cuff pressure mmHg gives K_f or the filtration coefficient
P_{vi}	The cuff pressure that must be applied before the filtration will occur in the limb. This is called the isovolumetric venous pressure. This is calculated by regression analysis of the Filtration Coefficient K_f against cuff pressure and is the point of intercept.

Table 2.1. The results that can be measured and calculated from a stepwise inflation protocol of venous occlusion plethysmography of the limb.

D) Whole Limb Compliance

In order to calculate whole limb compliance, the volume – pressure relationship for the decrease in limb volume during controlled cuff deflation at 1 mmHg/sec was constructed using the limb volume signal recorded to the DRT4 computer. The DRT4 Moorsoft programme sampled data from the strain gauges at 10 Hz. The advantage of this machine was that it allowed for an accurate pressure trace alongside the volume changes. During deflation this gave the protocol required for calculation of compliance described below. The data was transferred to Microsoft Excel for analysis (Figure 2.17).

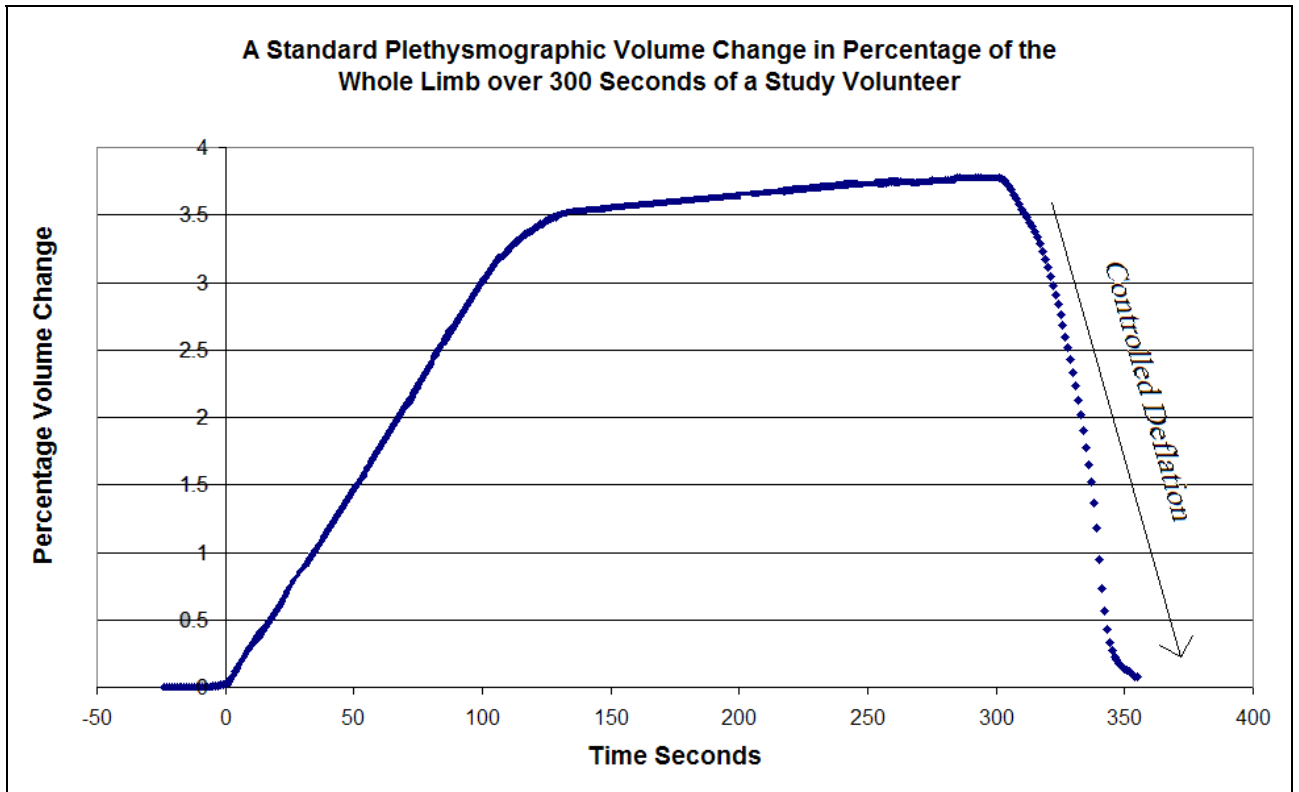


Figure 2.17 A standard plethysmographic trace showing controlled deflation.

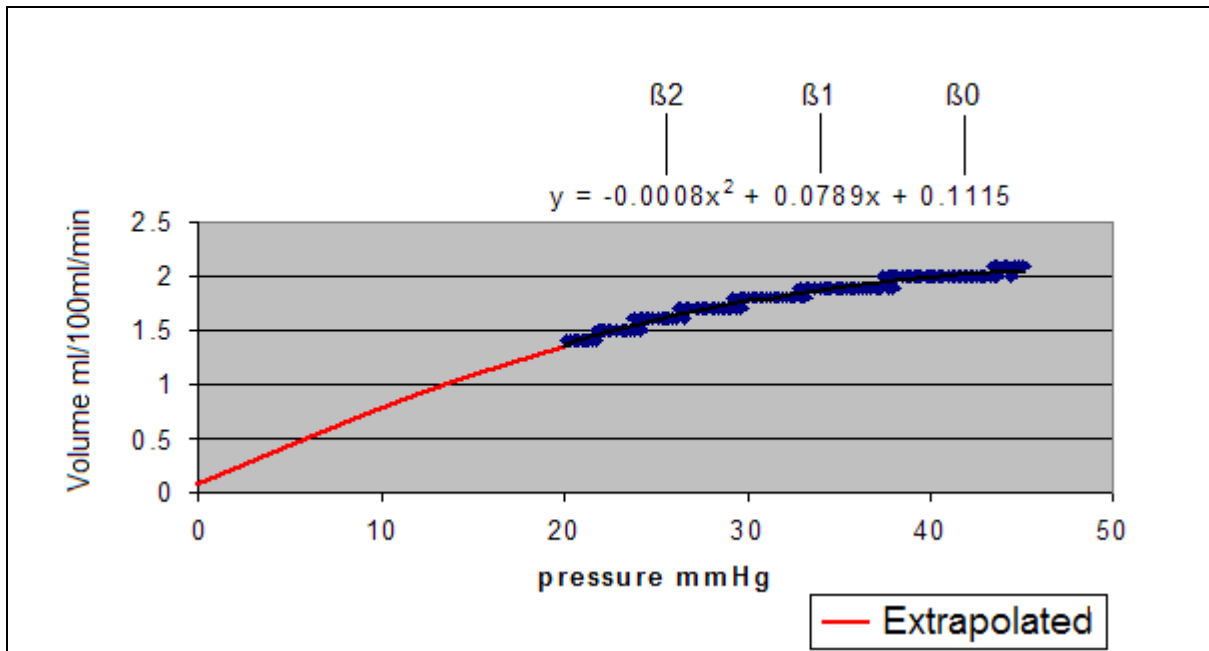


Figure 2.18 Polynomial regression analysis of deflation protocol. The β_0 , β_1 and β_2 constants were extracted and used in the calculation of whole limb and venous compliance.

Each individual's deflation pressure-volume curve (Figure 2.18) was then fitted by polynomial regression analysis using Excel such that the quadratic equation - change in limb volume = $\beta_0 + \beta_1 \times (\text{cuff pressure}) + \beta_2 \times (\text{cuff pressure})^2$ - described the relationship. β_0 reflects the volume of the limb with no pressure in the cuff (Y axis intercept on the graphical representation). β_0 is highly variable and dependent on venous tone, arterial inflow, fluid filtration and hysteresis effect of venous emptying and is therefore difficult to interpret. β_1 and β_2 reflect the slope of the curve at different pressures. Therefore, as described by Halliwill et al. (1999), Meendering et al. (2005), limb compliance can be calculated at each pressure using the formula $\text{compliance} = \beta_1 + 2 \cdot \beta_2 \cdot (\text{cuff pressure})$, and plotted against pressure to give a compliance / pressure slope. In other words, compliance was defined as the derivative of the pressure-volume curve. The slope of this line measures the rate of change of compliance in an individual and it is this rate of change in venous compliance over a range of pressure which

can then be used for comparison between individuals (Figure 2.19) and this is the figure used for analysis in the Results section.

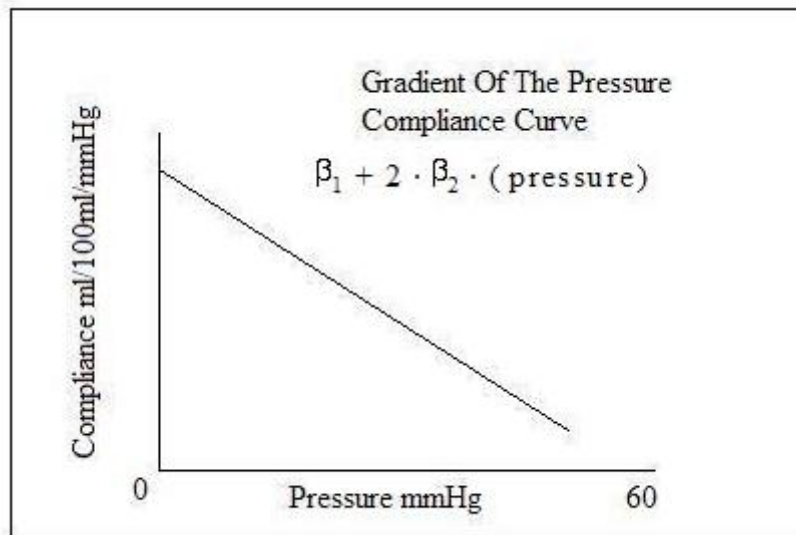


Figure 2.19. Compliance pressure curve using the β_1 and β_2 constants derived by quadratic analysis of distension. Here the compliance of the calf or vein decreases with increasing venous pressure.

In total the limb underwent three periods of cuff inflation and deflation for the measurement of compliance. The first cycle provided data from a control period of venous distension and compliance of the whole limb was derived during cuff deflation. The second and third venous distensions were used to image the Great Saphenous Vein and Popliteal Vein respectively and derive their compliance during cuff deflation. Between each period, 10 minutes of leg elevation at rest was to allow a chance for venous emptying and restoration of normal limb volume.

2.2.4 Great Saphenous Vein and Popliteal Vein diameter and compliance measured by ultrasound imaging

During the 2nd and 3rd period of venous distension, a 3-11 MHz linear probe attached to the ultrasound (Enviosior©) machine was used to image the GSV and the popliteal respectively. The GSV was marked with the patient standing 5 cm above the medial femoral condyle prior to positioning the patient in the leg elevated position. The vein was imaged in longitudinal section in B mode ultrasound. The video signal was routed from the ultrasound machine into a PC running Vascular Imaging Analysis (VIA) Software©. Originally designed by Newey & Nassiri, (2002) to measure arterial diameter during flow mediated dilation (Sidhu et al. 2002), this detects the interface between two different echogenic substances. VIA software was written together with the virtual US scanner using National Instruments LabVIEW and IMAQ (Austin, Texas, USA) and uses a neural network to interpret the samples taken and ascertain the position of the blood / vessel wall boundary. By calculating the difference between the anterior and posterior wall boundaries the vessel diameter can be calculated, see figure 2.20. Initially the operator is required to interpret the image on the computer display and define the region of interest (ROI) for the analysis software. Once the wall edge is detected the software carries out an analysis of its position, sampling at 25 frames per second. The data is stored online as a continuous read out of the vessel diameter throughout the distension. For analysis of data, a macro program running in Excel was created to reduce the output by averaging diameters first for each second and then for each 10 sec. This data could then be displayed against time to give a graphical representation of change in vein diameter.

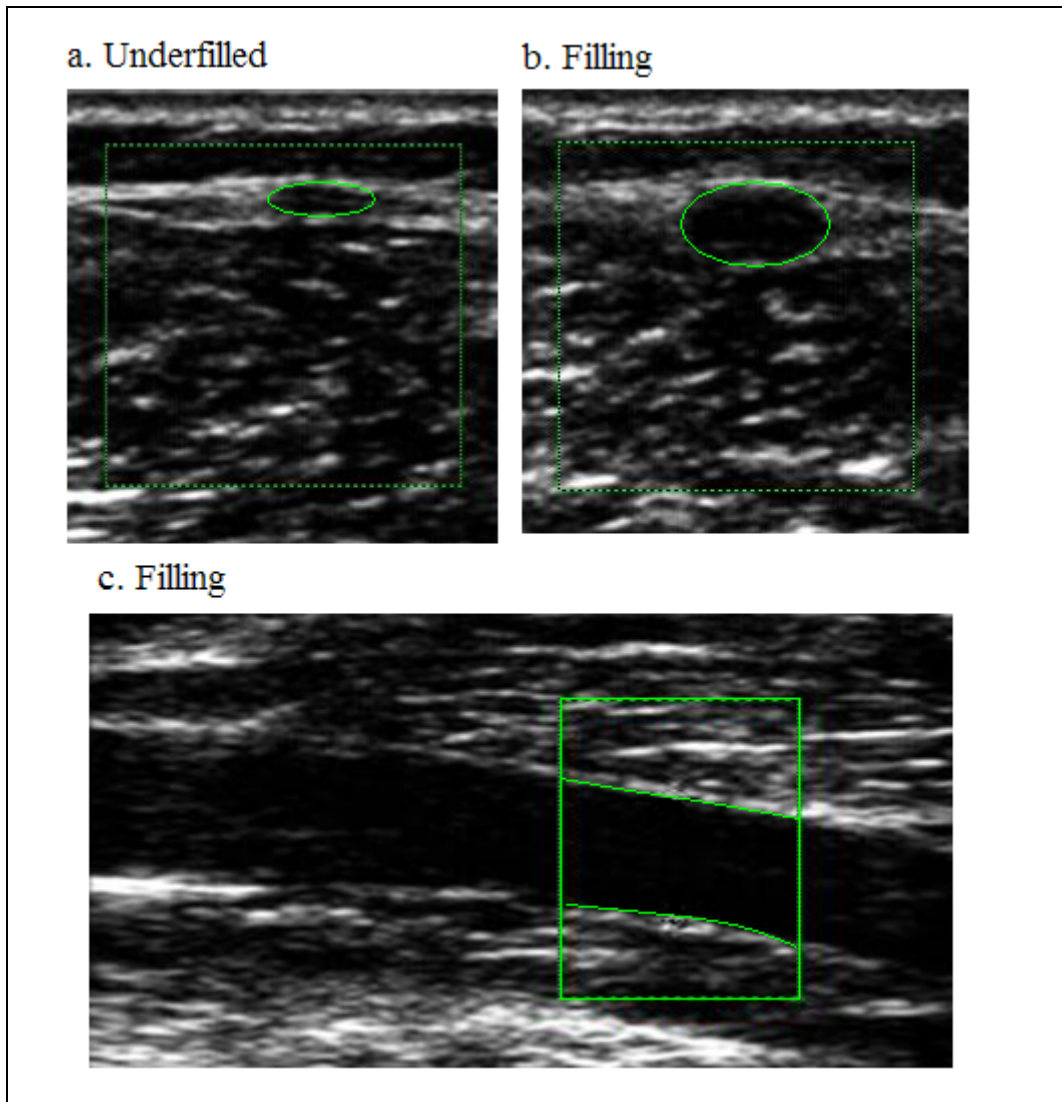


Figure 2.20. Images taken using ultrasound displaying the GSV in cross section when a) underfilled and b) filling. Image c) shows the region of interest measured by the VIA software displaying the near and far borders (in green) of the great saphenous vein wall.

During venous distension, the point of maximum vein filling at the end of the 5 min. was recorded as filled vein capacity for further analysis. Diameter values over the next 50 sec during deflation were noted. Any outlying points (an occasional complication of the sampling error due to movement artifact) were removed. A line of polynomial regression was fitted to

obtain a quadratic equation for the vein diameter / pressure deflation curve as described above for the whole limb, with the intercept taken as β_0 , and β_1 and β_2 coefficients describing the line of slope. From this a compliance / pressure curve was plotted using the formula: Compliance = $\beta_1 + 2 \times \beta_2 \times$ (cuff pressure) as previously described. The gradient of this line is the value used for analysis as previously described for whole limb compliance.

The VIA software was initially designed to be used with arterial flow mediated dilatation where the continuous changes of the cardiac cycle means that arterial diameter is rarely constant. This situation does not exist in the venous system and with occlusion plethysmography there is initial filling of the vein and, if present, some gradual viscoelastic creep of the vessel wall. This means that any movement is operator artifact. Attempts were made to support the ultrasound probe in the positions required with mechanical devices but this was found impractical and hand-held ultrasound was eventually used.

2.2.5 Blood Sampling and Insulin HOMA index calculation

Blood samples (24mls) were taken from the antecubital vein using aseptic techniques and divided into five separate tubes:

- 4ml in a grey top Sodium Fluoride/Potassium Oxalate Vacuette® tube for analysis of fasting blood glucose levels.
- 4ml in a yellow top Serum separator clot activator Vacuette® tube for analysis of sodium, potassium, urea, creatinine, albumin, alkaline phosphatase, alanine transaminase, aspartate transaminase and bilirubin.
- 4ml in a purple top K₃EDTA Vacuette® tube for analysis of haemoglobin, white blood cell count, platelets, haematocrit and mean cell volume.

- 6ml in a red top serum clot activator Vacuette® tube for analysis of 17 β -estradiol and progesterone levels.
- 6ml in a red top serum clot activator Vacuette® tube for analysis of fasting blood insulin levels.

All samples taken above were processed by the University Hospital Birmingham NHS Foundation Trust in the Clinical Chemistry and Haematology laboratories of the Queen Elizabeth Hospital of the UHBNHSFT, Birmingham, England except the fasting glucose level which was carried out at the University of Birmingham School of Sport and Exercise Science's laboratory.

Analysis of insulin levels was carried out using the DRG Human Insulin EIA-2935© assay technique and two samples were analysed to test reproducibility. Once the results of the samples of fasting blood insulin levels were known, they were used with the fasting blood glucose levels to calculate the HOMA index of insulin resistance original devised by Matthews et al. (1985) and updated to HOMA2 (Levy et al. 1998 & Wallace & Matthews, 2002) by the same group in Oxford to allow for the calculation of their individual insulin resistance levels. The computer program HOMA Calculator Version 2.2.2 © Diabetes Trial unit, University of Oxford <http://www.dtu.ox.ac.uk/homa> was downloaded and the data was inputted as displayed in figure 2.21. The output IR was then used as the insulin resistance of the volunteers.

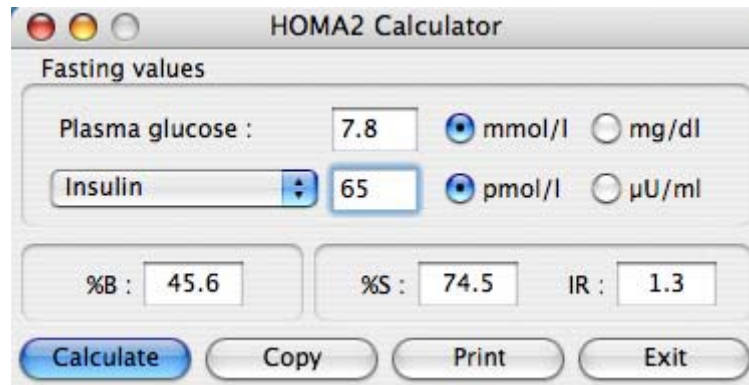


Figure 2.21. An example of the input and output fields in the HOMA2 calculator.

2.2.6 Gynaecology Questions

All volunteers completed a questionnaire on the aspects of their gynaecological history. Two primary measures were used in the study. The total number of full term pregnancies as reported by the volunteers and the length of time for which the volunteer had used the oral contraceptive pill throughout their lifetime reported in months. These results were both used as they were reported.

2.3 PROTOCOLS

Each subject participated in two testing sessions, one in each of two locations. The first took place at the University of Birmingham School of Sport and Exercise Vascular Laboratory (1). The second took place at the Wellcome Clinical Research Facility at the Queen Elizabeth University Hospital Birmingham (2). Participants informed the principal investigator on the first day of their menstrual period. Arrangements were then made for testing to take place within the next two days to maintain parity of female hormone levels throughout the population study group. They were asked to fast from midnight (no food, no caffeinated drinks, no sugary drinks) on the morning that they attended for study. They were asked to

drink 500mls of water on waking on the morning of the study to obtain a satisfactory level of hydration as per the euhydration protocol (Veldhuijzen van Zanten et al. 2005). Participants had been given the questionnaires (Weight Recall and Gynaecological history) at the screening visit. A full explanation of what was required to complete the form was given at this time. Filled questionnaires were recovered from participants when they attended for testing sessions. Room temperature was maintained at $21\pm 1^{\circ}\text{C}$ in both testing locations and subjects were allowed to acclimatize for 20 minutes after arrival in both venues (Abraham et al. 1994 & Jorfeldt et al. 2003).

2.3.1 Test Session 1: University of Birmingham School of Sport and Exercise Science (Appendix G)

Basic data was recorded on arrival as follows:- the study number was assigned, gender was checked, date of birth, age and dominant limb recorded. The subjects were required to re-confirm their consent for the study and any questions raised were answered at this point. Anthropometric data – height, weight, waist / hip / limb circumferences – were measured and recorded. Following inspection and palpation of the limb, a CEAP score for evidence of venous disease was determined as per Table 1.2.

Subjects moved to an examination couch for measurements of abdominal obesity. The Sacro abdominal/Sacro patella index (SA/SP) was measured whilst they sat upright with legs outstretched straight in front. Whilst supine, their sagittal-abdominal diameter (SAD) was recorded. Durometry measures of tissue hardness were then made at previously marked sites on the abdomen, both thighs and calves, in supine and sitting positions. Skin thicknesses were measured at these same sites using Harpenden calipers and ultrasound imaging. Venous refill times were determined following dorsiflexion using the Huntleigh Vascular Assist Device©.

With subjects lying supine, the femoral vein was located in the dominant limb in the thigh, 10cm distal to the groin skin crease and imaged with colour duplex in cross section to verify anatomical position and circular vein profile (the limb was held in approximately 15° of external rotation). In longitudinal B mode image, vein diameter was measured using the ultrasound machine inbuilt calipers. In pulse Doppler mode, resting femoral flow velocity (averaged over 15 cardiac cycles) was recorded in duplicate as mean velocity, time-averaged velocity mean, systolic and diastolic peak velocities. Peak femoral velocity during calf compression was then measured in triplicate. All resting and peak femoral velocities were then repeated with subjects seated upright at 90° with legs externally rotated at 15 ° on the couch.

Leg blood flow and filtration and venous function (capacity, compliance) were then measured using a combination of venous occlusion plethysmography and ultrasound imaging. Systolic, diastolic and mean arterial blood pressures were recorded by a Portapres© finger cuff on the non-dominant hand and heart rate was derived from this trace. Subjects were positioned with their limbs as shown in figure 2.10. Three measurements of calf resting blood flow were made followed by three 5 min periods of venous distension (cuff inflation to 50 mmHg) and emptying (cuff deflation at 1 mmHg/sec). The first distension (control) provided calf plethysmographic data and the 2nd and 3rd yielded ultrasound imaging data on the GSV and popliteal vein respectively to estimate venous capacity, filled vein diameter (GSV, popliteal), limb fluid filtration, and calf (limb) and vein compliance. Microvascular fluid filtration was then determined using the small incremental pressure step protocol and the Filtrass machine as described above (Section 2.2.3C).

2.3.2. Test Session 2: Wellcome Research Facility University Hospitals Birmingham

(Appendix H)

Following the measurements above, participants moved (still fasted) from the University of Birmingham Sport and Exercise Sciences building to the Wellcome Research Facility at the Queen Elizabeth University Hospital, a journey of 0.25 mile taking approximately 15 minutes. Subjects were admitted onto the Wellcome Trust computer system and a UHBNHSFT unit number was generated or retrieved. They were required to re-confirm their consent for the study and any questions raised were answered at this point.

Blood samples were taken from an arm vein as described in section 2.2.5. Subjects then underwent a Dual Energy X-ray Absorptiometry scan performed by the onsite DEXA qualified radiographer. Once this was completed they lay supine on a electronic hospital bed in the study room with ambient temperature set at 21°C. Mean arterial blood pressure and pulse were monitored on a Hewlett Packard Propaq© throughout the testing period.

After a period of 5 minutes rest the previously marked sites on the bilateral thigh and calves were located so that durometry measures could be taken in both the supine and upright seated positions. Following a further rest period, tissue pressure was measured at the groin by manometer in supine and seated positions. This concluded the test session.

2.4 DATA ANALYSIS AND STATISTICS

2.4.1 Reduction of Bias and Sampling Error

All readings taken in the study were made under the same controlled conditions in an attempt to maintain comparability of data. All subjects were studied in the same phase of the menstrual period (Barwin et al. 1977), the same room temperature (Jorfeldt et al. 2003), and fasted for at least 8 hours prior to the study which included the exclusion of caffeine. Volunteers were hydrated during their fast as per the euhydration protocol designed to maintain normal blood volume (van Zanten et al. 2005). All anthropometric, venous and whole limb function measures were taken twice by one investigator so that reproducibility could be assessed. Patients were encouraged to remain as relaxed as possible throughout to reduce movement artefact particularly for those measurements where it was important e.g. ultrasound imaging. Where there was concern raised by any member of the testing team as to the validity of a reading taken the test was reset and repeated after a necessary time delay to allow for return of normal tissue function if required.

2.4.2 Medical statistics software

Microsoft® Office Excel 2003 was used to collate data at the time of investigation. Descriptive statistics were derived by the inbuilt analysis software and all graphical data were produced using this program. Two different statistics packages were used in analysing and interpreting the data. These were chosen because the investigator had experience using them prior to commencing the study. MedCalc® Version 10.1.4.0 and StatView® for Windows version 5 were used to perform comparisons as described below.

2.4.3 Review of data

Data from the study was reviewed with input from the University Hospital Birmingham NHS Foundation Trust statistician Dr Peter Nightingale. All data underwent Kolmogorov-Smirnov testing for closeness of fit to the normal distribution (MedCalc® Version 10.1.4.0) and were found to be parametric (Appendix I). However, the study contained small numbers (26 female) of volunteers and it was felt that to avoid making assumptions and Type II errors, it would be better to treat the data as non-parametric. Therefore all group, paired and correlative analyses were made using non-parametric tests.

2.4.4 Box and Whisker plots.

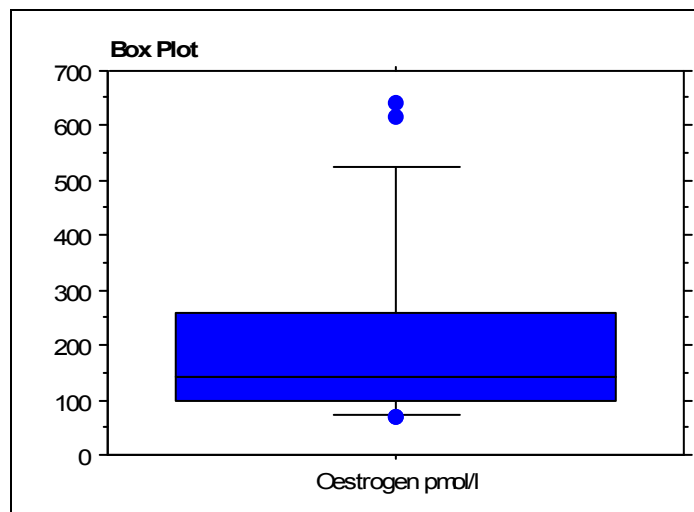


Figure 2.22. An example of a box and whisker plot showing the results of oestrogen taken from the study volunteers. Two points clearly lie beyond the 91st percentile of the patient population and these were removed from analysis to alter any skewing of the results.

Each individual variable was displayed for the whole study cohort on a box and whisker plot using StatView® for Windows version 5 to show the median, upper and lower quartile, 9th and

91st percentiles. The advantage of this type of graphical display is that it clearly demonstrates if a value lies outside the expected range for the sample population. The investigator can then decide whether to include or exclude this value from the analysis. Data analysis was supported by previously published results in the literature or known normal ranges described by laboratories.

2.4.5 Statistical Tests

The study population was described using the mean and standard deviation of measured variables. Comparison between the three BMI class groups - classes <25, 25-30 and >30 kg/m² – was made by the Kruskal-Wallis test. This was applied to all anthropometric data, all measures of abdominal adiposity, tissue pressure, durometry readings, vein sizes, capacities, vein compliance and flow, and blood levels of female hormones and metabolic indicators (glucose, insulin) as dependent variables with BMI group as the independent variable.

Correlational analysis was performed to test for associations between body size / dimensions and measures of abdominal adiposity, and between these variables and data on vein size (diameter, CSA, capacity), venous compliance, femoral vein flow with and without calf compression, calf blood flow and filtration. Results are presented in tabular form, giving Spearman's Rank Correlation coefficient Rho (ρ), and the level of significance. Blood concentrations of female hormones and metabolic indicators were also correlated with all of the above parameters to determine their influence. The possibility of a multiple regression model to test for key influences on venous function was considered but would not have been robust with the low n numbers involved.

Where duplicate or more readings were made of the same parameter, e.g. groin tissue pressure, skin thicknesses or vein flow values, reproducibility was determined by calculating

the coefficient of variance ($SD/mean * 100$). Where two different methods were applied to the same parameter, e.g. skin thickness by ultrasound or skinfold calipers, the limits of measurement agreement were estimated by correlation (Spearman's Rank) and by Bland Altman plot to illustrate any consistent bias.

Where measurements were made in both supine and seated position, e.g. femoral vein size and flow, groin tissue pressure, a paired comparison by the Wilcoxon test was performed to see if a significant alteration had occurred as a result of the change in posture.

Data are presented in the results section as mean and standard deviation unless otherwise stated. The level of significance was taken as $p=0.05$.

CHAPTER 3:

RESULTS

3.1 BODY COMPOSITION

3.1.1 Study Volunteer Characteristics

The “OBVIOUS” study ran from 1st April 2007 to 1st October 2008 at University Hospitals Birmingham NHS Foundation Trust and the University of Birmingham School of Sport and Exercise Sciences. Fifty-seven volunteers responded to advertisements and 50 females and 7 males were screened for entry into the study. Seven volunteers (6 female, 1 male) did not meet the inclusion criteria and 18 females were screened but unable to complete the study, leaving 26 female and 6 male participants. With these small numbers in the male group, it was deemed that there would be insufficient power for analysis of gender differences. Consequently, data was collected and will be reported from a total of 26 females. The consequences of this lower than expected response to request for study participants and small number included in the study will be dealt with in the Discussion.

At initial screening, arterial and venous haemodynamics were assessed. None of the 26 participants had venous reflux > than 0.5 sec at the sapheno-femoral or sapheno-popliteal junctions as measured by duplex ultrasound in standard clinical practice. Venous refill times (Sam et al. 2006) and ankle-brachial pressure index are shown below and fall within the normal range (Al-Qais et al. 2009).

The primary outcome measures from the study were indices of venous function that could be related to body composition and the presence of abdominal obesity. The demographic and physical characteristics of the participants are therefore shown for the whole group, and for the group divided in categories of BMI (normal, overweight, obese) as described in the

Methods. There were no differences in age or height between the BMI categories but weight was significantly greater in the overweight and obese individuals as would be expected.

	n	Mean (Standard Deviation)
Age (yrs)	26	41.2 (3.91)
Ankle Brachial Pressure Index	26	1.05 (0.09)
Venous Refill times (s)	26	40.9 (6.01)
Heart Rate (bpm)	26	70.9 (10.25)
Mean Arterial Blood Pressure mmHg	26	87.9 (11.71)
Height (cm)	26	167.0 (7.44)
Weight (Kg)	26	83.8 (19.22)
BMI (Kg/m ²)	26	30.0 (6.24)
Glucose (mmol/l)	20	4.9 (0.74)
Albumin (g/dl)	12	44.7 (3.94)
Haemoglobin (g/dl)	21	13.4 (0.90)
Haematocrit (fl)	21	0.4 (0.03)
Oestrogen (pmol/l)	18	167.9 (110.3)
Progesterone (nmol/l)	18	1.8 (1.02)
Insulin	22	9.8 (7.69)
HOMA index	17	1.34 (1.04)

Table 3.1. Demographic and physical characteristics of the 26 female volunteers. (No volunteers fell outside the normal range for any reading taken or they would have been excluded from the study.)

In the volunteer population no clinical parameter or biochemical reading taken was found to lie outside of the normal range either designated from the literature, e.g. ABPI, or designated by the laboratory performing the testing respectively. These data are displayed in table 3.1. In the volunteer population there were 23 (88.5%) right leg and 3 (11.5%) left leg dominant woman and 22 (84.6%) volunteers who had never smoked. 3 (11.5%) volunteers were present

smokers and 1 (3.8%) woman was an ex-smoker. CEAP classification demonstrated 14 (53.8%) class 0 volunteers and (46.2%) class 1 volunteers.

As previously discussed in methods the BMI was used as the reference range of body habitus for the volunteers. For analysis, the volunteers were divided into three groups according to BMI classifications of the World Health Organization (1995) as normal, overweight or obese. Physical characteristics of these groups are shown in Table 3.2.

		BMI Kg/m ²	Age	Height cm	Weight Kg
	n	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
BMI 20-25 normal	7	23.2 (1.6)	41.0 (5.7)	165.0 (8.4)	63.3 (7.5)
BMI 25.1-30 overweight	9	28.2 (1.3)	40.4 (3.4)	169.4 (7.3)	81.2 (6.7)
BMI 30+ obese	10	36.1 (4.9)	41.9 (3.0)	166.2 (7.0)	100.3 (17.7)

Table 3.2. BMI, age, height and weight are shown as mean (SD) for the 3 sub-groups according to BMI classification.

Kruskal Wallis testing revealed no significant difference between the groups in age ($p=0.72$) or height ($p=0.52$) although as expected the difference was significant between the weight ($p=0.0002$) and BMI ($p<0.0001$) by different BMI class.

Wrist and ankle circumferences were measured and correlated with BMI, height and weight. Table 3.3 shows that wrist circumference was correlated significantly with all three parameters, and ankle circumference with BMI and weight but not height.

	ρ	p
Wrist vs BMI	0.531	0.008
Wrist vs Height	0.576	0.004
Wrist vs Weight	0.735	0.0002
Ankle vs BMI	0.464	0.02
Ankle vs Height	0.257	0.200
Ankle vs Weight	0.560	0.005

Table 3.3. ρ and p value for the correlation of wrist or ankle circumference with BMI, height or weight for all participants (n=26).

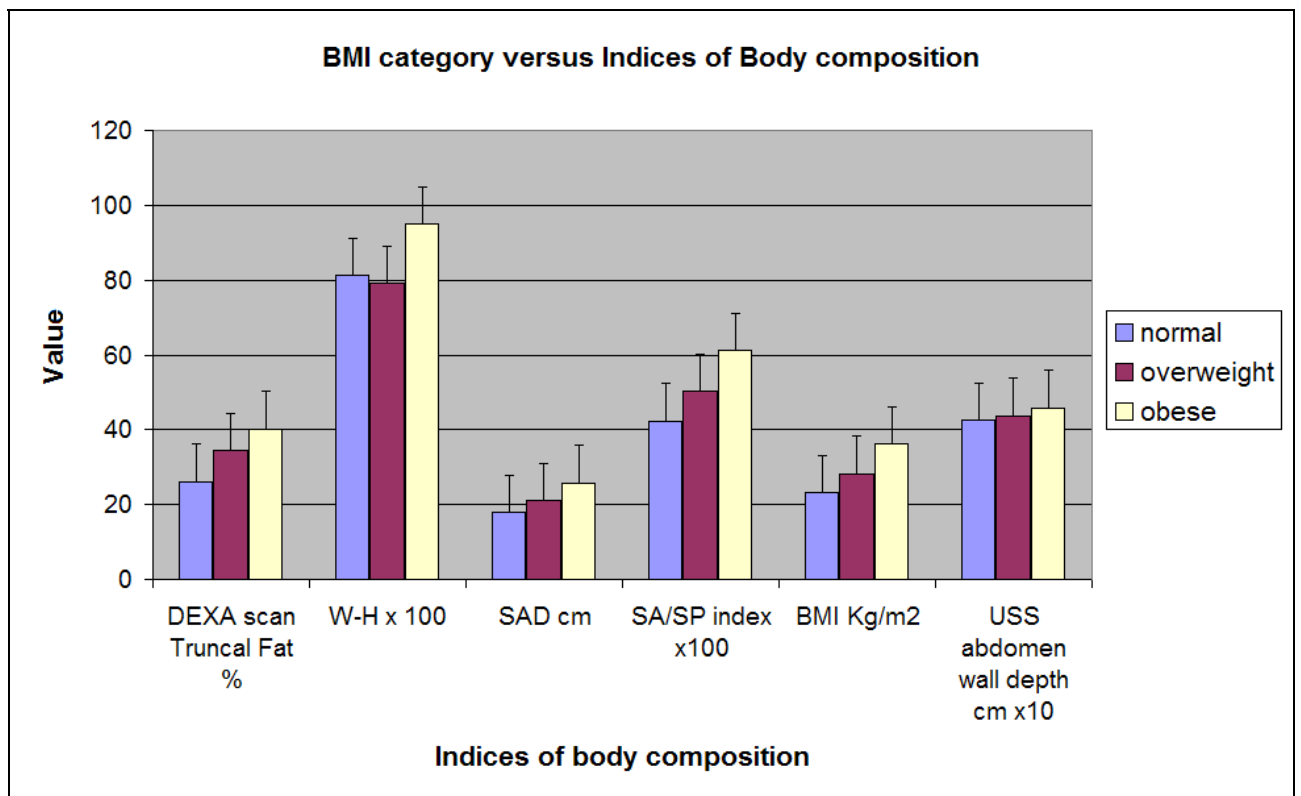


Figure 3.1. Demographic data, body mass index and indices of abdominal obesity in the normal (n=7), overweight (n=9) and obese (n=10) females grouped by BMI class. Values shown are mean \pm S.E.M. and the units for each variable are shown below the columns.

3.1.2 Validation of Sacro-Abdominal/Sacro-Patella index (SA/SP)

The most commonly-used simple non-invasive indices to evaluate central abdominal adiposity are sagittal-abdominal diameter (SAD), % truncal fat as determined by DEXA scan, waist circumference or waist-hip ratio (W-H), and subcutaneous tissue thickness measured by skinfold calipers or ultrasound. These measures are usually made with subjects in the supine position. In order to assess the impact of abdominal fat on venous return from the leg when seated, a new ratio – SA/SP – that measured the distance from sacrum to patella that is overhung by abdominal fat in the sitting position was devised (see Methods). This ratio was compared against the other established estimates of abdominal adiposity as illustrated in figure 3.1.

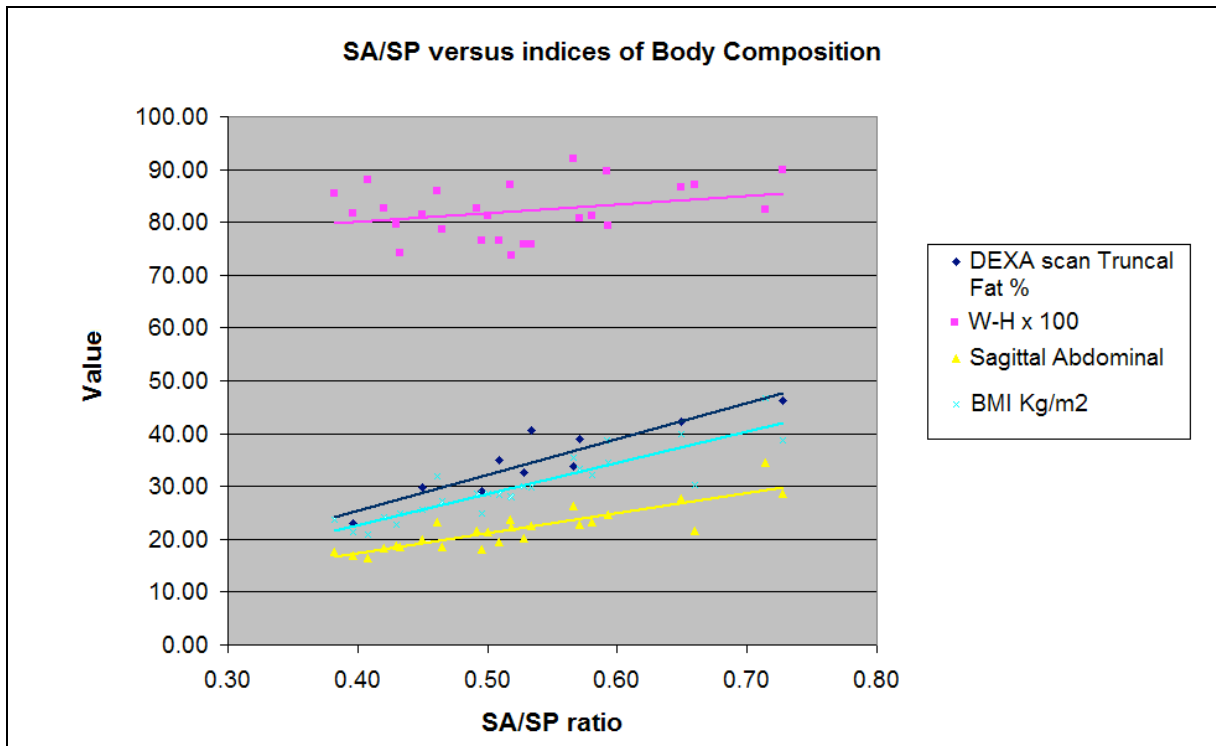


Figure 3.2. Scatter plot with regression lines to show correlation between SA/SP and other indices of abdominal fat (SAD, DEXA % truncal fat, waist-hip ratio) and body composition (BMI) in 26 females.

The SA/SP index was greater in obese > overweight > normal, as were BMI, SAD, and % truncal fat; W-H ratio did not distinguish between the groups. Within the study population as a whole, there was very good correlation between SA/SP and SAD ($\rho=0.85$, $p<0.0001$) or % truncal fat ($\rho=0.87$, $p=0.009$) but no association with W-H ratio ($\rho=0.19$, $p=0.3329$). SA/SP also correlated significantly with BMI ($\rho=0.90$, $p<0.0001$).

3.1.3 Comparison of Ultrasound and Skinfold Caliper in the measurement of abdominal and peripheral fat

Ultrasound measurements of subcutaneous fat thickness have been compared against the more traditional skinfold caliper technique at various body sites. Both methods displayed greater measurement error in obese than non-obese subjects but this was reported to be less apparent at the abdominal site (Demura & Sato, 2007). In order to characterize body fat distribution in the abdomen and legs in the present study, prior to consideration of its impact on venous function, an analysis was first made of the agreement between these two techniques in measuring at the abdomen, thigh and calf. Duplicate readings were made at each site using ultrasound and skin fold caliper. The coefficient of variance (SD as % mean) as a measure of reproducibility at abdomen, thigh and calf were 4.0%, 3.3% and 5.2% for ultrasound and 3.9%, 3.3% and 4.4% for caliper.

Figure 3.3 shows the correlation between caliper and ultrasound measurements with Spearman's rank correlation coefficients of $\rho=0.83$ for abdomen ($p<0.0001$), $\rho=0.77$ for thigh ($p<0.0001$) and $\rho=0.61$ for calf ($p<0.0001$). The readings obtained from the ultrasound were consistently lower than those from the calipers at all three sites (Table 3.6). The Bland-Altman plots (Fig. 3.4) demonstrate agreement between the two techniques at all three sites but with consistent differences (ultrasound – caliper) of -0.63 cm for abdomen, -1.23 cm for

thigh and -1.10 cm for calf. The difference between the two measurements was not related to magnitude for the abdomen and thigh, but in the calf, the difference increased with greater magnitude (Spearman's correlation coefficient -0.44, $p < 0.005$).

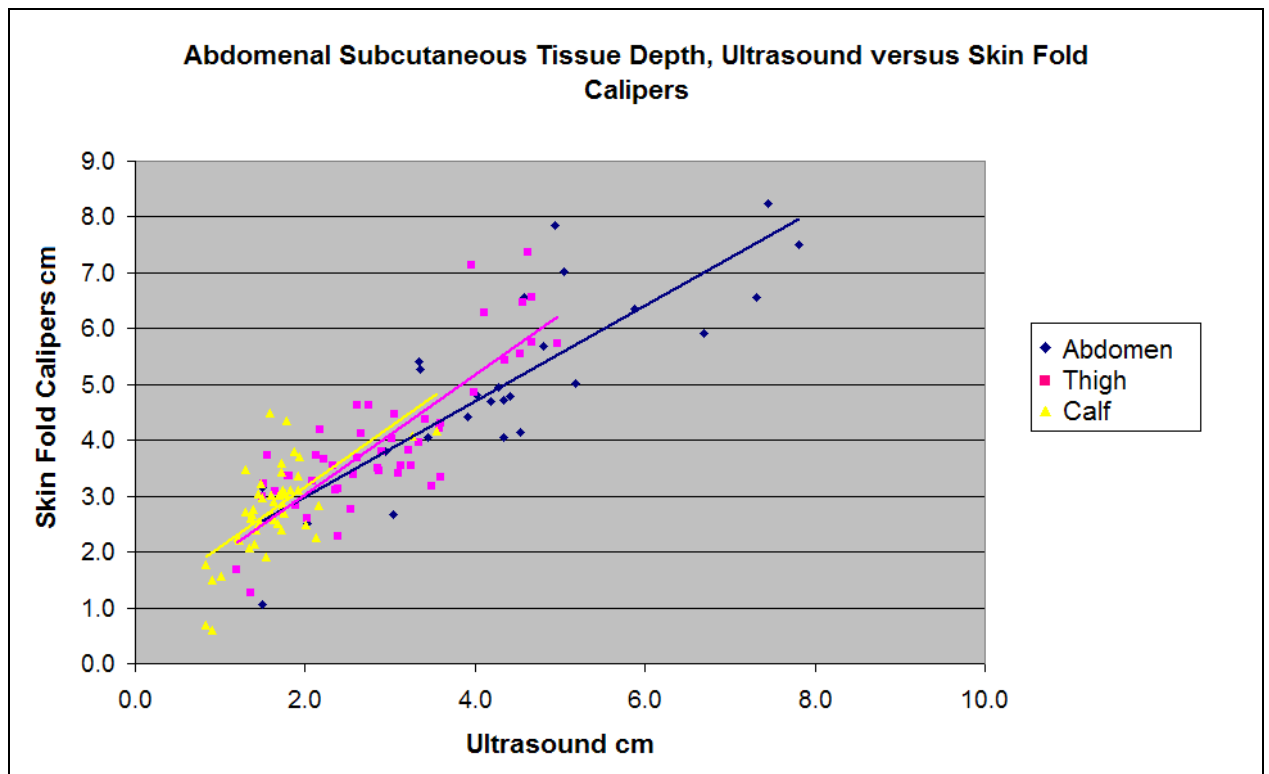


Fig 3.3. Scatter plots of relationship between caliper and ultrasound measurements of subcutaneous fat tissue thickness at the abdomen, thigh and calf in 26 female; data shown include duplicate measures at each site and values for both left and right legs

		Ultrasound cm	Calipers cm	
	n	Mean (standard deviation)	Mean (standard deviation)	P values
Abdomen	26	4.42 (1.65)	5.07 (1.68)	0.103
Thigh	26	3.04 (1.12)	3.93 (1.32)	0.006
Calf	26	1.67 (0.56)	2.65 (0.72)	<0.0001

Table 3.4. Mean (SD) values of skin thickness measured by ultrasound and skinfold calipers for the abdomen, thigh and calf in 26 females. P values are for paired Mann-Whitney test.

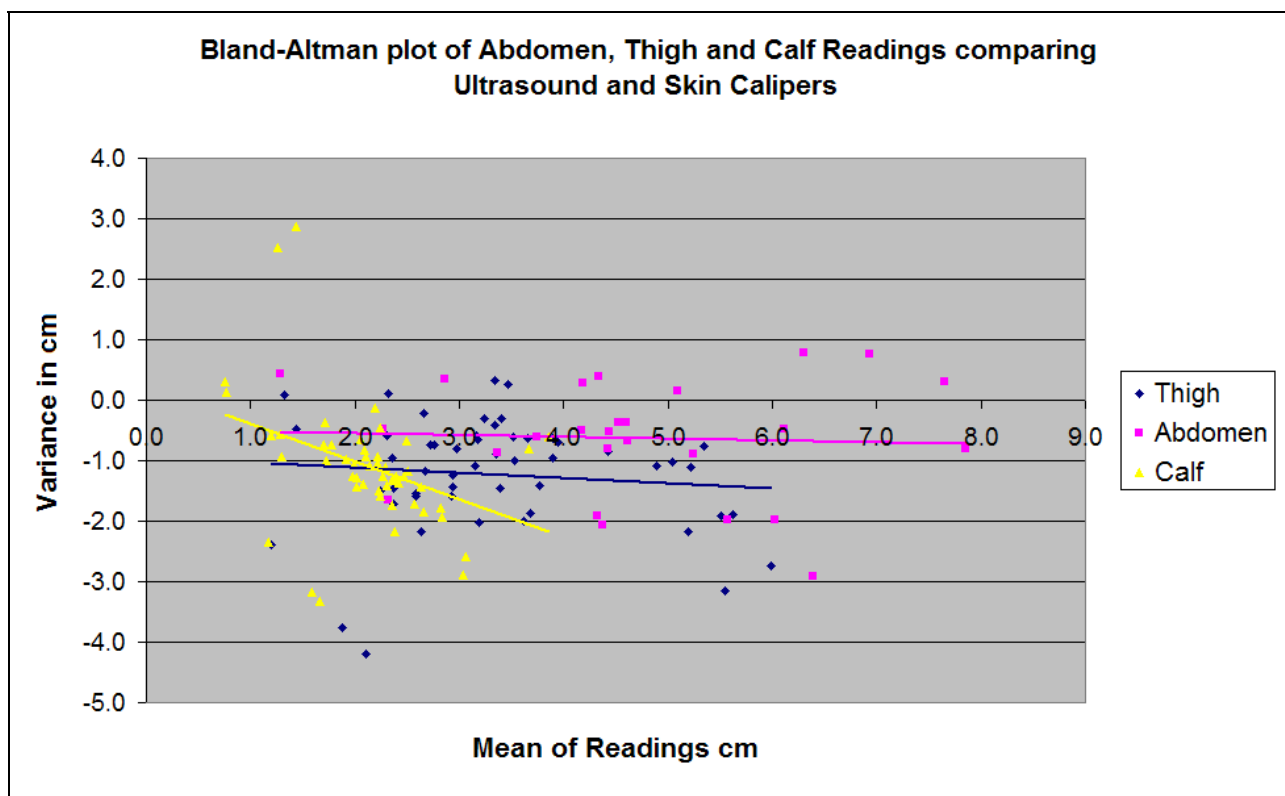


Fig 3.4. Bland-Altman plot of difference between DUS and caliper measurement of subcutaneous tissue thickness against mean of the two measurements in 26 females, including duplicate measures each site and values for both left and right legs.

Having established that abdominal fat thickness could be measured directly using ultrasound with good accuracy, the values were compared against the other indices of abdominal adiposity – SAD ($\rho=0.823$, $p<0.0001$), % truncal fat ($\rho=0.636$, $p=0.056$), W-H ratio ($\rho=0.375$, $p=0.609$) and the SA/SP ratio ($\rho=0.777$, $p=0.0001$) and against BMI ($\rho=0.847$, $p<0.0001$).

3.2. EFFECT OF BODY COMPOSITION AND ABDOMINAL ADIPOSITY ON INGUINAL TISSUE PRESSURE, FEMORAL VEIN FLOW

3.2.1 Measurement Inguinal Tissue Pressure

The presence of fat in the central abdominal region was considered to present potential impediment to femoral venous flow by providing mechanical hindrance in the groin region especially when seated. Inguinal tissue pressure was therefore measured directly by needle and manometer with participants lying supine (2 min) and then passively moved to an upright seating position with the legs extended (5 min). It can be seen from Fig. 3.5 that in the normal subjects, pressure readings showed a step increase, remaining stable at the higher value for the duration of sitting.

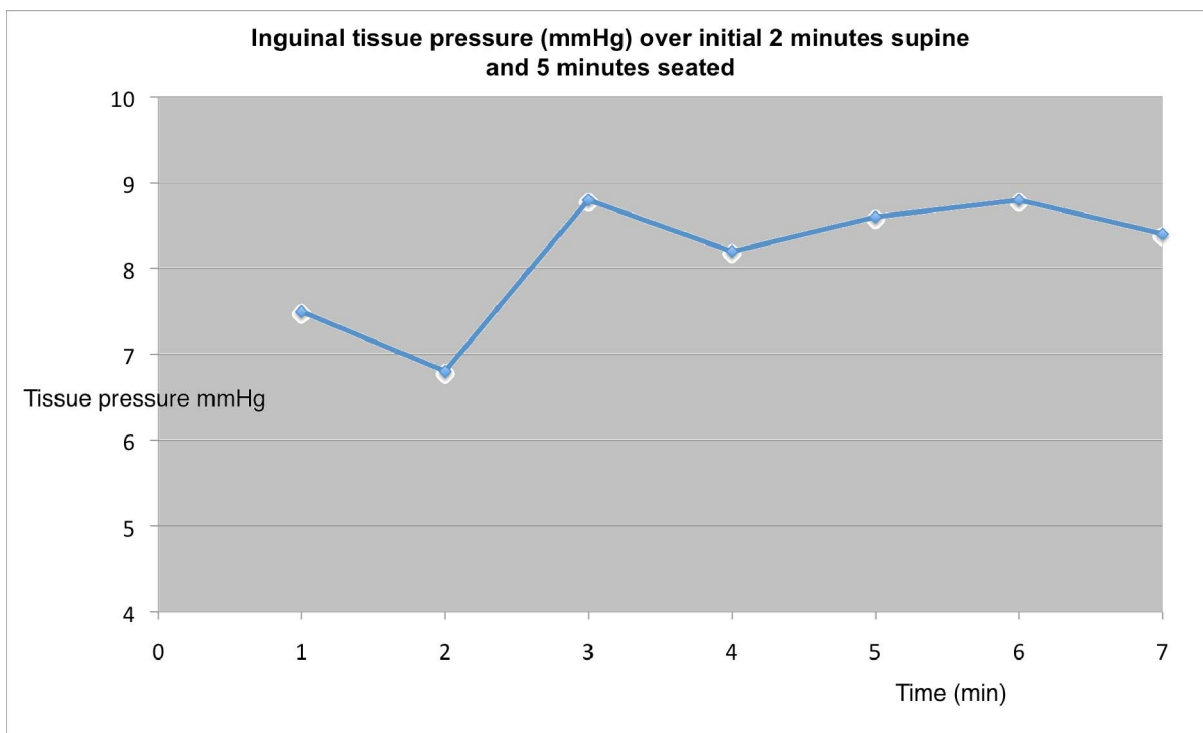


Figure 3.5. Inguinal tissue pressure (means) recorded each minute during 2 minutes of supine and 5 minutes of seated position in the normal BMI female volunteers.

The coefficient of variation of repeated inguinal tissue pressure measures for those of normal BMI in the supine position was 6.5% and in the seated position was 11.1%. This was calculated across the five supine and four seated measurement cycles. Inguinal tissue pressure data were obtained from 21 of the 26 participants in the study. No significant difference was demonstrated in supine tissue pressures between the BMI groups ($p=0.70$; figure 3.12). On moving to the seated position, pressures did change significantly for the normal ($p=0.0008$) and obese BMI group ($p=0.02$) with the greatest difference in the obese group, measured at 8.2 (3.5) mmHg. The difference from supine to seated was not quite significant in the overweight group ($p=0.07$). The relationship between inguinal tissue pressure and BMI is borne out by correlation, which was significant for seated values ($\rho=0.62$, $p=0.007$) but not supine ($\rho=0.25$, $p=0.27$).

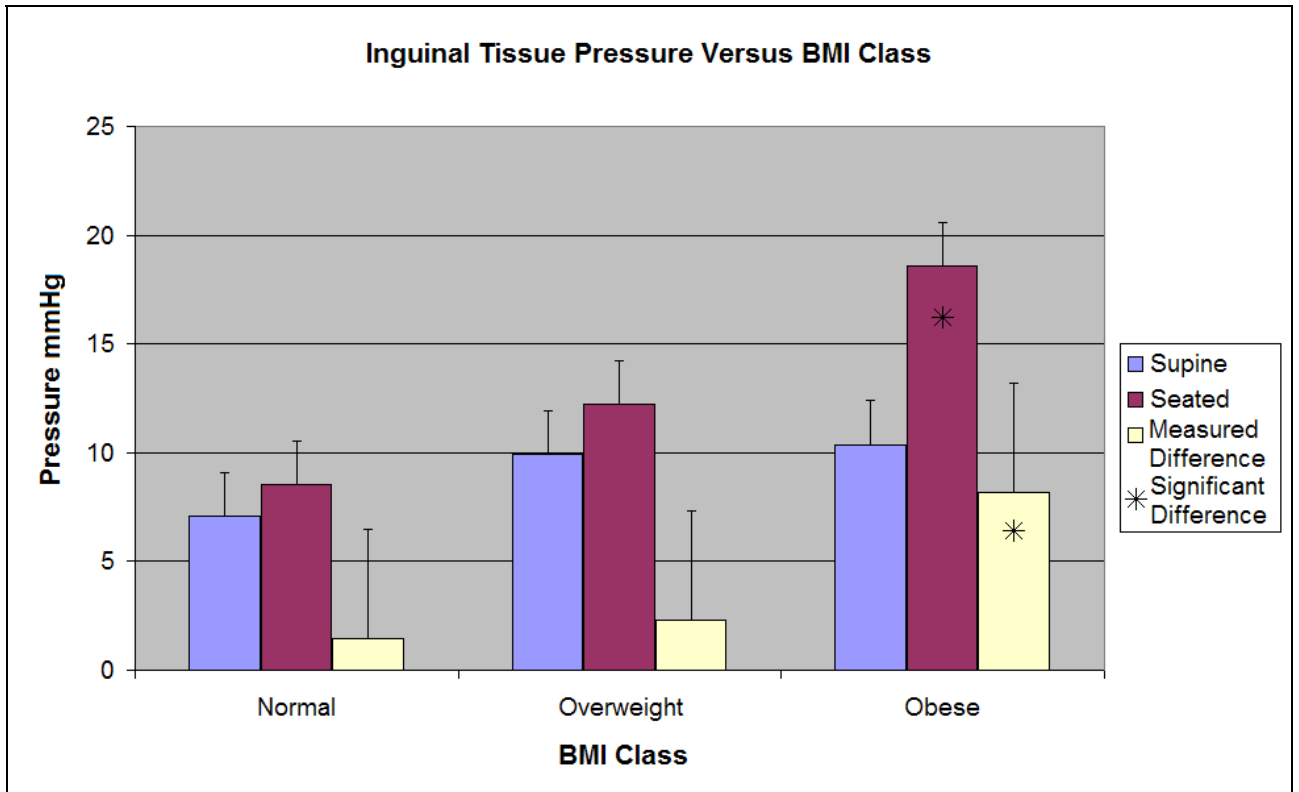


Figure 3.6. Inguinal tissue pressure (means) measured supine and seated in 21 females according to BMI class. The seated, supine and measured difference between them are shown with significant differences between BMI groups displayed between the obese seated and obese difference groups and their normal and overweight seated and difference measures.

Correlations between the seated pressure and the indices of abdominal adiposity were also noted (Table 3.5). They were particularly good for those indices that specifically detect abdominal fat i.e. SA/SP, ultrasound of the abdominal wall and sagittal abdominal diameter.

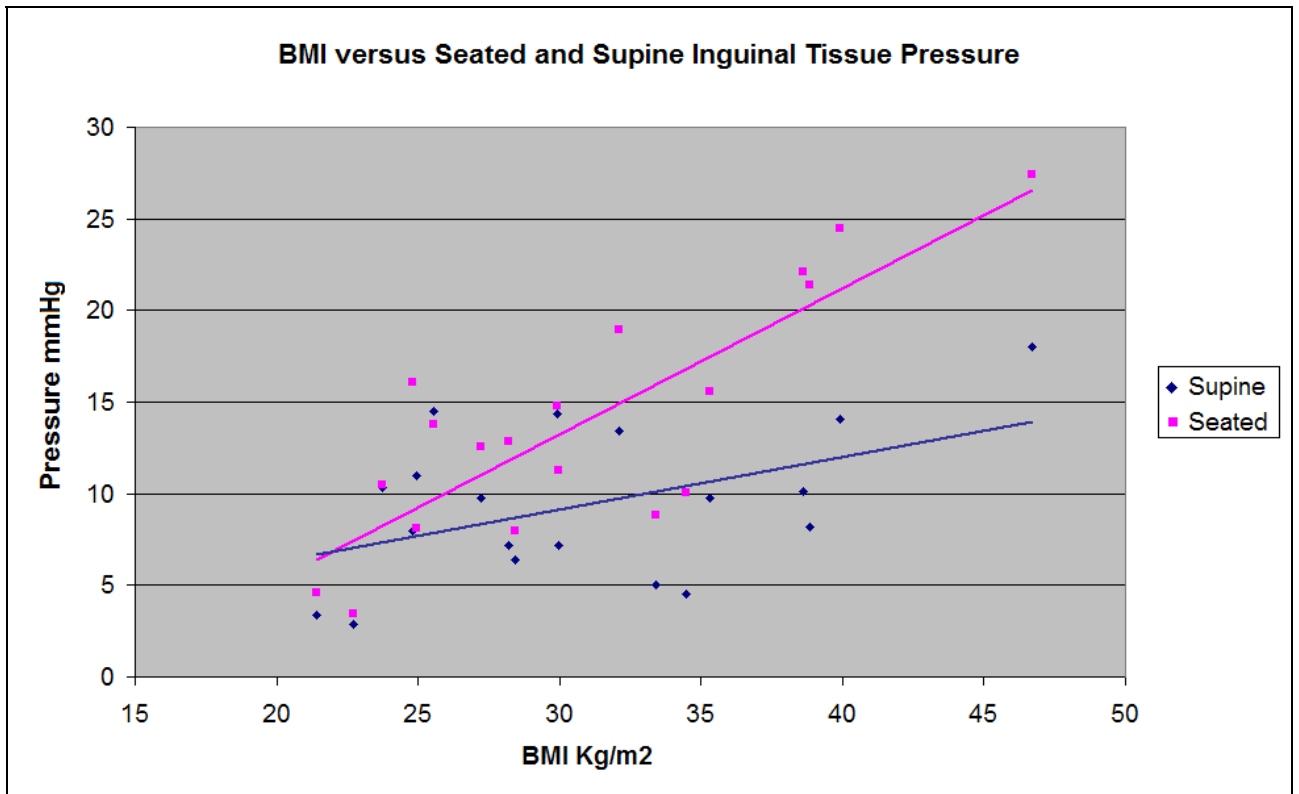


Figure 3.7. Correlation between BMI and seated and supine inguinal tissue pressure measures for 21 females.

	n	Seated Inguinal Tissue Pressure		Difference supine to seated	
		ρ	P	ρ	P
Body Mass Index (BMI) Kg/m ²	21	0.620	0.007	0.410	0.080
Supine Sagittal- Abdominal diameter cm (SAD)	18	0.647	0.008	0.311	0.200
DEXA percentage Truncal Fat	10	0.500	0.130	0.176	0.60
Waist-Hip Ratio	19	0.492	0.040	0.460	0.050
Sacro-Abdominal/Sacro-Popliteal index (SA/SP)	20	0.709	0.003	0.500	0.040
Ultrasound Abdomen wall subcutaneous tissue depth cm	19	0.700	0.03	0.440	0.060

Table 3.5. Correlations between inguinal tissue pressure and indices of abdominal adiposity.

Supine inguinal tissue pressure is not affected by any measure of body habitus. The seated inguinal tissue pressure can be seen to have a positive correlation with indices of body composition. The difference between these two readings is greatest and significant only in the obese group and this significance is seen with measures looking at the abdominal wall adiposity.

3.2.2 Relationships between body composition, abdominal adiposity and femoral vein size.

FV CSA was calculated from ultrasound measurements of diameter in the femoral triangle in the supine and seated position in 22 participants. The values obtained were similar to those published by De Groot et al. (2005), and pilot work in this laboratory (G. D. Bishop, PhD Thesis, University of Birmingham) has established that within-subject variability of vein CSAs calculated from diameter measured using VIA software yields a coefficient of variation of 2.6 – 6.1% for repeat measures within a session and 2.2 – 4.4% between sessions. There was no significant difference between the BMI classes comparing the supine FV CSA ($p=0.83$). Seated FV CSA was larger in the obese than the overweight or normal BMI groups but these differences were also not significant ($p=0.14$). The change in the size of FV CSA upon moving from supine to seated was significant in all three BMI groups (normal $p<0.0001$, overweight $p=0.018$ and obese $p=0.008$).

The percentage increase in CSA on changing position was also similar between the BMI groups ($p=0.13$). Although femoral vein size did not appear to be related to BMI across the three groups, there was a significant correlation between BMI and seated CSA ($\rho=0.50$, $p=0.02$) and the percentage increase in CSA ($\rho=0.55$, $p=0.01$) (fig 3.8). FV CSA was not

related to height in any postural position, implying that weight, and hence adiposity, play a greater role.

		Supine Femoral Vein CSA cm2	Seated Femoral Vein CSA cm2	% change from Supine to Seated
	n	Mean (SD)	Mean (SD)	Mean (SD)
BMI 20-25 normal	6	0.406 (0.244)	0.549 (0.220)	60.4 (57.5)
BMI 25.1-30 overweight	7	0.428 (0.132)	0.774 (0.291)	91.7 (66.5)
BMI 30+ obese	9	0.386 (0.173)	0.880 (0.480)	128.5 (47.9)

Table 3.6. Cross sectional area (CSA) of the femoral vein (means \pm SD) in 22 females when supine and seated and the percentage difference on changing position.

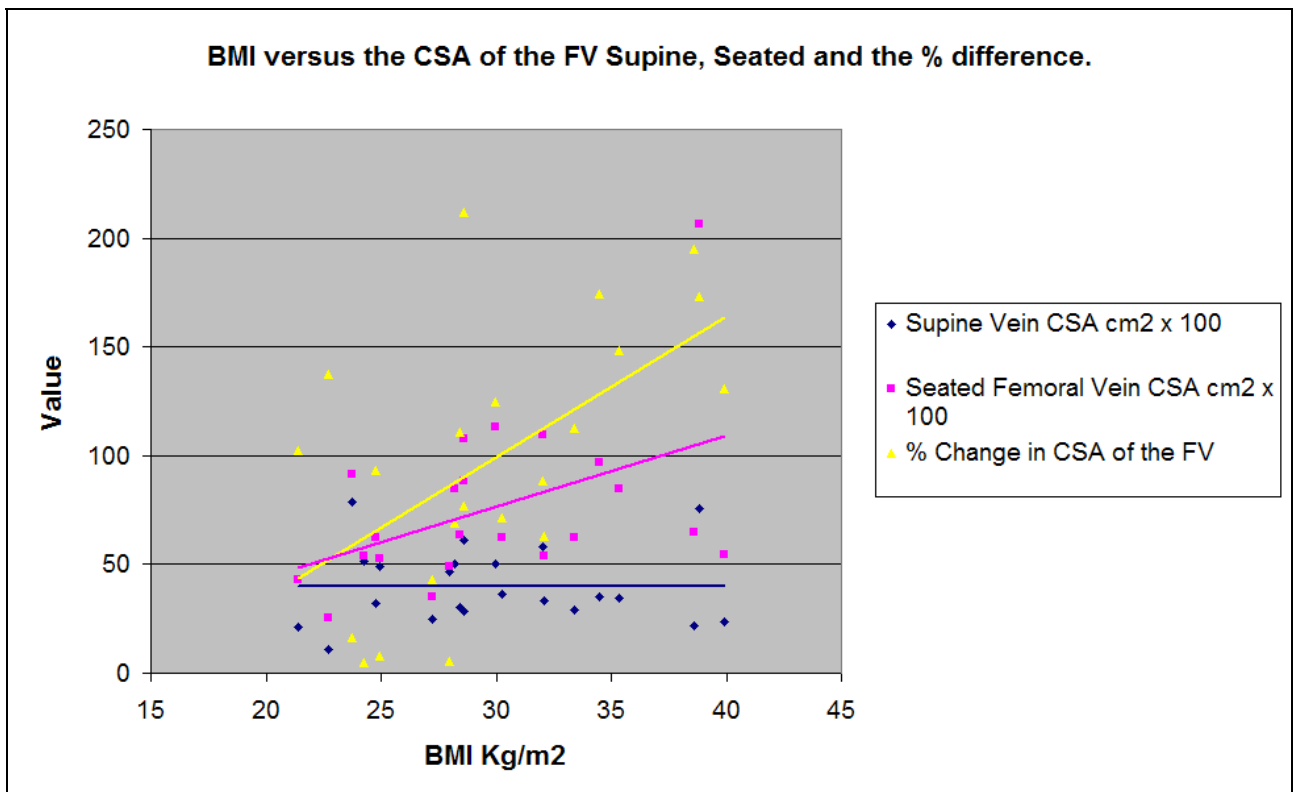


Figure 3.8. Scatter plot of the correlation of BMI against the supine and seated cross sectional area of the femoral vein. The percentage change between supine and seated is also shown.

To examine the effect of abdominal adiposity on femoral vein size, supine, seated and percentage change in CSA were correlated with SAD, % truncal fat, W-H ratio, SA/SP index and ultrasound abdominal tissue thickness. None of these indices were correlated with supine femoral vein CSA. Only SAD was marginally correlated with seated femoral vein CSA ($\rho = 0.41$, $p=0.07$). However, all indices except W-H ratio were significantly correlated with the percentage change in CSA on moving from supine to seated position (SAD $\rho = 0.42$, $p=0.05$; % truncal fat $\rho = 0.71$, $p=0.06$; SA/SP $\rho = 0.48$, $p=0.03$; ultrasound abdominal tissue thickness $\rho = 0.46$, $p=0.04$). Thus the greater the measure of abdominal fat, the larger the increase in femoral vein size when sitting.

In the previous section (3.2.1), seated inguinal tissue pressure was also shown to be correlated with abdominal adiposity assessed by W-H ratio, SA/SP index ultrasound tissue thickness and SAD but not % truncal fat. It was expected therefore that changes in femoral vein size would be associated with seated inguinal tissue pressure. However, neither seated femoral CSA nor percentage increase in the FV CSA were, related to seated inguinal tissue pressure ($\rho = 0.33$, $p=0.20$; $\rho = 0.24$, $p=0.34$ respectively) or change in pressure ($\rho = 0.05$, $p=0.85$; $\rho = 0.36$, $p=0.20$). Other body size factors may therefore impact upon the size of the femoral vein when sitting. One possibility is leg size because with the legs extended, thigh and calf tissues may act by compression to displace blood volume along the venous circulation.

Anthropometric estimations of leg size and composition were made using standard circumferential measures. Dominant thigh and calf CSA were calculated and, following correction of circumference for subcutaneous tissue thickness based on ultrasound / skinfold caliper measurement, lean CSA was also calculated for these two segments. Data were obtained from 23 of the female subjects yielding a mean (SD) thigh CSA of 224.1(55.6) cm²

and calf CSA 124.8(26.5) cm². Lean CSA (thigh 89.3(20.1) cm², calf 67.6(13.1) cm²) represent 39.8% and 54% of the leg CSA respectively.

None of the CSA values were related to supine FV CSA. However, seated FV CSA was significantly correlated with both thigh ($\rho = 0.53$, $p=0.02$) and calf ($\rho = 0.53$, $p=0.01$) CSA, as was the percentage change in FV CSA on moving from supine to sitting (vs thigh CSA $\rho = 0.56$, $p=0.01$; vs calf CSA $\rho = 0.59$, $p=0.07$).

Lean thigh CSA was not related to seated femoral CSA ($\rho = 0.10$, $p=0.63$) although there was association with lean calf CSA ($\rho = 0.43$, $p=0.05$). Figure 3.9 illustrates these relationships.

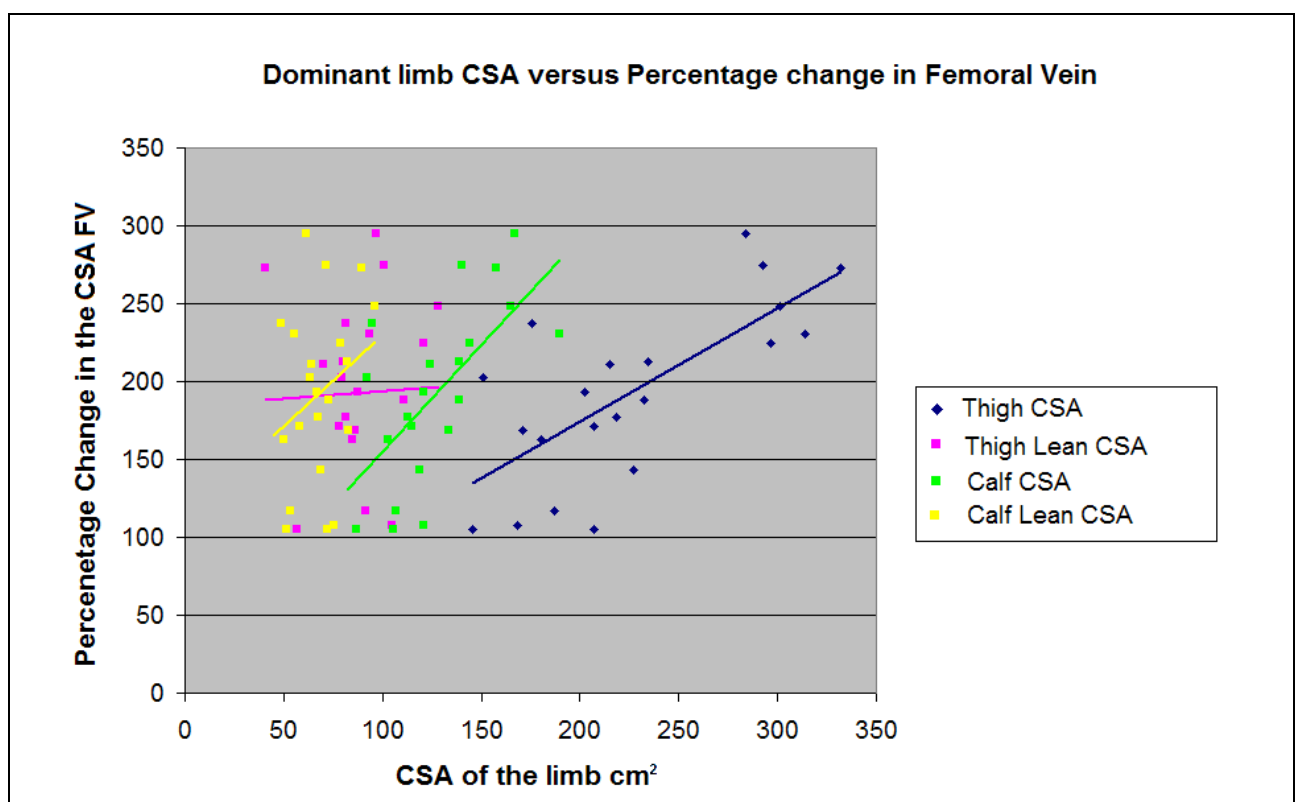


Figure 3.9. Scatter plot showing the correlation between percentage change in femoral vein cross sectional area CSA on moving from supine to sitting with legs extended and anthropometrically determined leg and lean CSA of the dominant thigh and calf in 23 females.

3.2.3 Relationships between body composition, abdominal adiposity and femoral vein flow

Resting femoral vein mean flow was estimated from ultrasound measured vein diameter and flow velocity averaged over 15 cardiac cycles in the supine and seated positions in 22 females. The coefficient of the variance of the repeated readings was 5.6%. Table 3.7 shows that there were no differences between BMI classes in mean flow either supine ($p=0.17$, Kruskal-Wallis) or seated ($p=0.28$), or in the percentage change in flow on changing position ($p=0.33$). Flow tended to decrease slightly on sitting in the normal and overweight groups on paired comparison of normal and overweight but not in the obese group. Since femoral vein diameter increased on sitting, a decrease in flow represents a decrease in flow velocity.

Peak femoral vein flow was estimated from resting diameter and peak flow velocity during application of rapid calf compression in supine and seated positions (Table 3.8). The coefficient of variance of the repeated readings was 3.4%. When supine, peak flow was 15-18 times higher than at rest but there was no difference between the BMI classes ($p=0.21$). On sitting, peak flow increased in all groups but the results were only significant in the overweight ($p=0.028$) and the obese group ($p=0.008$). There were no BMI class differences ($p=0.40$ for peak sitting flow, $p=0.91$ for percentage change in peak flow). The lack of association between BMI and femoral vein flow is confirmed by correlational analysis (Table 3.10).

Femoral mean and peak flows were also correlated against indices of abdominal adiposity and there were no associations in either supine or seated positions (Tables 3.9 & 3.10). The only factor impacting significantly on mean femoral flow was inguinal tissue pressure but this was evident only in the supine resting condition. Body composition and abdominal adiposity therefore appear to have little effect on the peak femoral flow as determined by ultrasound of

the femoral vein in either the seated or supine position or the change that occurs in the flow when a volunteer moves from the supine to seated position.

		Supine mean flow ml/min	Seated mean flow ml/min	Percentage Change in Mean flow Supine to Sitting
	n	Mean (SD)	Mean (SD)	Mean (SD)
BMI 20-25 normal	6	152.5 (96.4)	96.1 (39.8)	-9.8 (59.4)
BMI 25.1-30 overweight	7	212.5 (69.2)	152.2 (66.7)	-25.6 (31.3)
BMI 30+ obese	9	155.1 (82.6)	115.7 (40.8)	8.4 (51.6)

Table 3.7. Femoral vein mean volume flow (mean (SD)) in the supine and seated with legs extended position, and percentage change in flow on moving posture. Values are shown for 22 females and when grouped according to BMI class.

		Supine peak flow ml/min	Seated Peak Flow in ml/min	Percentage Change in Peak Flow Supine to Sitting
	n	Mean (SD)	Mean (SD)	Mean (SD)
BMI 20-25 normal	6	2690.2 (2180.0)	4978.8 (2339.6)	142.0 (89.3)
BMI 25.1-30 overweight	7	3201.4 (1080.7)	7153.0 (2954.6)	140.9 (108.0)
BMI 30+ obese	9	2550.4 (878.6)	5898.9 (2399.1)	151.3 (120.8)

Table 3.8. Femoral vein peak flow (mean (SD)) in the supine and seated with legs extended position, and percentage change in flow on moving posture. Values are shown for 22 females and when grouped according to BMI class.

	n	Supine Femoral Vein Mean Flow ml/min		Seated Femoral Vein Mean Flow ml/min		Percentage change from Supine to Seated	
		ρ	p	ρ	p	ρ	p
Body Mass Index (BMI) Kg/m ²	23	-0.62	0.80	0.30	0.18	0.18	0.43
Supine Sagittal-Abdominal diameter cm (SAD)	22	-0.006	0.98	0.23	0.31	0.21	0.36
DEXA percentage Truncal Fat	9	0.286	0.45	0.21	0.60	-0.25	0.54
Waist-Hip Ratio	23	-0.22	0.31	0.15	0.52	0.18	0.42
Sacro-Abdominal/Sacro-Popliteal index (SA/SP)	23	n/a	n/a	0.61	0.79	0.17	0.46
Ultrasound Abdomen wall subcutaneous tissue depth cm	23	-0.219	0.32	0.22	0.32	0.20	0.37
Supine Inguinal Tissue Pressure mmHg	17	0.57	0.029	n/a	n/a	n/a	n/a
Seated Inguinal Tissue Pressure mmHg	17	n/a	n/a	0.25	0.35	-0.20	0.46
Change in Inguinal Tissue Pressure mmHg	17	n/a	n/a	-0.21	0.42	0.67	0.80

Table 3.9. Correlational analysis of femoral vein mean flow supine and seated and percentage change in mean flow with BMI, indices of abdominal adiposity (SAD, % truncal fat, W-H ratio, SA/SP index, ultrasound abdominal tissue thickness) and inguinal tissue pressures (supine, seated and change on moving).

Values shown are Spearman's ρ and significance.

	Seated Femoral Vein Peak Flow ml/min		Seated Femoral Vein Peak Flow ml/min		Percentage change from Supine to Seated		
	n	ρ	p	ρ	p	ρ	p
Body Mass Index (BMI) Kg/m ²	23	-0.14	0.95	0.10	0.83	0.36	0.67
Sacro-Abdominal/Sacro-Popliteal index (SA/SP)	23	n/a	n/a	-0.04	0.85	-0.70	0.75
Waist-Hip Ratio	23	-0.34	0.88	0.13	0.58	-0.53	0.81
Supine Sagittal-Abdominal diameter cm (SAD)	22	0.17	0.44	0.06	0.80	-0.17	0.45
DEXA percentage Truncal Fat	9	-0.17	0.66	-0.14	0.73	0.25	0.54
Ultrasound Abdomen wall subcutaneous tissue depth cm	23	0.007	0.97	0.08	0.74	-0.11	0.64
Height cm	23	n/a	n/a	0.03	0.89	0.29	0.19
Supine Inguinal Tissue Pressure mmHg	17	0.22	0.40	n/a	n/a	n/a	n/a
Seated Inguinal Tissue Pressure mmHg	17	n/a	n/a	0.24	0.37	-0.86	0.75
Change in Inguinal Tissue Pressure mmHg	17	n/a	n/a	0.22	0.41	-0.25	0.36

Table 3.10. Correlational analysis of femoral vein peak flow supine and seated and percentage change in mean flow with BMI, indices of abdominal adiposity (SAD, % truncal fat, W-H ratio, SA/SP index, ultrasound abdominal tissue thickness) and inguinal tissue pressures (supine, seated and change on moving).

Values shown are Spearman's ρ and significance.

3.2.4 Tissue Durometry

From the previous sections, it has been shown that inguinal tissue pressure when seated is higher if individuals have a larger BMI and greater abdominal adiposity. Likewise, femoral vein CSA increases more on sitting with greater abdominal adiposity, but is also positively affected by size of the thigh and calf. On the other hand, femoral vein flow when seated is not influenced by BMI, abdominal adiposity or inguinal tissue pressure. Although flow in this conduit vein is not affected, the pressure effects of abdominal obesity may act upon venous microcirculation, possibly leading to fluid filtration into tissues at the thigh and causing tissue hardness. To investigate this, the novel technique of durometry was used. A hand-held device is allowed to rest under its own weight on the skin surface and provide a reading of indentation load or hardness. Tissue durometry has been shown to detect the increase in tissue hardness for patients with systemic sclerosis (Kissin et al. 2006).

Skin hardness may be influenced by skin thickness as well as elasticity and oedema. Previous studies reported a correlation between durometer readings and skin thickness measured by ultrasound in healthy controls and patients with systemic sclerosis in regions of the upper limb – hands, forearm, upper arm and validated in this condition by Kissin et al. (2006). To assess the impact of skin thickness on durometer readings in the present study population with high BMI and obesity, these two variables were compared in the supine position after twenty minutes rest and in the seated position after 5 min. The dominant thigh and calf in 23 females were examined. There was no correlation between durometry values (mean of the two readings) and ultrasound tissue thickness at either the thigh ($\rho=0.14$, $p=0.54$) or calf ($\rho=0.30$, $p=0.17$).

The coefficient of variance for repeated readings was 4.4% over both thigh and calf. No significant difference (increase) was demonstrated between the supine and seated thigh tissue

durometry readings in all three BMI groups (normal $p=0.22$, overweight $p=0.66$, obese $p=0.062$). In all three BMI groups there was a significant difference between the calf supine and seated tissue value with increase in values across the groups (normal $p=0.04$, overweight $p=0.01$, obese $p=0.006$).

	n	Change in Thigh Durometry Supine to Seated (units)	Change in Calf Durometry Supine to Seated (units)
		Mean (SD)	Mean (SD)
BMI 20-25 normal	6	2.0 (2.3)	3.0 (1.2)
BMI 25.1-30 overweight	7	2.2 (1.7)	3.6 (2.5)
BMI 30+ Obese	10	1.9 (2.7)	3.7 (4.8)

Table 3.11. Changes in skin hardness measured by durometry at thigh and calf on moving from supine to seated in 26 females.

Table 3.11 gives values for the change in durometry readings on moving from supine to sitting for all participants and according to BMI class. There was no significant difference between the different BMI classes when compared against each other using a Kruskal Wallis test (thigh $p=0.84$ and calf $p=0.71$).

The lack of effect of BMI on change in skin hardness with seating was also evident in correlational analysis (figure 3.11). Whereas the increase in inguinal tissue pressure was greater with larger BMI (as described in Section 3.2.1 above), there was no such relationship for the thigh ($\rho=0.05$, $p=0.85$) or calf ($\rho=0.29$, $p=0.22$). Neither did the durometry change correlate with seated inguinal pressure (thigh $p=0.88$; calf $p=0.86$), increase in inguinal tissue pressure on sitting (thigh $p=0.21$; calf $p=0.08$) or SA/SP (thigh $p=0.89$; calf $p=0.12$).

There is insufficient data to say that the BMI or the SA/SP ratio can explain the changes in lower limb tissue durometry. Height, being a major contributor to the lower limb orthostatic pressure demonstrated no correlation.

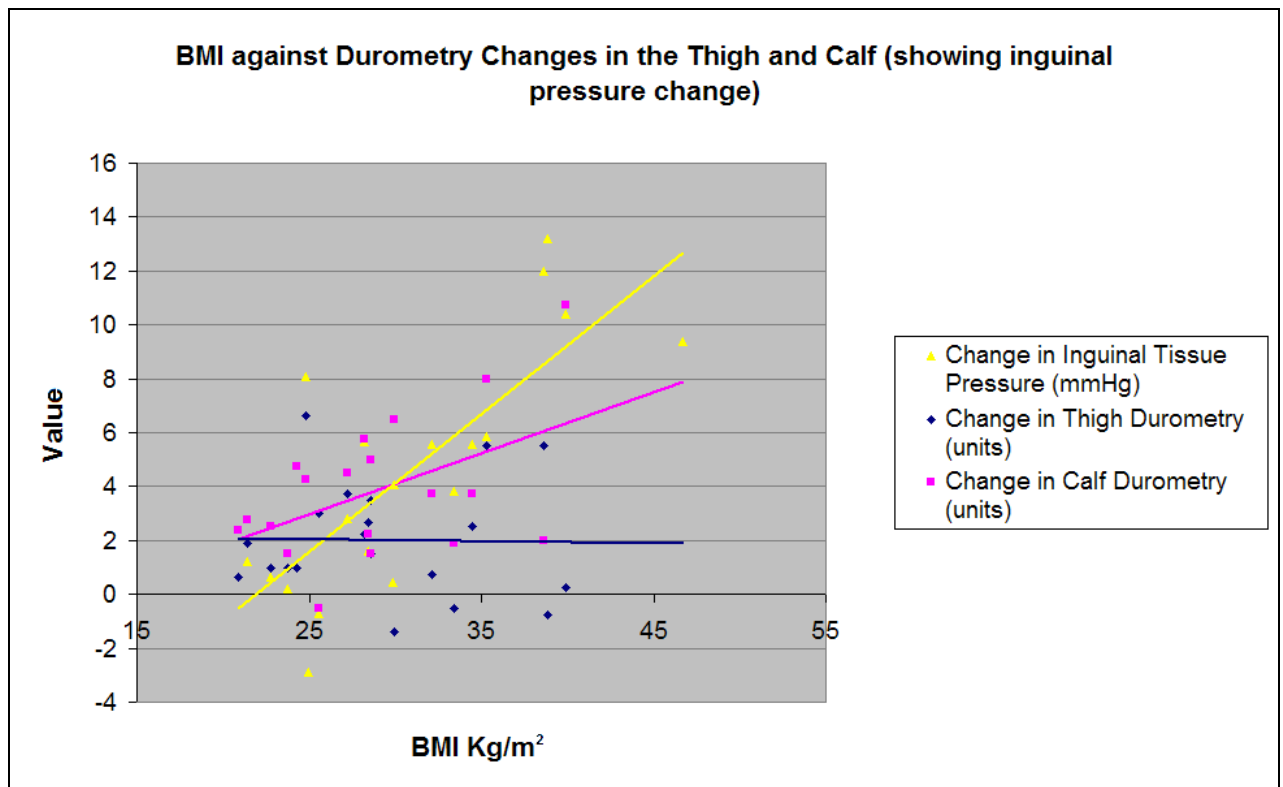


Figure 3.10. Scatter plot of the correlation between the change in skin hardness on moving from supine to seated, measured by durometry in thigh and calf against BMI in 23 females. Also included for comparison are data on the increase in inguinal tissue pressure on sitting.

3.3. EFFECT OF BODY COMPOSITION ON LOWER LEG VENOUS PROPERTIES (WHOLE LIMB CAPACITY, WHOLE LIMB AND VEIN SIZE)

The previous sections have demonstrated that body composition and in particular, abdominal adiposity, are related to femoral vein size but not to volume flow within the vein either supine or sitting. Adipose tissue as a percentage of the thigh is greater than that of the calf. This could mean that the support for the veins offered by the lean tissue of the limb would be less in the thigh compared to the calf. Described below are the studies evaluating venous function of the lower leg using a combination of strain gauge plethysmography (SGP) and ultrasound (US). With SGP and venous occlusion protocols, calf blood flow, venous vascular volume (capacity) and capillary filtration coefficient were determined and related to body size (BMI) and abdominal adiposity. Ultrasound was used evaluate changes in the diameter of the GSV and the Popliteal vein.

3.3.1 Calf blood flow

Calf blood flow values are shown in Table 3.12 and there was no difference between the BMI classes ($p=0.23$). There are several factors that can influence resting calf blood flow including limb size, anatomical vascular capacity and resistance vessel tone. Correlational analysis was carried out between calf blood flow and indices of body size, abdominal adiposity and calf size and composition. Although blood flow was not associated independently with age or height, it was clearly positively related to body size through BMI (Table 3.12). It also correlated with the SA/SP index with higher flow values linked to bigger abdominal adiposity. The impact of body fat on calf blood flow was further shown by significant correlations with thigh and calf CSA but not with lean CSA for these limb segments (Table 3.13). Further analysis of other independent variables is shown in section 3.5.

	n	Calf blood flow Mean (SD) ml/100ml/min
BMI 20-25 normal	7	1.70 (0.37)
BMI 25.1-30 overweight	9	1.98 (0.73)
BMI 30+ Obese	10	2.33 (0.72)

Table 3.12. Mean (SD) of calf blood flow for 26 females as a group and according to BMI class.

	Dominant Calf Mean Blood Flow		
	n	ρ	p value
Age years	26	0.28	0.18
Height cm	26	-0.16	0.43
BMI kg/m ²	26	0.46	0.02
SA/SP	26	0.52	0.01
Mean Arterial Blood pressure mmHg	24	0.13	0.53
Dominant Calf Cross Sectional Area cm ²	25	0.46	0.02
Dominant Calf Lean Cross Sectional Area cm ²	25	0.26	0.21
Dominant Calf Lean Cross Sectional Area as a percentage of whole calf CSA	22	-0.04	0.84
Dominant Thigh Cross Sectional Area cm ²	22	0.58	0.01
Dominant Thigh Lean Cross Sectional Area cm ²	22	0.16	0.45
Dominant Thigh Lean Cross Sectional Area as a percentage of whole thigh CSA	22	-0.62	<0.005

Table 3.13. Correlational analysis of resting calf blood flow with physical characteristics relating to body size and composition.

3.3.2 Venous Capacity

		Calf venous capacity after 2 min ml/100ml	Maximum calf capacity after 5 min ml/100ml
	n	Mean (SD)	Mean (SD)
BMI 20-25 normal	7	2.09 (0.69)	3.34 (1.16)
BMI 25.1-30 overweight	9	2.81 (0.81)	3.59 (1.08)
BMI 30+ Obese	10	2.32 (0.37)	2.62 (0.56)

Table 3.14 Mean (SD) calf venous capacity measured as the increase in limb volume either after 2 min of venous occlusion (rapid vascular filling phase) or after 5 min of venous occlusion (vascular filling + fluid filtration) for 26 females as a group and according to BMI class.

Venous capacity after 2 min and, more noticeably, the increase in calf volume after 5 min (capacity + fluid filtration) were different between the BMI classes ($p=0.08$ and $p=0.02$ respectively) with higher values in the overweight group than the normal or obese groups (Table 3.14). No correlation was noted between the indices of body composition and the venous filling capacity measured at 2 min. Venous capacity has been shown to be dependent on anatomical limb size, but no correlation was noted between the indices of body composition and the venous volume measured at 2 min. The effects of female hormones on venous capacity will be presented in section 3.5.

However, significant correlations were noted between indices of body composition and venous volume after 5 min. The correlation between this and BMI ($\rho=-0.617$, $p=0.002$) is shown in the figure 3.11, and correlations with SA/SP ($\rho=-0.516$, $p=0.010$), SAD ($\rho=-0.464$, $p=0.230$), ultrasound abdominal wall thickness ($\rho=-0.401$, $p=0.045$) and dominant thigh cross sectional area ($\rho=-0.605$, $p=0.006$) were also demonstrated. Since calf volume changes after

venous occlusion represents fluid filtration, once filled capacity is reached (2 min), this component was analysed separately.

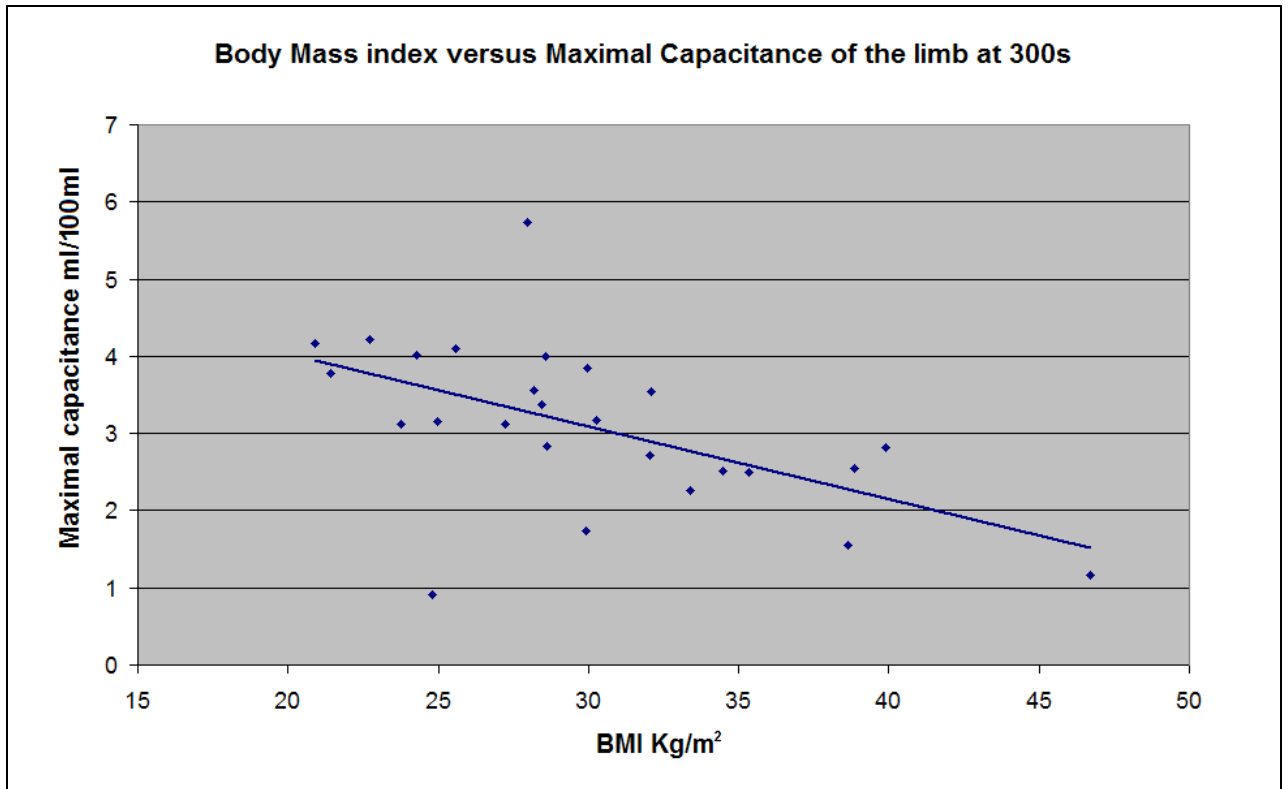


Figure 3.11 Body mass index versus the maximum limb capacity after 5 min of venous occlusion (vascular filling + fluid filtration) for 26 females.

3.3.3 Microvascular Filtration Capacity

Fluid filtration (Kf) measured from calf volume increases between 3-5 min after venous occlusion was associated with higher BMI and adiposity. Mean data for the BMI classes are shown in Table 3.15 and there was a significant difference between the three groups ($p < 0.05$). Filtration in overweight and obese individuals was lower than that of normal BMI participants. It was inversely correlated with BMI and with other indices of abdominal adiposity as shown in Table 3.16. Calf filtration is a product of permeability and

microvascular surface area for fluid exchange the effect of the latter is shown by the significant correlation of Kf with dominant calf total and lean CSA (Table 3.16). Limb size is therefore a factor in determining filtration capacity.

The protocol for venous occlusion by small incremental increases in thigh cuff pressure (see Methods section 2.2.3) allows an estimate of calf venous pressure (P_{vest}) to be obtained by extrapolating the volume-pressure relationship at each pressure step to zero volume change. The values of P_{vest} for the normal, overweight and obese BMI groups (Table 3.15) did not differ significantly (p=0.36) although in the overweight and obese group, pressures were double those in the normal BMI subjects. Another parameter derived from small step increases in venous occluding pressure is P_{vi}, the isovolumetric venous pressure at which point the hydrostatic and oncotic Starling forces are in balance and there is no net fluid filtration or absorption. These values (Table 3.15) were also similar in the three BMI groups (p=0.65), indicating no effect of body composition on this variable.

	Calf Fluid Filtration Kf ml/100ml/min/mmHg		P _{vi} mmHg		P _{vest}	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
BMI 20-25 normal	7	0.0031 (0.0012)	3	16.3 (9.2)	4	6.0 (4.9)
BMI 25.1-30 overweight	9	0.0015 (0.0013)	7	18.8 (6.8)	5	15.0 (9.9)
BMI 30+ Obese	8	0.0012 (0.0010)	8	17.3 (8.3)	9	12.2 (8.5)

Table 3.15 Mean (SD) calf fluid filtration, isovolumetric pressure (P_{vi}) and estimated venous pressure (P_{vest}) according to BMI class in 26 females.

	Capillary Filtration Kf (filtration) ml/100ml/min		
	n	ρ	p
BMI Kg/m ²	24	-0.55	0.005
Supine Sagittal- Abdominal diameter cm (SAD)	23	-0.442	0.038
SA/SP ratio	24	-0.514	0.014
Ultrasound Abdomen wall subcutaneous tissue depth cm	24	-0.412	0.048
DEXA percentage Truncal Fat	8	0.119	0.753
Waist-Hip Ratio	24	-0.079	0.706
Dominant Calf Cross Sectional Area cm ²	24	-0.434	0.041
Dominant Calf Lean Cross Sectional Area cm ²	24	-0.369	0.084
Dominant Thigh Cross Sectional Area cm ²	24	-0.451	0.049
Dominant Lean Thigh Cross Sectional Area cm ²	24	0.180	0.432

Table 3.16. Correlational analysis of calf fluid filtration against body composition and indices of abdominal adiposity

Significant negative correlation was found between the measures of adiposity and the measures of fluid filtration in the leg. Pvest measures of venous pressure increased with adiposity but did not reach levels of significance.

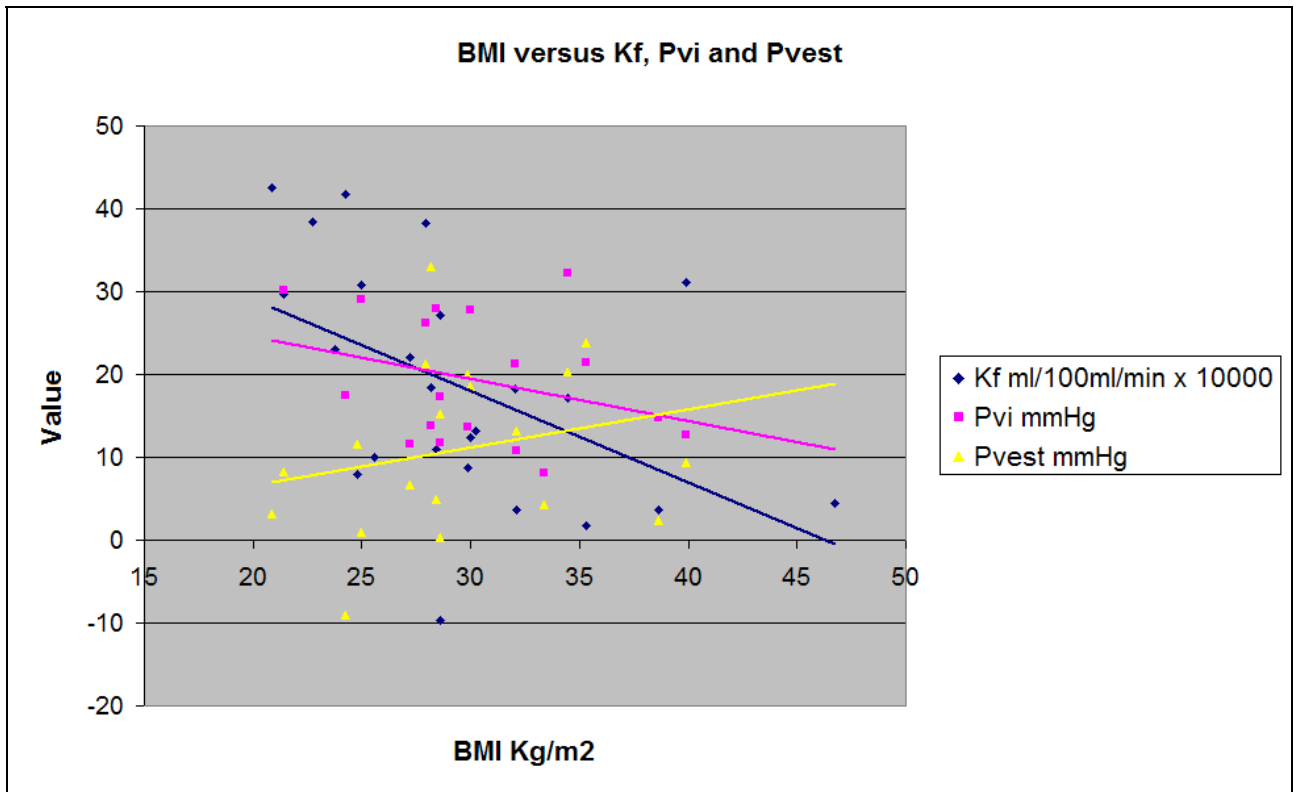


Figure 3.12. Scatter plot of correlation between calf filtration capacity and Pvi for 25 females.

3.3.4 Lower limb Vein Parameters.

The previous section has shown similar blood flow but impaired fluid filtration in the calves of overweight and obese females. Venous capacity determined from calf volume change during venous occlusion and rapid vascular filling was not different between BMI classes. On the other hand the femoral vein was distended to a larger CSA on sitting in obese and overweight than normal BMI individuals. Since plethysmographic change in calf volume represents filling of both large and small venous vessels, ultrasound was used to examine individual large veins of the lower leg and their properties during distension and emptying. Maximal filled diameters of the great saphenous vein (GSV) and popliteal vein were imaged by ultrasound after venous occlusion for 5 min. Mean (SD) diameters for the whole group were 3.8 (1.0) for the GSV and 9.3 (3.1) for the popliteal vein in a total of 13 subjects. Figure

3.13 illustrates maximal vein diameters separated by BMI group. Although the obese group tended to have large maximal popliteal diameters, there were no overall differences between the BMI classes ($p=0.56$). However, the GSV showed a graded increase in size with BMI and a significant difference between groups ($p=0.03$).

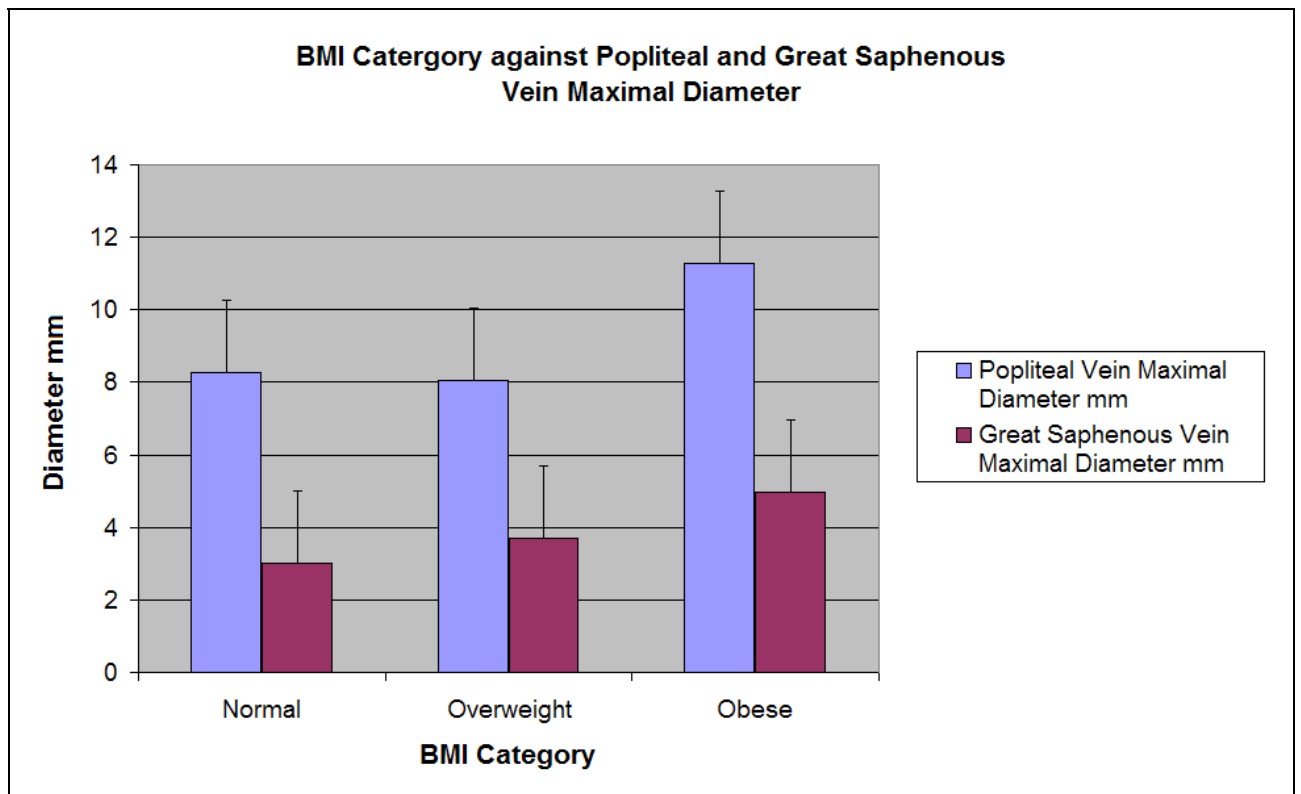


Figure 3.13. Maximal diameters (means \pm SD) of the popliteal and great saphenous veins during venous distension by thigh cuff inflation in BMI class groups (normal $n = 3$ and 5 for popliteal and GSV respectively, overweight $n = 5$ and 4 , obese $n = 5$ and 4).

The size of the popliteal vein was not significantly correlated with BMI ($\rho = 0.16$, $p=0.59$). The relationship is shown in figure 3.14. The popliteal vein was not related to calf size (calf whole CSA $\rho = 0.04$, $p=0.89$; calf lean CSA $\rho = 0.06$, $p=0.84$) or indices of abdominal

adiposity (SAD, W-H ratio, % truncal fat, SA/SP index, ultrasound abdominal thickness, thigh CSA).

In contrast, the GSV size was positively related to BMI ($\rho = 0.74$, $p=0.01$) shown in figure 3.14, and to leg size through both calf ($\rho = 0.70$, $p<0.02$) and thigh CSA ($\rho = 0.69$, $p<0.05$). It was also correlated with indices of abdominal adiposity, SAD ($\rho = 0.61$, $p<0.05$), SA/SP ($\rho = 0.76$, $p<0.01$) and ultrasound abdominal fat ($\rho = 0.51$, $p<0.07$) but not with % truncal fat or waist-hip ratio. Further analysis of independent variables oestrogen, OCP, glucose, insulin and HOMA2 is shown in section 3.5.

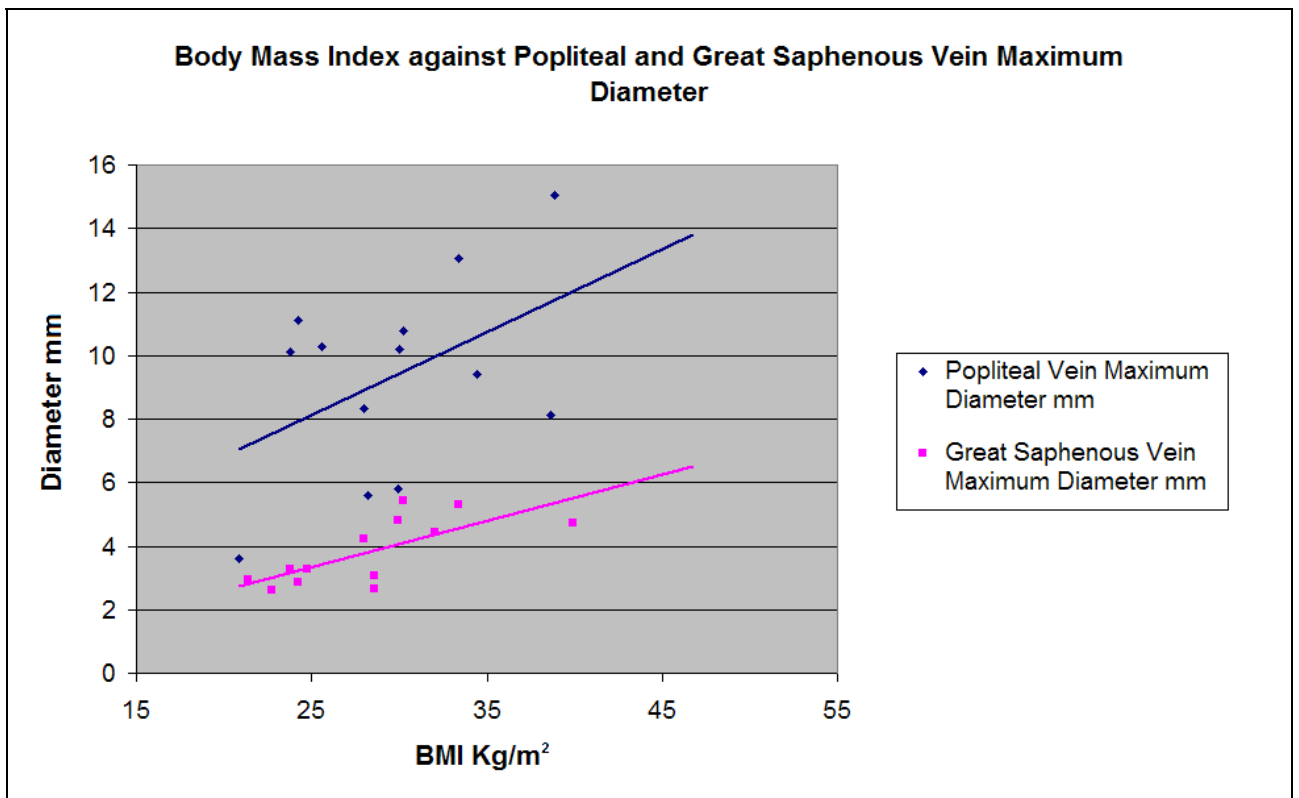


Figure 3.14 Body mass index plotted against Popliteal and Great Saphenous Vein Maximal Diameter.

3.4. EFFECT OF BODY COMPOSITION ON LOWER LEG VENOUS COMPLIANCE

Venous compliance has been assessed in two ways – one by measuring calf volume plethysmographically as a surrogate for venous vascular volume, and the other by imaging individual calf veins using ultrasound. With both approaches, compliance was evaluated during emptying of the filled venous system so that the derived volume / diameter – pressure curves represented the passive elastic recoil properties of the veins. The fitting of polynomial regression lines is described in the methods section 2.2.3.

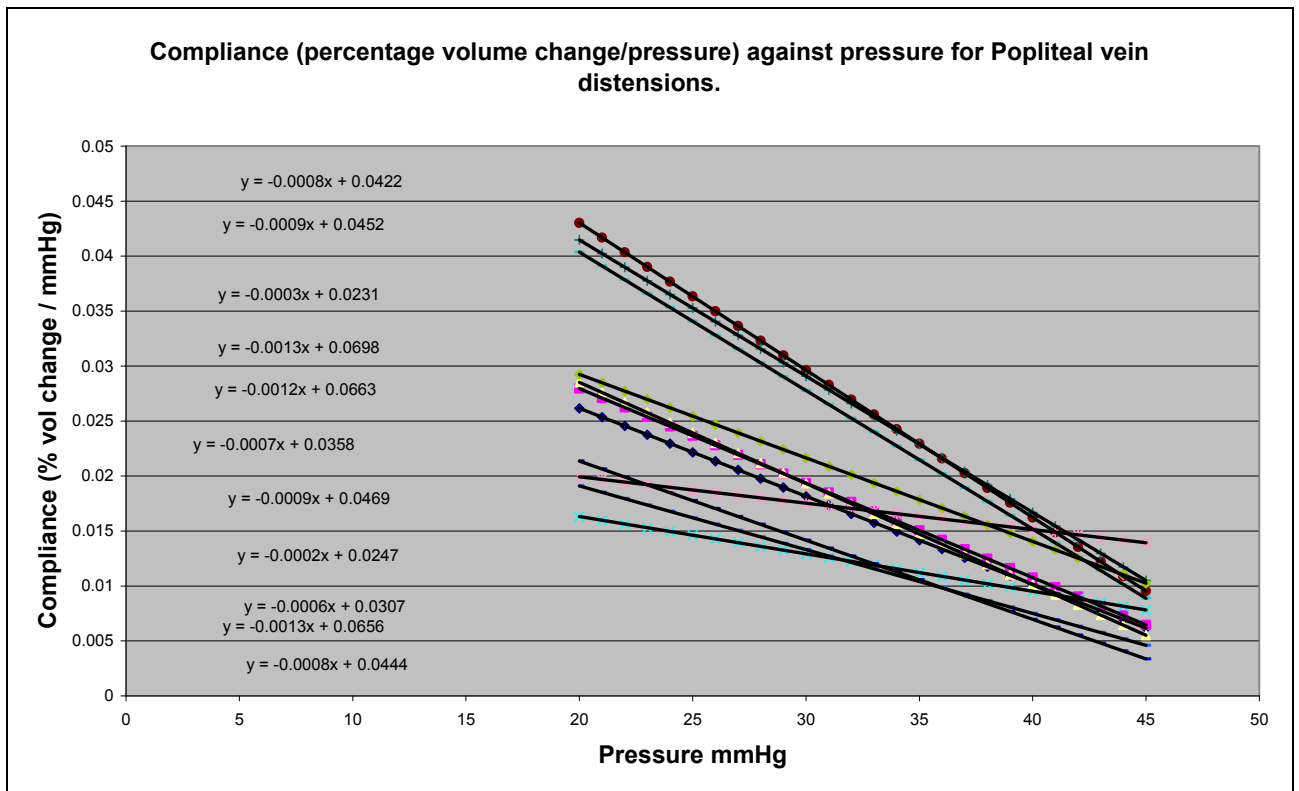


Figure 3.15. Plots of compliance of the whole calf, derived from diameter-pressure curves during emptying, against pressure for 14 female subjects.

	Whole Limb Compliance ml/100ml/mmHg/mmHg		Popliteal Vein Compliance in mm/mmHg/mmHg		Great Saphenous Vein Compliance in mm/mmHg/mmHg	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
BMI 20-25 normal	7	-0.0013 (0.0012)	4	-0.0022 (0.0019)	5	-0.00148 (0.0005)
BMI 25.1-30 overweight	6	-0.0015 (0.0008)	5	-0.0018 (0.0013)	4	-0.00065 (0.0018)
BMI 30+ Obese	8	-0.0012 (0.0010)	5	-0.00008 (0.0030)	4	-0.00030 (0.0010)

Table 3.17. Venous compliance (means \pm SD) of the whole calf, the popliteal vein and the GSV vein in different BMI groups.

Compliance of the calf measured plethysmographically did not differ with BMI ($p=0.31$, Table 3.18). Calf compliance was unrelated to indices of abdominal adiposity although it tended to be positively associated with larger leg size through calf and thigh CSA (Table 3.19.). In all three BMI groups, the compliance – pressure slopes were similar see figure 3.19. Although there was no overall significant difference between popliteal vein compliance the BMI groups ($p=0.45$) it was similar for the normal and overweight BMI classes but tended to be different in the obese group (Table 3.18). Figure 3.20 illustrates that, based on the group mean slopes and intercepts, popliteal compliance in the obese group did not decrease with increasing pressure, whereas it did in the normal and obese groups. Despite this difference, compliance of the popliteal vein did not significantly correlate with any measure of body size, body composition, abdominal adiposity or leg size (Table 3.19).

Similarly, GSV veins tended to show a decrease in compliance with increasing BMI (Table 3.18), but because of variability in this measure, the difference was not quite significant

between groups ($p=0.08$). For the obese group, GSV compliance plotted against pressure showed no decrease with increasing pressure compared to the other two groups shown in figure 3.21. The slope of the GSV compliance – pressure plot was positively correlated with BMI, was linked with abdominal adiposity through SAD and SA/SP index and was strongly associated with larger leg size (Table 3.19.). There was thus a mismatch between venous compliance determined for the calf and for the popliteal vein, which were not related to body size, composition or adiposity, and compliance of the GSV vein which was associated with all these factors and with leg size.

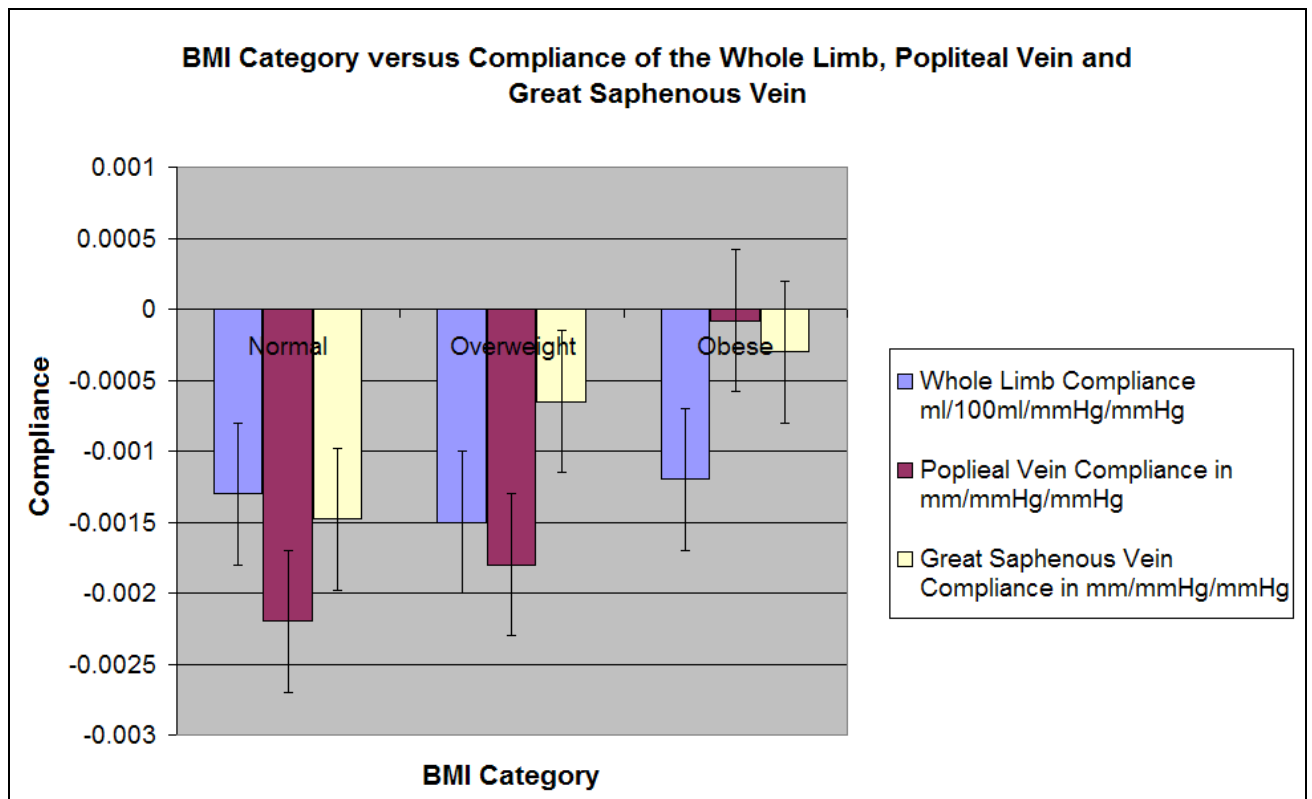


Figure 3.16. Bar chart depicting the compliance (means \pm SE) of the whole calf, the popliteal vein and the GSV vein in different BMI groups.

	n (Calf/Pop/ GSV)	Calf Compliance ml/100ml/mmHg/m mHg		Popliteal Vein Compliance in mm/mmHg/mmHg		GSV Compliance in mm/mmHg/mm Hg	
		ρ	P	ρ	P	ρ	P
BMI Kg/m ²	21/14/14	0.192	0.402	0.342	0.236	0.603	0.037
Age adjusted BMI	13/9/9	-0.122	0.685	-0.065	0.863	-0.167	0.659
SAD cm	20/13/14	-0.078	0.741	0.453	0.133	0.672	0.020
W-H ratio	21/14/14	0.346	0.131	-0.191	0.508	-0.291	0.313
SA/SP	21/14/14	-0.030	0.897	0.284	0.325	0.618	0.032
Ultrasound Abdominal wall thickness cm	21/14/14	-0.065	0.777	0.166	0.565	0.218	0.449
Truncal % fat measured by DEXA	8/5/6	0.170	0.678	0.400	0.488	0.600	0.230
Dominant calf CSA cm ²	21/14/14	0.401	0.080	0.304	0.293	0.580	0.045
Dominant lean Calf CSA cm ²	21/14/14	0.210	0.360	0.265	0.358	0.790	0.006
Dominant lean Calf CSA %	21/14/13	0.007	0.975	-0.036	0.896	0.023	0.936
Dominant Thigh CSA cm ²	19/9/13	0.425	0.080	0.606	0.069	0.715	0.018
Dominant Thigh Lean CSA cm ²	19/9/13	0.460	0.058	0.300	0.368	0.337	0.263
Dominant Thigh Lean CSA %	19/13/12	-0.137	0.560	-0.325	0.304	-0.383	0.204

Table 3.18. Correlational analysis of calf venous, popliteal and GSV vein compliance with body size and composition and other factors in females.

No correlation was noted between calf compliance and femoral vein cross-sectional area or inguinal tissue pressure. No correlation was noted between popliteal compliance and femoral vein cross-sectional area. No relationship was noted between GSV compliance and maximum CSA of the femoral vein, percentage change in the CSA of the femoral vein or change in inguinal tissue pressure.

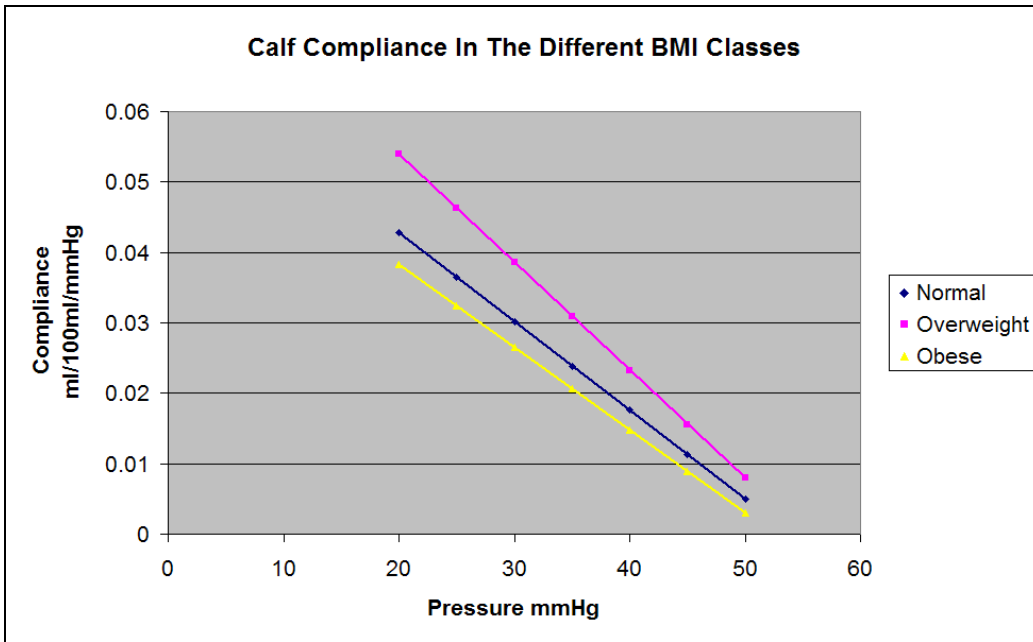


Figure 3.17. Compliance of the calf over a range of pressures for the three BMI classes.

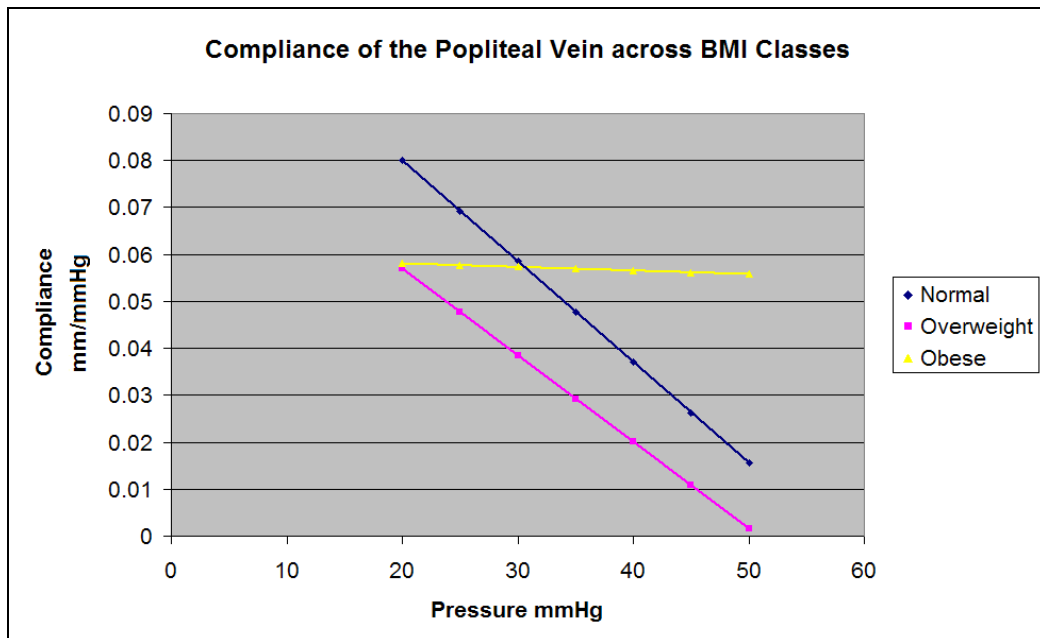


Figure 3.18. Compliance of the popliteal vein over a range of pressures for the three BMI classes.

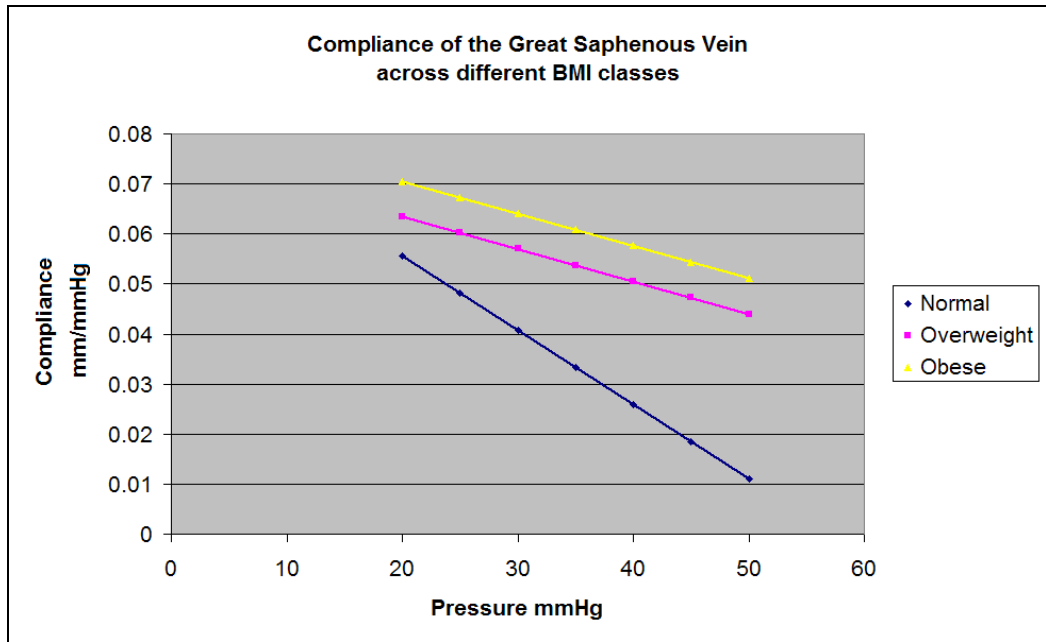


Figure 3.19. Compliance of the Great Saphenous Vein over a range of pressures for the three BMI classes.

3.5 OESTROGEN, ORAL CONTRACEPTIVE PILL, FULL TERM PREGNANCIES, HISTORICAL BMI, PHYSICAL ACTIVITY GLUCOSE, INSULIN AND HOMA2 INDEX

Epidemiological data has been used to try to explain the causative factors of venous disease. Several areas in the literature have highlighted investigation into these areas via various methods but using people with known disease process. This section looks at the various variables believed to have an effect on formation of venous disease in a population without any definable disease in an attempt to identify any patterns.

3.5.1 Blood Oestrogen levels and Oral Contraceptive Pill Usage

Table 3.20. shows no difference between the BMI groups in their level of blood oestrogen ($p=0.54$) or the length of oral contraceptive usage ($p=0.4474$). There was no significant correlation between BMI ($\rho= 0.013$, $p=0.97$) or SA/SP index ($\rho= 0.04$, $p=0.86$) and oestrogen levels. All females who completed the questionnaire in the study had used the oral contraceptive pill. There was no significant correlation between BMI ($\rho= -0.08$, $p=0.71$) or SA/SP index ($\rho= 0.02$, $p=0.91$) and oral contraceptive pill usage. Exposure to either endogenous or exogenous female reproductive hormones was thus independent of body size and abdominal adiposity.

	Blood Oestrogen pmol/l		Oral contraceptive pill usage (months)	
	n	Mean (SD)	n	Mean (SD)
BMI 20-25 normal	4	162.8 (48.5)	5	93.6 (76.6)
BMI 25.1-30 overweight	7	187.1 (167.0)	7	125.1 (61.6)
BMI 30+ obese	7	151.7 (68.5)	9	87.0 (70.0)

3.19. Blood oestrogen levels at the time of investigation and self reported use of the oral contraceptive pill.

	Blood Oestrogen Levels pmol/l		
	n	ρ	P
BMI Kg/m ²	18	0.100	0.967
SA/SP	18	0.042	0.862
Femoral vein diameter supine (mm)	18	-0.026	0.913
Femoral vein diameter seated (mm)	18	-0.084	0.737
% change femoral vein diameter from supine to seated	18	-0.133	0.595
% change in femoral vein mean flow from supine to seated	19	0.242	0.365
% change in femoral vein peak flow from supine to seated	19	0.313	0.241
Blood Flow ml/100ml/min	20	0.080	0.740
Capacitance at 120 seconds ml/100ml	18	0.317	0.179
Capacitance at 300 seconds ml/100ml	18	0.392	0.106
Kf fluid filtration ml/100ml/min	18	0.471	0.070
Pvi mmHg	17	0.124	0.642
Pvest mmHg	15	0.324	0.261
Popliteal vein maximum filled diameter (mm)	10	0.327	0.386
GSV maximum filled diameter (mm)	11	0.083	0.813
Whole limb compliance	12	0.015	0.963
Popliteal vein Compliance mm/mmHg/mmHg	12	0.092	0.795
Great Saphenous Vein compliance mm/mmHg/mmHg	12	0.015	0.964

Table 3.20. Spearman's rank correlation (ρ) co-efficient analysis of blood oestrogen levels against measures of venous function.

There were no demonstrable correlations between the blood oestrogen level at the time of investigation and the measured variables of venous function in the study (Table 3.21).

	Oral contraceptive pill usage (month)		
	n	ρ	p
BMI Kg/m ²	21	-0.082	0.713
SA/SP	21	0.024	0.914
Femoral vein diameter supine (mm)	19	-0.152	0.518
Femoral vein diameter seated (mm)	18	-0.178	0.464
% change femoral vein diameter from supine to seated	18	-0.041	0.867
% change in femoral vein mean flow from supine to seated	19	-0.301	0.260
% change in femoral vein peak flow from supine to seated	19	0.349	0.192
Blood Flow ml/100ml/min	21	0.150	0.490
Capacitance at 120 seconds ml/100ml	21	0.153	0.493
Capacitance at 300 seconds ml/100ml	21	0.175	0.435
Kf fluid filtration ml/100ml/min	19	0.166	0.481
Pvi mmHg	17	-0.058	0.553
Pvest mmHg	15	-0.547	0.582
Popliteal vein maximum filled diameter (mm)	12	0.070	0.817
GSV maximum filled diameter (mm)	10	0.418	0.210
Whole limb compliance	17	-0.099	0.711
Popliteal vein Compliance mm/mmHg/mmHg	17	0.194	0.561
Great Saphenous Vein compliance mm/mmHg/mmHg	17	0.129	0.715

Table 3.21. ρ analysis of oral contraceptive pill usage against measures of venous function.

There were no demonstrable correlations between oral contraceptive pill usage in the volunteer population and the measured variables of venous function in the study (Table 3.22). Thus, exposure to either endogenous oestrogen or exogenous female hormones did not impact upon any of the measured venous properties.

3.5.2 Pregnancy and Historical Body Mass Index

To investigate the impact of previous the number of full term pregnancies and the effect of previous body weight history the following data was collected. (Table 3.23).

	Full term Pregnancies number		Historical BMI Kg/m ²	
	n	Mean (SD)	N	Mean (SD)
BMI 20-25 normal	5	2.0 (0)	4	24.1 (4.8)
BMI 25.1-30 overweight	7	0.9 (1.2)	5	27.2 (3.6)
BMI 30+ obese	9	1.4 (1.0)	9	30.5 (4.1)

Table 3.22. Number of full term pregnancies and self reported historical BMI of the volunteer population.

There was no difference between the different BMI groups in the number of full term pregnancies ($p=0.213$). The historical BMI was significant in its correlation to present BMI ($p=0.003$) but not related to the SA/SP ratio. Demonstrated in the two tables below 3.24 and 3.25 are the Spearmans' rank correlation coefficients of the number of full term pregnancies and Historical BMI against the variables of venous function measured in the study. No significant correlations could be demonstrated.

	Full term Pregnancies number		
	N	ρ	P
BMI Kg/m ²	21	-0.009	0.969
SA/SP	21	-0.094	0.674
Femoral vein diameter supine (mm)	19	0.042	0.859
Femoral vein diameter seated (mm)	18	0.053	0.827
% change femoral vein diameter from supine to seated	18	0.135	0.579
% change in femoral vein mean flow from supine to seated	19	0.177	0.515
% change in femoral vein peak flow from supine to seated	19	0.346	0.196
Blood Flow ml/100ml/min	21	-0.153	0.493
Capacitance at 120 seconds ml/100ml	21	-0.204	0.361
Capacitance at 300 seconds ml/100ml	21	-0.175	0.435
Kf fluid filtration ml/100ml/min	19	0.160	0.945
Pvi mmHg	17	-0.018	0.949
Pvest mmHg	15	-0.089	0.757
Popliteal vein maximum filled diameter (mm)	11	0.093	0.768
GSV maximum filled diameter (mm)	10	0.076	0.820
Whole limb compliance	17	0.315	0.238
Popliteal vein Compliance mm/mmHg/mmHg	17	0.397	0.234
Great Saphenous Vein compliance mm/mmHg/mmHg	17	0.500	0.157

Table 3.23 ρ analysis of number of full term pregnancies against measures of venous function.

	Historical BMI Kg/m ²		
	N	ρ	p
BMI Kg/m ²	18	0.713	0.003
SA/SP	18	0.611	0.012
Femoral vein diameter supine (mm)	16	-0.038	0.880
Femoral vein diameter seated (mm)	15	0.204	0.446
% change femoral vein diameter from supine to seated	15	0.257	0.336
% change in femoral vein mean flow from supine to seated	17	0.181	0.536
% change in femoral vein peak flow from supine to seated	17	-0.214	0.458
Blood Flow ml/100ml/min	18	0.318	0.189
Capacitance at 120 seconds ml/100ml	18	0.086	0.722
Capacitance at 300 seconds ml/100ml	18	-0.065	0.789
Kf fluid filtration ml/100ml/min	16	-0.176	0.481
Pvi mmHg	16	-0.437	0.115
Pvest mmHg	13	0.077	0.790
Popliteal vein maximum filled diameter (mm)	9	0.524	0.166
GSV maximum filled diameter (mm)	9	0.167	0.659
Whole limb compliance	13	-0.076	0.794
Popliteal vein Compliance mm/mmHg/mmHg	9	0.070	0.840
Great Saphenous Vein compliance mm/mmHg/mmHg	8	-0.167	0.659

Table 3.24 ρ analysis of historical BMI against measures of venous function.

3.5.3 Physical Activity

The level of physical activity which the volunteers undertook was assessed by the means of a seven day recall questionnaire (Washburn et al. 2003)

	Physical Activity Kcal/day	
	n	Mean (SD)
BMI 20-25 normal	5	1728.0 (472.5)
BMI 25.1-30 overweight	7	1790.3 (245.0)
BMI 30+ obese	8	1965.8 (532.6)

Table 3.25 Self reported level of physical activity in the volunteer population.

There were no significant correlations identified between the physical activity levels of the volunteers and any measures of body habitus or the assessed measures of venous function in the study.

3.5.4 Fasting Blood Insulin and Glucose levels, and HOMA2 index

The HOMA2 data for the whole study group showed a normal distribution. Kruskal wallis testing showed no difference between the different BMI groups in the level of blood glucose (p=0.17), level of blood insulin (p=0.25). However there was a significant difference between the three BMI groups when the HOMA2 index was calculated on the volunteer population (p=0.023).

	Glucose mmol/l		Insulin μ IU/ml		HOMA2 index	
	n	Mean (SD)	n	Mean (SD)	N	Mean (SD)
BMI 20-25 normal	4	4.6 (0.2)	6	5.8 (3.4)	4	0.5 (0.2)
BMI 25.1-30 overweight	6	4.7 (0.5)	7	9.2 (7.0)	5	1.3 (0.9)
BMI 30+ obese	8	5.0 (0.4)	9	12.9 (9.4)	8	1.8 (1.2)

Table 3.26 Fasting blood glucose and insulin levels of the study volunteer population and the calculated HOMA2 Index.

Fasting blood glucose levels did not correlate with the indices of venous function that were analysed in the study except for a significant correlation to GSV maximum filled diameter ($\rho=0.929$, $p=0.014$) and tended towards significance with the compliance of the GSV compliance measures. The implications of this are considered in the discussion.

The co-efficient of variation of the two readings of fasting insulin levels was 5.1%. The measurement of fasting blood insulin level did demonstrate any correlation with the measured indices of venous function analysed in the study. The insulin levels were all within the manufactures described normal range. There was significant positive correlation between fasting blood insulin levels and BMI ($\rho=0.446$, $p=0.040$) and SA/SP index ($\rho=0.507$, $p=0.020$) see figure 3.22 below).

	Glucose mmol/l		
	n	ρ	p
BMI Kg/m ²	18	0.375	0.122
SA/SP	18	0.436	0.072
Femoral vein diameter supine (mm)	16	-0.359	0.165
Femoral vein diameter seated (mm)	15	-0.046	0.862
% change femoral vein diameter from supine to seated	15	0.253	0.344
% change in femoral vein mean flow from supine to seated	15	0.010	0.970
% change in femoral vein peak flow from supine to seated	15	-0.246	0.358
Blood Flow ml/100ml/min	16	-0.016	0.947
Capacitance at 120 seconds ml/100ml	18	-0.147	0.544
Capacitance at 300 seconds ml/100ml	18	-0.330	0.174
Kf fluid filtration ml/100ml/min	17	-0.159	0.526
Pvi mmHg	13	-0.073	0.801
Pvest mmHg	13	0.232	0.421
Popliteal vein maximum filled diameter (mm)	10	-0.164	0.624
GSV maximum filled diameter (mm)	8	0.929	0.014
Whole limb compliance	14	-0.066	0.812
Popliteal vein Compliance mm/mmHg/mmHg	10	0.173	0.604
Great Saphenous Vein compliance mm/mmHg/mmHg	8	0.667	0.078

Table 3.27 ρ analysis of fasting blood glucose levels against measures of venous function.

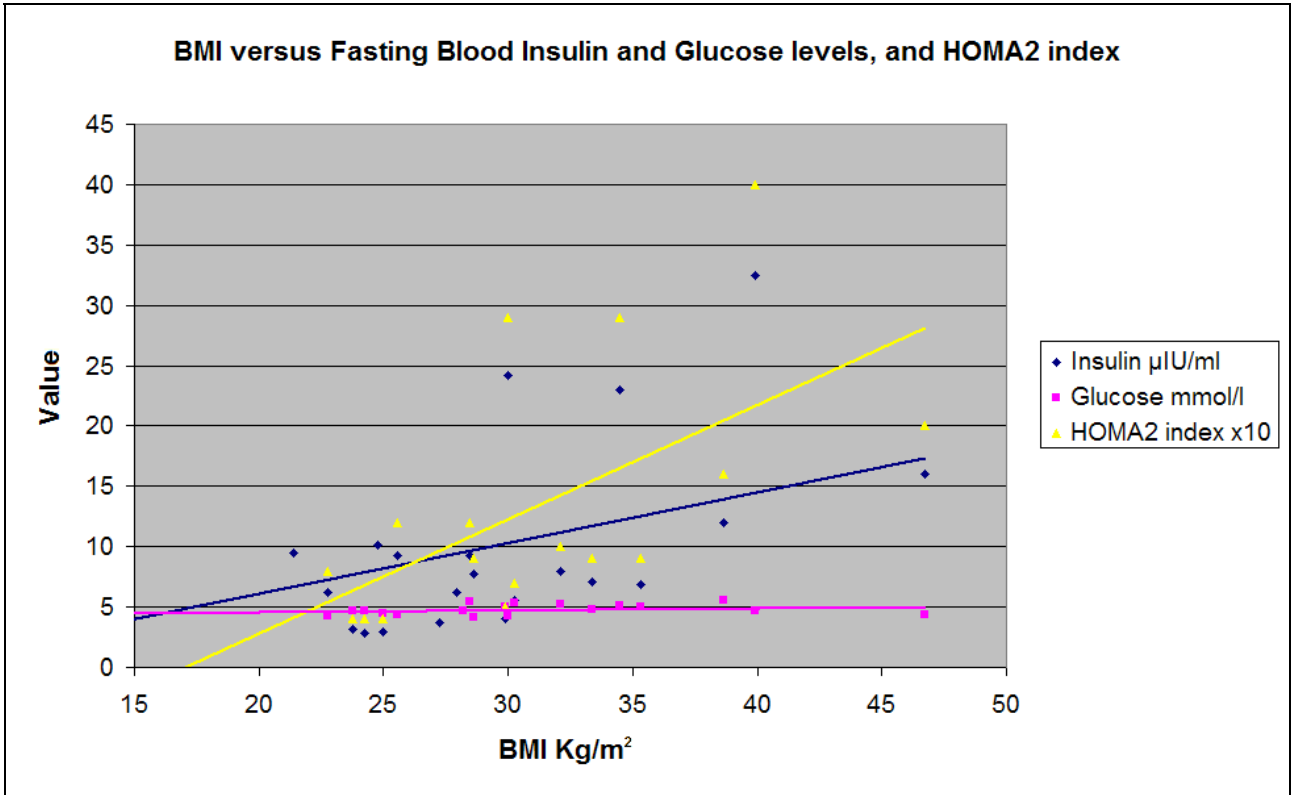


Figure 3.20. The Study volunteers BMI against fasting blood glucose, insulin and their calculated HOMA2 index.

	Insulin $\mu\text{IU/ml}$		
	n	ρ	p
BMI Kg/m^2	22	0.446	0.040
SA/SP	22	0.507	0.020
Femoral vein diameter supine (mm)	20	-0.406	0.077
Femoral vein diameter seated (mm)	19	0.136	0.564
% change femoral vein diameter from supine to seated	19	0.431	0.067
% change in femoral vein mean flow from supine to seated	19	0.275	0.243
% change in femoral vein peak flow from supine to seated	19	0.214	0.365
Blood Flow ml/100ml/min	22	0.388	0.757
Capacitance at 120 seconds ml/100ml	22	-0.049	0.822
Capacitance at 300 seconds ml/100ml	22	-0.300	0.169
Kf fluid filtration ml/100ml/min	21	-0.336	0.133
Pvi mmHg	17	0.255	0.308
Pvest mmHg	16	0.091	0.723
Popliteal vein maximum filled diameter (mm)	10	-0.152	0.650
GSV maximum filled diameter (mm)	13	0.070	0.808
Whole limb compliance	18	0.198	0.414
Popliteal vein Compliance mm/mmHg/mmHg	10	0.152	0.649
Great Saphenous Vein compliance mm/mmHg/mmHg	13	0.070	0.808

Table 3.28. ρ analysis of fasting blood insulin levels against measures of venous function.

HOMA2 index demonstrated a significant correlation with BMI ($\rho=0.681$, $p= 0.006$) and SA/SP ($\rho=0.632$, $p=0.011$) as would be expected. Only two variables could be demonstrated to have a significant correlation with the HOMA2 index, the percentage change in the CSA of the FV on moving from supine to seated ($\rho=0.698$, $p=0.012$) and calf blood flow ($\rho =0.607$, $p=0.015$) (see figure 3.23).

	HOMA2 index		
	n	ρ	P
BMI Kg/m ²	9	0.681	0.006
SA/SP	9	0.632	0.011
Femoral vein diameter supine (mm)	15	0.504	0.060
Femoral vein diameter seated (mm)	14	0.401	0.145
% change femoral vein diameter from supine to seated	12	0.698	0.012
% change in femoral vein mean flow from supine to seated	14	0.379	0.172
% change in femoral vein peak flow from supine to seated	14	0.311	0.262
Blood Flow ml/100ml/min	17	0.607	0.015
Capacitance at 120 seconds ml/100ml	17	0.194	0.439
Capacitance at 300 seconds ml/100ml	17	-0.197	0.430
Kf fluid filtration ml/100ml/min	16	-0.218	0.398
Pvi mmHg	12	0.028	0.926
Pvest mmHg	12	0.416	0.168
Popliteal vein maximum filled diameter (mm)	9	0.040	0.890
GSV maximum filled diameter (mm)	8	0.060	0.875
Whole limb compliance	13	0.040	0.890
Popliteal vein Compliance mm/mmHg/mmHg	10	0.648	0.052
Great Saphenous Vein compliance mm/mmHg/mmHg	8	0.506	0.181

Table.3.29. ρ analysis of the calculated HOMA2 index against measures of venous function.

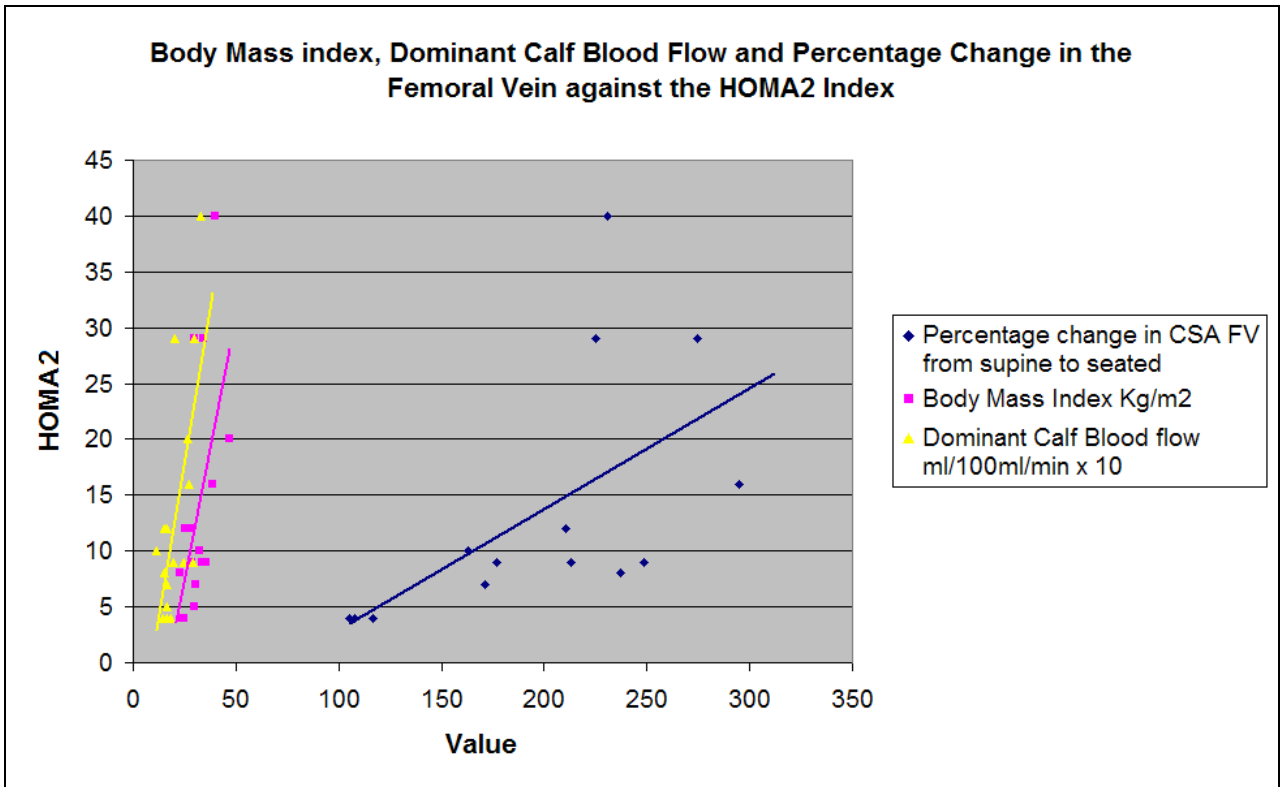


Figure 3.21. Body Mass Index, the percentage change in femoral vein cross sectional area and dominant calf blood flow against the HOMA2 index of study volunteers.

The analysis of fasting blood glucose and insulin levels and the HOMA2 index has yielded results that suggest that glucose may affect the distensibility and compliance of the GSV. Insulin levels relate to the level of obesity and this may be a summative factor in the effects of obesity on venous function. HOMA2 index and insulin resistance may give rise to a change in the distensibility of the FV and this may in part be due to an increase in inflow.

CHAPTER 4:

DISCUSSION

Venous disease is a major contributor to the burden of health care provision. An understanding of the risk factors that cause venous disease has been attempted through epidemiological studies. On the basis that venous hypertension makes a major contribution to venous wall and valvular damage in the lower limbs, resulting in the classic signs of venous disease, skin changes and ulceration, factors likely to exacerbate raised venous pressure have been of particular interest. The evidence for an association between increased body size (height, weight, BMI) and incidence of venous disease has been derived from large population-based studies where the subjects already exhibit overt venous disease to varying degrees. However, since the effects of venous hypertension must accrue over time before chronic disease is diagnosed, it is not yet known if changes in venous structure and function prior to this are also associated with body size and obesity. Aside from a large BMI and high levels of body fat, there is also evidence that fat specifically in the abdominal region could contribute to venous hypertension and disease particularly when in the sitting position through mechanical compression in the abdominal compartment and restriction of venous return. In addition high body fat levels might be associated with hormonal changes that affect the vein wall.

The aim of the work presented in this thesis was to observe a normal middle-aged healthy female population and determine if risk factors previously identified have any association with venous properties in the absence of venous disease. Using a combination of ultrasound imaging and plethysmographic techniques, specific measures were made of lower limb venous function to ascertain the impact of a sitting position on FV size and flow, calf venous volume, and GSV and popliteal vein size and compliance. These were related to indices of

body size and composition, and to descriptive measures of abdominal obesity so that associations could be determined. The study comprised relatively small numbers of volunteers, which reduced the power of statistical comparisons. The key findings summarised below must take this into account before conclusions can be drawn:

- A new measure of abdominal adiposity when seated, the SA/SP ratio, was devised to assess mechanical impediment to venous return from the legs. It correlated well with BMI, the most common proxy for human body fat, and with other measures of subcutaneous abdominal fat (SAD, ultrasound thickness) but less well with % truncal fat by DEXA scan. Since the latter includes visceral as well as subcutaneous fat, SA/SP more closely reflects the presence of subcutaneous as opposed to visceral abdominal adipose tissue.
- In the sitting position, the size of the distended FV was positively associated with increasing obesity, described by BMI. Furthermore, the greater the measure of abdominal fat, the larger the increase in FV size and the bigger the reduction in vein flow velocity when sitting. FV distension on sitting also correlated with thigh and calf cross sectional areas and this relationship was dependent on the presence of subcutaneous fat.
- Inguinal tissue pressure on sitting also increased to higher values as abdominal adiposity increased. There was no evidence, however, that this presented impediment to femoral venous outflow because the increase in vein size compensated for the reduced velocity such that volume flow was not affected. The increase in thigh tissue hardness on sitting was also unrelated to body size or abdominal fat levels in this small sample size.

- Lower leg filled venous capacity determined by calf plethysmography – a surrogate measure of venous vascular volume – and popliteal vein size measured by ultrasound were not related to BMI, abdominal adiposity or leg size. However, the cross sectional area of the GSV was significantly positively correlated with all three measures of body composition.
- The compliance of the popliteal vein showed no significant differences when compared against measures of BMI and abdominal obesity although there was a tendency for it to be raised in obese individuals. GSV compliance, on the other hand, did show significant correlation with measures of abdominal adiposity and body size but the sample size was too small to detect differences between BMI classes. These data suggest that there may be some changes to vein wall elasticity in obesity that are consistent with alterations in wall structure allowing for greater dilatation.
- In contrast, compliance of the calf venous circulation was not associated with BMI or the presence of abdominal fat indicating possible heterogeneity of changes in large versus smaller vessels or results from differing testing methodology.
- Contrary to expectation, there was no association between lower leg venous distensibility and circulating levels of oestrogen or progesterone or with duration of oral contraceptive use. Neither was it associated with the number of full term pregnancies in the female volunteers studied or with body weight history, possibly reflecting paucity of data collection in this area.
- Physical activity assessed by recall questionnaire showed no correlation to obesity or to the measures of venous function taken in the study.
- Metabolic status as assessed by HOMA2 index did not correlate with an increase in leg vein size on positional movement. No relationship was noted with venous

compliance or calf venous capacity. Positive significant associations were noted between increasing abdominal adiposity, increasing body size and with higher calf blood flow, the latter most likely due to elevated insulin levels in the obese individuals.

4.1 STUDY POPULATION

Since the EVS (Evans et al. 1999) identified incidence of venous disease to be more prevalent in those aged greater than 55 (men 25.3% and women 12.3%) than under 55 (men 2.5-7.7% and women 3.8-7.9%), this study aimed to investigate healthy men and women aged between 30-50 years without venous disease. The choice of a healthy population was so that the impact of abdominal obesity *per se* on leg venous haemodynamics could be examined before the pathological changes known to occur in the diseased vein wall are established. Since the completion of work in this thesis, two new studies have confirmed an obstructive effect of abdominal obesity on femoral vein flow in healthy individuals (Willenberg et al. 2010, 2011). Despite the perception that women are more prone to venous disease than men, the EVS reported a higher incidence of clinical disease in men as shown above. During subject recruitment for the current study, it became apparent that far fewer men were volunteering for screening (6 males and 54 females). The reason for this was most likely the method of recruitment, which involved travelling to slimming clubs, advertisement on the intranet of UHB, and recruitment from the hospital wards/theatre staff. With such small numbers of males there was a lack of statistical power to make any association within this group and it was therefore decided to analyse only the population of 26 females.

The numbers required for the study in normal weight, overweight and obese BMI categories were based on power calculations using published data on femoral vein flow velocity (Fromy

et al. 1997) and with totals of 7, 9 and 10 in these groups, fell well short of the estimated 30 per group. Accordingly, the study did not reach sufficient power for statistically significant results to be detected.

For the 26 women included in the final analysis, CEAP classification was 0 for 54% and 1 for 46% of the volunteers. These values are lower than the level expected for the age range as reported in the EVS for the prevalence of varicose veins in a similar population (see data in paragraph above) but the recruitment information did stress that volunteers were needed who did not have varicose veins or known venous disease. With this low incidence of venous symptoms, the study population can be classified as essentially healthy and free from venous disease. The exclusion criteria applied by history-taking and bedside testing prevented entry into the study of volunteers with co-morbidities (known to the volunteer or identified) likely to affect the venous and vascular function. The fact that all blood tests performed in the study reported values within laboratory normal limits reinforces the study population as healthy. BMI ranged from 21 to 47 across all three BMI matched groups (7 normal, 9 overweight, 10 obese). Biggest variation in BMI was in the obese group, as shown by the standard deviation, mainly due to one individual of very high BMI. Even without this subject, the BMI categories were statistically distinguishable so as to enable comparisons between them.

Since the study population was entirely female and pre-menopausal, hormonal status and parity had to be considered as possible influences on venous function. Raised oestrogen and progesterone during the menstrual cycle, OCP use or pregnancy have been shown to increase venous distensibility (Goodrich & Wood 1966, Barwin & Roddie, 1976, Walter & Sheilds, 1977, Edouard et al. 1998). In order to control for this, all measurements were made during the menstrual phase of the normal cycle, which corresponds to the time of lowest levels of

endogenous hormones (Hirshoren et al. 2002), and to the ‘withdrawal phase’ of OCP use when serum concentrations of the active ingredients ethinylloestradiol and levonorgestrel are also lowest (Endrikat et al. 2002). Plasma levels of oestrogen and progesterone in the study population were within the normal ranges for this period of the menstrual cycle with no differences between BMI groups. The relationship between female hormonal status and venous compliance is more complicated with no effect of menstrual cycle or OCP use on lower leg compliance (Meendering et al. 2005) but an increased venous tone together with decreased compliance identified in rats with multiple pregnancies (Dhawan et al. 2005). The incidence of varicose veins has been associated with the number of full-term pregnancies (Jukkola et al. 2006) but the current study population displayed no differences between the BMI classes for the number of pregnancies or the length of use of the OCP even though the latter covered a large range from 3-240 months. Overall, it can be concluded that the participants in the study were comparable with regard to hormonal status.

Physical activity levels were assessed using the Stanford 7 day Physical Activity Recall Questionnaire (Washburn et al. 2003). The validity of self-report for assessment of energy expenditure has been debated by Westerterp et al. (2008), with questionnaires showing relatively low agreement with the gold-standard doubly labeled water method. However, Westerterp stated that recall questionnaires are reliable when compared against each other and across various weight ranges and are therefore suitable as an activity-ranking instrument for group comparisons. Daily energy expenditure – around 1800 kcal/day – was slightly lower than levels reported for moderately overweight young women (2633 kcal/day; Washburn et al. 2003) but higher than in obese women before they undertook a weight loss program (1145 kcal/day; Racette et al. 1995). Such variation is due in part to the need for aggressive follow-up and persistent questioning in order to gain an accurate picture of the physical activity

levels in all recall studies. There were, however, no differences noted between the levels of physical activity among the BMI groups in the current study and hence this was not a confounding factor in group comparisons.

With respect to metabolic status, glucose levels in the study population fell within the normal fasting range (4-6.8mmol/l) as recommended by Diabetes UK and although there was an increase across the BMI groups this was not significant. Fasting plasma insulin levels in the study population also lay within the range described in the literature (Vogeser et al. 2007). Insulin resistance status (IR) used to be determined by HOMA1 model calculation but more recently has been redefined by the HOMA2 (Levy et al. 1998) as this shows better correlation to gold-standard physiological tests of insulin sensitivity and β -cell function i.e. glycaemic clamp or fasting glucose testing. The calculated HOMA2 levels in the current study are reflective of normal ranges for a healthy population based on published values (Stepniakowski & Egan 1995, Vogeser et al. 2007) and the online calculator as described in methods section 2.2.5. A recent article by Geloneze et al. (2009) on a Brazilian study (BRAMS) reported a cut-off point for defining insulin resistance at a HOMA2 value of >1.8 in healthy individuals. In the present study higher values of HOMA2 were correlated with increasing BMI, and with a mean of 1.8 in the obese group, this may be reflective of early stage insulin resistance in these individuals. The increase across the range of BMI was felt to suggest a possible difference across the study population. It was felt that analysis in the study was warranted and not just used as an establishment of volunteer normality.

4.2 ESTABLISHMENT OF THE DISTRIBUTION OF ABDOMINAL ADIPOSITY.

Obesity is a relatively permanent characteristic as described by Seidell et al. (1986). BMI is a universal measure of overall body fat applicable to adults but there are other methods which specifically evaluate fat distribution in the abdominal region. Of these, the SAD is a measure of 'supine abdominal height' that reflects obesity. It has been shown to predict insulin resistance and cardiovascular mortality (Després et al. 2007) with better precision than computed tomography (CT) of abdominal compartment fat (Guzzaloni et al. 2009). SAD measures also correlate with regional-specific DEXA scanning of fat (Bertin et al. 2000) whereas waist-hip ratio shows least agreement with a criterion measure of total abdominal fat by CT (Clasey et al. 1999). This is borne out by the present data which showed distinction between BMI groups in SAD and % truncal fat (DEXA) but not W-H ratio. The measurement of abdominal adiposity is important as previously research has demonstrated that this can be linked to an increase risk of hypertension, cardiovascular disease and the development of non-insulin dependent diabetes, reviewed by Haffner (2007).

Although SAD and % truncal fat agreed well with BMI in the study population, both these indices of abdominal fat were measured in the supine position and therefore do not indicate the effects of abdominal adiposity in presenting mechanical obstruction to venous outflow from the legs in a sitting position. To address this, a novel method of assessing abdominal adiposity was devised, namely the sacro-abdominal/sacro-popliteal (SA/SP) index. This specifically set out to evaluate the degree of 'overhang' of abdominal fat along the legs in the seated position. SA/SP values showed good correlation with BMI, SAD and % truncal fat, supporting the notion that the new seated index defines abdominal obesity as such. This is confirmed because the correlation of SA/SP with BMI was only through weight, not height,

and SA/SP was unrelated to W-H ratio, which incorporates hip size that does not contribute to the former.

While the SA/SP index was derived to assess abdominal fat that might contribute to mechanical impairment of venous return from the lower leg, adiposity in the abdominal region is not confined to the subcutaneous compartment. There is also a significant amount of intra-abdominal visceral fat (Snijder et al. 2006) that could also play a role in obstruction of venous flow. To separate out these two compartments, ultrasound imaging of abdominal subcutaneous fat thickness was undertaken, and values correlated highly with SAD and SA/SP index, which both detect subcutaneous abdominal wall adiposity albeit in supine and seated positions respectively. Association with % truncal fat was less good, consistent with the fact that the latter includes both subcutaneous and visceral fat. The ultrasound abdominal thicknesses determined in the present female study population were larger than those reported for women of similar age by Murakami et al. (1997) (26.8 mm compared to 21.1 mm); however, their population was of a smaller BMI range (21.8 - 23.25 kg/m²) than group of the same age.

To validate the ultrasound method for this study, abdominal thickness was compared against measurements obtained using the traditional skinfold caliper technique. Values correlated highly, as shown in other studies (Black et al. 1988, Demura & Sato, 2007). There was, however, a persistently lower reading (by 0.63 cm) for ultrasound than calipers, a systematic error that has been noted previously in abdominal (Demura & Sato 2007) and calf (Weits et al. 1986) fat measurements. This occurs possibly because imaging measures thickness at a single plane through the tissue whereas the skinfold encompasses a greater tissue sample area, averaging out regional variations. Studies have reported closer agreement between caliper measurements with the criterion measure computed tomography (CT) than ultrasound,

particularly at the abdominal region (Orphanidou et al. 1994, Stevens-Simon et al. 2001, Gradmark et al. 2010). Despite this, ultrasound values were better able than calipers to predict body density in a population of young men (Weits et al. 1986), and there was no evidence from Bland-Altman analysis in the present study that the systematic error in abdominal fat thickness was altered in obesity.

To further validate ultrasound against calipers and provide information on peripheral limb obesity, subcutaneous fat thicknesses at the thigh and calf were recorded using both methods. These are not the classical measuring points over which skin calipers are used i.e. triceps or lower end of scapulae. Over the legs, the tissue is not as lax as the points above and it was anticipated that the calipers would have difficulty obtaining accurate readings as they require a “good” pinch of skin in order for readings to be made. Indeed, this was found to be the case as the difference between ultrasound and caliper values was greater for thigh and calf than for the abdomen, and became more pronounced with increasing tissue thickness consistent with the increase in skin tightness on moving from trunk to calf. In future the author expects opinion of Fomage et al. (1993) to be the norm; i.e. that high resolution ultrasound is used for assessment of subcutaneous tissue depth due to its ease of use and flexibility for use in all areas of the body and the contrary to CT does not involve radiation.

4.3 LEG VEIN SIZE AND CALF VENOUS CAPACITY IN RELATION TO BODY DIMENSION AND ADIPOSITY

The maximum filled size of leg veins - femoral, popliteal and GSV - was determined by ultrasound, and calf venous vascular volume – capacity of entire venous circulation - by plethysmography in order to investigate their relationship to body size and composition.

4.3.1 Vein Size

Ultrasound imaging has become the technique of choice for investigation of large superficial and deep veins of the lower limb (Cavezzi et al. 2006). Diagnostic scanning of leg veins is normally carried out in the standing position since this allows for venous filling and observation of reflux if present. In the laboratory, many tests of venous characteristics are carried out supine, and under this condition veins do not necessarily have a circular profile due to incomplete filling. Studies examining vein size therefore adopt procedures that induce filling to maximum size, such as Valsalva's manoeuvre or head-up or –down tilting. In this study the seated position was used when imaging the FV, and a venous congesting cuff for the popliteal, GSV and calf volume. Vein CSAs were calculated from intra luminal diameter measures.

In the study population of healthy females aged 34-49 years, seated FV maximum diameters were slightly larger (mean 9.6 mm) than those reported for healthy volunteers of mixed gender and all ages by Jeanneret et al. (1999) (mean 7.3 mm during a Valsalva manoeuvre) but similar to those of Hertzberg et al. (1997) and Haenen et al. (1999) of 9.2 mm. Likewise, popliteal vein diameter (mean 9.3 mm) corresponded with that of De Groot et al. (2005), mean ~ 10 mm), both during venous congestion., with 8.9 mm measured during sitting by Haenen et al. (1999) and 8.8 mm during Valsalva manoeuvre by Hertzberg et al. (1999). The

GSV diameter (mean 3.8 mm) was also similar to that reported during sitting (mean 4.0 mm, Haenen et al. 1999) and standing (mean 3.2 mm, Seidel et al. 2005). These observations indicate that the protocol for measuring vein size, which involved careful control of the ultrasound probe angle to the vessel for optimal imaging, and the edge-detection VIA software that measured wall-to-wall distance, yielded appropriate data on the maximum filled diameter. The measurement locations were also standardized along the limb to minimize variations because vein size decreases by about 11 mm from the proximal common femoral to the distal tibial vessels (Haenen et al. 1999, Hertzberg et al. 1999).

Maximal FV and GSV size in the present study correlated significantly with BMI across the three groups. An association between vein size and body dimensions has been described previously in normal weight healthy subjects (Mortensen et al. 1990) with bigger diameters of both FV and GSV when BMI was $> 22.5 \text{ kg.m}^2$ (Jeanneret et al. 1999). Kröger et al. (2003) also showed that FV and GSV CSA on standing were graded with BMI from slim (<20) to obese (>30) in a mixed age and gender group but with CEAP classification of zero. Even in patients of CEAP 5-6, FV diameters on standing were larger in those weighing $> 90 \text{ kg}$ (mean 13.3 mm) than those weighing $< 90 \text{ kg}$ (mean 10.2 mm) (Van Rij et al. 2008). The scaling of vein size with BMI in the present study was through the effect of body weight because there was no correlation of FV or GSV CSA, either supine or seated, with height, and femoral size was unrelated to other indices of body size such as wrist or ankle circumference. By contrast, popliteal vein dimensions were not significantly associated with body size, although showing a slight tendency to increase with BMI. Moreover, whereas both FV and GSV showed correlation of their maximal size with leg size through thigh and calf CSA, there was no such correlation for the popliteal vein. Before considering the reasons for this difference, the impact of abdominal and peripheral obesity on vein size will be discussed.

It was hypothesized that fat in the abdominal region could impede venous outflow from the legs when seated by mechanical hindrance, and that this would cause greater distending pressure to be transmitted to the leg veins. A significant difference in FV CSA was noted in all BMI groups on moving from the supine to seated position. It increased 1.6-fold in normal BMI subjects, 1.9-fold in overweight, and 2.3-fold in obese individuals. All measures of abdominal adiposity (SA/SP, SAD, % truncal fat, ultrasound abdominal wall thickness) except W-H ratio (positive but not significant) demonstrated positive correlation with percentage change in the FV CSA. This does not, however, clarify whether larger vein size is related simply to body weight or specifically to ‘overhang’ and compression from abdominal adiposity. When standing, body weight is one of the main determinants of foot vein pressures (Kügler et al. 2001) and higher venous pressures were recorded on standing in obese (> 90 kg, 110 mmHg) than normal weight (< 90 kg, 93 mmHg) individuals (Van Rij et al. 2008). Although in the latter study the majority of subjects already had CEAP class 5 disease, of interest is that foot vein pressure increased on moving from lying to sitting with a straight back, similar to the postural move employed in the present study, and that the increase was greater in the obese (68 mmHg) than in the normal weight subjects (59 mmHg). Moreover, when subjects were sitting but leaning forwards 45°, foot vein pressure increased a further 2 mmHg in the normal weight subjects but by 8 mmHg in the obese (Van Rij et al. 2008). This implies that mechanical obstruction by abdominal fat when seated can present additional impediment to outflow and induce venous hypertension. A similar situation can be envisaged for the GSV since its maximal size was also correlated with SAD, SA/SP and ultrasound abdominal wall thickness but not % truncal fat or W-H ratio. Again, a contradictory finding was that there was no relationship between the size of the popliteal vein and measures of abdominal adiposity.

In seated posture, abdominal fat ‘overhang’ projects along the upper thigh, and the SA/SP index specifically estimated the extent of this. It is possible that peripheral adiposity in the thigh region also contributes to mechanical compression in this position and hence vein sizes were compared with leg dimensions and measures of peripheral fat. The FV CSA was correlated with thigh total size CSA and ultrasound fat thickness but not lean CSA. Greenleaf et al. (2004), in a review of the evidence for deep venous thrombosis in long haul flights/seated position, considered the evidence for thigh compression as a risk factor for limb swelling, decreased venous flow and alteration in lymphatic drainage. Pottier et al. (1969) showed that when only the posterior of the thigh was compressed that there was an increase in foot swelling compared to a normal rested dependent leg, and Olszewski et al. (1977) demonstrated decreased venous flow with thigh compression at level sufficient to induce lymphatic but not venous occlusion. They concluded that the presence of increased pressure on the back of the thigh while seated increases venous and lymphatic congestion and subsequently decreases lymphatic clearance of fluid filtrate, thus facilitating oedema. In the present study, the obese individuals had greater peripheral fat thickness in the thigh and it can be assumed that this contributes to additional compression and FV distension.

In a similar way, the GSV maximum size was significantly correlated with calf and thigh total CSA and with calf and thigh ultrasound fat thickness. These measures were made during venous occlusion rather than the seated position but illustrate that the GSV, which is invested on the outside of the deep compartments of the leg, surrounded by a loose fascia (Caggiati et al. 1999), may also be distended more when there is a higher proportion of soft tissue in the calf. Again, the popliteal vein displayed different characteristics, being unrelated in size to calf or thigh total CSA or calf or thigh ultrasound fat thickness.

Why does the popliteal vein show no association with body size, leg size or abdominal adiposity whereas the FV and GSV do? It is unlikely to be because of the different methods used to induce maximum vein size. FV size was measured in the seated position allowing the hydrostatic pressure in the venous system to fill the FV from the level of the heart. This was acceptable as the position seated with legs straight out is comfortable for short periods. Popliteal vein and GSV size was measured during inflation of a thigh cuff to 50 mmHg in supine subjects. Christ et al. (1997) have shown that the cuff pressure is transmitted to the deep venous system and under ultrasound, it could be clearly seen that there was no flow in GSV once the cuff was applied and minimal or static flow in the popliteal vein. Although measurement conditions were different for the FV and GSV, they were both dependent on body dimensions while the popliteal was not.

Other reasons may lie in the anatomical and haemodynamic connections between the GSV, FV and popliteal vein. The popliteal vein lies deep in the popliteal fossa behind the knee joint and is the confluence of the venae committantes of the anterior and posterior tibial veins and receives venous return from the calf muscle pump via the sural veins (Browse et al. 1998). It lies deep to the artery between the two heads of the gastrocnemius muscle which, in combination, offer it structural support. The GSV, as already stated, lies superficially and is far more easily compressible. One of the ways of identifying this vein for ultrasound imaging is a compression test – light pressure exerted with the transducer probe – which squashes the vein profile to a more elliptical shape whereas arterial vessel profiles are unchanged (Bérczi et al. 2005).

According to Recek, (2000), the normal direction of flow within the calf during ambulatory muscular contraction is from the superficial system (GSV) to the deep (popliteal in this case) veins. In the standing position, pressure in the popliteal vein was equal (80-100mmHg) to that

of the hydrostatic pressure due to gravity. On activation of the calf muscle pump, pressure in the superficial system fell by nearly 40 mmHg while popliteal vein pressure remained constant. This would suggest that the popliteal vein is under longer periods of sustained high pressure in the upright posture while the GSV experiences fluctuating levels. Chronic exposure to high pressure e.g. during arterial grafting induces significant structural remodelling of the GSV (Loesch & Dashwood, 2009, Monos et al. 1995) but the popliteal vein wall seems less susceptible to pressure, in keeping with its resistance to aneurysms (Roche-Nagle et al. 2009). There are no data, to the best of the author's knowledge, that describe the exact histology of the popliteal vein compared to other segments but it is possible that it acts purely as a large conduit. In support of this, it does not respond to interventions that evoke sympathoexcitation (Young et al. 2008, Phillips, 2010) indicating a lack of innervation and ability to regulate vascular tone.

The FV, on the other hand, is a proximal continuation of the popliteal vein and shows the same maintenance of pressure during ambulation as the latter while the superficial lower leg vein pressure lowers because of the muscle pump (Recek, 2000). However, it will also be subject to any mechanical forces arising because of abdominal and / or thigh-related pressures which the popliteal vein does not experience. Whether this may or may not explain why the femoral but not popliteal vein is related to body dimensions will be explored further in a subsequent section. The presence of female hormones or metabolic status cannot explain the disparity between the different veins because circulating factors would impact on all vessels.

4.3.2 Venous Capacity

With regard to calf venous capacity, plethysmography has been the modality of choice for the investigation of vascular function in the limbs due to its wide availability. Venous capacity of the whole limb is the volume of arterial inflow which is required to fill all the venous vessels, estimated from red cell scintigraphy as 10% of total calf volume (Vissing & Nielsen, 1988). The study demonstrated values of 2.39 ml/100ml venous filling at 120 seconds similar to those reported for the calf by Lindenberger et al. (2007) (2.24ml/100ml) and Monahan et al. (2001) (3.0ml/100ml). Although the latter data were obtained in young women while the present study group were approximately 40 years old, there is no decrease in capacity with age in females (Lindenberger & Länne, 2007), and values are therefore as expected.

For the present study, despite the wide range of body size, there was no association of calf venous capacity with BMI. Venous capacity was also unrelated to any measure of abdominal adiposity. It has been reported previously that venous capacity was lower in the obese forearm than in normal weight healthy individuals (Stepniakowski & Egan, 1995) but Ianuzzi et al. (2002) found no association between calf venous volume and BMI in post-menopausal women even though some of them had overt venous disease. Of the calf filled venous volume, only 30 % is due to filling of the major large superficial and deep veins (Cirovic et al. 2006), the rest residing in small veins and venules. Therefore while large veins such as the GSV are scaled in size to body dimensions, it appears that the rest of the venous circulation is not.

Monahan et al. (2004) demonstrated that men with larger calf volumes have a greater venous capacity than women, suggesting an anatomical effect of calf size. However, calf venous capacity did not relate to leg size (calf total or lean CSA or ultrasound fat thickness). The proportions of skin and subcutaneous fat in the calf, based on the calculated lean and total calf CSA, were 43% and 41% in normal weight and overweight groups, but 51% in the obese

group. Although there are differences in the vascularity of these soft tissue components (skin, fat, skeletal muscle) (Elia & Kurpad, 1993) the variation in proportions does not affect venous capacity.

The time taken for venous filling is debated in the literature but is dependent on the venous volume to be filled and the rate of arterial inflow (Hollenberg et al. 1972). Brown et al. (1966) found disparate rates of individual vein filling in the forearm but noted that 120 seconds was sufficient to allow venous circulation to reach capacity under normal conditions, and Halliwell et al. (1999) estimated filling time for the calf also as 120 seconds. This was the time point used in the present study to measure venous capacity. There was a significant positive correlation between BMI and resting calf blood flow, and increasing limb blood flow by heating has been shown to increase the rate of limb volume expansion on venous occlusion (Brown et al. 1966, Jorfeldt et al 2003). Even if blood flow and hence venous filling were more rapid in the obese group, a greater blood flow into a greater limb volume gives an equivalent increase in percentage volume – the units of plethysmographic measurements - to a smaller flow into a smaller limb, and this may account for the similar venous capacities of all BMI groups.

There is a considerable body of evidence demonstrating that filled venous volume in the limbs is greater under the influence of female hormones, oestrogen in particular. For example, Goodrich et al. (1966) demonstrated an increase in venous distensibility with administration of exogenous oestrogen, with oral contraceptive use and during pregnancy. The present study found no association between venous capacity and blood oestrogen levels but this may be because it was designed to minimize variations in oestrogen by testing in the menstrual phase of the cycle and correlation was hence performed over a limited range of values. However, a lack of effect of female hormones is also supported by non-significant correlations of capacity

with OCP use or number of pregnancies. Ciardullo et al. (2000) observed greater calf venous volume to be associated positively with oestrogen levels in post-menopausal women but both parameters were also correlated with the incidence of varicose veins and it is thus not possible to determine cause or effect from this study. The current finding suggests that prior to the development of venous disease, there is no evidence that oestrogen exposure increases calf venous distensibility. A very similar conclusion was drawn for young healthy women by Meendering et al. (2005).

Physical activity levels can also increase venous capacity. Boutcher & Boutcher, (2005) demonstrated larger calf venous capacity in trained athletes than untrained volunteers. In the present study, there was no relationship between daily physical activity derived from the 7 day recall questionnaire and capacity, but the study was not intended to recruit highly trained individuals and the overall low activity levels of the subject cohort may explain why no effect was observed.

4.4. LOWER LEG VEIN AND CALF VENOUS COMPLIANCE IN RELATION TO BODY DIMENSIONS AND ADIPOSITY

As explained in the Introduction, venous compliance defines the relationship between applied pressure and either individual vein size or limb venous volume. Whilst this can be derived during venous filling to indicate distensibility, the measurement of compliance during emptying of a distended full limb/vein in small pressure steps by a controlled deflation protocol allows for the more accurate assessment of the elastic passive recoil properties of the vein without the effects of hysteresis. These can be related to vein wall structure and the balance of the major structural proteins elastin and collagen and smooth muscle tone (Risk et al 2003). Risk mapped an initial rapid filling of the vein under the influence of elastic properties (elastin) when small pressure increases causes large volume changes. This gives way to a slower period of filling beyond a break point when the pressure in the lumen is stretching against the structural proteins (collagen). Understanding the relationship between the two phases enable the investigator to understand balance of the structural proteins that make up the venous wall.

4.4.1 Calf Venous Compliance

The compliance of the whole calf will be discussed first as there are far more published data using the same protocol with which to compare the present findings. Compared to data reported by Halliwill et al. (1999) obtained in young men, the slopes of the calf compliance – pressure relationship of the normal weight group were slightly flatter (slope -0.0013 vs. -0.00204 ml/100ml/mmHg), i.e. compliance was lower especially in the lower pressure range. This may be partly a gender difference because Meendering et al. (2005) found lower compliance slopes in young women than men, and partly due to age difference because

Young et al. (2006) showed lower calf compliance in old than young subjects. In relation to body size, however, no significant difference in calf compliance was found in the present study between the three BMI groups and no correlation observed with any other measure of body size (height, weight, ankle size) or calf size (total or lean). Convertino et al. (1988) reported an inverse relationship between calf compliance and calf cross-sectional area based on computed tomography, suggesting that a large muscle mass would provide structural limitation to expansion of the venous circulation. However, that study measured compliance during venous filling rather than emptying and would therefore be subject to variation because of delayed hysteresis. Moreover, subjects were all men who have a greater proportion of muscle than subcutaneous tissue in the calf than women (Abe et al. 2003).

Calf compliance was also not significantly correlated to the recorded measures of obesity, abdominal or peripheral, or any other factors examined in the study such as female hormones, physical activity levels or metabolic status. Based on plethysmography, there is therefore no evidence for any structural changes in venous properties in relation to obesity that could be taken as indication of subsequent venous disease. It is not surprising that compliance was unrelated to oestrogen levels. Meendering et al. (2005) also found no effect of menstrual cycle phase or OCP use on calf compliance. Dhawan et al. (2005) demonstrated decreased venous compliance with multiple pregnancies in the splanchnic circulation of multiparous rats but this is difficult to extrapolate to human limbs. Hernandez & Franke, (2004) found higher calf compliance in fitter individuals irrespective of age but the present study population showed no difference in levels of physical activity. There was no association of compliance with measures of insulin resistance. Bell et al. 1988 showed a decrease in venous compliance in diabetes of duration greater than 15 years but used different assessment from the deflation protocol used in this study.

4.4.2 Vein Compliance

Previously published data on the compliance of the popliteal and GSV is limited and it is only relatively recently that attempts have been made to measure compliance of individual veins using ultrasound. De Groot et al. (2005) used CSA of the popliteal vein to derive compliance-pressure plots based on quadratic regression coefficients from the original diameter-pressure curves during deflation. This correlated with whole calf plethysmographic compliance in healthy and spinal cord-injured subjects. Young et al. (2008), on the other hand, reported that popliteal vein compliance was higher than, and did not agree with, calf compliance in young healthy individuals. Popliteal compliance-pressure plots for the normal weight group in the present study (Figure 3.20) are similar to those shown by Young et al. (2008) with slopes of -0.0021 and -0.0017 respectively. For the whole study population, popliteal compliance did not correlate with whole calf compliance ($\rho = 0.31$, $p=0.36$) and there was no significant difference between the BMI groups. However, comparison of the plots which are based on group mean values does indicate differences related to body size. The overweight group had a similar slope (-0.0018) to normal weight albeit shifted slightly downward indicating a lower compliance over the whole pressure range. The obese group plot was completely flat demonstrating lower compliance at low pressures and a lack of decrease in compliance as pressure increased, such as would be seen in an infinitely elastic tube.

The author has been unable to locate any previously published data on GSV compliance measured using a comparable deflation protocol using ultrasound. In patients with venous disease Zamboni et al. (1998) compared GSV compliance based on diameter versus pressure plots during postural changes (lying, sitting, standing) with that of vein segments in vitro after stripping. They were able to show that compliance in vivo reduced in relation to severity of CEAP classification, but there are no data from deflation protocols. In the present normal

weight group, GSV compliance slopes (Figure 3.21) were similar to those of the popliteal vein and again were unrelated to whole calf compliance. Although not quite significant between the three BMI groups, it is clear from Figure 3.21 that GSV compliance does alter with increasing body size, showing the same flattening of slope in the overweight and obese group as the popliteal vein. Overall, GSV compliance showed a significant negative correlation with BMI, abdominal adiposity and calf size (total and lean CSA), but not calf fat thickness. It is difficult to say if calf muscle plays a significant role in GSV compliance as the absence of correlation with calf adiposity means interpretation of the remaining tissues is relying on muscle and non-reactive substances.

These changes in individual leg vein passive elastic recoil are notable given that the study group displayed no overt signs of any venous disease. Risk et al. (2003) pointed out that compliance at low pressures is more dependent on elastin and SMC components of a vessel wall while at high pressures, it is determined by collagen content. Reduced venous compliance is recognized as a consequence of established disease such as varicose veins, and the post-thrombotic state (Neglén & Raju, 1995, Molnár et al. 2006). Both elastin and collagen III have been noted to decrease in VV and old age (Venturi et al. 1996, Sansilvestri-Morel et al. 2001) accompanied by an increase in the structural elements of the vein wall provided by collagen I (Sansilvestri-Morel et al. 2001, Gandhi et al. 1993) and disorganization of the structure of the internal and external architecture of the intimal smooth muscle cells (Venturi et al. 1996). The decreasing compliance of the GSV and popliteal vein in this study suggests that similar changes in the vein wall may already be taking place in this asymptomatic obese group, supported by the increase in vein size during maximum distension. This would suggest that the forces or process that has caused the increase of diameter is the same as those causing a decrease in compliance.

Without histological evidence, it is not possible to say which wall elements are most affected. It is also not possible to determine what causes the changes which were associated with body dimensions in one case (GSV) but not in the other (popliteal). The only additional factor that showed a borderline significant relationship to popliteal compliance was the HOMA2 index. This could be explained by the increase in blood flow noted with increasing BMI discussed later as the popliteal vein acts only as a passive conduit to the transfer of blood from the limb.

4.5. EFFECT OF ABDOMINAL ADIPOSITY ON VENOUS OUTFLOW FROM THE LEG

In Section 4.3 above, it was established that FV CSA was similar in the three BMI groups when supine but CSA increased significantly in each group on moving to the seated position with legs straight out. The extent of change was highest in the obese > overweight > normal weight group and if it is assumed that the hydrostatic pressure at the inguinal region from the postural change was similar between groups because standing heights were the same, greater vein size with obesity could be due to i) impedance to outflow due to abdominal fat causing the vein to dilate as an adaptive change, ii) additive compressive effects of thigh tissue, or iii) greater inflow into the vein. To investigate the first of these, the effects of the seated position on inguinal tissue pressure, FV flow, and thigh tissue hardness were studied.

4.5.1 Inguinal Tissue Pressure

One aim of the study was to determine if the ‘overhang’ generated by abdominal adiposity when seated could be demonstrated to have an effect on the subcutaneous tissue pressure measured in the groin through which the venous and lymphatic drainage must pass. Accurate measurement of interstitial fluid pressure is technically difficult. Guyton et al. (1971) described a method, which involved the placement of a perforated capsule in the subcutaneous tissue of the leg which could fill with fluid over time and found pressures of approximately -6mmHg. Christenson et al. (1986) used needle manometry to measure subcutaneous and intramuscular pressures in normal and post-phlebotic limbs (showing an increase in pressure in phlebotic limbs) with pressure ranging from -2 to +4 mmHg in the supine position. It has also been used clinically to measure pressures in the intramuscular compartments in patients suspected of compartment syndrome. Olsen et al. (1998) reported

normal resting intramuscular pressures of approximately 5-9 mmHg and stated that this combined fluid and solid tissue pressure. The manometer technique was preferred over Guyton's capsule method as it would allow for immediate measurement of change in pressure in real-time. To the author's knowledge, this method has not been applied to the inguinal region before.

Baseline values recorded in the supine position (approximately 5-10 mmHg) were stable over several minutes in either supine or seated position and were nearly always positive. This may be related to the use of a simple needle rather than a slit-catheter. For example, Moed et al. (1993) reported that the simple needle gave a consistently higher pressure by 18 mmHg in the antero-lateral compartment of the dog hind-limb than a slit catheter or side-ported needle. There is approximately 18 mmHg difference between the resting pressures obtained in this study and the -6mmHg described in Guyton's method, suggesting a similar effect. Although in absolute terms these values may be over-stated, their consistency over time, demonstrated by a coefficient of variation of 6.5% on supine pressures and 11.1% on seated pressures during 5 repeated trials for normal weight subjects lends credibility to their use for demonstrating relative changes on change in posture.

A significant change of approximately 8 mmHg that stabilised within 1 min of moving from supine to sitting was observed but only in the obese subjects. There were no pressure differences between the BMI groups when supine, but the changes in pressure on sitting were significantly correlated with BMI and the measures of subcutaneous abdominal fat contributing to seated 'overhang' (SAD, SA/SP, ultrasound thickness) but not % truncal fat which includes visceral fat. It appears that a SAD of > 22 cm or a SA/SP of 0.5, the minimum values for the obese group, are required before raised inguinal tissue pressure on sitting is evident. Despite this relationship, there was only a weak positive but non significant

correlation between the increase in pressure when seated and the increase in size of the FV. This was contrary to expectation and suggests other factors may be involved. The needle for measuring pressure was carefully placed in the femoral triangle next to the FV under ultrasound guidance. This does not, however, enable determination of intra-abdominal pressures which are more commonly measured using a bladder catheter (Malbrain, 2004). The hypothesis that abdominal adiposity causes a raise in intra-abdominal pressure was put forward by Sugerman et al. (1997) and Cobb et al. (2005) and Arfvidsson et al. (2005) showed that morbidly obese patients with elevated intra-abdominal pressure also had raised ileo-femoral pressures. There is no information on whether inguinal tissue pressure matches intra-abdominal pressure. However, consistent with the present study, intra-abdominal pressures have been reported to be positive and higher in obese (8-14 mmHg) than normal weight individual (5-7 mmHg) and to be increased on moving from supine to a head-elevated position (Cheatham et al. 2009). It is likely, therefore, that the obese subjects in the current work experienced raised intra-abdominal as well as inguinal pressure, which may have contributed to the larger vein size.

4.5.2 Femoral Vein Flow

What is the likely impact of abdominal adiposity and raised inguinal tissue pressure on venous outflow from the lower limbs? To investigate potential impedance, FV flow was derived from flow velocity and vein diameter supine and seated, and during calf compression. Supine femoral velocities were lower (7.5 cm/s) than those reported by others - 13 cm/s in healthy men and women (Fromy et al. 1997), 14.6 cm/s in females (Fronek et al. 2001), 37.3 cm/s in healthy supine men and women of all ages (Ashby et al. 1995). This may be due to differences in the manner in which the readings were taken. The present study recorded

average velocity over 15 cardiac cycles determined by ECG and derived from the internal software of the Envisor ultrasound machine. Fromy et al. (1997), on the other hand, took average peak velocity only during an 8 second period while other papers do not give details of measurement period. It is important to collect data over a sufficient time period to take account of cardiac rhythm since Abu-Yousef et al. (1997) showed variation in femoral velocity of -1.8 to 12 cm/s between diastolic and systolic phases. Also respiration changes femoral velocity with a 53% increase during deep regular breathing (Kwon et al. 2003) and variations in the proportion of flow occurring during inspiration from 65% to -11% depending on the type of respiration (Miller et al. 2005). Calculated femoral volume flow values (152-212ml/min) were also considerably lower than published in the data e.g. 864ml/min (Fromy et al. 1997), 800 ml/min (Miller et al. 2005) but the latter were measured at the common FV, not in the femoral triangle as in the present study. However, on the basis that arterial inflow and venous outflow to the leg should be comparable, the current vein flows are in keeping with superficial femoral artery flow measured by Thijssen et al. (2008) as 184ml/min in healthy young men.

It was found that there was no significant change in volume flow in the FV when moving from the supine to seated position because vein diameter had increased and velocity decreased. All three BMI groups showed the same effects. There was also no correlation with abdominal adiposity measures or inguinal tissue pressure and it therefore appears that, despite fat 'overhang' and raised inguinal tissue pressure, there was no evidence of impedance to leg venous outflow. However, this was achieved through greater distension of the FV in the obese than overweight than normal weight groups which implies greater pressure to distend because of abdominal fat, consistent with the findings of Van Rij et al. (2008) of higher leg vein pressures with obesity in seated patients with venous disease. A larger vein size as a result of

adaptation over time to higher pressures coupled with similar volume flows as in normal sized veins leads to a greater venous reservoir in the limbs which may itself cause problems by allowing stagnation and stasis..

An additional test of potential femoral flow was made by recording peak flow velocity during calf compression with a cuff inflated briefly to 80mmHg. Peak velocities when supine (~ 120 cm/s) were similar to those found by Delis et al. 2000 when they applied clinical compression devices to the lower leg (120cm/s). They increased significantly on sitting in all BMI groups to a similar extent, and as with resting femoral flow, there was no relationship to abdominal adiposity, inguinal tissue pressure or leg size.

Supine position equates to an underfilled venous system as resistance to outflow is reduced (removal of hydrostatic pressure). The veins at this point have the ability to absorb sharp rises in venous pressure or flow by distension. The ability to distend means that mechanical force given by the cuff to generate the peak flow is not only being seen in increased flow of blood but also in the venous distension. In the seated position the veins are filled. The amount of distensibility in the veins is now reduced. When mechanical force is applied this will be converted more into flow than into venous distensibility. An increase in flow was noted on moving from supine and seated in the peak flow and this may reflect this description.

4.5.3 Lower Limb Tissue Durometry

Increased pressure in the groin may not be sufficient to have an increased effect over and above that of intra-abdominal pressure on venous flow but may be sufficient to disrupt the flow of lymphatic fluid from the limb (Guyton et al. 1971). Intra-abdominal hypertension has indeed been associated with impedance to lymph flow in intensive care patients on positive pressure ventilation. (Malbrain et al. 2007). With impedance of lymphatic outflow, the limb

would be in a persistent state of lymphatic congestion akin to lymphoedema. This would have the long term consequences to the tissues of the complications seen in lymphatic disease (eczema, haemosiderin deposit, lipodermatosclerosis and ulceration). Durometry as an assessment tool is in its infancy and has initially been used by Falanga et al. (1993) and Romanelli et al. (1995) to evaluate skin hardness in systemic sclerosis and venous disease. Both studies showed a high correlation between disease severity and tissue hardness and have recommended its use for assessment of these conditions. Impedance to lymphatic outflow from abdominal adiposity may cause an increase in tissue turgor leading to increased durometry readings

It was found that calf, but not thigh, skin increased significantly in hardness after relatively short periods of moving to the seated position, and that the change was greatest in the obese group, although not this significant against the other BMI groups. Changes in skin hardness were unrelated to subcutaneous tissue depth and therefore represent true changes in the properties of the tissue underneath the durometry probe. Standing alone has been demonstrated to increase lower limb tissue hardness in healthy individuals and increasing BMI was correlated with this through weight (Wall et al. 2010). In seated subjects in the present study the increase in hardness only in the calf may in part be due to the tightness of the lower limb skin. The calf readings were higher than at the thigh when supine, and a small volume increase into the calf which has less capacity to expand would be expected to be transferred more quickly to the skin than the same increase in the thigh which may have a greater capacity to absorb volume increase. It may also be that the time allowed for changes to occur was too short and with longer durations, thigh hardness may also have increased.

4.6 BLOOD FLOW AND FILTRATION

4.6.1 Blood Flow

Although the main focus of this work was the venous circulation, limb blood flow was also estimated because of its potential influence upon venous filling characteristics in the calf. Resting calf blood flow by strain gauge plethysmography was positively correlated with BMI and abdominal obesity. Data comparing basal limb blood flow in obese and lean subjects are contradictory, with reports of no difference in young (femoral flow, Limberg et al. 2010), middle-aged (calf SGP, Vollenweider et al. 1994) or elderly obese individuals (calf SGP, Acree et al. 2007). On the other hand, Ribeiro et al. (2001) found lower basal forearm blood flow by SGP in middle-aged females associated with higher levels of muscle sympathetic nerve activity, but others found it to be higher in heavier individuals (Stepniakowski & Egan, 1995) or not different (Vollenweider et al 1994).

Some of this variation may be due to the fact that SGP measures flow from skin and subcutaneous fat as well as skeletal muscle. Estimates of blood flow by xenon clearance to forearm skin (9.1 ml/min/100g) and adipose tissue (3.8 ml/min/100g) were higher than for skeletal muscle (1.6 ml/min/100g), (Elia & Kurpad, 1993) and a greater proportion of the former two tissues in the limb will give rise to higher plethysmographic flow. This is supported by correlation found between calf blood flow and total but not lean calf cross sectional area.

Furthermore, in obesity these anatomical differences may be confounded by alterations in vascular tone and reactivity because of systemic and hormonal changes. Hypertension, which is associated with reduced limb blood flow, is unlikely to be involved as the subject cohort were normotensive. Oestrogen is a vasodilator that has been shown to increase forearm blood

flow (Volteranni et al. 1995). Peripheral conversion of oestrogen has been demonstrated to be higher in the presence of a raised BMI (Quinkler et al. 2004) and long term exposure to HRT in post menopausal females has been shown by Vehkavaara et al. (2000) to increase blood flow over non-users. Although oestrogen levels at the time of study did not correlate with calf blood flow, life time exposure to higher levels of oestrogen may account for the higher readings of calf blood flow. However, there was no association with OCP use or pregnancy data.

Another potential vasodilator is insulin and its presence at increased levels might explain an increase in flow (Birkeland et al. 1995). Blood insulin was found to be higher with increasing BMI and associated with a rising but normal glucose level across the groups. The HOMA2 index for the obese population put them within the published levels for Insulin Resistance (IR) as defined in a normal population by Geloneze et al. (2009). Insulin infusion increases leg blood flow (Birkeland et al. 1995) and Vollenweider et al. (1994) showed that chronic long term high levels of insulin are just as effective at producing increased calf blood flow and decreased calf resistance as pulses of insulin. A raised blood glucose level has been noted to have the same dilator effect by Straznicky et al. (2009). In the presence of IR (calculated from insulin and glucose) they also demonstrated a greater blood flow at rest than in insulin-sensitive volunteers, associated with decreased glucose uptake into skeletal muscle (Laakso et al. 1990). Irving et al. (2001) showed higher fasting plasma glucose was associated with lower capillary density but increased capillary blood velocity in the skin. If these three observations are taken together, a situation where increased inflow to the tissues is matched by lower peripheral limb resistance, shown in obesity Messerli et al. (1981), due to chronic exposure to insulin manifesting as a reduced transit time through the capillary bed can be envisaged. The correlation between HOMA2 index and basal calf blood flow is consistent

with this scenario. Vehkavaara et al. (2000) studied interactions between insulin and oestrogen, both known to increase blood flow (de Jongh et al. 2004), but concluded that they are not additive.

4.6.2 Capillary Filtration

Estimation of capillary filtration coefficient (Kf) with the multi-step pressure protocol devised by Gamble 2002¹⁶² enable derivation of not only Kf but also Pvi, the isovolumetric pressure in the microcirculation at which fluid filtration occurs, and Pvest, the pressure in the venous system at rest. The results obtained for Kf in the normal weight group were in keeping with those previously published by Gamble et al. (1998, 2002) 0.0030 ml/100ml/min/mmHg) and Lindenberger et al. (2007) (0.0045 ml/100ml/min/mmHg) even these studies were performed on younger study populations. There was a decrease in Kf with increasing BMI, and a similar inverse correlation with measures of abdominal obesity. Considering the finding that the obese volunteers had a greater blood flow than the normal and overweight group, how is it that the Kf of the obese group is lower?

L'Hermitte et al. (2003) looked at the effect of adiposity on the capillary filtration of albumin and lower limb lymphatic function. Obese volunteers had an expanded circulating volume (reinforced by Messerli et al. 1981) with greater extra-cellular fluid volume. This may suggest that the limbs are “loaded” with excess volume before measurements commenced and that the results reflect limited additional fluid movement into an already full leg. However, the study data does not support this as inguinal tissue pressure and tissue durometry – an indicator of tissue turgor due to fluid accumulation - did not correlate at rest with any indices of abdominal composition.

Lindenberger et al. (2007) noted that there was higher Kf in females than males which raises the possibility that sex hormones are involved. Although not significant, there was a positive correlation between oestrogen levels and Kf in the present study, although oestrogen was not related to BMI. Turzyniecka et al. (2009) assessed calf capillary filtration in the same way as in the present study in an obese non-diabetic older population and noted a decreased Kf with increasing insulin resistance and abdominal adiposity. These authors suggested that there is capillary rarefaction occurring in this situation, providing less surface area for fluid exchange to occur. Having demonstrated IR in the study obese individuals, it is possible that they are demonstrating early changes in their microcirculation. A similar structural microvascular deficit has also been suggested by Noon et al. (1997) in young men predisposed to hypertension which the obese group, although normotensive, are at risk of developing.

Another explanation for the decrease in capillary filtration seen in the obese group in the face of higher calf blood flow relates to the role of insulin in control of blood flow through limb skeletal muscle. Rattigan et al. (2006) propose that muscle capillaries are laid out in two parallel circuits, one which supplies nutrients to the muscle when required (nutritive), and another that bypasses the muscle capillary bed (non-nutritive) and that insulin, as a vasodilator, plays a key role in directing flow preferentially through skeletal muscle. In IR states, however, this does not occur and flow bypasses the capillary bed so that less capillary surface area will be exposed for fluid filtration to occur across. It must be emphasized at this point that the concept of parallel capillary pathways and insulin-mediated capillary recruitment has been challenged by others in the field of muscle microcirculation (Poole et al 2008).

A raised isovolumetric venous pressure, Pvi, has been associated with conditions of inflammation and leucocyte adhesion in the microcirculation (Anim-Nyame et al. 2003) but

the study did not demonstrate any difference in the Pvi of the BMI groups. It is interesting to note that inflammatory changes including leucocyte adhesion are evident in veins chronically exposed to pressure (Bergan et al. 2006) and hence implicated in the pathogenesis of venous disease (Somers & Knaapen, 2006, Lim & Davies, 2009). The absence of an obesity-related increase in Pvi derived from whole calf plethysmography is consistent with the lack of effect of body size or abdominal adiposity on calf venous capacitance. The contrast between this and the changes seen in the large leg veins with obesity highlights the discrepancy between what is happening to the small venules and venous microcirculation as opposed to the large vessels. It may be the case that smaller veins do not yet display alterations that are evident in the large vessels.

Supine venous pressure at calf level, Pvest, showed a slight but not significant correlation with BMI but a stronger positive relationship with abdominal fat measures (SAD $p=0.06$, ultrasound abdominal thickness $p=0.07$). In agreement with the study hypothesis, this is indicative of a degree of venous hypertension that would be expected to increase when sitting or standing. and could be a contributor to the changes seen in

4.7 PREGANANCY AND WEIGHT HISTORY

This area of the study was dependent on the self-reporting of the study volunteers. It is probably safe to assume that the volunteers were aware of the number of full term pregnancies they had undergone and that this is an accurate result. Historical BMI is more open to retrospective interpretation by the volunteers. The study could not demonstrate any correlations between the parameters of venous function measured and the number of pregnancies or the historical BMI. No correlation was demonstrated between BMI or historical BMI and the number of pregnancies.

The literature reports that those females whom have had pregnancies increase their weight and this maybe a confounding factor in large population based studies i.e. Jukkola et al. (2006). This may have also had an effect in this study but numbers were insufficient to demonstrate this.

4.8 LIMITATIONS OF THE STUDY

4.8.1 Study Design

The aim of the study was to examine the effect of obesity on venous function in a healthy population. It would also have been possible to compare the relationship of obesity to vein function in subjects with and without established venous disease. However, examination of venous parameters in subjects prior to development of pathological changes rather than comparison of normal and diseased veins was felt to be appropriate for several reasons. A varicose vein wall is different from the wall in non-diseased vein due to imbalance in the structural proteins (Sansilvestri-Morel et al. 2001) and also the structure and number of smooth muscle cells (Venturi et al. 1996). This alters the mechanical properties of the vein as described by Travers et al. (1996) and reinforced by Krasiński et al. (2010). Studying the vein when it is diseased would complicate the assessment of venous functioning as it is not able to react to the stresses of filling as normal veins do (van Rij et al. 2008) and it is difficult to quantify the degree to which the vein has been affected. Furthermore, as described in the Introduction section, work has demonstrated that the vein is not uniform in its disease (Labropoulos et al. 1999, Abu-Own et al. 1994) and therefore, during imaging, it would be difficult to ascertain if the assessment was of diseased or normal vein segment.

4.8.2 Recruitment and Small Sample Size

The power calculations initially used for the establishment of the number of volunteers in this study were designed to detect a difference in the femoral vein flow velocity following postural movements. No data was available for the analysis of venous compliance using ultrasound measurement and hence power calculations were not based on these measures. The

recruiting difficulties in this study are dealt with in the Discussion section and the low number of volunteers that this yielded made interpreting the results achieved much more difficult. Following discussion with Dr Peter Nightingale regarding data interpretation all data were analysed with non-parametric testing, which did not require assumption of normal distribution. Correlational analysis was used for the majority of data interpretation as the small numbers in each group meant that multivariate regression analysis was not possible. The author hopes that the data presented can highlight for the reader the correlations demonstrated in the population. The small numbers in each BMI group did allow for assessment between the BMI groups but the standard deviation in each group was larger due to small sample sizes, increasing the likelihood of a Type II error. Larger numbers would be required to determine significant differences in the groups.

4.8.3 Gender Considerations

The mode of advertisement used for the study meant that the majority of volunteers were female. Small numbers of males were recruited but were insufficient for analysis. This means that the author has not been able to produce baseline data for the male population or make any comment on the functioning of male lower limb veins through this study. Although no confounding effects of female hormones on venous haemodynamics were seen in the female study population, the complex interactions of oestrogen and progesterone with veins may mean that the genders display differing effects in venous function through their exposure to obesity.

4.8.4 New Technology

The assessment of any new investigative techniques requires two criteria to be satisfied. Is the test acceptable to the volunteers and is the test accurate in individual assessment and reproducible across an investigated population? Several of the measures in the study e.g. femoral vein cross sectional area, height, weight etc. were not repeated during the test sessions because their validity has been established in previous studies and hence the time commitment of each volunteer could be kept to a minimum. One omission for the study was the establishment of the reproducibility of venous occlusion plethysmography in this subject cohort. Occlusion plethysmography as a technique is well established but in the study the technical challenges of simultaneous venous imaging and whole limb readings are described elsewhere in the discussion but a failure of this study is that these measures were not able to be repeated and therefore the variability of this test could not be confirmed. The inguinal tissue pressure measures were only performed at one meeting as the test was invasive. The repeatability of this test was described in the Results section and had a coefficient of variation of 6.5% when supine and 11.1% when seated. This was a measure of the whole group readings and suggests that the test could be used in the future for investigation of the tissue pressure. The possible limitations of this technique are presented in the Discussion section.

CHAPTER 5:

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis set out to investigate whether being overweight or obese in middle age is associated with deleterious changes in leg vein properties even before venous disease can be diagnosed. Abdominal adiposity was considered as a key factor likely to be imposing mechanical hindrance to venous outflow from the legs and / or increased venous pressure. This study has suggested that in an obese population with no clinical venous disease there is demonstrable enlargement in the size of lower limb veins in the deep and superficial systems accompanied by a loss of venous compliance. These changes were linked with the presence of greater levels of abdominal fat both subcutaneous and visceral, which correlated with raised inguinal tissue pressure especially in the sitting position. The most likely scenario is one whereby venous hypertension is induced by central obesity over and above the normal hydrostatic forces due to gravity and weight, and this is illustrated in Figure 5.1 below. Even though inguinal compression of the femoral vein alone using the fingers is capable of increasing femoral vein size (Kim et al. 2008) by a similar degree as seen with obesity in the present study, it is likely that higher intra-abdominal pressure due to visceral fat also plays a role in accounting for femoral vein distension when seated. It was surprising in some respects that weight history, which documented loosely the duration of obesity, was not related to vein distension even though the average weight gain for the group since the age of 20 years was approximately 20 kg. Nor was the number of pregnancies a significant factor even though this has been suggested to impose abdominal pressure and pelvic venous congestion.

In terms of obstruction to venous outflow, no difference was observed between normal weight and obese individuals in femoral volume flow irrespective of posture, but this was accommodated by the bigger size of the vein in obesity. Whether there are changes in the

femoral vein wall composition as a result of obesity-induced distension remains to be determined. The novel finding of lower compliance of the GSV with increasing BMI in a non-disease state implies that structural changes to the vein wall may already be underway. This could only be confirmed by direct histological examination of vein tissue, which is not ethical in healthy subjects. Krasinski et al. (2010) have demonstrated differing tensile strengths between GSV in health and disease and compared it to healthy FV harvested at post-traumatic amputation. Such an approach allied to obesity measures may provide further evidence of a systemic structural deficiency as described by Sansilvestri-Morel et al. (2007).

It was interesting to note that a tendency for raised venous pressure in the calf – albeit estimated indirectly – was not associated with a higher isovolumetric pressure, which would have indicated an inflammatory state. Given that venous disease is clearly linked with an inflammatory cascade affecting the valves and vein wall (Bergan et al. 2008), it may be that this will ensue at a later time point. There is no way of knowing from this study whether the venous changes associated with obesity would have progressed to overt venous disease in time. This could only be ascertained from a longterm cohort study over the next 10-20 years. This would likely require a large multicentre population based approach. Of consideration but not within the scope of this study is the pro-inflammatory condition present in morbid obesity and metabolic syndrome and whether this may play a role in the venous parameters studied.

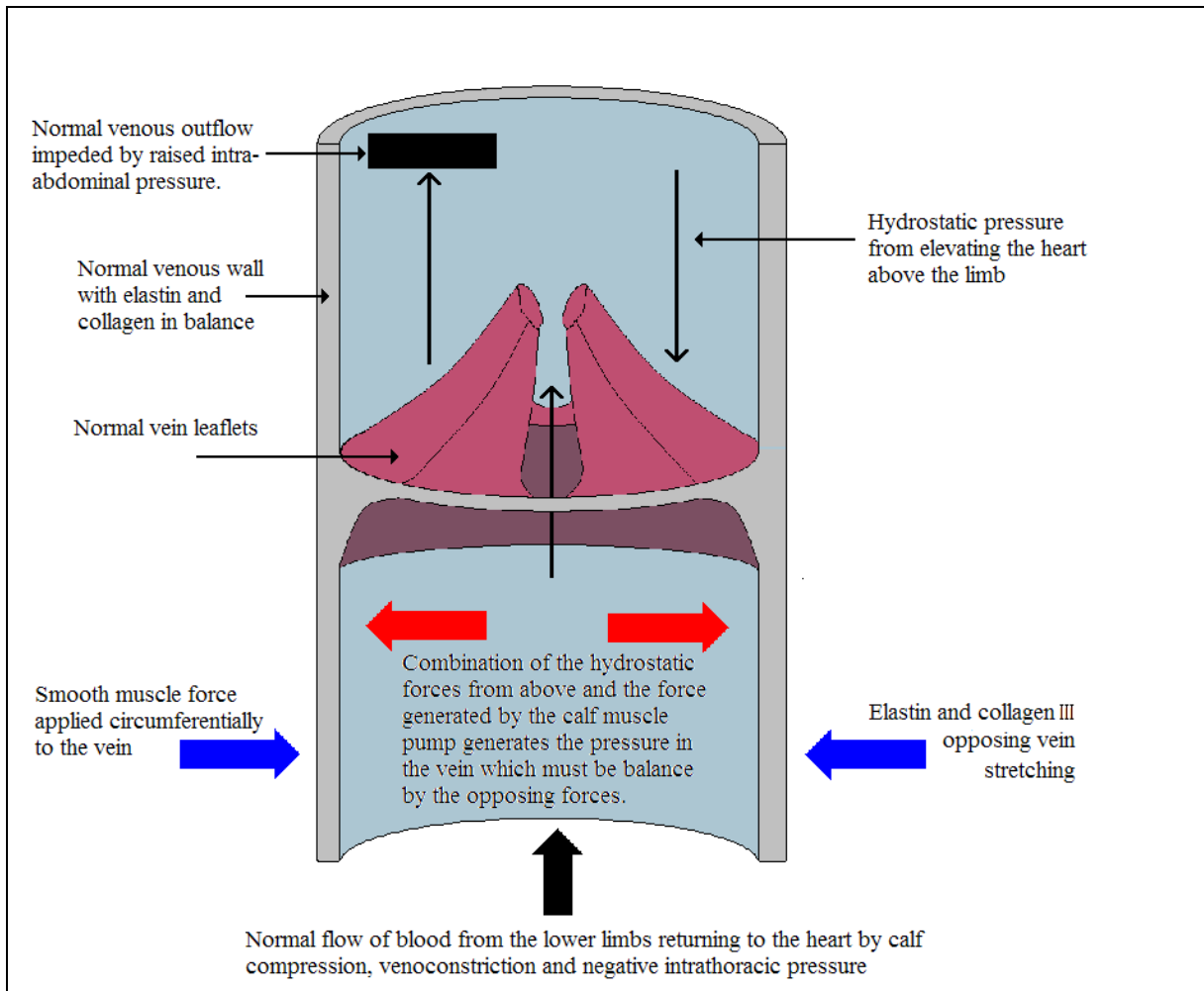


Figure 5.1 Diagram of the forces applied to the vein in the study volunteers where there was no demonstrable clinical venous reflux or disease.

Despite correlational analysis, it remains impossible to establish definitive cause and effect between being overweight, having abdominal obesity and changes in venous properties from the small population studied. There are various animal models that could be used to shed light on this, such as surgical venous ligation techniques to induce hypertension (Bergan et al. 2008¹⁹³) or the approach used by Monos et al. (1995) where rat hindlimb venous pressure was chronically elevated by maintaining animals in a head-up posture leading to saphenous vein enlargement. To date, none of these have been applied to obese animal strains to investigate

interactions with body size and adiposity. From the human perspective, the study would have been strengthened by intravenous pressure monitoring in different postures, but this invasive approach was not felt to be warranted in healthy individuals. Future scope for assessment of femoral venous haemodynamics without intervention will be possible. Deformation analysis using ultrasound to calculate intravenous pressure and 3-D ultrasound allowing for venous flow analysis could both be of benefit. This may give a better understanding of the flow dynamics in the vein and may be used to produce models of venous blood flow. This could help in the development of reconstructive deep venous disease such as the use of in-stent venous valve devices. (Geselschap et al 2006)

A surprising aspect of the findings was that there appeared to be no relationship between vein properties and oestrogen or progesterone levels despite a considerable body of evidence showing both arterio- and veno-dilator effects of these hormones. This may simply be a reflection of the fact that menstrual phase was tightly controlled and that OCP use was well monitored, albeit by self-report. Although it would involve confounding effects of age, a more stringent examination of the association between endogenous or exogenous oestrogen and vein characteristics in a 'free from venous disease' post-menopausal sample of women with and without hormone replacement therapy might help to resolve this issue. Previous evidence has shown an improvement in venous function when exogenous oestrogen has been administered to post menopausal woman Ciardullo et al. (2000).

The metabolic syndrome of insulin resistance is well known for its link to obesity but no connection was found between glucose / insulin levels and vein properties other than higher resting calf blood flow. Insulin has been shown to reduce venous constrictor tone, an effect attenuated by obesity (Feldman et al. 1995). Indeed, there are many other vasoactive substances that can modify venomotor tone (see Appendix A) and it may be that an impaired

ability of veins to counter distending pressure by myogenic constriction or changes in vein response to venodilators / constrictors – catecholamines, endothelin, endothelial-dependent factors etc. – plays a role in the development of wider vessels and loss of compliance. Such effects would have to be vein-specific to account for the differential adaptation of the saphenous versus popliteal vein. It is clear that different leg veins react in different ways. For instance, the popliteal vein has been shown not to be responsive to sympathetic neural activation with a cold pressor test (Young et al 2008) whereas the whole limb and the GSV vein demonstrate increased venous tone (Philips, 2010). Therefore the degree of adrenergic innervation of the popliteal vein may be different from the small deep veins / the superficial venous system. It can be seen from figures 3.19, 3.20 and 3.21 that there is a marked difference between the compliance of individual veins and the whole limb/calf. In the freely-moving human, interactions between pressure effects within the superficial and deep systems and factors that influence venous tone are inevitably complex (Ludbrook, 1962, Ludbrook et al. 1964) and full understanding of how this fails in venous disease must await future investigation.

Not only were there disparate changes to large vein properties within the superficial and deep systems but venous capacity and compliance based on calf plethysmography showed no overall effects from obesity. It becomes clear from this study and published literature that when examining lower limb venous function the method of experimental approach is fundamentally important. Lower limb plethysmography has long been used as a surrogate for venous function based on venous vascular volume but this technique has recently been challenged by Young et al. (2008) when they demonstrated a difference in the compliance of the popliteal vein from that of whole limb. With whole limb plethysmography, volume changes during filling include not only expansion of deep veins (Buckey et al. 1988) but also

a significant contribution from small venous vessels (Cirovic et al. 2006) as well as soft tissue compliance, hysteresis and 'creep'. It is therefore less easy to ascribe changes to one or other of these components definitively. The use of ultrasound for assessment of lower limb venous parameters offers direct vision of the venous vessels for measurement of capacity and compliance (De Groot et al. 2005, Young et al. 2008) but is, of course, limited to veins of a certain size compatible with the resolution of the imaging. Increasing accuracy of cross sectional and in-line ultrasound investigation into venous parameters of the limb suggests the modality of choice for the future and researchers should not rely on whole limb plethysmography as a surrogate marker.

The study did not demonstrate any clear association between venous function in obesity and signs of skin changes although in disease, the pathway from venous hypertension through microvessel inflammation, oedema, skin hardening and impaired oxygen delivery to ulceration is well documented. However, the reduced capillary filtration in the face of raised arterial inflow in the calf suggests that microvascular disturbances have occurred that presage deleterious nutritive perfusion. This may well also involve skeletal muscle, which is the major contributor to calf cross-sectional area encompassed by a strain gauge. In advanced venous disease, calf muscle shows changes indicative of a failure of the adequate delivery of nutrients to the tissues and / or failure of the tissue to clear waste and breakdown products. For example, Qiao et al. (2005) noted pathological changes in the gastrocnemius muscle of varicose vein patients, correlated with ambulatory venous hypertension. Disruption of nutritive perfusion in either tissue is therefore a consequence of obesity to be aware of.

A caveat to all the findings of the present study is that the data are derived from a relatively small number of subjects. Despite substantial efforts on the part of the author in the recruitment for this study over a 16 months period, the number of female volunteers remained

only 26. Due to some difficulties in scheduling testing sessions and occasional failures in certain measurement procedures, a complete set of data was not obtained from each subject, as indicated by the smaller n number shown against values in the Results section. This led to the data being treated as non-parametric for analytic purposes. The power to determine the difference between the groups is reduced due to the low numbers and the correlation is less robust when relying on these small numbers to demonstrate relationships between the variables. Despite the small numbers, it has been shown in the Discussion section that the data acquired are in line with previously published studies. This study therefore provides baseline data on a small population sample from which larger cohort studies could be designed.

Since commencement of the thesis papers by Willenberg et al. (2010, 2011) have been published studying the effect of obesity on venous flow either in an obese population free from venous disease or simulated obesity with abdominal cuffs. The data displayed in these papers correlate closely on the measures with the findings of this thesis on the size of the femoral vein and reduction in venous velocity. Willenberg et al. surmised from their data that obesity was creating mechanical impedance to lower limb venous flow and suggested this may give rise to an increase risk of chronic venous disease.

Venous disease remains one of the major health care costs. 2% of the NHS budget is spent on venous ulceration and as this study is suggesting, the venous disease is going to increase in prevalence due to obesity then this cost would be expected to rise. The question of future research then turns to see if the situation can be recovered by a change in body habitus. This was noted by Sugerma (et al 2001) who showed healing of lower limb ulceration and resolution of venous/congestive ulceration post weight reduction surgery. A study designed to examine the metabolic and venous parameters pre and post gastric bypass/banding may help to go some way to demonstrate if the situation of loss of venous compliance examined in this

thesis could be reversed. Allied to this would be an understanding of the changes in the skin and muscular vasculature to help aid explanation of chronic changes induced by venous stasis. Ultimately tissue sampling of venous wall may be required to gain full understanding of the pathophysiological changes induced by obesity in the healthy individual. This could have major implications for future health prevention and care. If the financial burden of venous disease could be reduced through weight reduction then it makes a powerful argument for more aggressive interventions in controlling obesity at an earlier stage, looking to reduce future health burden.

APPENDICES

Appendix A: Vasoactive Substances

Multiple pharmacological agents are known to affect the venous system. Listed in the appendix below are the substances and there known to have an effect (Aellig, 1994).

Pharmacological Group	Effect on the Venous System
α_1 - α_2 Adrenoreceptor Agonists	Cause venoconstriction at 1000 times the concentration required to cause an alteration in the systolic blood pressure. Evidence that both receptors are present in the venous system but adrenaline appears to have greatest effect suggesting α_1 predominates.
5-HT-Receptors Agonists	Shown to cause venoconstriction and potentiate the effects of adrenoreceptor agonists.
Ergot Compounds	Has a venoconstrictive effect that can be shown to remain present and even increase post infusion for up to 30 minutes. This was suggested to represent a deeper receptor compartment delaying maximal response activated by diffusion.
Angiotensin 1,2 and ACE Inhibitors	Angiotensin 1 and 2 produces dose dependent response in administration into peripheral hand vein of venoconstriction. There is evidence that conversion from less potent angiotensin 1 to 2 also occurs in venous segments which shows the presence of ACE in the endothelium of peripheral venous tissue.
Endothelin	A venoconstrictor which acts via calcium channel activation.
Effects of Prostaglandins	Can be shown that different compounds effect the arterial and venous systems differently A_1 , A_2 , E_1 and E_2 were shown to produce a venodilatation in precontracted veins. B_1 and $F_{2\alpha}$ produce venoconstriction likely to be due to stimulation of Thromboxane A_2 .
β -Adrenoreceptor Agonists	Reports suggest that this class of drugs cause venodilatation when infused into superficial arm veins. This can be inhibited by β -adrenoreceptor blockers. Venous distensibility can be shown to reduce in patients who have taken propranolol for 2 weeks. This is

	reversible.
Muscarinic Receptor Agonists	At low doses they cause venodilation in pre constricted veins due to release of Nitrous oxide (Endothelium Derived Relaxing Factor, EDRF). At higher doses they cause vasoconstriction thought to be due to release of noradrenaline controlled by muscarinic receptors.
Nitrates	Best known and most widely used venodilators. Shown to cause venodilation in the hand vein pre-constricted with noradrenaline. Action is via the release of Nitric Oxide (EDRF).
Calcium Channel Antagonist	Shown to cause venodilation in precontracted hand vein. With a greater effect on post-junctional extrasynaptic α_2 -adrenoreceptors than in post-synaptic α_1 -adrenoreceptors. Very little report of clinical orthostatic intolerance suggesting that calcium channel antagonists have little effect on the neuronal control of venous tone which may be higher in hypertensive patients.

Appendix B: Screening Visit
SCREENING VISIT ASSESSMENT SHEET

Date: _____

Name: _____

Date of Birth _____

Study No: _____

GP Practice: _____

Dominant Leg _____

Height _____

Weight _____

LMP in females _____

Could the subject be pregnant *YES / NO*

Past Medical History

ABPI L _____
 R _____

TBPI L _____
 R _____

Exclusion Criteria:

	Yes	No
DVT		
Post thrombotic syndrome		
Post menopausal		
Type 1 Diabetes Mellitus		
Pregnancy		
Peripheral arterial Disease		
Heart Failure		
Exercise limiting Arthritis		
Lymphoedema		
Liver Failure		
Clinical use of Steroids		

USS duplex of leg: _____

Acceptable for study: *YES / NO*

If yes give patient info leaflet, questionnaires and arrange for next visit:

Next visit set at _____ at the _____

Appendix C: Weight Recall

Below is a copy of the weight recall questionnaire used in the study to ascertain the volunteers' weight throughout their adult life. A mean of the results returned was used with the volunteers' present height to calculate the historical BMI.

WEIGHT QUESTIONNAIRE

Age _____ Weight _____ Height _____ BMI _____

Weight at 20 _____

Weight at 25 _____

Weight at 30 _____

Weight at 35 _____

Weight at 40 _____

Weight at 45 _____

Weight at 50 _____

Appendix D: Gynaecology History

GYNAECOLOGY QUESTIONNAIRE

- Age of first Period _____
- Date of last period (first day) _____
- Do you have regular periods? YES / NO
- Do you suffer from premenstrual symptoms such as bloating or cramping abdominal pain? YES / NO

- Is there a chance you maybe pregnant? (there is a small risk with the radiation from the DEXA scan) YES / NO
- Have you ever used the oral contraceptive pill? YES / NO

If yes for how long in total throughout your life time? (it does not have to have been continuous) _____

If yes which different makes of pill have you used and for how long each one?

- Have you ever had a full term pregnancy? YES / NO

If Yes: How many full term deliveries? _____

What age/s were you when your child/dren were born?

- Have you had any premature children? YES / NO

If Yes: How many premature deliveries? _____

How many weeks were the deliveries? _____

What age/s were you when your child/dren were born?

- Have you ever had any failed pregnancies? YES / NO

If Yes: How many failed pregnancies? _____

How many weeks was / were your pregnancy/ies?

What ages were you at the start of these pregnancies?

- Have you ever breastfed your child/dren? YES / NO

If Yes For how long in total? _____

- Have you ever had any gynaecological surgery? YES / NO

If yes What operation/s was performed? _____

How long ago was this done? _____

- Have you had ever had any menopausal symptoms (such as hot flushes or period irregularity)? YES / NO

Appendix E: Stanford Seven Day Activity Recall Questionnaire

The Stanford Seven Day Recall Questionnaire is a validated questionnaire for assessment of daily activity (Washburn et al 2003¹⁶³) The volunteers are required to recall and record the different levels of physical activity they undertake over how long each day for a continuous 7 day period and this data is used to calculate the energy expenditure per day.

PAR#: 1 2 3 4 5 6 7 Participant _____

Interviewer _____ Today is _____ Today's Date _____

1. Were you employed in the last seven days? 0. No (Skip to Q#4) 1. Yes

2. How many days of the last seven did you work? _____ days

3. How many total hours did you work in the last seven days? _____ hours last week

4. What two days do you consider your weekend days? _____ (mark days below with a squiggle)

WORKSHEET

		DAYS						
SLEEP		1	2	3	4	5	6	7
M O R N I N G	Moderate							
	Hard							
	Very Hard							
A F T E R N O O N	Moderate							
	Hard							
	Very Hard							
E V E N I N G	Moderate							
	Hard							
	Very Hard							
Total Min Per Day	Strength:	_____	_____	_____	_____	_____	_____	_____
	Flexibility:	_____	_____	_____	_____	_____	_____	_____

4a. Compared to your physical activity over the past three months, was last week's physical activity more, less or about the same?

1. More
2. Less
3. About the same

Worksheet Key:	Rounding: 10-22 min.=.25 23-37 min.=.50 38-52 min.=.75 53-1:07 hr/min. =1.0	1:08-1:22 hr/min.=1.25
-----------------------	---	---------------------------

An asterisk (*) denotes a work-related activity.
A squiggly line through a column (day) denotes a weekend day.

Appendix F: Patient information leaflet

The effects of OBesity on Venous Impedance and Outflow measured by UltraSound “OBVIOUS STUDY”

Participants Information Leaflet

Research Study

We would like to invite you to take part in a research study examining how the blood flow in our legs is affected by our differing body shapes. Before you decide, it is important for you to understand why the research is being undertaken and what it will involve. Please take the time to read the following information carefully. Feel free to ask questions if there is anything that is not clear or if you would like more information.

Thank you for reading this information sheet.

What is the purpose of the research?

Problems with the veins that drain the blood from our legs affect about 1% of people. They can lead to varicose veins, eczema and eventually ulcers. If we want to prevent this happening and develop treatments, we need to understand how blood moves out of the legs. We think that being overweight or using oral contraceptives may change the way the veins do this, especially when we are sitting.

Why am I right for the study?

We would like to study men and women, who may or may not have used oral contraceptives in the past, of all different body shapes and sizes. If you have volunteered and been chosen for the study it is because you have no other medical problems which would interfere with our investigation and, based on a leg ultrasound scan, you have a normal venous system draining your legs.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this sheet to keep for reference and asked to sign a consent form. If you do decide to take part, you are free to withdraw at any time and without giving a reason. This decision will not in any way affect any present or future care.

What will happen to me if I take part?

Having volunteered you will be asked to attend an assessment at the Vascular Outpatients, Selly Oak Hospital, where we will ask questions about your general health and carry out an ultrasound scan of the veins of your legs. We will then be able to inform you if you are suitable for the study.

The next visit is arranged to take place at the Wellcome Clinical Research Facility at the Queen Elizabeth Hospital, Edgbaston, Birmingham where you will sign the consent form to enter the study. We will then:

1. check your pulse and blood pressure
2. inspect your legs for any signs of problems with your veins
3. measure blood pressure in your legs
4. measure your waist, hip, wrist and ankle circumference
5. measure your leg length and stomach depth, lying and sitting

You will be asked to fill out questionnaires on your weight since you were age 20, your daily physical activity and, if you are female, a history of contraceptive use and pregnancies up until the present time and your present position in the menstrual cycle (to help determine the date of your next visit). We will then:

6. ask you to lie down and then sit up while we measure how long it takes your leg to refill with blood. This involves three cuffs and a painless sensor placed around your leg and the cuffs inflated for short periods at low pressure
7. ask you to lie down and sit up whilst a painless probe is placed on your leg to measure its firmness.
8. ask you to lie down and have a small injection in your groin with a needle to numb the skin. A needle will then be placed in the tissue below the skin to measure the pressure and you will be asked to sit up to see if the pressure goes up or down.
9. ask you to undergo a DEXA scan. This is a very low intensity X-ray which measures the composition of the different materials of your body (muscle, bone and fat).

A final visit will be arranged at the School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, Birmingham where we will:

1. take 15 ml of blood (less than an egg cup)
2. measure the depth of tissue around your arms, trunk and legs with an ultrasound probe and calipers.
3. carry out an ultrasound scan of your leg veins to examine the flow of blood and how they fill up after emptying (by being raised up). This uses cuffs that inflate and deflate around the leg. The pressure used is less than taking normal blood pressure in the arm.

What do I have to do?

Transportation will be arranged to and from all visits. In total, the study will take 8 hours of your time. All visits will be set in and around your schedule to cause minimal disruption to you. The night before and the morning of your visits we will ask you to control the amount you drink and ask you to not eat and avoid caffeinated drinks.

What are the benefits?

Although the study may be of no personal benefit for you, we hope to gain a greater understanding of the problems associated with leg veins and how they occur.

What are the risks?

Blood taking can result in bruising of the arm. A needle placed in the groin may have the same effect and runs a very small risk of bleeding that might require prolonged pressure to stop. There is also a very small risk of infection with both procedures. The dose from a DEXA scan is equal to one normal days background radiation dose (less than half a standard chest X-ray in casualty).

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you wish to complain or have been in anyway harmed by any action taken during the study, the normal National Health Service complaints procedure will be available to you. Information will be supplied as required.

What will happen to the information gathered?

All the gathered information in the study is strictly confidential. No identifiable information will be given out. The data once analysed will be publish in medical journals or presented at medical meetings, again with no personal identifiers. All information will be stored behind hospital password-protected computer systems.

Who is organising the study?

The research is being arranged jointly between the University Hospital Birmingham Vascular Department and the University of Birmingham School of Sport and Exercise

Sciences. There is no official financial sponsor for the project, funding coming jointly from the above organisations.

Who has reviewed the study?

The study has been reviewed and Approved by the Dudley and Wolverhampton Research Ethics committee.

Contact for further information:

Mr Michael Wall Telephone
(Lead researcher) Fax
Mobile



Mr Malcolm Simms Telephone
(Vascular Surgeon and Chief Investigator)



Appendix G: Test Session 1: University of Birmingham School of Sport and Exercise Science

The effects of OBesity on Venous Impedance and Outflow measured by UltraSound “OBVIOUS STUDY”

PROTOCOL SHEET

Date:

Subject information:

ID

Gender: M / F

Date of birth:

(Age yrs)

Dominant leg L / R

Anthropometry

Height (cm)
(kg).....

Weight

Waist circumference (cm)

Hip circumference (cm).....

Wrist circumference (L / R cm)

Ankle circumference (L / R cm)

Thigh circumference (L / R cm)

Calf circumference (L / R cm)

[Mark positions on skin of: lower leg 15 cm above medial malleolus
Thigh 15 cm above femoral condyle
Abdomen 3 cm below umbilicus]

CEAP Clinical classification of chronic lower extremity venous disease (Porter & Moneta 1995)

Clinical signs	Class	Tick appropriate class
No visible or palpable signs of venous disease	0	
Teleangiectases, reticular veins, malleolar flare	1	
Varicose veins	2	
Edema without skin changes	3	
Skin changes ascribed to venous disease (pigmentation, venous eczema, lipodermatosclerosis)	4	
Skin changes (as defined above) in conjunction with healed ulceration	5	
Skin changes (as defined above) in conjunction with active ulceration	6	

Sagittal abdominal diameter

Subject to lie flat on couch and rest. Place caliper under back, ask subject to breathe out slowly and make measurement

SAD (cm).....

Wall-Simms index

Subject to sit upright with legs outstretched. Measure distance from sacrum to superior border of patella. Measure distance from sacrum to final point of contact between abdomen and thigh.

Sacrum – patella (cm) Sacrum – abdomen (cm)

Durometry

Record 2 measures of skin hardness at sites 1, 2 & 3 above.

	Reading 1	Reading 2	Mean
Abdomen			
Thigh left			
Thigh Right			
Calf Left			
Calf Right			

Ultrasound

Measure depth of subcutaneous tissue at sites 1, 2 and 3 above. Use vein setup

	Reading 1	Reading 2	Mean
Abdomen			
Thigh left			
Thigh Right			
Calf Left			
Calf Right			

Harpender calipers

Measure depth of subcutaneous tissue at sites 1, 2 and 3 above.

	Reading 1	Reading 2	Mean
Abdomen			
Thigh left			
Thigh Right			
Calf Left			
Calf Right			

Ultrasound flow velocities

Fit regular arm-sized Hokansen© cuff around lower leg (R or L, dominant). Set up cuff inflator to 80 mmHg.

Attach ECG electrodes to subject.

Use [Mike] venous DUS setup (LE vein + Adaptive Doppler + autotrace + average over 15 cycles).

Locate femoral vein in colour Doppler. Switch to Pulse Wave and set up for recording velocity. Switch to spectral mode. Press measure to obtain autotrace (blue / black outlines) or use Waveform softkey on/off (left hand under screen). Check scale for velocity (usually less than 100 cm/s).

Ask subject to breath quietly and regularly. Allow to record 15 cycles and freeze image as soon as velocity data is displayed. Repeat.

SUPINE resting venous flow velocities:

1. Mean velocity (cm/s)..... TAVM (cm/s) S velocity (cm/s) D velocity (cm/s)
2. Mean velocity (cm/s)..... TAVM (cm/s) S velocity (cm/s) D velocity (cm/s)

After short rest period, inflate cuff around calf to 80 mmHg for 4 secs. Upon release note peak velocity. Repeat.

SUPINE Peak venous flow velocities:

1. Peak (cm/s)
2. Peak (cm/s)
3. Peak (cm.s)

Repeat all measures in sitting position.

SITTING resting venous flow velocities:

1. Mean velocity (cm/s)..... TAVM (cm/s) S velocity (cm/s) D velocity (cm/s)
2. Mean velocity (cm/s)..... TAVM (cm/s) S velocity (cm/s) D velocity (cm/s)

After short rest period, inflate cuff around calf to 80 mmHg for 4 secs. Upon release note peak velocity. Repeat.

SITTING Peak venous flow velocities:

1. Peak (cm/s)
2. Peak (cm/s)
3. Peak (cm.s)

Resting calf blood flows

Remove calf cuff. Place 2 Hokansen thigh cuffs around L and R thighs. Measure calf circumference and select strain gauge 2 cm smaller. Fit gauges to both calves. Fit small Hokansen cuffs around ankles. Set cuff inflator to 50 mmHg.

Set up Chart / Powerlab© to record volumes. Set up Portapres© to record finger blood pressure (and heart rate) and display on Chart.

Inflate ankle cuffs manually to systolic BP + 50 mmHg

Inflate thigh cuffs to 50 mmHg for 8-10 sec. Record calf volume increase. Repeat twice more.

Venous distension

Remove thigh cuff from dominant leg and replace with cuff from DRT4. Leave other thigh cuff on. Attach LDF probe from DRT4 to non-dominant leg close to strain gauge. Check recording via DRT4© onto PC using Moor software©. Subject to position feet on bench and secure using bean bag pad. Check BP recording OK from Portapres©. If necessary, use heating blanket.

Re-locate femoral vein with DUS and image in longitudinal section. Set up VIA to track diameter. Set up Chart and DRT4 to measure volume. Check PORH setup on DRT4 to be 50 mmHg for 300 sec.

Control distension

When ready, press start on DRT4 to give 20 sec countdown to cuff inflation.

At start of cuff inflation / distension, press start on clock.
At 2 min 40 sec, inflate Hokansen 50 mmHg for 8-10 sec to get blood flow from non-dominant leg
At 2 min 55 sec, countdown to START OF INTERVENTION PERIOD.
At 3 min 40 sec, inflate Hokansen 50 mmHg for 8-10 sec to get blood flow from non-dominant leg
At 3 min 55 sec, countdown to END OF INTERVENTION PERIOD.
At 5 min, cuff will deflate automatically. Keep imaging until full deflation (30 sec).

CPT distension

When ready, press start on DRT4 to give 20 sec countdown to cuff inflation.
At start of cuff inflation / distension, press start on clock.
At 2 min 40 sec, inflate Hokansen 50 mmHg for 8-10 sec to get blood flow from non-dominant leg.
At 2 min 55 sec, countdown to START OF INTERVENTION PERIOD.
SUBJECT TO PLACE HAND UP TO WRIST IN ICE WATER.
At 3 min 40 sec, inflate Hokansen 50 mmHg for 8-10 sec to get blood flow from non-dominant leg.
At 3 min 55 sec, countdown to END OF INTERVENTION PERIOD.
SUBJECT TO REMOVE HAND FROM ICE WATER.
At 5 min, cuff will deflate automatically. Keep imaging until full deflation (30 sec).
Stop and save DRT4 and all Chart recordings.

Appendix H: Test Session 2: Wellcome Research Facility University Hospitals Birmingham

The effects of OBesity on Venous Impedance and Outflow measured by UltraSound “OBVIOUS STUDY”

Admission onto Wellcome trust system and DEXA Scanning

Set up pressure transducer and draw up lignocaine and saline flush

Name: _____

Next of Kin _____

Date of Examination: _____

DOB: _____

Check Consent into study: _____ Allergies _____

Check in Female the LMP: _____ Check Pregnancy status: _____

Collect Questionnaires: _____

Lie down on bed

Oxygen Saturations: _____

Blood Pressure: _____

MAP: _____

Pulse: _____

BP Left arm	
BP Right arm	
BP Left Leg	
BP Right Leg	
TP Left Leg	
TP Right leg	

ABPI: _____

TBPI: _____

	Circumference in cm
Wrist	

Waist	
Hip	
Ankle	

Wall-Brown-Simms ratio: _____

Position	Tissue hardness	
	Left	Right
Thigh 15 cm from medial femoral condyle		
Calf 15 cm from medial malleolus		
Abdomen 3 cm below umbilicus		

Introduce lignocaine

Introduce pressure transducer into groin under ultrasound guidance.

Flush line				
Start Stop Watch	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Record resting pressure at 1 min				
Record resting pressure at 2 min				

Sit patient up to 90 degrees				
Pressure at 1 minute				
Pressure at 2 minutes				
Pressure at 3 minutes				
Pressure at 4 minutes				
Pressure at 5 minutes				

Tissue Hardness	Left	Right
After first Cycle		
Thigh		
Calf		

Remove transducer and apply pressure to the wound:

Venous refill time:

Dominant Limb _____

Take Bloods:

Completion of examination session date _____

Lead investigator: _____

Research nurse: _____

Appendix I: Kolmogorov-Smirnov Test for Normality of Data.

Observed and calculated data were analysed using a Kolmogorov-Smirnov to establish if the variables that were to be future analysed were normally distributed. The results are displayed below. The majority of the data was normally distributed.

Vascular Parameters

Variable	Kolmogorov-Smirnov test p=0.05	Normal Distribution
Mean Arterial Pressure	0.975	Yes
Ankle Brachial pressure index	0.646	Yes
Dominant limb arterial blood flow	0.305	Yes
Venous Refill time	0.012	No

Body composition

Variable	Kolmogorov Smirnov test p=0.05	Normal Distribution
Age	0.625	Yes
Height	0.974	Yes
Weight	0.746	Yes
BMI	0.698	Yes
Age Adjusted BMI	0.032	No
Left Wrist	0.008	No
Right Wrist	0.180	Yes
Left Ankle	0.653	Yes
Right Ankle	0.323	Yes
Left Thigh	0.008	No
Right Thigh	0.487	Yes
Left Calf	0.742	Yes
Right Calf	0.946	Yes
SA/SP index	0.789	Yes
Sagittal Abdominal Diameter	0.312	Yes
% Truncal Fat as determined by DEXA	1.000	Yes
Waist-Hip ratio	0.962	Yes

Variable	Kolmogorov Smirnov test p=0.05	Normal Distribution
Ultrasound Anterior Abdominal Wall subcutaneous tissue thickness	0.739	Yes
Ultrasound Thigh subcutaneous tissue thickness	0.833	Yes
Ultrasound Calf subcutaneous tissue thickness	1.000	Yes
Caliper Anterior Abdominal Wall subcutaneous tissue thickness	0.999	Yes
Caliper Thigh subcutaneous tissue thickness	0.643	Yes
Caliper Calf subcutaneous tissue thickness	0.752	Yes
Dominant Calf Cross sectional area	0.868	Yes
Dominant Calf Radius	0.974	Yes
Dominant Calf lean Cross Sectional area	0.100	Yes
Dominant Calf lean Cross sectional area as a percentage of whole calf	0.043	No
Oestrogen levels	0.076	No
7 day Activity Questionnaire	0.993	Yes
Age at Menarch	0.212	Yes

Inguinal tissue pressure and femoral vein flow.

Variable	Kolmogorov Smirnov test p=0.05	Normal Distribution
Difference in Inguinal Tissue Pressure from Supine to Seated	0.844	Yes
Supine femoral vein diameter	0.853	Yes
Supine femoral vein radius	0.767	Yes
Supine femoral vein Cross Sectional Area	0.493	Yes
Supine resting mean femoral flow velocity per second	0.622	Yes
Supine resting time average velocity mean femoral flow velocity per second	0.766	Yes
Supine resting systolic femoral flow velocity per second	0.572	Yes
Supine resting diastolic femoral flow velocity per second	0.762	Yes
Supine peak femoral vein velocity per second	0.810	Yes
Supine resting mean femoral flow velocity per min	0.420	Yes
Supine peak femoral flow velocity per min	0.952	Yes
Seated femoral vein diameter	0.468	Yes
Seated femoral vein radius	0.460	Yes
Seated femoral vein Cross Sectional Area	0.292	Yes
Seated resting mean femoral flow velocity per second	0.346	Yes
Seated resting time average velocity mean femoral flow velocity per second	0.606	Yes
Seated resting systolic femoral flow velocity per second	0.696	Yes
Seated resting diastolic femoral flow velocity per second	0.177	Yes
Seated peak femoral vein velocity per second	0.988	Yes
Seated resting mean femoral flow velocity per min	0.017	No
Seated peak femoral flow velocity per min	0.706	Yes
Change in femoral vein Cross sectional area supine to seated	0.979	Yes
Percentage change in the mean femoral vein flow from supine to seated	0.268	Yes
Percentage change in the mean femoral vein flow from supine to seated	0.633	Yes

Durometry

Variable	Kolmogorov Smirnov test p=0.05	Normal Distribution
Durometry Thigh	0.763	Yes
Durometry Calf	0.925	Yes
Difference in Thigh Durometry supine to seated	0.426	Yes
Difference in Calf Durometry supine to seated	0.839	Yes

Lower leg venous properties (whole limb capacity, capillary filtration, vein size)

Variable	Kolmogorov Smirnov test p=0.05	Normal Distribution
Venous Capacitance at 120 seconds	0.693	Yes
Max Venous Capacitance after 300 seconds	0.914	Yes
Filtration determined from Chart analysis	0.939	Yes
Filtration determined from filtrass analysis	0.812	Yes
PV _i	0.467	Yes
PV	0.993	Yes
Popliteal Vein Maximum Diameter	0.958	Yes
Popliteal vein Maximum Cross Sectional Area	0.830	Yes
Great Saphenous Vein Maximum Diameter	0.421	Yes
Great Saphenous Vein Maximum CSA	0.313	Yes

Lower leg venous compliance

Variable	Kolmogorov Smirnov test p=0.05	Normal Distribution
Whole Limb Compliance	0.149	Yes
Popliteal Vein Compliance	0.537	Yes
Great Saphenous Vein Compliance	0.895	Yes

Fasting Blood Glucose, Insulin and HOMA2 index

Variable	Kolmogorov Smirnov test p=0.05	Normal Distribution
Glucose mmol/l	0.830	Yes
Insulin μ UI/ml	0.093	Yes
HOMA2 Index	0.238	Yes

REFERENCES

- Abe T, Kojima K, Kearns CF, Yohena H, Fukuda J. Whole body muscle hypertrophy from resistance training: distribution and total mass. *Br J Sports Med.* 2003; 37(6): 543-5.
- Abraham P, Leftheriotis G, Desvaux B, Saumet M, Saumet JL. Venous return in lower limb during heat stress. *Am J Physiol.* 1994; 267(4 Pt 2): H1337-40.
- Abramowitz HB, Queral LA, Finn WR, Nora PF Jr, Peterson LK, Bergan JJ, Yao JS. The use of photoplethysmography in the assessment of venous insufficiency: a comparison to venous pressure measurements. *Surgery* 1979; 86(3): 434-41.
- Abramson JH, Hopp C, Epstein LM. The epidemiology of varicose veins *J Epidemiol Community Health* 1981; 35: 213-17.
- Abu-Own A, Scurr JH, Coleridge Smith PD. Saphenous vein reflux without incompetence at the sapheno-femoral junction. *Br J Surg* 1994; 81: 1452-4.
- Abu-Yousef MM, Mufid M, Woods KT, Brown BP, Barloon TJ. Normal lower limb venous Doppler flow phasicity: is it cardiac or respiratory? *Am J Roentgenol.* 1997; 169(6): 1721-5.
- Acree LS, Comp PC, Whitsett TL, Montgomery PS, Nickel KJ, Fjeldstad AS, Fjeldstad C, Gardner AW. The influence of obesity on calf blood flow and vascular reactivity in older adults. *Dyn Med.* 2007; 26(6):4.
- Aellig WH. Clinical pharmacology, physiology and pathophysiology of superficial veins-1. *Br J Clin Pharmacol* 1994; 38: 181-96.
- Aellig WH. Clinical pharmacology, physiology and pathophysiology of superficial veins-2. *Br J Clin Pharmacol* 1994; 38: 289-305.

- Ahti TM, Mäkivaara LA, Luukkaala T, Hakama M, Laurikka JO. Effect of family history on the incidence of varicose veins: a population-based follow-up study in Finland. *Angiology*. 2009; 60(4): 487-91.
- Al-Qais M, Nott DM, King DH, Kaddoura S. Ankle Brachial Pressure Index (ABPI): An update for practitioners. *Vasc Health Risk Manag*. 2009; 5: 833-41.
- Allison MA, Cushman M, Callas PW, Denenberg JO, Jensky NE, Criqui MH. Adipokines are associated with lower extremity venous disease: the San Diego population study. *J Thromb Haemost*. 2010; 8(9): 1912-8.
- Anim-Nyame N, Gamble J, Sooranna SR, Johnson MR, Steer PJ. Microvascular permeability is related to circulating levels of tumour necrosis factor-alpha in pre-eclampsia. *Cardiovasc Res*. 2003 1; 58(1): 162-9.
- Ando S, Imaizumi T, Harada S, Hirooka Y, Takeshita A. Atrial natriuretic peptide increases human capillary filtration and venous distensibility. *J Hypertens* 1992; 10: 451-7.
- Arbeille P, Kerbeci P, Mattar L, Shoemaker JK, Hughson R. Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. *Am J Physiol Heart Circ Physiol*. 2008; 295(5): H1846-54.
- Arfvidsson B, Eklof B, Balfour J. Iliofemoral Venous Pressure Correlates with Intra-abdominal Pressure in Morbidly Obese Patients. *Vasc Endovascular Surg* 2005; 39(6) 505-9.
- Armstrong P, Wastie ML. Introduction ed. Armstrong P, Wastie ML, *Diagnostic imaging fourth edition* Blackwell publishing 2003 pages 1-16.

- Ashby EC, Ashford NS, Campbell MJ. Posture, blood velocity in the common femoral vein, and prophylaxis of venous thrombo-embolism. *Lancet* 1995; 345: 419-21.
- Barwin BN, Roddie IC. Venous distensibility during pregnancy determined by graded venous congestion. *Am J Obstet and Gynecol* 1976; 125(7): 921-3.
- Beaglehole R, Prior IAM, Salmond CE, Davidson F. Varicose veins in the South Pacific. *Int j Epidemiol* 1975; 4(4): 295-9.
- Beebe-Dimmer J, Pfeifer J, Engle J, Schottenfeld D. The epidemiology of Chronic Venous Insufficiency and Varicose Veins. *Ann Epidemiology* 2005; 15: 175-184.
- Belin de Chantemèle E, Pascaud L, Custaud MA, Capri A, Louisy F, Ferretti G, Gharib C, Arbeille P. Calf venous volume during stand-test after a 90-day bed-rest study with or without exercise countermeasure. *J Physiol.* 2004 1; 561(Pt 2): 611-22.
- Bell D, Collier A, Nicoll JJ, Jackson M, Millar AM, Clarke BF, Muir AL. Reduced venous compliance and increased transcapillary escape of protein in insulin-dependent diabetic patients. *Diabet Med.* 1988; 5(5): 454-8.
- Benigni JP, Cazaubon M, Tournerocche A, Achhammer I, Mathieu M. Is obesity an aggravating factor in chronic venous disease? Results of a French epidemiological study in male patients. *Int Angiol.* 2006; 25(3): 297-303.
- Bérczi V, Molnár AA, Apor A, Kovács V, Ruzics C, Várallyay C, Hüttl K, Monos E, Nádasy GL. Non-invasive assessment of human large vein diameter, capacity, distensibility and ellipticity in situ: dependence on anatomical location, age, body position and pressure. *Eur J Appl Physiol.* 2005; 95(4): 283-9.
- Bergan JJ, Pascarella L, Schmid-Schönbein. Pathogenesis of primary chronic venous disease: Insights from animal models of venous hypertension. *J Vasc Surg* 2008 47(1): 183-92.

- Bergan JJ, Schmid-Schönbein GW, Coleridge Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Mechanism of disease: Chronic Venous Disease *N Engl J Med* 2006; 355(5): 488-98.
- Bertin E, Marcus C, Ruiz JC, Eschard JP, Leutenegger M. Measurement of visceral adipose tissue by DXA combined with anthropometry in obese humans. *Int J Obes Relat Metab Disord.* 2000; 24(3): 263-70.
- Birkeland KI, Hanssen KF, Strandén E, Falch JA, Vaaler S. Insulin-mediated increase in calf blood flow is not related to blood pressure levels in patients with type 2 diabetes mellitus. *Blood Press.* 1995; 4(2):80-4.
- Black D, Vora J, Hayward M, Marks R. Measurement of subcutaneous fat thickness with high frequency pulsed ultrasound: comparisons with a caliper and a radiographic technique. *Clin Phys Physiol Meas.* 1988; 9(1): 57-64.
- Boutcher YN, Boutcher SH. Limb vasodilatory capacity and venous capacitance of trained runners and untrained males. *Eur J Appl Physiol.* 2005; 95(1):83-7.
- Brand FN, Dannenberg AL, Abbott RD, Kannel WB. The epidemiology of varicose veins: the Framingham Study. *Am J Prev Med.* 1988; 4(2): 96-101.
- Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J clin Invest* 2007; 117: 1219-1222.
- Brinsuk M, Tank J, Luft FC, Busjahn A, Jordan J. Heritability of venous function in humans. *Arterioscler Thromb Vasc Biol.* 2004; 24(1):207-11.
- Brown E, Greenfield DM, Goei JS, Plassaras G. Filling and emptying of the low-pressure blood vessels of the human forearm. *J Appl Physiol.* 1966; 21(2): 573-82.

- Browse NL, Burnand KG, Irvine AT, Wilson NM. Embryology and radiographic anatomy. In: Diseases of the Veins 2nd Edition. London: Arnold Publishers, 1999; 3: 223-47.
- Browse NL, Burnand KG, Irvine AT, Wilson NM. Physiology and functional anatomy. In: Diseases of the Veins 2nd Edition. London: Arnold Publishers, 1999; 3: 248-65.
- Browse NL, Burnand KG, Irvine AT, Wilson NM. Varicose Veins: Pathology In: Diseases of the Veins 2nd Edition. London: Arnold Publishers, 1999; 5: 145-158.
- Buckey JC, Peshock RM, Blomqvist CG. Deep venous contribution to hydrostatic blood volume change in the human leg. *Am J Cardiol.* 1988; 62(7): 449-53.
- Buján J, Gimeno MJ, Jiménez JA, Kielty CM, Mecham RP, Bellón JM. Expression of elastic components in healthy and varicose veins. *World J Surg.* 2003; 27(8): 901-5.
- Caggiati A, Phillips M, Lametschwandtner A, Allegra C. Valves in Small Veins and Venules. *Eur J Vasc Endovascular Surg* 2006; 32: 447-452.
- Caggiati A. Fascial relationships of the long saphenous vein. *Circulation.* 1999; 100(25): 2547-9.
- Callam MJ, Harper DR, Dale JJ, Ruckley CV. Chronic ulcer of the leg: clinical history. *Br Med J (Clin Res Ed).* 1987; 30: 294(6584):1389-91.
- Cameron JD, Cruickshank JK. Glucose, insulin, diabetes and mechanisms of arterial dysfunction. *Clin Exp Pharmacol Physiol* 2007; 34: 677-682.
- Carpentier PH, Maricq HR, Biro C, Ponçot-Makinen CO, Franco A. Prevalence, risk factors and clinical patterns of chronic venous disorder of lower limbs: A population-based study in France. *J Vasc Surg* 2004; 40: 650-9.

- Cavezzi A, Labropoulos N, Partsch H, Ricci S, Caggiati A, Myers K, Nicolaidis A, Smith PC, Duplex Ultrasound Investigation of the veins in chronic venous disease of the lower limbs-UIP consensus document. Part II. Anatomy. *Eur J Vasc Endovasc Surg* 2006; 31: 288-299.
- Ceballos C, Ribes C, Amado JA, Pérez J, García Unzueta MT, de Berrazueta JR. Venous endothelial function in postmenopausal women who are receiving long-term estrogen and progestagen therapy. *Fertil Steril*. 2000; 74(2): 268-73.
- Chauveau M, Bassez S, Tra S, Scherrer B. Area-pressure relationship of the lower limb main veins in man. *VASA* 2006; 35: 59-66.
- Cheatham ML, De Waele JJ, De Laet I, De Keulenaer B, Widder S, Kirkpatrick AW, Cresswell AB, Malbrain M, Bodnar Z, Mejia-Mantilla JH, Reis R, Parr M, Schulze R, Puig S; World Society of the Abdominal Compartment Syndrome (WSACS) Clinical Trials Working Group. The impact of body position on intra-abdominal pressure measurement: a multicenter analysis. *Crit Care Med*. 2009; 37(7): 2187-90.
- Christ F, Bauer A, Brügger D, Niklas M, Gartside IB, Gamble J. Description and validation of a novel liquid metal-free device for venous congestion plethysmography. *J Appl Physiol*. 2000; 89(4): 1577-83.
- Christ F, Gamble J, Baschnegger H, Gartside I. Relationship between venous pressure and tissue volume during venous congestion plethysmography in man. *J Physiol* 1997; 503: 643-7.
- Christ F, Gamble J, Baschnegger H, Gartside IB. Relationship between vein pressure and tissue volume during venous occlusion plethysmography in man. *J Physiol* 1997; 503: 463-8.

- Christenson JT, Al-Hassan HK, Shawa NJ. Subcutaneous and intramuscular pressures in the post-phlebotic limb. *Scandinavian Journal of Clinical Laboratory Investigation* 1986; 46: 137-41.
- Christopoulos D, Nicolaides AN, Cook A, Irvine A, Galloway JM, Wilkinson A. Pathogenesis of venous ulceration in relation to the calf muscle pump function. *Surgery*. 1989; 106(5): 829-35.
- Ciardullo AV, Panico S, Bellati C, Rubba P, Rinaldi S, Iannuzzi A, Cioffi V, Iannuzzo G, Berrino F. High endogenous estradiol is associated with increased venous distensibility and clinical evidence of varicose veins in menopausal women. *J Vasc Surg*. 2000; 32(3): 544-9.
- Cirovic S, Walsh C, Fraser WD, Gulino A. Venous filling and elastance in the calf positioned above and below heart level. *Aviat Space Environ Med*. 2006; 77(10): 1009-14.
- Clarke H, Smith SRG, Vasdekis SN, Hobbs JT, Nicolaides AN. Role of venous elasticity in the development of varicose veins. *Br J Surg* 1989; 76: 577-580.
- Clasey JL, Bouchard C, Teates CD, Riblett JE, Thorner MO, Hartman ML, Weltman A. The use of anthropometric and dual-energy X-ray absorptiometry (DXA) measures to estimate total abdominal and abdominal visceral fat in men and women. *Obes Res*. 1999; 7(3): 256-64.
- Cobb WS, Burns JM, Kercher KW, Matthews BD, James Norton H, Todd Heniford B. Normal intra abdominal pressure in healthy adults. *J Surg Res*. 2005; 129(2): 231-5.
- Convertino V, Doerr D, Flores J, Wyckliffe Hoffler G, Buchanan P. Leg size and muscle functions associated with leg compliance. *J Appl Physiol* 1988; 64(3): 1017-21.

- Cornu-Thenard A, Boivin P, Baud JM, De VI, Carpentier PH. Importance of the familiar factor in varicose disease: clinical study of 134 families. *J Dermatol Surg Oncol* 1994; 20: 318-26.
- Cotton LT. Varicose veins. Gross anatomy and development. *Br J Surg.* 1961; 48: 589-98.
- Criqui MH, Denenberg JO, Bergan J, Langer RD, Fronck A. Risk Factors for chronic venous disease: The San Diego Population study. *J Vasc Surg* 2007; 46: 331-7.
- Criqui MH, Jamosmos M, Fronck A, Denenberg JO, Langer RD, Bergan J, Golomb BA. Chronic venous disease in an ethnically diverse population: The San Diego Population Study. *Am J Epidemiol* 2003; 158: 448-56.
- Daintree Johnson H, Pflug J. The systemic circulation In: *The Swollen Leg.* London William Heineman Medical Books Ltd 1975; 4: 39-47.
- Danielsson G, Eklof B, Grandinetti A, Kistner RL. The influence of obesity on chronic venous disease. *Vasc Endovascular Surg.* 2002; 36(4): 271-6.
- Danielsson G, Eklof B, Kistner RL Association of venous volume and diameter of incompetent perforator veins in the lower limb—implications for perforator vein surgery. *Eur J Vasc Endovasc Surg.* 2005 ;30(6): 670-3.
- De Groot PC, Bleeker MW, Hopman MT. Ultrasound: a reproducible method to measure conduit vein compliance. *J Appl Physiol.* 2005; 98(5): 1878-83.
- De Jongh RT, Clark AD, IJzerman RG, Serne EH, de Vries G, Stehouwer CD: Physiological hyperinsulinaemia increases intramuscular microvascular reactive hyperaemia and vasomotion in healthy volunteers. *Diabetologia* 2004; 47: 978– 986.

- Delaney EP, Young CN, DiSabatino A, Stillabower ME, Farquhar WB. Limb Venous Tone and Responsiveness in Hypertensive Humans. *J Appl Physiol* 2008; 105: 894-901.
- Delis KT, Knaggs AL, Khodabakhsh P. Prevalence, anatomic patterns, valvular competence, and clinical significance of the Giacomini vein. *J Vasc Surg* 2004; 40: 1174-83.
- Delis KT, Knaggs AL, Sonecha TN, Zervas V, Jenkins MP, Wolfe JH. Lower limb venous haemodynamic impairment on dependency: quantification and implications for the “economy class” position. *Thromb Haemost.* 2004; 91(5): 941-50.
- Delis KT, Slimani G, Hafez HM, Nicolaides AN. Enhancing Venous Outflow in the Lower Limb with Intermittent Pneumatic Compression. A Comparative Haemodynamic Analysis on the Effect of Foot vs. Calf vs. Foot and Calf Compression. *Eur J Vasc Endovasc Surg* 2000; 19: 250–260.
- Delis KT. Leg perforator vein Incompetence: Functional Anatomy. *Radiology* 2005; 235(1): 327-34.
- Demura S, Sato S. Suprailiac or abdominal skinfold thickness measured with a skinfold caliper as a predictor of body density in Japanese adults. *Tohoku J Exp Med.* 2007; 213(1): 51-61.
- Després JP. Cardiovascular disease under the influence of excess visceral fat. *Crit Pathw Cardiol.* 2007; 6(2): 51-9.
- Dhawan V, Brookes Z, Kaufman S. Repeated pregnancies (multiparity) increase venous tone and reduces compliance. *Am J Physiol Regul Integr Comp Physiol.* 2005 ;289(1): R23-8.

- Doorenbos CJ, Blauw GJ, van Brummelen P. Arterial and venous effects of atrial natriuretic peptide in the human forearm. *Am J Hypertens*. 1991; 4(4 Pt 1): 333-40.
- Edouard DA, Pannier BM, London GM, Cuche JL, Safar ME. Venous and arterial behavior during normal pregnancy. *Am J Physiol*. 1998; 274(5) (2): H1605-12.
- Edwards JE, Edwards EA. The saphenous valves in varicose veins. *Am J Heart* 1940; 19: 338-51.
- Eiken O, K oleg ard R Comparison of vascular distensibility in the upper and lower extremity. *Acta Physiol Scand*. 2004; 181(3): 281-7.
- Elia M, Kurpad A. What is the blood flow to resting human muscle? *Clin Sci (Lond)*. 1993; 84(5): 559-63.
- Elsharawya MA, Naimb MM, Abdelmaguidc EM, Al-Mulhim AA. Role of saphenous vein wall in the pathogenesis of primary varicose veins. *Interact CardioVasc Thorac Surg* 2007; 6: 219-224.
- Endrikat J, Blode H, Gerlinger C, Rosenbaum P, Kuhnz W. A pharmacokinetic study with a low-dose oral contraceptive containing 20 microg ethinylestradiol plus 100 microg levonorgestrel. *Eur J Contracept Reprod Health Care*. 2002; 7(2): 79-90
- Evans CJ, Fowkes FGR, Ruckley CV, Lee AJ, Prevalence of varicose veins and chronic venous insufficiency in men and women in the general population: Edinburgh Vein Study. *J Epidemiol Community Health* 1999; 53:149-53
- Evcimen ND, King GL. The role of protein kinase C activation and the vascular complications of diabetes. *Pharmacol Res* 2007; 55: 498-501.
- Fagrell B, Jorneskog G, Intaglietta M. Disturbed microvascular reactivity and shunting – a major cause for diabetic complications. *Vasc Med* 1999; 4: 125-127.

- Falanga V, Bucalo B. Use of durometer to assess skin hardness. *J Am Acad Dermatol* 1993; 29(1): 47-51.
- Fawer R, Dettling A, Weihs D, Welti H, Schelling JL. Effect of the menstrual cycle, oral contraception and pregnancy on forearm blood flow, venous distensibility and clotting factors. *Eu J Clin Pharmacol* 1978; 13: 251-7.
- Fegan PG, Tooke JE, Gooding KM, Tullett JM, MacLeod KM, Shore AC. Capillary pressure in subjects with Type 2 diabetes and hypertension and the effects of antihypertensive therapy. *Hypertension* 2003; 41: 1111-17.
- Feldman RD, Bierbrier GS. Insulin-mediated vasodilation: impairment with increased blood pressure and body mass. *Lancet* 1993; 342: 707-709.
- Feldman RD, Hramiak IM, Finegood DT, Behme MT. Parallel Regulation of the Local Vascular and Systemic Metabolic Effects of Insulin. *J Clin Endocrinol Metab* 1995; 80: 1556-9
- Fitzpatrick MA, Hinderliter AL, Egan BM, Julius S. Decreased venous distensibility and reduced renin responsiveness in hypertension. *Hypertension* 1986; 6: Suppl 2 36-43.
- Fornage BD. Sonography of the skin and subcutaneous tissues. *Radiol Med.* 1993; 85(5 Suppl 1): 149-55.
- Fowkes FG, Lee AJ, Evans CJ, Allan PL, Bradbury AW, Ruckley CV. Lifestyle risk factors for lower limb venous reflux in the general population: Edinburgh Vein Study. *Int J Epidemiol.* 2001; 30(4): 846-52.
- Fromy B, Legrand MS, Abraham P, Leftheriotis G, Cales P, Saumet JL. Effects of positive pressure on both femoral venous and arterial blood velocities and the cutaneous microcirculation of the forefoot. *Cardiovasc Res.* 1997; 36(3): 372-6.

- Fronck A, Criqui MH, Denenberg J, Langer RD. Common femoral vein dimensions and haemodynamics including Valsalva response as a function of sex, age, and ethnicity in a population study. *J Vasc Surg* 2001; 33: 1050-6.
- Fronck A, Denenberg JO, Criqui MH, Langer RD. Quantified duplex augmentation in healthy subjects and patients with venous disease: San Diego population study. *J Vasc Surg* 2003; 37: 1054-8.
- Fronck A, Kim R, Curran B. Non-invasively determined ambulatory venous pressure. *Vas med* 2000; 5: 213-6.
- Fronck A, Minn C, Kim R. Venous outflow and inflow resistance in health and venous disease. *J Vasc Surg* 2000; 31: 472-6.
- Gamble J, Christ F, Gartside IB. Human calf pre-capillary resistance decreases in response to small cumulative increases in venous congestion pressure. *J Physiol* 1998; 507: 611-17.
- Gamble J, Gartside IB, Christ F J. A reassessment of mercury in silastic strain gauge plethysmography for microvascular permeability assessment in man *Physiol*. 1993; 464: 407-22.
- Gamble J. Realisation of a Technique for the Non-Invasive, Clinical Assessment of Microvascular Parameters in Man; The KM Factor. *Eur Surg Res* 2002; 34: 113-22.
- Gandhi RH, Irizarry E, Nackman GB, Halper VJ, Mulcar RJ, Tilson MD. Analysis of the connective tissue matrix and proteolytic activity of primary varicose veins. *J Vasc Surg* 1993; 18: 814-20.
- Gascho JA, Fanelli C, Zelis R. Aging reduces venous distensibility and venodilatory response to nitroglycerin in normal subjects. *Am J Cardiol* 1989; 63: 1267-70.

- Gelman S. Venous function and central venous pressure. *Anaesthesiology* 2008; 108: 735-748.
- Geloneze B, Vasques AC, Stabe CF, Pareja JC, Rosado LE, Queiroz EC, Tambascia MA; BRAMS Investigators. HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). *Arq Bras Endocrinol Metabol*. 2009; 53(2): 281-7.
- Giribela CR, Rubira MC, de Melo NR, Plentz RD, de Angelis K, Moreno H, Consolim-Colombo FM. Effect of a low-dose oral contraceptive on venous endothelial function in healthy young women: preliminary results. *Clinics*. 2007; 62(2): 151-8.
- Goodrich SM, Wood JE. The effect of estradiol-17 β on peripheral venous distensibility and velocity of venous blood flow. *Am J Obstet Gynaecol* 1966; 96(3): 407-12.
- Gosling RG, Beasley MG. Effect of Oral contraceptives on resting blood flow in the leg. *Angiology*. 1977; 28(9): 588-98.
- Gourgou S, Dedieu F, Sancho-Garnier H. Lower limb venous insufficiency and tobacco smoking; a case-control study. *Am J Epidemiol* 2002; 155(11): 1007-15.
- Gradmark AM, Rydh A, Renström F, De Lucia-Rolfe E, Sleigh A, Nordström P, Brage S, Franks PW. Computed tomography-based validation of abdominal adiposity measurements from ultrasonography, dual-energy X-ray absorptiometry and anthropometry. *Br J Nutr* 2010; 7: 1-7.
- Grassi G, Quarti-trevano F, Seravalle G, Dell'Oro R. Cardiovascular risk and adrenergic overdrive in the metabolic syndrome. *Nutr Metab Cardiovasc Dis* 2007; 17: 473-481.

- Greenleaf JE, Rehrer NJ, Mohler SR, Quach DT, Evans DG. Airline chair-rest deconditioning: induction of immobilisation thromboemboli? *Sports Med.* 2004; 34(11): 705-25.
- Guyton AC, Granger HJ, Taylor AE. Interstitial fluid pressure. *Physiol Rev.* 1971; 51(3): 527-63.
- Guyton AC, Granger HJ, Taylor AE. Interstitial fluid pressure. *Physiol Rev.* 1971; 51(3): 527-63.
- Guyton AC. A concept of negative interstitial pressure based on pressures in implanted perforator capsules. *Circ Res* 1963; 3: 70.
- Guzzaloni G, Minocci A, Marzullo P, Liuzzi A. Sagittal abdominal diameter is more predictive of cardiovascular risk than abdominal fat compartments in severe obesity. *Int J Obes (Lond).* 2009; 33(2): 233-8.
- Haas E, Meyer MR, Schurr U, Bhattacharya I, Minotti R, Nguyen HH, Heigl A, Lachat M, Genoni M, Barton M. Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. *Hypertension.* 2007; 49(6): 1358-63.
- Haderslev KV, Haderslev PH, Staun M. Accuracy of body composition measurements by dual energy x-ray absorptiometry in underweight patients with chronic intestinal disease and in lean subjects. *Dyn Med.* 2005; 4(1): 1.
- Haenen JH, Janssen MC, Brakkee AJ, Van Langen H, Wollersheim H, De Boo TM, Skotnicki SH, Thien T. Venous reflux has a limited effect on calf muscle pump dysfunction in post-thrombotic patients. *Clin Sci (Lond).* 2000; 98(4): 449-54.

- Haenen JH, van Langen H, Janssen MC, Wollersheim H, van't hof MA, van Asten WN, Skotnicki SH, Thien T. Venous duplex scanning of the leg: range, variability and reproducibility. *Clin Sci (Lond)*. 1999; 96(3): 271-7.
- Haffner SM. Abdominal adiposity and cardiometabolic risk: do we have all the answers? *Am J Med*. 2007 Sep;120(9 Suppl 1):S10-6.
- Halliwill JR, Minson CT, Joyner MJ. Measurement of limb venous compliance in humans: technical considerations and physiological findings. *J Appl Physiol* 1999; 87: 1555-1563.
- Henriksen O. Sympathetic reflex control of blood flow in human peripheral tissues. *Acta Physiol Scand Suppl*. 1991; 603: 33-9.
- Hernandez J, Franke W. Age-and fitness-related differences in limb venous compliance do not affect tolerance to maximal lower body negative pressure in men and women. *J Appl Physiol* 2004; 97(3): 925-9.
- Hertzberg BS, Kliewer MA, DeLong DM, Lalouche KJ, Paulson EK, Frederick MG, Carroll BA. Sonographic assessment of lower limb vein diameters: implications for the diagnosis and characterisation of deep venous thrombosis. *Am J Roentgenol*. 1997; 168(5): 1253-7.
- Hewlett AW, van Zwaluwenburg JG. The rate of Blood Flow in the arm. *Heart* 1909; 1: 87-97.
- Higman DJ, Greenhalgh RM, Powell JT. Smoking impairs endothelium-dependent relaxation of saphenous vein. *Br J Surg*. 1993; 80(10):1242-5.
- Higman DJ, Strachan AM, BATTERY L, Hicks RC, Springall DR, Greenhalgh RM, Powell JT. Smoking impairs the activity of endothelial nitric oxide synthase in saphenous vein. *Arterioscler Thromb Vasc Biol*. 1996; 16(4): 546-52.

- Hirai M, Naiki K, Nakayama R. Prevalence and risk factors of varicose veins in Japanese women. *Angiology*. 1990; 41(3): 228-32.
- Hirshoren N, Tzoran I, Makrienko I, Edoute Y, Plawner MM, Itskovitz-Eldor J, Jacob G. Menstrual cycle effects on the neurohumoral and autonomic nervous systems regulating the cardiovascular system. *J Clin Endocrinol Metab*. 2002; 87(4): 1569-75.
- Hollenberg NK, Boréus LO. The influence of rate of filling on apparent venous distensibility in man. *Can J Physiol Pharmacol* 1972; 50: 310-16.
- Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med*. 2000; 30(1): 1-15.
- Iannuzzi A, Panico S, Ciardullo A, Bellati C, Cioffi V, Iannuzzo G, Celentano E, Berrino F, Rubba P. Varicose veins of the lower limb and venous capacitance in postmenopausal women: Relationship to obesity. *J Vasc Surg* 2002; 36: 965-8.
- Irving RJ, Walker BR, Noon JP, Watt GC, Webb DJ, Shore AC. Microvascular correlates of blood pressure, plasma glucose, and insulin resistance in health. *Cardiovasc Res*. 2002; 53(1): 271-6.
- Jeanneret C, Jäger KA, Zaugg CE, Hoffman U. Venous Reflux and Venous Distensibility in Varicose and Healthy Veins. *Eur J Vasc Endovasc Surg* 2007; 34: 236-42.
- Jeanneret C, Labs KH, Aschwanden M, Bollinger A, Hoffmann U, Jäger K. Physiological reflux and venous diameter change in the proximal lower limb veins during a standardised Valsalva manoeuvre. *Eur J Vasc Endovasc Surg* 1999; 17(5): 398-403.

- Jorfeldt L, Vedung T, Forsström E, Henriksson J. Influence of leg position and environmental temperature on the segmental volume expansion during venous occlusion plethysmography. *Clin Sci* 2003; 104: 599-605.
- Joyner MJ, Dietz NM, Shepherd JT. From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol*. 2001; 91(6): 2431-41.
- Jukkola TM, Mäkivarra LA, Luukkaala T, Hakama M, Laurikka J. The effects of parity, oral contraceptive use and hormone replacement therapy on the incidence of varicose veins. *J Obstet Gynaecol* 2006; 26(5): 448-51.
- Karas RH, Baur WE, van Eickles M, Mendelsohn ME. Human vascular smooth muscle cells express and oestrogen receptor isoform. *FEBS Letters* 1995; 377: 103-08.
- Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation* 1994; 89(5): 1943-50.
- Keates JS, Fitzgerlad DE. Limb volume and blood flow changes during the menstrual cycle. 2 Changes in blood flow and venous distensibility during the menstrual cycle. *Angiology* 1969; 20(10): 624-7.
- Kenney WL, Armstrong CG. The effect of aerobic conditioning on venous pooling in the foot. *Med Sci Sports Exerc* 1987. 19:474-479.
- Kim J, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006; 113: 1888-1904.
- Kim JT, Lee NJ, Na HS, Jeon Y, Kim HS, Kim CS, Kim SD. Ultrasonographic investigation of the effect of inguinal compression on the cross-sectional area of the femoral vein. *Acad Emerg Med*. 2008; 15(1): 101-3.

- King ES. The genesis of varicose veins. *Aust N Z J Surg.* 1950; 20(2): 126-33.
- Kirsch D, Dienes HP, Kuchle R, Duschner H, Wahl W, Böttger T, Junginger T. Changes in the extracellular matrix of the vein wall – the cause of primary varicosities. *Vasa* 2000; 29(3): 173-7.
- Kissin EY, Schiller AM, Gelbard RB, Anderson JJ, Falanga V, Simms RW, Korn JH, Merkel PA. Durometry for the assessment of skin disease in systemic sclerosis. *Arthritis Rheum.* 2006 15; 55(4):603-9.
- Knaapen MW, Somers P, Bortier H, De Meyer GR, Kockx MM. Smooth muscle cell hypertrophy in varicose veins is associated with expression of estrogen receptor- β . *J Vasc Res.* 2005; 42(1): 8-12.
- Komsuoğlu B, Göldeli O, Kulan K, Cetinarslan B, Komsuoğlu SS. Prevalence and risk factors of varicose veins in an elderly population. *Gerontology.* 1994;40(1):25-31.
- Krasiński Z, Biskupski P, Dzieciuchowicz Ł, Kaczmarek E, Krasińska B, Staniszewski R, Pawlaczyk K, Stanisić M, Majewski P, Majewski W. The Influence of Elastic Components of the Venous Wall on the Biochemical Properties of Different Veins Used for Arterial Reconstruction. *Eur J Vasc Endovasc Surg* 2010 (epub ahead of print).
- Kröger K, Ose C, Rudofsky G, Roesener J, Weiland D, Hirche H. Peripheral veins: influence of gender, body mass index age and varicose veins on cross-sectional area. *Vasc Med* 2003; 8(4): 249-55.
- Kügler C, Strunk M, Rudofsky G. Venous Pressure Dynamics of the healthy Human Leg. *J Vasc Res* 2001; 38: 20-9.

- Kwon OY, Jung DY, Kim Y, Cho SH, Yi CH. Effects of ankle exercise combined with deep breathing on blood flow velocity in the femoral vein. *Aust J Physiother.* 2003; 49(4): 253-8.
- Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J Clin Invest.* 1990; 85(6): 1844-52.
- Labropoulos N, Giannoukas A, Stavridis G, Bailey D, Glenville B, Nicolaides A. Insights in the Development of primary venous reflux. *Vasc Endovascular Surg.* 1999; 33: 191-6.
- Laing W. Chronic venous diseases of the leg: Report no. 108. London UK: Office of Health Economics; 1992; 28: 1-44.
- Lake M, Pratt GH, Wright IS. Arteriosclerosis and varicose veins: Occupational activities and other factors. *JAMA* 1942; 119 696-701.
- Lee A, Evans C, Allan P, Vaughan Ruckley C, Fowkes FGR. Lifestyle factors and the risk of varicose veins: Edinburgh Vein study. *J Clin Epi* 2003; 56: 171-179.
- Lee AJ, Evans CJ, Ruckley CV, Fowkes FGR. Does lifestyle really affect venous disease? In: Ruckley CJ, Fowkes FGR, Bradbury AW (eds). *Venous Disease: Epidemiology, Management and Delivery of Care.* London, Springer 1999; 4: 32-41.
- Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care.* 1998; 21(12): 2191-2.
- L'Hermitte F, Behar A, Pariès J, Cohen-Boulakia F, Attali JR, Valensi P. Impairment of lymphatic function in women with gynoid adiposity and swelling syndrome. *Metabolism.* 2003; 52(7): 805-9.

- Lim CS, Davies AH. Pathogenesis of primary varicose veins. *Br J Surg* 2009; 96: 1231–1242.
- Lindenberger M, Länne T. Decreased capillary filtration but maintained venous compliance in the lower limb of aging women. *Am J physiol Heart Circ Physiol* 2007; 293: H3568-74
- Lindenberger M, Länne T. Sex-related effects on venous compliance and capillary filtration in the lower limb. *Am J Physiol Regul Integr Comp Physiol* 2007; 292: R852-9.
- Loesch A, Dashwood MR. On the sympathetic innervation of the human greater saphenous vein: relevance to clinical practice. *Curr Vasc Pharmacol.* 2009; 7(1): 58-67.
- Ludbrook J. Functional aspects of the veins of the leg. *Am Heart J.* 1962; 64: 706-13.
- Ludbrook J, Loughlin J. Regulation in postarteriolar vessels of the lower limb. *Am Heart J.* 1964; 67: 493-507.
- MacKenzie RK, Allan PL, Ruckley CV, Bradbury AW. The effect of long saphenous vein stripping on deep venous reflux. *Eur J Vasc Endovasc Surg.* 2004; 28(1): 104-7.
- Mahy IR, Tooke JE, Shore AC. Capillary pressure during and after incremental venous pressure elevation in man. *J Physiol.* 1995; 15 (485) (Pt 1):213-9.
- Malbrain ML. Different techniques to measure intra-abdominal pressure (IAP): time for a critical re-appraisal. *Intensive Care Med.* 2004; 30(3): 357-71.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985; 28(7): 412-9.

- Meendering JR, Torgrimson BN, Houghton BL, Halliwill JR, Minson CT. Effects of menstrual cycle and oral contraceptive use on calf venous compliance. *Am J Physiol Heart Circ Physiol*. 2005; 288(1): H103-10.
- Mercurio G, Balloi C, De Candia G, Panzuto MG, Zoncu S, Cagnacci A, Melis GB, Cherchi A. Effects of Transdermal Estrogen Administration on Peripheral Vascular Responsiveness in Menopausal Women. *International Journal of Angiology* 1997; 6: 237-240.
- Messerli FH, Christie B, DeCarvalho JG, Aristimuno GG, Suarez DH, Dreslinski GR, Frohlich ED. Obesity and essential hypertension. Hemodynamics, intravascular volume, sodium excretion, and plasma renin activity. *Arch Intern Med*. 1981; 141(1): 81-5.
- Miller JD, Pegelow DF, Jacques AJ, Dempsey JA. Effects of augmented respiratory muscle pressure production on locomotor limb venous return during calf contraction exercise. *J Appl Physiol*. 2005; 99(5): 1802-15.
- Moed BR, Thorderson PK. Measurement of intracompartmental pressure: a comparison of the slit catheter, side-ported needle, and simple needle. *J Bone Joint Surg Am*. 1993; 75(2): 231-5.
- Molnár AA, Apor A, Kristóf V, Nádasy GL, Préda I, Hüttl K, Acsády G, Monos E, Bérczi V. Generalized changes in venous distensibility in postthrombotic patients. *Thromb Res*. 2006; 117(6): 639-45.
- Moloney MC, Lyons GM, Egan M, Wallis F, Burke PE, Kavanagh E, Grace PA. Does size matter? The impact of calf muscle volume on venous return in patients with venous leg ulcers. *Phlebology*. 2007; 22(2): 65-9.

- Monahan JD, Ray CA. Gender affects calf venous compliance at rest and during baroreceptor unloading in humans. *Am J Physiol Heart Circ Physiol* 2004; 286: H895-901.
- Monahan KD, Dinunno FA, Seals DR, Halliwill JR. Smaller age-associated reductions in leg venous compliance in endurance exercise-trained men. *Am J Physiol Heart Circ Physiol* 2001; 281: H1267-73.
- Monos E, Bérczi V, Nádasy G. Local control of veins: biomechanical, metabolic, and humoral aspects. *Physiol Rev.* 1995; 75(3): 611-66.
- Mortensen JD, Talbot S, Burkart JA. Cross-sectional internal diameters of human cervical and femoral blood vessels: relationship to subject's sex, age, body size. *Anat Rec.* 1990; 226(1): 115-24.
- Muntinga JHJ, Schut JK, Visser KR, Zijlstra WG. Age-related differences in elastic properties of the upper arm vascular bed in healthy adults. *J Vasc Res* 1997; 34: 137-47.
- Murakami M, Arai S, Nagai Y, Yamazaki K, Iizuka S. Subcutaneous fat distribution of the abdomen and buttocks in Japanese women aged 20 to 58 years. *Appl Human Sci.* 1997; 16(4): 167-77.
- Naik J, Bello AM, Scriven JM, Hartshorne T, London NJM (2000) Lower limb ulceration: a detailed study of aetiology in 555 patients. *Br J Surg* 2000; 87: 502.
- Naoum J, Hunter G, Woodside K, Chen C. Current advances in the pathogenesis of Varicose Veins. *J Surg Res* 2007; 141: 311-6.
- Neglén P, Raju S. Compliance of the normal and post-thrombotic calf. *J Cardiovasc Surg (Torino).* 1995; 36(3): 225-31.

- New Diagnostic Criteria for Diabetes.
http://www.diabetes.org.uk/About_us/Our_Views/Care_recommendations/New_diagnostic_criteria_for_diabetes.
- Newey VR, Nassiri DK. Online artery diameter measurement in ultrasound images using artificial neural networks. *Ultrasound Med Biol*. 2002; 28(2): 209-16.
- Noon JP, Walker BR, Webb DJ, Shore AC, Holton DW, Edwards HV, Watt GC. Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. *J Clin Invest*. 1997 15; 99(8): 1873-9.
- Olsen H, Länne T. Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. *Am J Physiol Heart Circ Physiol* 1998; 275: 878-86.
- Olszewski W, Engeset A, Jaeger PM, Sokolowski J, Theodorsen L. Flow and composition of leg lymph in normal men during venous stasis, muscular activity and local hyperthermia. *Acta Physiol Scand*. 1977; 99(2): 149-55.
- Ono T, Bergan JJ, Schmid-Schönbein GW, Takase S. Monocyte infiltration into venous valves. *J Vasc Surg* 1998; 27: 158-66.
- Ooué A, Ichinose TK, Inoue Y, Nishiyasu T, Koga S, Kondo N. Changes in blood flow in conduit artery and veins of the upper arm during leg exercise in humans. *Eur J Appl Physiol*. 2008; 103(3): 367-73.
- Ooué A, Ichinose-Kuwahara T, Shamsuddin AK, Inoue Y, Nishiyasu T, Koga S, Kondo N. Changes in blood flow in a conduit artery and superficial vein of the upper arm during passive heating in humans. *Eur J Appl Physiol*. 2007; 101(1): 97-103.

- Orphanidou C, McCargar L, Birmingham CL, Mathieson J, Goldner E. Accuracy of subcutaneous fat measurement: comparison of skinfold calipers, ultrasound, and computed tomography. *J Am Diet Assoc.* 1994; 94(8): 855-8.
- Padberg F Jr, Cerveira JJ, Lal BK, Pappas PJ, Varma S, Hobson RW 2nd. Does severe venous insufficiency have a different etiology in the morbidly obese? Is it venous? *J Vasc Surg.* 2003; 37(1): 79-85.
- Padberg FT. Classification and clinical diagnostic evaluation of patients with chronic venous disorders : In Cronenwett JL, Gloviczki P, Johnston KW, Krupski WC, Ouriel K, Sidawy AN (Eds). *Rutherford Vascular Surgery 6th Edition*, 2005. Philadelphia, Elsevier Saunders. 155: 2230-40.
- Pang CC. Measurement of body venous tone. *J Pharmacol Toxicol Methods.* 2000; 44(2): 341-60.
- Pappas PJ, Lal BK, Cerveira JJ, Duran WN. 2005. The Pathophysiology of Chronic Venous Insufficiency. In Cronenwett JL, Gloviczki P, Johnston KW, Krupski WC, Ouriel K, Sidawy AN (Eds). *Rutherford Vascular Surgery 6th Edition*. Philadelphia, Elsevier Saunders. 2005; 154: 2220-9.
- Perrot-Applanat M, Cohen-Solal K, Milgrom E, Finet M. Progesterone Receptor Expression in Human Saphenous Veins. *Circulation* 1995; 92: 2975-83.
- Phillips CJ. Changes in veno-motor tone during sympatho-excitation: influence of gender and female cycle phase. Feb 2010 [awaiting library reference].
- Planken RN, Keuter XH, Kessels AG, Hoeks AP, Leiner T, Tordoir JH. Forearm cephalic vein cross-sectional area changes at incremental congestion pressures: towards a standardized and reproducible vein mapping protocol. *J Vasc Surg* 2006; 44(2): 353-8.

- Pollack AA, Wood EH. Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. *J Appl Physiol*. 1949; 1(9): 649-62.
- Poole DC, Brown MD, Hudlicka O: Counterpoint: there is not capillary recruitment in active skeletal muscle during exercise. *J Appl Physiol* 2008; 104: 891– 893.
- Porter JM, Moneta GL, International Consensus Committee on Chronic Venous Disease Reporting standards in venous disease: an update , *J Vasc Surg* 1995; 21: 635-45.
- Porter JM, Moneta GL. Reporting standards in venous disease: an update. International Consensus Committee on Chronic Venous Disease. *J Vasc Surg*. 1995 Apr;21(4):635-45. CEAP classification
- Pottier M, Dubreuil A, Monod H. The effects of sitting posture on the volume of the foot. *Ergonomics* 1969; 12(5): 753-8.
- Prerovsky I. Disease of the veins. World Health Organization, internal communication. MHO-PA 10964.
- Purewal TS, Goss DE, Watkins PJ, Edmonds ME. Lower limb venous pressure in diabetic neuropathy. *Diabetes Care* 1995; 18: 377-381.
- Qiao T, Liu C, Ran F. The impact of gastrocnemius muscle cell changes in chronic venous insufficiency. *Eur J Vasc Endovasc Surg*. 2005; 30(4): 430-6.
- Quinkler M, Sinha B, Tomlinson JW, Bujalska IJ, Stewart PM, Arlt W. Androgen generation in adipose tissue in women with simple obesity—a site-specific role for 17beta-hydroxysteroid dehydrogenase type 5. *J Endocrinol*. 2004; 183(2): 331-42.
- Racette SB, Schoeller DA, Kushner RF, Neil KM, Herling-Iaffaldano K. Effects of aerobic exercise and dietary carbohydrate on energy expenditure and body

- composition during weight reduction in obese women. *Am J Clin Nutr.* 1995; 61(3): 486-94.
- Raju S, Fredericks R, Lishman P, Neglén P, Morano J. Observations on the calf venous pump mechanism: Determinants of post exercise pressure. *J Vasc Surg* 1993; 17(3): 459-69.
 - Rattigan S, Bradley EA, Richards SM, Clark MG. Muscle metabolism and control of capillary blood flow: insulin and exercise. *Essays Biochem.* 2006; 42: 133-44.
 - Recek C, Pojer H. Ambulatory pressure gradient in the veins of the lower extremity. *VASA* 2000; 29: 187-90.
 - Recek C. Conception of the venous hemodynamics in the lower extremity. *Angiology* 2006; 57: 556-563.
 - Ribeiro MM, Trombetta IC, Batalha LT, Rondon MU, Forjaz CL, Barretto AC, Villares SM, Negrão CE. Muscle sympathetic nerve activity and hemodynamic alterations in middle-aged obese women. *Braz J Med Biol Res.* 2001; 34(4): 475-8.
 - Risk MR, Lirofonis V, Armentano RL, Freeman R. A biphasic model of limb venous compliance: a comparison with linear and exponential models. *J Appl Physiol* 2003; 95: 1207-15.
 - Roche-Nagle G, Oreopolous G. Extracranial carotid artery aneurysms. *Am J Surg.* 2009; 197(6): e64-5.
 - Rokitansansky. A manual of pathological anatomy. Vol 4 London , Sydenham Society 1852.
 - Romanelli M, Falanga V. Use of durometer to measure the degree of skin induration in lipodermatosclerosis. *J Am Acad Dermatol* 1995; 32(2 pt 1): 188-91

- Rothe CF. Physiology of venous return. An unappreciated boost to the heart. *Arch Intern Med.* 1986; 146(5): 977-82.
- Rothe CF. Reflex control of veins and vascular capacitance. *Physiol Rev* 1983; 63(4): 1281-342.
- Safar ME, London GM. Arterial and venous compliance in sustained essential hypertension. *Hypertension* 1987;10(2): 133-9.
- Sam RC, Darvall KA, Adam DJ, Silverman SH, Bradbury AW. A comparison of the changes in generic quality of life after superficial venous surgery with those after laparoscopic cholecystectomy. *J Vasc Surg.* 2006; 44(3): 606-10.
- Sam RC, Darvall KAL, Adam DJ, Silverman SH, Bradbury AW. Digital venous photoplethysmography in the seated position is a reproducible noninvasive measure of lower limb venous function in patients with isolated superficial venous reflux. *J Vasc Surg* 2006; 43: 335-41.
- Sam RC, MacKenzie RK, Paisley AM, Ruckley CV, Bradbury AW. The effect of superficial venous surgery on generic health-related quality of life. *Eur J Vasc Endovasc Surg.* 2004; 28(3): 253-6.
- Sansilvestri-Morel P, Fioretti F, Rupin A, Senni K, Fabiani JN, Godeau G, Verbeuren TJ. Comparison of extracellular matrix in skin and saphenous veins from patients with varicose veins: does the skin reflect venous matrix changes. *Clinical Science* 2007; 112: 229-39.
- Sansilvestri-Morel P, Rupin A, Badier-Commander C, Kern P, Fabiani J-N, Verbeuren TJ, Vanhoutte PM. Imbalance in the synthesis of collagen type 1 and collagen 111 in smooth muscle cells derived from human varicose veins. *J Vasc Res* 2001; 38: 560-68.

- Sansilvestri-Morel P, Rupin A, Jaisson S, Fabiani JN, Verbeuren TJ, Vanhoutte PM. Synthesis of collagen is dysregulated in cultured fibroblasts derived from skin of subjects with varicose veins as it is in venous smooth muscle cells. *Circulation*. 2002 Jul 23; 106(4): 479-83.
- Sansilvestri-Morel P, Rupin A, Jullien ND, Lembrez N, Mestries-Dubois P, Fabiani JN, Verbeuren TJ. Decreased Production of Collagen type III in culture smooth muscle cells from varicose vein patients is due to a degradation by MMPs: Possible interaction of MMP-3. *J Vasc Res* 2005; 42: 388-98.
- Schmitt M, Broadley AJM, Nightingale AK, Payne N, Gunaruwan P, Taylor J, Lee L, Cockcroft J, Struthers AD, Frenneaux MP. Atrial Natriuretic Peptide Regulates Regional Vascular Volume and Venous Tone in Humans. *Arterioscler Thromb Vasc Biol* 2003; 23: 1833-8.
- Scott TE, LaMorte WW, Gorin DR, Menzoian JO. Risk factors for chronic venous insufficiency: a dual case-control study. *J Vasc Surg*. 1995; 22(5): 622-8.
- Seidel AC, Miranda F, Juliano Y, Novo NF. Relationship between the diameter of GSV and BMI. *J Vasc Br* 2005; 4(3): 265-69.
- Seidell JC, Bakx JC, De Boer E, Deurenberg P, Hautvast JGAJ. Fat distribution of overweight persons in relation to morbidity and subjective health. *Int J Obes* 1985; 9: 363-74.
- Seidell JC, De Groot LCPGM, van Sonsbeek JLA, Deurenberg P, Hautvast JGAJ. Associations of moderate and severe overweight with self-reported illness and medical care in Dutch adults. *Am J Public Health*. 1986; 76(3): 264-9.

- Serné EH, de Jongh RT, Eringa EX, Ijzerman RG, Stehouwer CDA. Microvascular dysfunction: a potential pathophysiological role in the metabolic syndrome. *Hypertension* 2007; 50: 204-211.
- Sidhu JS, Newey VR, Nassiri DK, Kaski JC. A rapid and reproducible on line automated technique to determine endothelial function. *Heart*. 2002; 88(3): 289-92.
- Simon G, Franciosa JA, Cohn JN. Decreased venous distensibility in essential hypertension: Lack of Systemic Hemodynamic correlates. *Angiology* 1979; 30(3): 147-59.
- Sisto T, Reunanen A, Laurikka J, Impivaara O, Heliövaara M, Knekt P, Aromaa A. Prevalence and risk factors of varicose veins in lower extremities: Mini-Finland Health Survey. *Eur J Surg* 1995; 161:405-14.
- Snijder MB, van Dam RM, Visser M, Seidell JC. What aspects of body fat are particularly hazardous and how do we measure them? *Int J Epidemiol*. 2006; 35(1): 83-92.
- Song D, Hutchings SR, Pang CC. Impaired in vivo venous constriction in conscious obese Zucker rats with metabolic syndrome. *Naunyn Schmied Arch Pharmacol* 2006; 373: 451-456.
- Sparey C, Haddad N, Sissons G, Rosser S, de Cossart L. The Effect of Pregnancy on the Lower-limb Venous System of Women with Varicose Veins. *Eur J Vasc Endovasc Surg* 1999; 18: 294-99.
- Stanhope JM. Varicose veins in a population of Lowland New Guinea. *Int J Epidemiol* 1975; 4: 221-5.
- Stepniakowski K, Egan B. Additive effects of obesity and hypertension to limit venous volume. *Am J Physiol* 1995. 268; R562-R568

- Stevens-Simon C, Thureen P, Stamm E, Scherzinger A. A comparison of four techniques for measuring central adiposity in postpartum adolescents. *J Matern Fetal Med.* 2001; 10(3): 209-13.
- Stick C, Jaeger H, Witzleb E. Measurements of volume changes and venous pressure in the human lower leg during walking and running. *J Appl Physiol.* 1992; 72(6): 2063-8.
- Straznicky NE, Lambert GW, Masuo K, Dawood T, Eikelis N, Nestel PJ, McGrane MT, Mariani JA, Socratous F, Chopra R, Esler MD, Schlaich MP, Lambert EA. Blunted sympathetic neural response to oral glucose in obese subjects with the insulin-resistant metabolic syndrome. *Am J Clin Nutr.* 2009; 89(1): 27-36.
- Streeten DH. Role of impaired lower-limb venous innervation in the pathogenesis of the chronic fatigue syndrome. *Am J Med Sci.* 2001; 321(3): 163-7.
- Struckmann JR. Assessment of venous muscle pump function by ambulatory calf volume strain gauge plethysmography after surgical treatment of varicose veins: a prospective study of 21 patients. *Surgery.* 1987; 101(3): 347-53.
- Sugerman H, Windsor A, Bessos M, Wolfe L. Intra-abdominal pressure, sagittal abdominal diameter and obesity correlation. *Journal of internal medicine* 1997; 241: 71-9.
- Sugerman HJ, Sugerman EL, Wolfe L, Kellum JM Jr, Schweitzer MA, DeMaria EJ. Risks and benefits of gastric bypass in morbidly obese patients with severe venous stasis disease. *Ann Surg.* 2001; 234(1): 41-6.
- Sugerman HJ. Effects of increased intra-abdominal pressure in severe obesity. *Surg Clin North Am* 2001; 81(5): 1063-75.

- Sung BH, Ching M, Izzo J, Dandona P, Wilson MF. Insulin-mediated venodilation is impaired in patients with high cholesterol. *Hypertension* 1998; 31: 1266-1271.
- Takase S, Pascarella L, Bergan JJ, Schmid-Schönbein GW. Hypertension-induced venous valve remodeling. *J Vasc Surg* 2004; 39: 1329-34.
- Takase S, Pascarella L, Lerond L, Bergan JJ, Schmid-Schönbein GW. Venous Hypertension, Inflammation and Valve Remodeling. *Eur J Vasc Endovascular Surg* 2004; 28: 484-493.
- Takeshita A, Mark AL. Decreased venous distensibility in borderline hypertension. *Hypertension* 1979; 1(3): 202-6.
- Thijssen DH, Bleeker MW, Smits P, Hopman MT. Reproducibility of blood flow and post-occlusive reactive hyperaemia as measured by venous occlusion plethysmography. *Clin Sci (Lond)*. 2005; 108(2): 151-7.
- Thijssen DH, Kooijman M, de Groot PC, Bleeker MW, Smits P, Green DJ, Hopman MT. Endothelium -dependent and -independent vasodilation of the superficial femoral artery in spinal cord-injured subjects. *J Appl Physiol*. 2008; 104(5): 1387-93.
- Tomei F, Baccolo TP, Tomao E, Palmi S, Rosati MV. Chronic venous disorders and occupation. *Am J Ind Med*. 1999; 36(6): 653-65.
- Turzyniecka M, Wild SH, Krentz AJ, Chipperfield AJ, Gamble J, Clough GF, Byrne CD. Skeletal muscle microvascular exchange capacity is associated with hyperglycaemia in subjects with central obesity. *Diabet Med*. 2009; 26(11): 1112-9.
- Van Den Broek TA, Kuijper CF, Rauwerda JA, van Rij GL, Dijkstra HJ. Use of passive function testing in venous insufficiency: a prospective study. *Surgery*. 1991; 110(5): 860-5.

- Van Rij AM, De Alwis CS, Jiang P, Christie RA, Hill GB, Dutton SJ, Thomson IA. Obesity and venous function. *Eur J Vasc Endovasc Surg* 2008; 35: 739-744.
- Varbiro S, Vajo Z, Nadasy GL, Monos E, Acs N, Lorant M, Felicetta J, Szekacs B. Sex hormone replacement therapy reverses altered venous contractility in rats after pharmacological ovariectomy. *Menopause* 2002; 9(2): 122-6.
- Vehkavaara S, Westerbacka J, Hakala-Ala-Pietilä T, Virkamäki A, Hovatta O, Yki-Järvinen H. J Clin Effect of estrogen replacement therapy on insulin sensitivity of glucose metabolism and preresistance and resistance vessel function in healthy postmenopausal women. *Endocrinol Metab.* 2000; 85(12): 4663-70.
- Veldhuijzen van Zanten JJ, Thrall G, Wasche D, Carroll D, Ring C. The influence of hydration status on stress-induced hemoconcentration. *Psychophysiology.* 2005 Jan;42(1):98-107.
- Venturi M, Bonavina L, Annoni F, Colombo L, Butera C, Peracchia A, Mussini E. Biochemical Assay of Collagen and Elastin in the Normal and Varicose Vein Wall. *J Surg Res* 1996; 60: 245-248.
- Vissing SF, Nielsen SL. Regional blood volume in man determined by radiolabelled erythrocytes. *Clin Physiol.* 1988; 8(3): 303-8.
- Vogeser M, König D, Frey I, Predel HG, Parhofer KG, Berg A. Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. *Clin Biochem.* 2007; 40(13-14): 964-8.
- Vollenweider P, Randin D, Tappy L, Jéquier E, Nicod P, Scherrer U. Impaired insulin-induced sympathetic neural activation and vasodilation in skeletal muscle in obese humans. *J Clin Invest.* 1994; 93(6): 2365-71.

- Volterrani M, Rosano G, Coats A, Beale C, Collins P. Estrogen acutely increases peripheral blood flow in postmenopausal women. *Am J Med.* 1995; 99(2): 119-22.
- Wali MA, Eid RA. Intimal changes in varicose veins: an ultrastructural study. *J Smooth Muscle Res.* 2002; 38(3): 63-74
- Wall ML, Nicolson AM, Choh CH, Simms MH. My feet are killing me. *B J Surg* 2010; 97(2): S2 1-18 [Abstract]
- Wallace TM, Matthews DR. The assessment of insulin resistance in man. *Diabet. Med.* 2002; 19: 527–534.
- Walters WAW, Shields L. Venous distensibility during the menstrual cycle. *British Journal of Obstetrics and Gynaecology* 1977; 84: 697-700.
- Washburn RA, Jacobsen DJ, Sonko BJ, Hill JO, Donnelly JE. The Validity of the Stanford Seven Day Physical Activity Recall in Young Adults. *Med Sci Sports Exerc* 2003; 35(8): 1374-80.
- Watenpaugh DE, Vissing SF, Lane LD, Buckey JC, Firth BG, Erdman W, Hargens AR, Blomqvist CG. Pharmacologic atrial natriuretic peptide reduces human leg capillary filtration. *J Cardiovasc Pharmacol* 1995; 26(3): 414-9.
- Weits T, van der Beek EJ, Wedel M. Comparison of ultrasound and skinfold caliper measurement of subcutaneous fat tissue. *Int J Obes.* 1986; 10(3): 161-8.
- Westerterp KR. Assessment of physical activity: a critical appraisal. *Eur J Appl Physiol* 2009; 105(6): 823-8.
- WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series 854. Geneva: World Health Organization, 1995.

- Widmer LK. Peripheral Venous Disorders. Prevalence and socio-medical importance. Observations in 4529 apparently Healthy Persons. Basle 111 study. Bern: Hans Huber, 1978.
- Wilkins RW, Bradley SE. Changes in arterial and venous blood pressure and flow distal to a cuff inflated on the human arm. *Am J Physiol* 1946; 147: 260-9.
- Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol* 2001; 52: 631-46.
- Yang D, Vandongen YK, Stacey MC. Effect of exercise on calf muscle pump function in patients with chronic venous disease. *Br J Surg.* 1999; 86(3): 338-41.
- Yki-Jarvinen H, Westerbacka J. Insulin resistance, arterial stiffness and wave reflection. *Adv Cardiol* 2007; 44: 252-260.
- Young CN, Prasad RY, Fullenkamp AM, Stillbower ME, Farquhar WB, Edwards DG. Ultrasound assessment of popliteal vein compliance during a short deflation protocol. *J Appl Physiology* 2008; 104: 1374-1380.
- Young CN, Stillabower ME, DiSabatino A, Farquhar WB. Venous smooth muscle tone and responsiveness in older adults. *J Appl Physiol* 2006; 101: 1362-67.
- Zamboni P, Portaluppi F, Grazia Marcellino M, Manfredini R, Pisano L, Liboni A. Ultrasonographic assessment of ambulatory venous pressure in superficial venous incompetence. *J Vasc Surg* 1997; 26: 796-802.
- Zsotér T, Cronin RFP. Venous Distensibility in Patients with Varicose Veins. *Can Med Ass J* 1966; 94: 1293-7.

PUBLISHED WORK

Wall ML, Philips CJ, Brown MD. "The effect of abdominal adiposity on venous outflow from the lower limbs in healthy women." Physiology Society Cambridge July 15th 2008. Proc Physiol Soc 11, PC61.

Wall ML, Philips CJ, Davies RSM, Hobbs S, Brown MD, Simms MH. "The effect of abdominal adiposity on venous outflow from the lower limbs." European Society of Vascular Surgery Nice September 5-9th 2008

ML Wall, CJ Phillips, RSM Davies, SD Hobbs, MD Brown MH Simms. "Loss Of Great Saphenous Vein Compliance With Increasing Obesity In Females" Vascular Surgical Society 12-14th November 2008.

CTP Choh, ML Wall, MH Simms. "Use of Durometry in Assessment of Venous Disease." West Midlands Surgical Society 7th November 2008.

CTP Choh, ML Wall, MH Simms. "Use of Durometry in Assessment of Venous Disease." Venous Forum, Royal Society of Medicine 2009.

Wall ML, Davies RSM, SD Hobbs, CJ Philips, MD Brown, MH Simms. "Raised Body Mass Index is Associated with Poor Capillary Function" ASGBI May 2009.

Wall ML, Davies RSM, MH Simms. "Raised Body Mass Index is Associated with Poor Capillary Function" ESSR 2010.

Choh CT, Wall ML, Brown MD, Nicolson AM, Simms MH. Use of durometry in assessment of venous disease. *Phlebology*. 2010; 25(2): 94-9.