The role of peroneal nerve electrical neuromuscular stimulation in the augmentation of lower limb circulatory physiology in lower limb arterial and venous disease

Rachel Barnes

BSc(Hons), MBChB, MA (Merit), FRCS

PG Certificate in Diagnostic Imaging (Distinction)

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University of Hull and the University of York

Hull York Medical School

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Abstract

Introduction: Studies in healthy volunteers have demonstrated that peroneal nerve stimulation augments blood flow. The studies described within this thesis aimed to establish whether use of the geko™ neuromuscular stimulation device was effective and augments arterial venous and microcirculatory flow in patients with lower limb arterial and venous disease. The fibrinolytic and angiogenic activity of electrical stimulation within this cohort was also examined.

Methods:
Ethical approval was obtained for all aspects of the described studies. Participants with claudication, varicose veins or post- operative infrainguinal bypass grafts were assigned to receive either unilateral active stimulation or sham.
Duplex assessments of arterial and venous volume flow were performed at baseline and following stimulation. Laser Doppler flowmetry measurements of microcirculatory flow were made continuously throughout the study period. ELISA analysis was undertaken of plasma samples drawn at baseline and following stimulation to determine the effect on vascular endothelial growth factor(VEGF), tissue plasminogen activator(t-PA) and plasminogen activator inhibitor 1(PAI-1).

Results:
The geko™ device was effective at producing visible muscle contraction in only 59% patients tested due to a combination of oedema and neuropathy.
77 participants were recruited in total to either a proof of concept study or the initial phase of a randomised trial: 30 claudicants (25 active, 5 control), 25 post infra-inguinal bypass (19 active, 6 control) and 22 varicose veins (17 active, 5 control).
Arterial volume flow increased significantly in all patient groups receiving active stimulation: claudicants 60 ml/ min($P<0.001$); infra-inguinal bypass grafts 70ml/min($P<0.001$) and varicose veins 80 ml/min($P<0.001$).
Venous volume flow significantly increased in all patient groups: claudicants 30 ml/min($P=0.001$); infra-inguinal bypass grafts 30 ml/min($P=0.004$) and varicose veins 80 ml/min($P<0.001$).

Microcirculatory flow increased in all patient groups: claudicants 22.2 flux units($P<0.001$); infra-inguinal bypass grafts 21.8 flux units($P<0.001$) and varicose veins 28.4 flux units($P<0.001$).
ELISA analysis demonstrated no statistically significant change in VEGF or t-PA antigen levels when comparing the active with the passive and control limbs. Peroneal nerve stimulation in the active limbs resulted in a significant decrease in PAI-1 of 34 ng/ml($P<0.001$).

Conclusion: Transcutaneous peroneal nerve stimulation with the geko™ device is potentially beneficial in patients with lower limb arterial and venous disease. This is primarily due to its ability to augment venous, arterial and microcirculatory flow. The results of this study were influential in the NICE guidance approving its use in vascular patients as a means of DVT prophylaxis.
The enhanced fibrinolytic effect, by decreasing levels of PAI-1, warrants further investigation.

Peer reviewed publications

- Shahin Y, Barakat H, Barnes R, Chetter IC. The Vicorder device compared to SphygmoCor in the assessment of carotid- femoral pulse wave velocity in patients with peripheral arterial disease. *Hypertens Res* 2013 Mar;36(3):208-12. (IF 2.576)

- Barnes, R., Shahin, Y., Gohil, R., Chetter, I.C. Electrical stimulation versus standard care for chronic ulcer healing: A systematic review and meta-analysis of randomised controlled trials. *European Journal of Clinical Investigations* 2014 Apr;44(4):429-40.

- Barnes, R., Madden, L.A., Chetter, I.C. Fibrinolytic effects of peroneal nerve stimulation in patients with lower limb vascular disease. *Blood, Coagulation and Fibrinolysis* 2016 Apr;27(3):275-80

Published abstracts

- Barnes, R., Shahin, Y., Tucker, A.T., Chetter, I.C. "Haemodynamic efficacy of the geko™ electrical neuromuscular stimulation device in claudicants" *British Journal of Surgery* 2014; 101(s4): 8 (Abstract: Presented at ASGBI 2013)

- Barnes, R., Barakat, H., Tucker, A.T., Chetter, I.C. Prospective observational series to evaluate the efficacy of the geko™neuromuscular electrical stimulation device to produce muscle contraction in vascular patients. *International Journal of Surgery*, 2014; 12, S110 (Abstract: presented at ASIT 2014)

- Barnes, R.,Shahin, Y., Tucker, A.T. Chetter, I.C. Haemodynamic Augmentation in Patients with Peripheral Arterial Occlusive Disease with the GekoTM Transcutaneous Neuromuscular Electrical Stimulation Device. *BJS* 2015; 102 S1 (Abstract: presented at ASGBI May 2014)

- Barnes R, Barakat H, Tucker, A.T., Chetter, I.C. Prospective observational series to establish the efficacy of the GekoTM Electrical Stimulation Device to produce visible muscle twitch in vascular patients.

Br J Surg 2015; **102** (Suppl. 1): 2 (Abstract: presented at ASGBI May 2014).

- Barnes, R., Shahin, Y., Tucker, A.T., Chetter, I.C. Haemodynamic efficacy of the geko™ electrical neuromuscular stimulation device in claudicants. SARS 2014

- Barnes, R., Barakat, H., Shahin, Y., Tucker, A.T., Chetter, I.C. Prospective observational series to establish the efficacy of the geko™ electrical stimulation device to produce visible muscle twitch in vascular patients. ASGBI 2014

- Barnes, R., Shahin, Y., Tucker, A.T., Chetter, I.C. Haemodynamic augmentation in patients with peripheral arterial occlusive disease with the geko™ transcutaneous neuromuscular electrical stimulation device. ASGBI 2014

- Barnes, R., Tucker, A.T., Chetter, I.C. Haemodynamic flow augmentation in patients with superficial venous insufficiency utilising the geko™ electrical neuromuscular stimulation device. RSM Venous Forum

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List of abbreviations

ABPI	Ankle brachial pressure index
ADP	adenosine diphosphate
ANOVA	Analysis of Variance
CI	Confidence interval
CIN	Contrast induced nephropathy
CLI	Critical limb ischaemia
CO	Cardiac output
CRP	C reactive protein
CT	Computed tomography
CV	Coefficient of variance
CVI	Chronic Venous Insufficiency
ECM	Extracellular matrix
ELISA	Enzyme-linked immunosorbent assay
EVLA	Endovenous laser ablation
FGF	Fibroblast growth factor
GCP	Good Clinical Practice
GSV	Great saphenous vein
HIF	Hypoxia inducible factor
HR	Heart rate
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
MHRA	Medicines and Healthcare Products Regulatory Agency
MMPs	Matric metalloproteases
MRI	Magnetic resonance imaging
NHS	National Health Service
NHS R&D	National Health Service Research and Development Unit
NO	Nitric oxide
PAI-1	Plasminogen activator inhibitor
PAF	Platelet activating factor

PAOD	Peripheral arterial occlusive disease
PDGF	Platelet derived growth factor
PI	Principal Investigator
PIS	Patient Information Sheet
PTFE	polytetrafluoroethylene
QoL	Quality of Life
RFA	Radiofrequency ablation
SCS	Spinal cord stimulation
SD	Standard deviation
SE	Standard error
SFJ	Saphenofemoral junction
SV	Stroke volume
SVR	Systemic vascular resistance
TENS	Transcutaneous electrical nerve stimulation
TGF β	Transforming growth factor β
TNF	Tumour necrosis factor
t-PA	Tissue plasminogen activator
uPA	urokinase plasminogen activator
VEGF	Vascular endothelial growth factor
VTE	Venous thromboembolism
vWF	Von Willebrand factor

Firstly I wish to thank Professor Chetter for giving me the opportunity to carry out this research, his supervision and his belief in me.

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Declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources.

I confirm that any patient information obtained to produce this piece of work has been appropriately anonymised.

Rachel Barnes

December 2015

Chapter One: Introduction

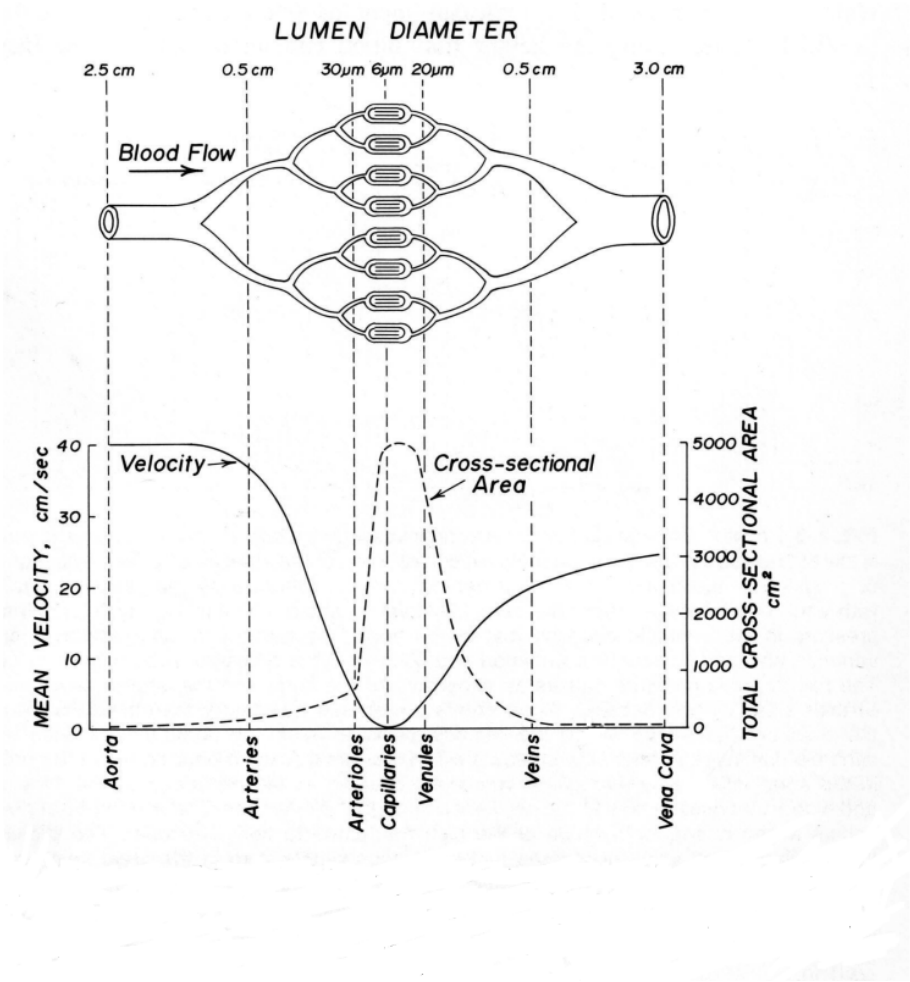
This thesis will examine the effects of peroneal nerve stimulation (PNS) on the peripheral vascular system. To explain the modulating effects of PNS, the research measurements and potential clinical benefits of its use, we first need to consider the physiology and pathophysiology of the cardiovascular system.

1.1 The circulatory system

The mammalian circulatory system is a closed loop transport system beginning and ending at the heart and comprises a system of muscles and vessels. Its functions include the delivery of nutrients and oxygen to the tissues, transfer of waste products of metabolism to the excretory organs, thermoregulation, contributing to the immune system as well as transport of hormones, electrolytes etc. The heart functions as the central muscular pump which drives the circulatory system. The action of the heart is divided into two phases termed systole and diastole. In systole the ventricles contract increasing the left ventricular pressure to approximately 120mmHg, resulting in the opening of the aortic valve and blood is expelled into the aorta. The aortic valve then closes and the ventricles relax, termed diastole, resulting in a reduction in the pressure, although this does not drop to 0 due to elastic recoil in the vessels. The difference between the systolic and diastolic pressure is referred to as the pulse pressure.

The blood passes through the arteries which divide and subdivide into smaller and smaller arteries and finally arterioles. These lead to the dense network of capillaries which supply the tissues and are the site of gaseous exchange. At the end of the capillary networks the vessels begin to converge and form venules which in turn merge to form veins.

Figure 1: Cross sectional area and mean velocity of systemic blood vessels(1)



The blood is returned to the right atrium of the heart in the inferior and superior venae cavae. Relaxation of the tricuspid valve allows passage of blood into the right ventricle. When this contracts, during systole, blood is driven through the pulmonary valve into the pulmonary circulation.

1.1.1 Cardiac performance

The function of the heart is to generate a sufficiently high arterial blood pressure to ensure adequate perfusion of the end organs.

Blood pressure = cardiac output (CO) x systemic vascular resistance (SVR)

Vascular resistance to blood flow is controlled by the arterioles. Vasodilation reduces the peripheral resistance returning more blood to the venous circulation i.e. venous return, which in turn reduces after load. This results in

an increase in cardiac output. Conversely an increase in peripheral resistance retains blood in the arterial circulation, reducing venous return and hence increasing after load. Cardiac output is therefore decreased.

Cardiac output (CO) = Heart rate (HR) x Stroke volume (SV)

Heart rate is controlled by the actions of the sinoatrial node. This has an intrinsic firing rate of between 100 and 115 beats per minute which is modified by neuro-hormonal factors. The actions of the vagus nerve decrease this intrinsic rate, whilst an increase is caused by decreased vagal tone and increased sympathetic nerve activity. Changes in body temperature, levels of catecholamines and circulating thyroxine also impact upon heart rate.

The stroke volume refers to the volume of blood expelled by the heart per contraction. It is determined by:

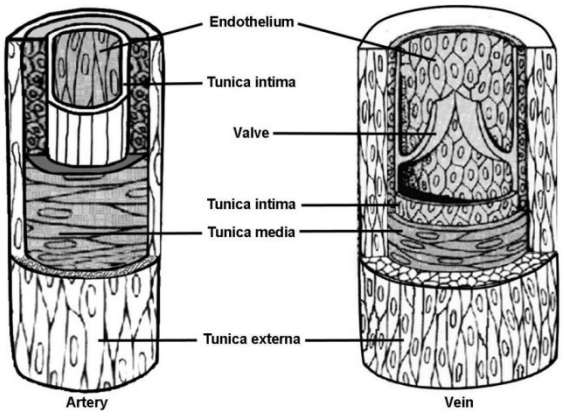
1. Preload: This is the length of the muscle fibres prior to contraction and is dependent on the end diastolic volume or degree of ventricular filling. It is related to the pressure in the right atrium or central venous pressure and is determined by venous return
2. After load: This refers to the arterial pressure against which the heart muscles must contract. As such the after load of the right ventricle is related to the pressure in the pulmonary artery and the after load of the left ventricle is related to aortic pressure.
3. Contractility: This refers to the ability of the heart muscle to eject a stroke volume at a given preload and after load.

The Frank-Starling law of the heart or Maestrini heart law(2), states that the stroke volume of the heart is increased by increasing the preload (end diastolic volume). This is due to the increased load stretching the cardiac muscle fibres which increases the binding of calcium to troponin C and as such increases the number of actin myosin bonds which form.

1.1.2 Structure of vessels

The structure of arteries and veins is generally similar with the walls comprising three concentric layers. The tunica adventitia is the strong outermost layer of both veins and arteries. It comprises connective tissue, collagen and elastin as well as nerves which supply the vessel and its nutrient vessels.
The tunica media is the middle, thickest layer and contains a circular arrangement of elastin, connective tissue and particularly in arteries, vascular smooth muscle, which is responsible for the calibre of the vessel.
The tunica intima is the thinnest layer and in arteries this layer is composed of an internal elastic membrane lining and a single layer of smooth endothelial cells. The difference between veins and arteries is that veins do not contain the elastic membrane lining and in some veins the tunica intima is folded back on itself to form valves.

Figure 2: Structure of vessel walls (reproduced with permission of BiologyonlineUS(3)



1.1.3 Function of vessels

Arteries are responsible for carrying blood away from the heart; they can be subdivided into the pulmonary arteries which are responsible for carrying deoxygenated blood from the heart to the lungs and the systemic arteries, responsible of carrying oxygenated blood from the heart to the body. The systemic arteries can be further subdivided into the elastic conducting and

muscular distributing arteries. The elastic arteries are located close to the heart, they are thick walled, with a large lumen and accommodate changes in blood pressure. The muscular arteries are found distal to the elastic arteries and are responsible for delivering blood to specific tissues and organs. They have a thick media wall which contains smooth muscle.

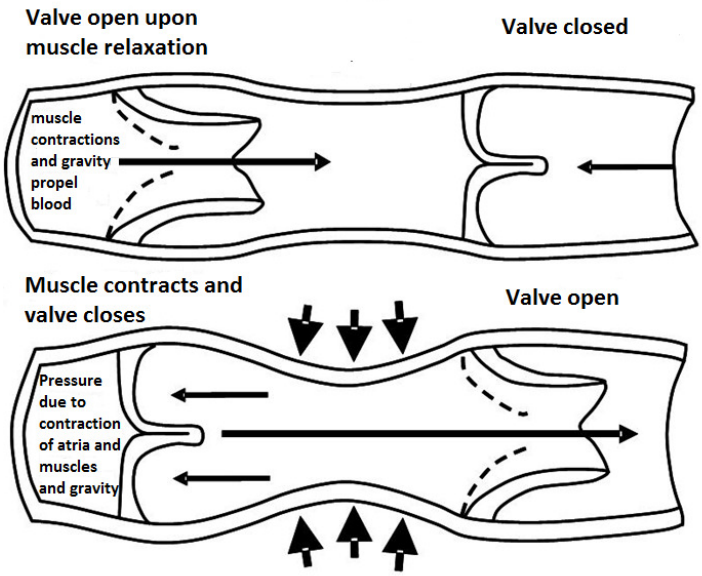
Arterioles interconnect arteries and capillaries and are responsible for the regulation of flow through constriction and dilatation under the control of the sympathetic nervous system and local tissue factors. Arteriole walls are predominantly comprised of smooth muscle.

Capillaries are narrow vessels with thin walls and form networks in most organs and tissues. The walls are only one cell thick, comprising of the tunica intima only, permitting the diffusion of nutrients and oxygen into tissues and the diffusion of waste products out into the blood. They interconnect arterioles and venules. The pressure within the capillaries ranges from around 25mmHg at the arterial end to 15mmHg at the venous end

Venules are small vessels which interconnect capillaries and veins. Their walls comprise little muscle and a thin externa. By constricting they can increase resistance and impact upon the transport of fluid and substances between the tissues and blood.

Veins transmit blood back to the heart. They have larger lumens and thinner walls containing less muscle than arteries. As a result they are very distensible and typically contain up to 70% of the circulating blood. By constricting they can return large volumes of blood to the heart and arteries and as such increase cardiac output. This enables control of blood pressure in the instance of blood loss or haemorrhage. Folds of interna form valves which prevent the back flow of blood.

Figure 3: Venous valve function(adapted from BiologyonlineUS(3)



1.1.4 The endothelium

The endothelium is a monolayer of endothelial cells which lines all vessels, lymphatics and the heart. It is an important regulator of a variety of physiological functions required for normal functioning of blood vessels.

1.1.4.1 Haemostasis

The endothelium provides a non-thrombogenic surface to the circulating platelets. The release of prostacyclin and nitric oxide by the endothelium inhibits platelet activation. Aggregation of platelets is inhibited by the expression of ecto-adenosine diphosphatase which breaks down adenosine diphosphate(4).

The endothelial surface contains heparin sulphate which acts as a cofactor in the activation of antithrombin III. This forms complexes with coagulation factors (excepting factor VII) inactivating them. The activity of protein C is enhanced by the presence of thrombomodulin on the endothelial surface which acts as a site for the binding of thrombin(5).

Several anticoagulant factors are secreted by the normal endothelium including tissue plasminogen activator which activates the fibrinolytic pathway, proteoglycans and protein S.

Conversely the endothelium also has a role in preventing blood loss through the production of pro-coagulant factors e.g. Tissue factor, von Willebrand's factor, fibronectin and type IV collagen(6).

Table 1: Regulation of coagulation by the endothelium: adapted from Shireman and Pearce(6)

Procoagulant	Anticoagulant
Thromboxane	Prostacyclin
Von Willebrand's factor	Protein C
Interleukin-1	Protein S
Platelet-activating factor	Thrombomodulin
Phospholipids	Heparan sulphate
Tissue factor	Tissue factor pathway inhibitor
Plasminogen activator inhibitor type I	Urokinase
	Tissue type plasminogen activator

1.1.4.2 Barrier function

The endothelium is semi-permeable barrier to the passage of molecules and fluid between the blood and interstitial fluid. The majority of dissolved gases, solutes etc. are small molecules which pass through the endothelium by passive diffusion, down the concentration gradient. The vessel wall restricts the passage of high molecular weight substances as these are not so readily used by the tissues and contribute to intravascular volume. Certain plasma proteins such as albumin, permeate the vascular wall and act as transport molecules for substances such as hormones and fatty acids which are hydrophobic. The degree of differentiation of the endothelial cells is related to which organ the vessels are supplying. Those in the glomerulus of the kidney and at the blood brain barrier have adapted to perform special filtering functions(7).

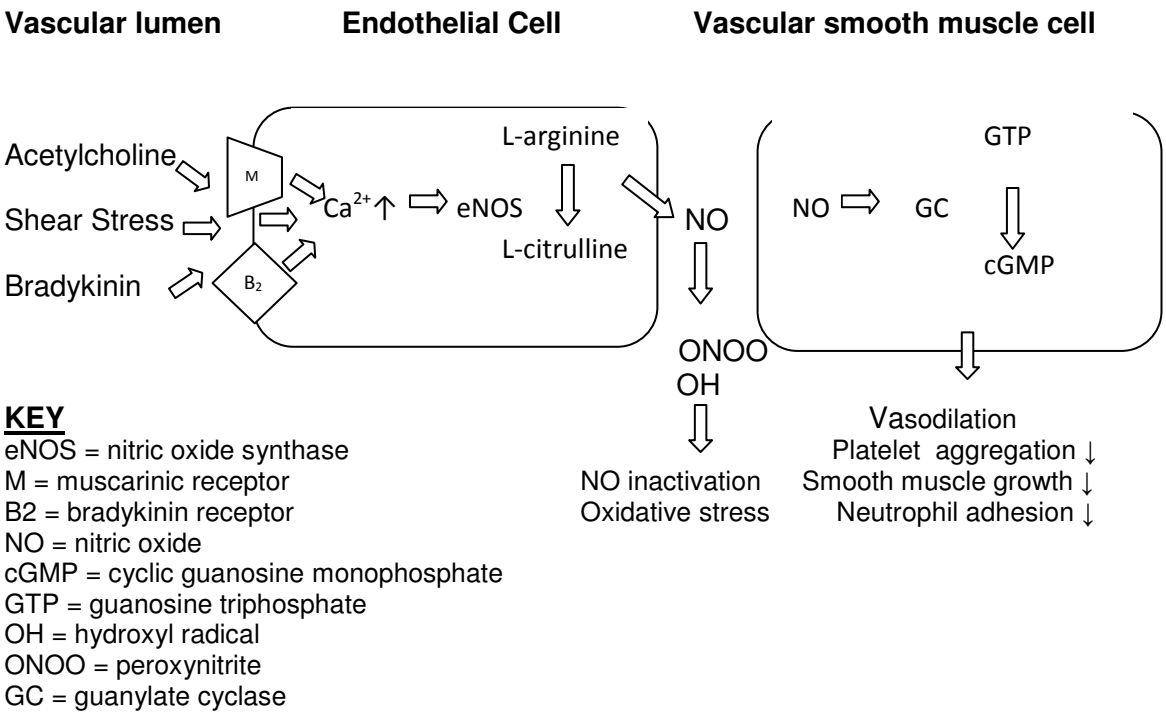
Disruption of the endothelium leads to an increase in its permeability to plasma proteins and therefore can result in oedema.

1.1.4.3 Vascular tone

The endothelium plays an important role in the control of vascular tone through both its response to circulating mediators such as bradykinin and thrombin as well as the production and release of vasoactive substances. Furchgott and Zawadski(8) first demonstrated the existence of nitric oxide (NO) and its vasodilatory effects. The action of endothelial nitric oxide synthase on L-arginine leads to the generation of NO which diffuses to the vascular smooth muscle cells. NO activates guanylate cyclase resulting in an accumulation of cGMP and increased calcium uptake by the smooth endoplasmic reticulum and as such vasodilation.

In normal physiological environments the release of NO is activated by changes in shear stress to ensure that organ perfusion remains relatively constant, despite changes in cardiac output. The generation of nitric oxide can also be mediated by bradykinin, adenosine, vascular endothelial growth factor (released in response to hypoxia) and serotonin(9).

Figure 4: The vasodilatory role of nitric oxide



KEY

eNOS = nitric oxide synthase
M = muscarinic receptor
B2 = bradykinin receptor
NO = nitric oxide
cGMP = cyclic guanosine monophosphate
GTP = guanosine triphosphate
OH = hydroxyl radical
ONOO = peroxynitrite
GC = guanylate cyclase

1.1.5 Lower limb arterial tree

The femoral artery is a direct continuation of the external iliac artery. The external iliac becomes the common femoral artery when it passes beneath the inguinal ligament and enters the femoral triangle. Within the femoral triangle, the common femoral artery bifurcates into the profunda femoris (deep femoral) and the superficial femoral arteries. The profunda supplies the head and neck of the femur and several muscles of the posterior, medial and lateral thigh.

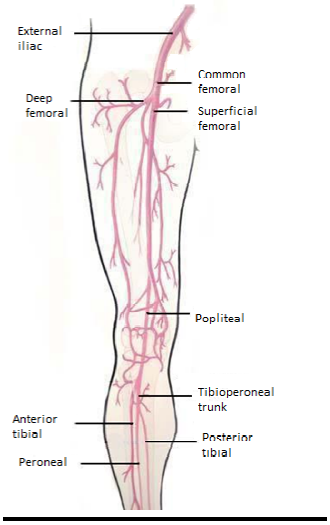
The superficial femoral artery continues down the anterior surface of the thigh via the adductor canal, supplying the anterior thigh muscles. It exits the adductor canal through the adductor hiatus and travels posteriorly becoming the popliteal artery.

The popliteal artery gives off several genicular branches which supply the knee. At the inferior border of the popliteus muscle it terminates by dividing into the anterior tibial artery and tibioperoneal trunk.

The anterior tibial artery passes anteriorly, above the interosseus membrane between the tibia and fibula. It descends down the anterior compartment of the leg supplying all the muscles in the compartment and onto the dorsum of the foot, where it becomes the dorsalis pedis artery.

The tibioperoneal truck quickly divides into the posterior tibial artery, which remains on the postero-medial aspect of the leg and the peroneal artery which supplies the postero- lateral side of the leg. The posterior tibial continues distally and passes posterior to the medial malleolus before dividing into the lateral plantar artery, which forms the plantar arch, and the medial plantar artery.

Figure 5: Arterial supply of the lower limb- reproduced with permission: http://vascularultrasound.net/wp-content/uploads/2010/08/leg-arteries3.jpg



1.2 Haemostasis

The term haemostasis is derived from the Greek *heme,* blood and *stasis,* halt. It refers to a complex series of reactions which take place to stop blood flow. Haemostasis depends on the interplay of the vascular system, through vasoconstriction, coagulation factors, platelets and the fibrinolytic system. The process is divided into two phases: primary and secondary haemostasis.

1.2.1 Primary haemostasis

Primary haemostasis refers to the mechanism by which a platelet plug forms at the site of injury and usually occurs within 20 seconds of an injury. Failure of this pathway typically results in haemorrhage or prolonged bleeding. Vascular endothelium provides a protective barrier which separates blood and tissues. It also produces several factors which prevent excessive haemostasis such as nitric oxide and Prostaglandin I_2 which inhibit platelet activation.

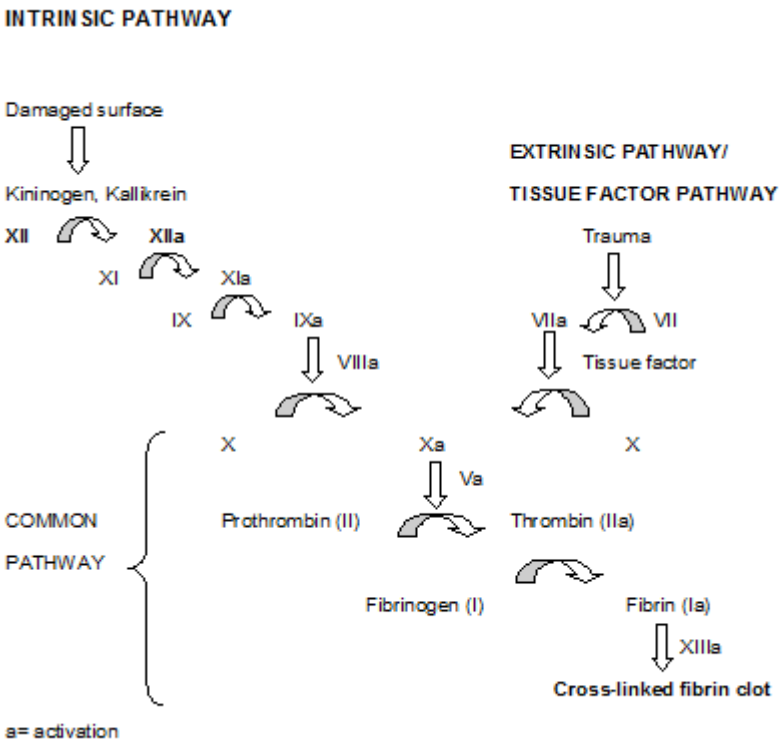
Following vascular injury with endothelial disruption the initial response is vasoconstriction, which reduces blood loss and flow in the vessel to enable

platelet adhesion. Platelets become anchored to the subendothelium mediated by von Willebrand factor (vWF) and fibrinogen to varying extents. Once adhered the platelets are activated through their contact with collagen by adenosine diphosphate (ADP) and release thromboxane, and platelet activating factor (PAF). These, in combination with ADP and serotonin, activate and recruit additional platelets from the blood which bind to the already adherent platelets through fibrinogen bridges(10).

1.2.2 Secondary haemostasis

Secondary haemostasis refers to the mechanisms responsible for the stabilisation of the platelet plug and the maintenance of vasoconstriction. Vasoconstriction is under the control of serotonin, prostaglandin and thromboxane which are secreted by the activated platelets. Fibrin formation to stabilise the clot is triggered by the secretion of procoagulant factors from platelets including fibrinogen, factor V and vWF, by tissue factor (extrinsic pathway) and contact activation (intrinsic pathway). The fibrin binds the platelets together and to the vessel(10).

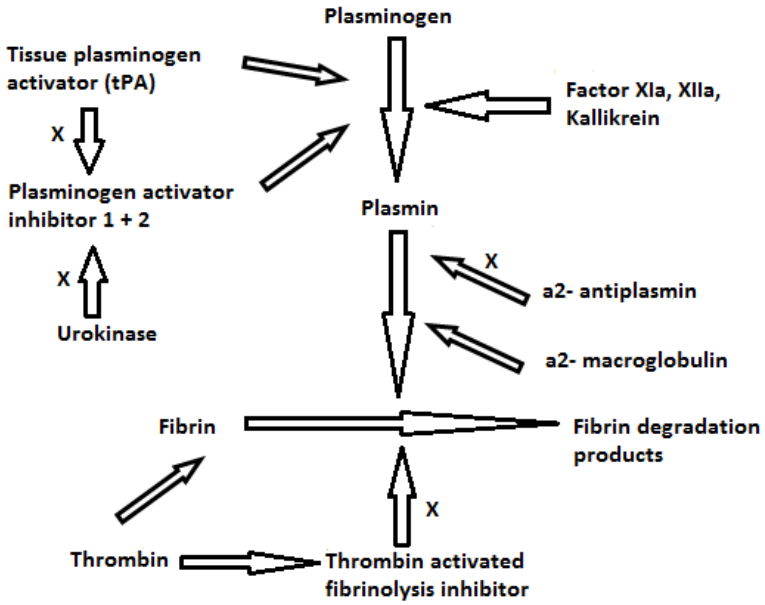
Figure 6: The clotting cascade



1.3 Fibrinolysis

The coagulation and fibrinolytic pathways work together, under normal physiologic conditions, to ensure blood flow whilst preventing blood loss. The final stage in the coagulation cascade is the conversion of fibrinogen to fibrin. Plasminogen is cleaved to plasmin, a fibrinolytic protease under the control of tissue plasminogen activator (t-PA) and urokinase (uPA). A positive feedback mechanism exists whereby plasmin then initiates the breakdown of t-PA and uPA. Fibrin self regulates by binding t-PA and plasminogen enhancing the production of plasmin. Plasmin cleaves fibrin producing soluble degradation products. The dissolution of fibrin is also controlled by plasminogen activator inhibitor-1 (PAI-1) and α_2-plasmin inhibitor(11).

Figure 7: Fibrinolytic pathway (adapted from Cesarman-Maus, Haijar 2005(11)



X = inhibition

1.4 Thrombosis

A thrombus is a solid collection of blood constituents which is formed in flowing blood within a vessel and impedes normal flow. An equilibrium normally exists between the factors promoting and retarding the coagulation processes to stop formation of thrombi. Virchow's triad is used to explain the patho-physiological mechanisms responsible for the formation of thrombus. The three responsible features are: (1) Abnormalities in blood flow; (2) Changes in blood constituents and (3) Changes to the vessel wall.

1.4.1 Changes in flow

Laminar flow describes the normal flow of blood through the circulatory system and refers to concentric layers of blood moving in parallel down the blood vessel length. The highest rate of flow is found in the centre of the vessel where there is lowest resistance. Disruption of laminar flow leads to turbulence.

Arterial branches and bifurcations cause changes in the normal flow by reducing wall shear stress and increasing turbulence (12). Reduced wall shear stress is associated with reduced nitric oxide production and increased leukocyte adhesion and as such these points are more susceptible to the development of atherosclerosis.

The rate of flow through a vessel relates to the force pushing the blood through i.e. the strength of cardiac contractility and the resistance of the vessel.

Poisseuille's Law states that the resistance to flow in a vessel is proportional to the length of the vessel and the viscosity of the blood and inversely proportional to the radius of the vessel to the fourth power.

$$R \alpha \frac{L.\eta}{r^4}$$

It is therefore easy to see how even small changes in the radius of the vessel such as with atherosclerotic deposits or stenoses have a significant impact on the resistance and hence flow in a vessel. Such stenoses precipitate turbulent flow within the vessel and as such increase the energy required to drive the flow.

Figure 8: Ultrasound duplex of laminar flow in a healthy vessel

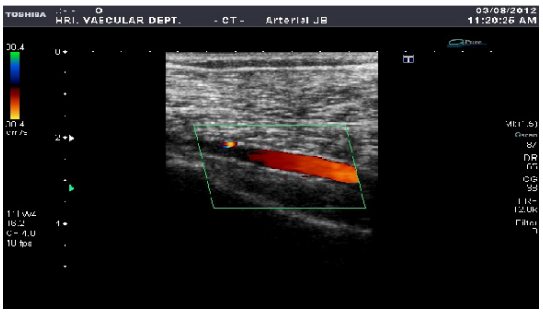
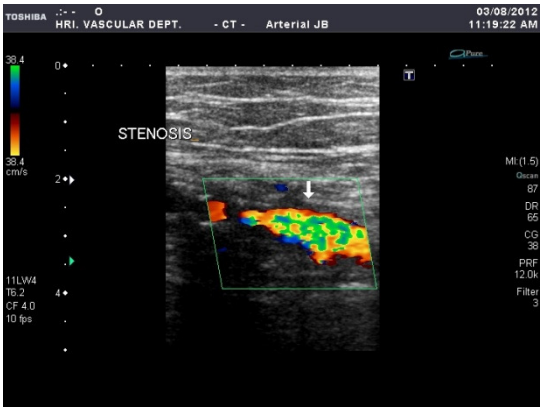


Figure 9: Ultrasound duplex of turbulent flow secondary to a stenosis



1.4.2 Changes in blood constituents

Endothelial dysfunction, caused by the risk factors for peripheral arterial occlusive disease (PAOD), results in increased platelet activation and aggregation. Fibrinogen levels also increase leading to stabilisation of the platelet plug and its adherence to the endothelium. It is known to play a major role in inflammation through the facilitation of leukocyte adherence and the resultant chemotactic response.

Tissue plasminogen activator is slowly released by the endothelium following vascular injury promoting fibrinolysis through the conversion of plasminogen to plasmin. The action of t-PA is usually inhibited by plasminogen activator inhibitor 1 and 2 however activation of the endothelium results in an imbalance and the creation of a pro-coagulable state. PAI-1 reduces fibrinolysis and increases thrombosis.

Endothelial dysfunction and atherosclerosis result in the increase of inflammatory markers such as IL-6 and CRP. CRP inhibits the release of nitric oxide by the endothelium and increases PAI-1 production via the action of cellular adhesion molecules. IL-6 is one of the factors responsible to activating the endothelial cells and as such leukocyte binding and platelet production and adhesion. It is also responsible for increased expression of fibrinogen, factor VIII and vWF(13).

1.4.3 Changes to the vessel wall/ arterial function

The risk factors for vascular disease such as smoking, hypertension, hyperglycaemia etc. play an important role in the initial processes involved in the formation of atheroma through endothelial injury. The presence of oxidative stress, risk factors and inflammation result in injury to the endothelium. This in turn releases less vasodilators such as nitric oxide and disrupts the normal vascular homeostatic equilibrium. An inflammatory, prothrombotic state results which promotes atherosclerosis(14).
The process also results in intimal-medial thickening leading to arterial stiffness(15). In healthy individuals the stiffness of arteries increases the more distal from the aorta. As such the anterograde pulse wave is reflected back to the aorta enhancing coronary perfusion. Increased arterial stiffness leads to faster pulse wave propagation and the retrograde pulse is reflected back faster and increases systolic blood pressure and reduces coronary perfusion pressure(14). Arterial stiffness has been shown to be an independent risk factor for cardiovascular disease(16).

1.5 t-PA

Tissue plasminogen activator (t-PA) is predominantly produced by vascular endothelial cells(17). It is responsible for the conversion of plasminogen to plasmin and as such the breakdown of fibrin, dissolving clots. As a result it has therapeutic uses in clinical medicine to treat thromboses and emboli. Its activity is regulated by fibrin levels. t-PA and its antigen's levels increase in the presence of inflammation and antigen levels appears to be correlated to the levels of inflammatory markers such as C reactive protein and leucocyte count(18, 19).
t-PA antigen levels are a marker of the formation of t-PA and PAI-1 complexes and is associated with higher incidence of vascular disease(18). In a study by Smith *et al* levels of t-PA antigen and leucocyte elastase were

found to be predictive of myocardial infarction(20). Strong correlations exist between t-PA antigen levels and PAI-1 activity(21).

1.6 Plasminogen activator inhibitor

Plasminogen activator inhibitor (PAI-1) is a serine protease inhibitor and under normal physiological conditions is released by liver and smooth muscle cells, adipocytes and platelets. In the presence of inflammation, tumour cells and endothelial cells can release large volumes of PAI-1. The inflammatory mediators Tumour Necrosis Factor (TNF) α and interleukin (IL) 1 increase tissue factor activity and as such increase thrombin generation which in turn increases PAI-1 activity(22).

PAI-1 inhibits the activity of both t-PA and urokinase plasminogen activator (U-PA). Its activity is autoregulated by transforming growth factor β (TGFβ) and is in turn activated by U-PA.

Deficiency of PAI-1 leads to bleeding tendencies, whilst over expression leads to increased tendencies towards thrombosis. Indeed increased levels have been shown to be a risk factor for the development of coronary artery disease, myocardial infarctions and deep vein thrombosis. It can therefore be seen that PAI-1 plays an important role in thrombotic vascular disease (23, 24). Clot lysis studies have also shown that the vironectin in platelets may activate PAI-1 and as such PAI-1 may be a factor in the resistance of thrombi, rich in platelets to thrombolysis(22).

It is believed that insulin, free fatty acids and triacylglycerol may be mediators for PAI-1 elevation and as such that PAI-1 maybe a link between insulin resistance and arterial disease. Studies have also shown that weight loss is associated with a significant reduction in PAI-1 levels(25).

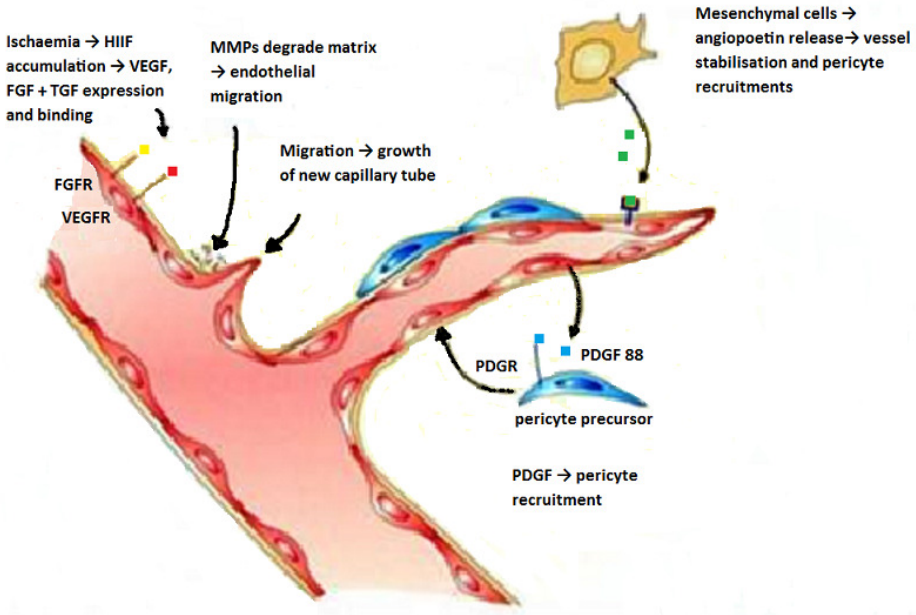
1.7 Angiogenesis

During development a process mediated by vascular endothelial growth factor, called vasculogenesis leads to the development of new blood vessels from angioblasts. The development of new vessels from pre-existing blood vessels is called angiogenesis and is required for healing and reproduction. Hypoxia is the primary stimulus for angiogenesis via the release of the transcription factor, hypoxia-inducible factor (HIF). It induces the expression of the angiogenic growth factors including vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), angiopoietin and fibroblast growth factor (FGF)(26). In turn these attract endothelial and inflammatory cells increasing vascular permeability and cell migration.

The release of endothelial platelet derived growth factor B from endothelial cells leads to the recruitment of pericytes. They are necessary for the structural support of the new vessels and promote endothelial survival. Pericytes aid the directionality of the new vessels and produce VEGF. Transforming growth factor is responsible for both forming and strengthening the new vessel wall by promoting the differentiation of the mural cells(27). The endothelial cells secrete MMPs (matrix metalloproteases) which are critical for the remodelling of the extracellular matrix (ECM). When the endothelial cells migrate, in a process called tubulogenesis, from existing blood vessels towards an angiogenic stimuli such as VEGF, they need to pass through the ECM(27).

Continuous blood flow must be restored and as such the new vessels originating from arterioles must anastomose with those originating from venules.

Figure 10: Angiogenesis- adapted from Klagsbrun *et al*(28)



1.8 VEGF

As early as 1932 it was postulated that a 'blood vessel growth stimulating factor' may exist in tumours(29). Algire *et al*'s observation in 1945 that tumour growth is preceded by a local increase in vascular density only served to reinforce this theory(30). This led to further work being carried out to determine the nature and origin of the responsible factor. In 1968 studies were undertaken which demonstrated that tumour angiogenesis is mediated by diffusible factors which are released from the tumour cells (31, 32). Folkman *et al* (1971) reported how tumours acquire nutrients in both a prevascular and vascular state and hypothesised that a 'tumor angiogenesis factor' was responsible for this transformation. This led to the proposition that developing a way to impede angiogenesis could be a strategy for the treatment of cancer(33). As a result a vast amount of research was carried out to identify factors responsible for new vessel growth (34). In 1983 a protein was partially isolated by Senger *et al* which was shown to induce vascular leakage and was named "tumour vascular permeability factor". Unfortunately at this time total protein isolation was not possible in order to further establish its mode of action and effect(35). In 1989 Ferrara and Henzel isolated an endothelial cell specific mitogen which they named

"vascular endothelial growth factor"(36). Subsequent studies revealed that "vascular permeability factor" and "vascular endothelial growth factor" were in fact the same molecule. VEGF includes four isoforms which are named according to the number of amino acids they contain: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$. $VEGF_{165}$ is the most common form but all the isoforms are mitogenic for endothelial cells and increase vascular permeability(37).

Some of the earliest evidence that VEGF is an angiogenic factor in vivo and is associated with blood vessel growth was reported in 1990. Phillips *et al* demonstrated that VEGF mRNA is only present in low levels in the avascular granulose cells in the ovary whilst the highly vascular corpus luteum displayed up regulation(38). It was also determined that high affinity VEGF binding sites are selectively expressed in endothelial cells in vivo (39).

This in vivo and in vitro evidence, that the binding sites were found exclusively in endothelial cells and that the expression of VEGF mRNA is correlated to blood vessel growth, combined to establish the role of VEGF as a regulator of angiogenesis. This has since been confirmed by the application of anti- VEGF antibodies and VEGF inhibitors, which have been shown to retard growth of human cancer cell lines by inhibiting angiogenesis (40, 41).

It is clear that whilst VEGF has a role in normal physiological angiogenesis it also has a role in several pathological conditions such as tumour growth, ischaemia and diabetic ocular disorders.

Whilst produced and secreted by stromal and epithelial tumour cells, platelets contain large stores, in their α granules, of angiogenic factors including VEGF (42). It is, as such believed that platelets act as transporter cells for VEGF.

The potential for VEGF to stimulate therapeutic angiogenesis has also been the driving force behind much research into VEGF. It has been postulated that administration of VEGF could increase the vascularity of patients suffering from vascular insufficiency with resultant limb ischaemia. This theory was supported by early animal studies which suggest that administration of VEGF leads to the development of collateral vessels and functional endothelial recovery (43, 44). More recently VEGF has been shown to prevent coronary restenosis in pigs, by the '*prevention of adventitial*

microvessel regression, enhanced adventitial elastin accumulation, reduced adventitial myofibroblast numbers, and a pronounced adventitial inflammatory response considered as a part of arterial healing'(45). This suggested that therapeutic VEGF administration may be a treatment option to prevent re-stenosis following percutaneous angiographic interventions. To date, however, despite encouraging results from animal studies, high level evidence in human subjects remains elusive (46).

Some small non- placebo controlled trials in humans, carried out in the 1990's reported an increase in angiogenesis and improvement of distal blood flow in patients with limb ischaemia following administration of VEGF (47, 48). However the large, placebo controlled study of 174 patients carried out by Henry *et al* failed to show a clinical benefit when $VEGF_{165}$ was administered into the coronary circulation. This implied that the placebo effect may be greater than previously thought, even in patients with severe cardiac dysfunction (49).

It has been hypothesised that the difference seen between animal and human studies may be that the healthy animals are better '*able to mount an effective endogenous angiogenic response that can be maximised by an additional stimulus..... whereas patients with extensive atherosclerotic disease may have an impaired response to endogenous and exogenous factors*'(50).

Since then studies have concentrated on a more persistent exposure to VEGF to establish whether this results in a more prolonged expression of the new vessels formed and whether administration of other pro- angiogenic factors in combination with VEGF may be more successful in resulting in stable vessel formation (51, 52).

Safety concerns do exist that increasing levels of VEGF may result in vascular malformations, tumour formation, oedema or haemorrhage however no such effects have been reported in the literature to date.

1.8.1 Measuring VEGF levels

As previously stated platelets act as a store for angiogenic factors such as VEGF. Serum VEGF levels are therefore several times higher than in plasma due to the release of VEGF during clotting (53). This led to the suggestion that plasma levels should be measured as standard (54). However the lower plasma levels of VEGF lie close to the lower limits of ELISA sensitivity and as such serum VEGF, normalised by platelet count is reported by some to be the optimal measurement(55, 56).

1.9 Peripheral arterial occlusive disease

1.9.1 Pathophysiology

Peripheral arterial occlusive disease is a progressive disorder resulting from the accumulation of atherosclerotic plaques in the arterial wall with associated luminal narrowing/ obliteration. The aorta, visceral and the lower limb arteries are the most commonly affected. Ischaemia occurs when critical luminal narrowing results in inadequate perfusion, such that the transport of nutrients and oxygen and the removal of waste products does not meet the demands of the tissues.

The development of atherosclerosis occurs in four stages:

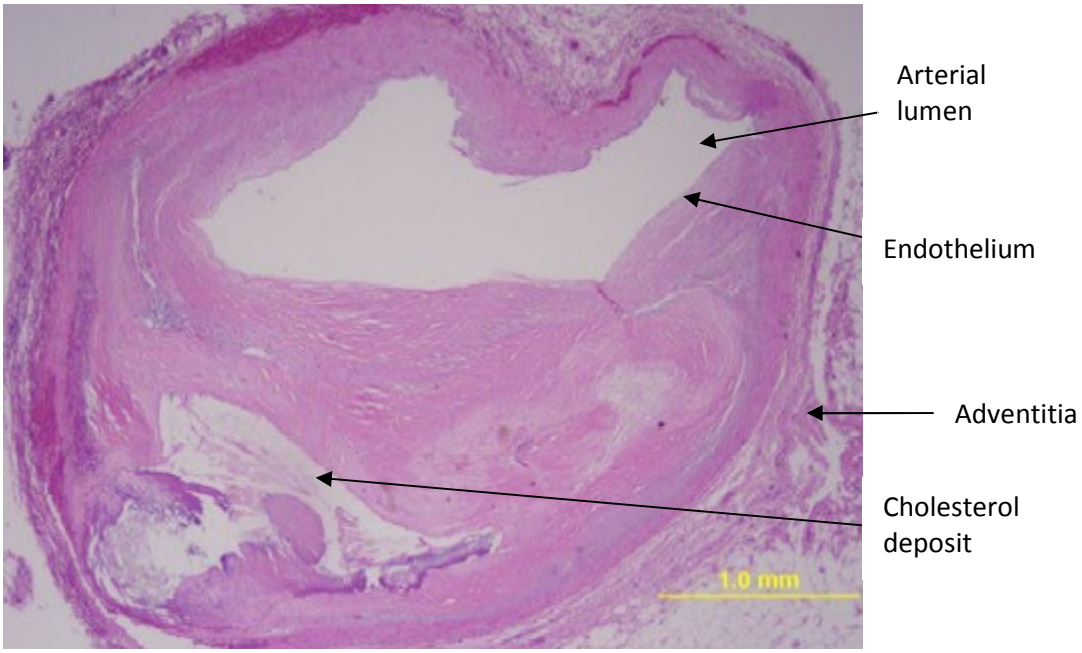
The endothelium is responsible for vessel permeability and the diffusion and active transport of substances in and out of the blood. Endothelial injury can occur secondary to hypertension, hyperlipidaemia, inflammation and oxidative stress i.e. the production of superoxide anion radicals seen in diabetes, hypertension and hyperlipidaemia. This results in increased vessel permeability, activation of cytokines and recruitment of leucocytes.

The exposure of blood components to the vessel wall, following endothelial injury result in cell mitosis and transformation. The oxidization process accelerates this and also results in inflammation, platelet aggregation with activation of the intrinsic coagulation system and vasoconstriction(57, 58).

The formation of fatty streaks results when monocytes and leucocytes adhere to endothelial cells initiating the release of cytokines and other growth factors. The leucocytes migrate into the intima where the monocytes mature into macrophages and take up lipid. Smooth muscle cells migrate into the intima and proliferate resulting in hypertrophy of the vascular wall. Growth factor expression also induces the deposition of substances such as collagen, calcium and proteins, leading to the development of atherosclerotic plaques. These lesions develop into what is termed the advanced lesion, comprised of endothelial and smooth muscle cells, inflammatory cells and a lipid core. It is covered by a fibrous cap, which can rupture and stimulate thrombus formation (57, 58). Atheroma causes reduced vessel wall elasticity and luminal narrowing/ occlusion reducing the blood supply to the tissues and organs.

Atherosclerotic plaques can be broadly divided into those which are stable and those more prone to rupture or fissuring. Stable, asymptomatic plaques have a higher proportion of smooth muscle cells and extracellular matrix, whilst unstable plaques have a higher foam cell and macrophage proportion and a weaker fibrous cap(59). Rupture of a plaque exposes thrombogenic material to the circulation leading to the formation of thrombi, which may detach and occlude more distal vessels.

Figure 11: Microscopic cross-section of atheromatous plaque within a coronary artery (reproduced with permission HYMS)



1.9.1.2 Arterial stiffness

Several methods exist for measuring or quantifying the degree of arterial stiffness. Pulse wave velocity (PWV) between the carotid and femoral artery is the most commonly utilised because it is simple, accurate and reproducible (60, 61). PWV is defined as the distance travelled by the wave divided by the time taken for the wave to travel that distance.

The Moens-Kortweg equation (62) links PWV, strain on the vessel, pulse pressure and blood density:

$$PWV = (\rho \times \text{distensibility})^{-1/2}$$

where ρ = blood density and distensibility = the luminal area change over the local pulse pressure.

As the degree of arterial stiffness increases so too does the PWV.

The presence of points of resistance to flow within the arterial tree i.e. bifurcations, atherosclerotic plaques or simply increased arterial stiffness results in part of the wave being reflected back to the heart. Increased PWV can result in wave reflections which augment systole and may result in cardiac hypertrophy, failure and thromboses. Reduced PWV conversely

results in augmentation of diastole and as such improved coronary perfusion (63).
The augmentation index (AI) is the ratio of aortic pressure and the central pulse pressure and relates to the degree of wave reflection due to arterial stiffness. It has been shown to correlate well with PWV(64).

There are several factors which are known to affect pulse wave analysis, i.e. augmentation index and pulse wave velocity. Arterial walls are known to become stiffer with increasing age due to loss of elastin and intimal thickening. Individuals under the age of 20 show AI values of -2 to 5% increasing to 30-37% in those between 80 and 90 years of age (65). Studies have also shown that height and AI have an inverse relationship (64). Changes in heart rate can impact upon AI with one study reporting a 5.6% decrease in AI with every 10 beat/ minute increase in heart rate (66), thus medications which reduce heart rate can decrease AI (67).
Intima-medial thickness (IMT) is a recognised marker of atherosclerosis and increases in IMT are associated with increased PWV (68). Diabetes mellitus is also known to increase arterial stiffness(69).
Several medications are known to impact on pulse wave analysis and velocity such as ACE inhibitors and antihypertensives(70, 71).

1.9.2 Epidemiology

Symptomatic peripheral arterial occlusive disease (PAOD) is common affecting 12-14% of the population. The prevalence increases with age and affects up to 20% of those over 75 years (72). The prevalence of asymptomatic PAOD is at least three times greater than symptomatic forms (73).
PAOD often co-exists with coronary artery disease and cerebro-vascular disease. Bhatt *et al* 2005 demonstrated that 63% of patients with PAOD will have polyvascular disease(74). Patients with large vessel disease have a relative risk of 3.1 (95% CI 1.9-4.9) for death from all causes compared to those with none and 5.9 (95% CI 3.0- 11.4) for all deaths from cardiovascular

disease (75). Further studies have also shown that progression of PAOD, as determined by serial ABPI (ankle brachial pressure indices) is significantly and independently associated with an increased mortality from cardiovascular causes (76).

Diseases of the circulatory system are known to be a major cause of mortality in England and Wales. The 5 year mortality rate is around 30% versus 10% in aged matched controls, with 75% due to cardiovascular events (77).

Table 2: Mortality Statistics: Deaths registered in England and Wales (Series DR), 2010

	Male	Female
Total	237,916	255,326
Neoplasms	74,267 (31.2%)	67,179 (26.3%)
Diseases of circulatory system	77,260 (32.5%)	80,824 (31.7%)
Diseases of respiratory system	31,563 (13.3%)	35,713 (14.0%)
Diseases of digestive system	12,164 (5.1%)	13,498 (5.3%)
Other	42,662 (17.9%)	58,112 (22.6%)

1.9.3 Symptomatology

Patients with peripheral arterial occlusive disease may be asymptomatic. The most common presenting symptom is intermittent claudication. This refers to pain felt in the muscles distal to the affected vessels which occurs on mobilisation or exertion and is alleviated by rest. The distance at which the symptoms occur is measurable by performing treadmill tests.

In chronic critical limb ischaemia patients may experience numbness or pain in the forefoot at rest and in particular whilst elevated in bed at night. The pain may be alleviated by hanging the leg out of bed or standing and occasionally walking. This can lead to dependant oedema. Tissue loss or ulceration attributable to arterial occlusive disease is a sign of severe disease.

Acute limb ischaemia is a vascular emergency which typically occurs due to rupture of an atherosclerotic plaque and resultant thrombus formation, or distal migration (embolisation) from a proximal location such as the heart or aorta. The clinical presentation is typically characterised by the “6 P’s”: pain, pallor, pulseless, paralysis, paraesthesia and perishing cold.

1.9.4 Classification of PAOD

The disease can be graded according to the degree of severity utilising either the Rutherford classification or Fontaine stages. PAOD presents clinically as intermittent claudication, the severity of which depends upon the site and extent of the lesion and the efficiency of the collateral circulation (Stages 1-3). More severe forms of this disease result in critical leg ischaemia which is defined as rest pain (Stage 4) for more than 2 weeks, or ulceration/ gangrene (Stages 5-6), and an ankle pressure of <50mmHg or a toe pressure of <30mmHg(78).

The natural history of intermittent claudication is that the majority of patients will improve or remain stable, however 7% will undergo infra-inguinal bypass surgery, 4% major amputation and 16% a worsening of their symptoms(74). The main risk factors for disease progression include continued smoking, diabetes and low initial ankle brachial pressure index (79).

Table 3: Classification of PAOD *(80)*

Rutherford Classification	Fontaine stage	Symptoms	Criteria
0	0	Asymptomatic	Normal treadmill or reactive hyperaemia
1	I	Mild intermittent claudication	Post exercise AP >50mmHg but 20mmHg lower than resting
2	IIa	Moderate claudication (≥ 200 meters)	Between 1 + 3
3	IIb	Severe claudication (< 200 metres)	AP post exercise <50mmHg
4	III	Rest pain	Resting AP <40mmHg or TP <30mmHg
5	IV	Ischemic ulceration not exceeding ulcer of the digits of the foot	Resting AP <60mmHg, TP <40 mmHg
6	V	Severe ischemic ulcers or frank gangrene	Resting AP <60mmHg, TP <40 mmHg

1.9.5 Risk factors

1.9.5.1 Diabetes

Patients with diabetes are more likely to suffer from PAOD, however the presence of peripheral neuropathy means sufferers may not present until the development of a complication, such as an ulcer or gangrene. Both vessel structure and function are affected by the disease process as the reduced levels of nitric oxide result in endothelial dysfunction, whilst hyperglycaemia results in increased apoptosis of vascular smooth muscle cells making the atherosclerotic plaques more unstable. Hyperglycaemia also causes platelet dysfunction (81). Calcification of the tunica media, seen most frequently in the lower limb vessels, is more prevalent in diabetic patients, termed Monckeberg's sclerosis, and is associated with poor prognosis. The calcification causes increased arterial stiffness and increased pulse pressure, resulting in high ABPI values (82).

In the UK Prospective Diabetes Study it was determined that 1.2% of newly diagnosed Type 2 diabetics have PAOD (diagnosed by ABPI <0.8) but the prevalence increases to 12.5% 18 years following diagnosis(81). The study also showed a 28% increase in the risk of developing PAOD with each 1% increase in HbA1c.

1.9.5.2 Smoking

Studies have shown that current smoking doubles the risk of developing peripheral arterial occlusive disease when compared with non-smokers (81). Smoking is known to not only increase the risk of PAOD but is also responsible for earlier onset of symptoms by up to a decade when compared to non-smokers (58).

Smokers are much more likely to develop severe forms of the disease such as critical limb ischaemia and as such have a much higher risk of amputation. Surgical interventions in patients who smoke have lower success rates and a meta-analysis concluded that continued smoking following infra-inguinal bypass graft surgery results in a threefold increase in graft failure rate (83). A study by Shammas *et al* demonstrated that smoking is an independent risk factor for the need for urgent interventions to revascularise the lower limbs following an initially successful treatment (84).

It is thought that smoking increases the atherosclerotic process through its effects on the structural and elastic properties of the arteries, oxidative stress and reduced levels of nitric oxide, which is known to cause vasodilation (85).

1.9.5.3 Hypercholesterolaemia/ hyperlipidaemia

Dislipidaemia is known to significantly increase the risk of peripheral arterial occlusive disease. The prevalence of familial hypercholesterolaemia increases the prevalence of PAOD from 5 fold to 10 fold in non- sufferers (74).

1.9.5.4 Hypertension

Hypertension was shown to be a positive risk factor for PAOD in a study conducted by Selvin and Erlinger in 2004 (OR 1.75, 95% CI 0.97-3.13) (86). The Rotterdam study demonstrated, on multivariate analysis, that every

10mmHg increase in systolic blood pressure confers an increased risk of PAOD (87). Control of hypertension in these patients is also known to result in a reduction in the incidence of cardiovascular events. As such the guidelines advocate that persistently elevated blood pressure, >140/90, in patients with pre-existing cardiovascular disease should be treated(88).

1.9.5.5 Obesity

Metabolic syndrome is the combination of certain risk factors which are known to increase the risk of cardiovascular, cerebro-vascular and peripheral vascular disease and diabetes. One of the most important risk factors is obesity. It is characterised by elevated levels of inflammatory markers such as C - reactive protein and excessive coagulation due to increased fibrinogen (89). An increased waist to hip ratio above the median has been shown to increase the prevalence of PAOD by a factor of two (90). It has also been shown that the distance at which patients experience claudication symptoms decreases with increasing body weight(91).

1.9.6 Diagnosis

The clinical diagnosis of peripheral arterial disease severity is known to be inaccurate. As aforementioned patients may be asymptomatic, however a detailed history should be taken to establish the presence, or absence of claudication and the distance at which onset of symptoms occurs.

A full examination of both cardiovascular and peripheral vascular systems should be undertaken for all patients.

On examination signs of PAOD may be apparent such as ulcers or gangrene. All pulses must be palpated but the accuracy of this is dependent on the experience of the examiners as well as the presence of oedema, body habitus etc.

Buerger's test is useful for patients with critical ischaemia in the absence of tissue loss or gangrene. The symptomatic leg is elevated above the heart and pallor should result, on lowering the leg a 'sunset red' appearance will occur as the capillaries of the foot dilate. In patients where Doppler

examination is not possible, i.e. patients with ulcers, or will not be helpful i.e. in patients with incompressible arteries the ischaemic angle can be calculated. The leg is elevated until the Doppler foot signals disappear and the level above the heart is measured (0.73mmHg = 1cm H_20) giving a crude value for ankle pressure(92). The diagnosis is usually confirmed by performing the Ankle Brachial Pressure Index (ABPI).

1.9.6.1 The ankle brachial pressure index

The ABPI is used as a quick and non-invasive bedside measure of peripheral arterial occlusive disease. It was first described by Yao *et al* in 1968 (93). A handheld Doppler ultrasound probe is utilised to detect the required pulse and a sphygmomanometer is inflated proximal to the probe until the pulse is no longer detected. The cuff is slowly deflated and the pressure at which the pulse is re-detected is the systolic pressure of that artery.
It is calculated by using the following formula.

$$ABPI_{LEG} = \frac{P_{LEG}}{P_{ARM}} = \frac{\text{systolic blood pressure at dorsalis pedis or posterior tibial artery}}{\text{systolic blood pressure at brachial artery}}$$

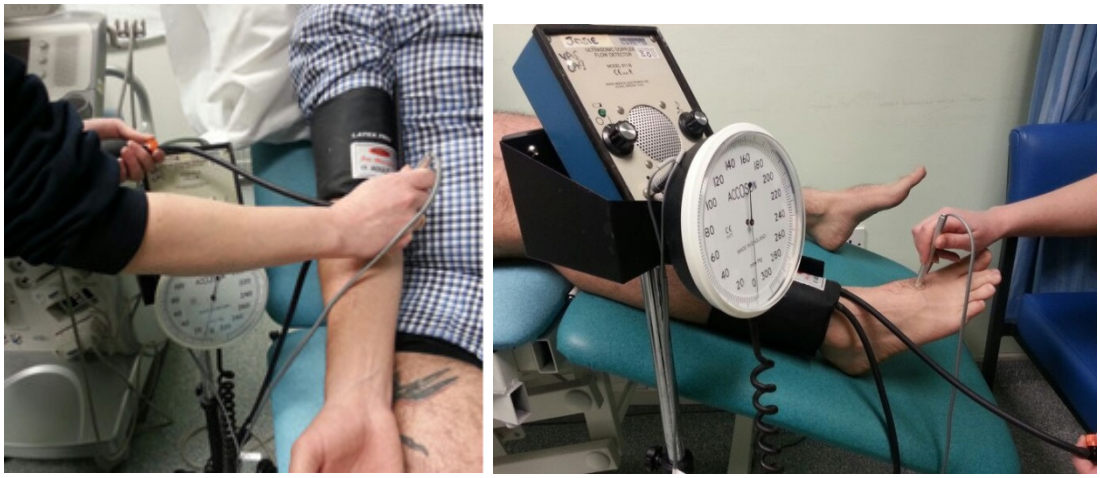
The normal range is quoted to be 0.91- 1.31 (94). ABPI can give an objective measure as to the disease severity and allows for identification of asymptomatic disease. The reproducibility has been shown to be dependent on the experience of the operator (95). The sensitivity of ABPI as a diagnostic tool in PAOD ranges from 80-95% and the specificity from 95-100% (94).
Studies have shown that very low (<0.7) and low (0.7- 0.9) ABPI's are associated with increased all cause and cardiovascular mortality and combine cardiovascular morbidity and mortality. It has also been shown that a high ABPI (≥1.4) is associated with an increased risk of cardiovascular events (76).

Table 4: Interpretation of ABPI value(96)

ABPI	Interpretation
>1.1	Normal but may be a sign of incompressible, calcified arteries i.e. in diabetes
0.9-1.1	Normal range
0.7-0.89	Mild to moderate peripheral arterial occlusive disease (patient's symptoms may range from asymptomatic to intermittent claudication)
<0.7	Moderate to severe peripheral arterial occlusive disease (patient's symptoms may range from intermittent claudication to rest pain +/- gangrene

ABPI's may not be accurate in patients with diabetes, chronic renal failure and the very elderly, due to a high prevalence of calcification of the medial arteries within these cohorts. The arteries are less compressible and as such there is a high false negative rate. ABPI is also an insensitive tool at detecting PAOD progression when compared to imaging (97).

Figure 12: Measuring the ABPI



Brachial systolic blood pressure measurements taken bilaterally and ankle systolic measurements taken bilaterally from both dorsalis pedis and posterior tibial arteries.

1.9.6.2 Imaging

In order to plan revascularisation accurate anatomical information pertaining to the site, number and severity of the lesions is imperative. Knowledge of the inflow and outflow to the area is also necessary particularly when planning a vascular reconstruction.

1.9.6.2.1 Ultrasound duplex

The 2005 AHA guidelines have stated that analysis of Doppler waveforms should be combined with visualisation of the arteries with ultrasound i.e. duplex ultrasound (98). A recent systematic review has reported sensitivity rate of 88% and a specificity of 94% (99). Duplex ultrasound scanning has the benefit of being widely available, inexpensive and non-invasive.

The quality of ultrasound duplex is known to be operator dependant and requires proper training. Some arterial segments are more difficult to visualise than others, in particular segments distal to a severe stenosis or occlusion (100) and proximal vessels i.e. the aorta and iliacs can be hard to visualise if obscured by bowel gas or in patients of large body habitus.

1.9.6.2.2 Computed tomography angiography

The diagnostic accuracy of CT has improved with advances in technology to 98-99% sensitivity and 96-99% specificity (94). The accuracy decreases the more distal the vessel and image interference can occur with heavily calcified arteries.

The average dose of radiation reported for a CTA is equivalent to 2-3 times the background annual radiation exposure(101). It has been suggested that within the cohort of patients with PAOD this is not of significant concern as the latent period of radiation induced malignancy is less than the typical life expectancy(102).

The contrast agents utilised are associated with the dose related risk of developing contrast-induced nephropathy (CIN). The overall risk for the development of CIN in high risk patients reported in the literature is 16.8% (103) of whom 1% require renal replacement therapy. Contrast-induced nephropathy has been shown to have an in-patient mortality rate of 34% versus 7% for patients without (104).

1.9.6.2.3 Magnetic resonance angiography

A 2010 meta-analysis has shown MRI scanning to have a pooled sensitivity of 95% and specificity of 96%(105). It has the advantage that patients are not exposed to ionising radiation and there is no risk of CIN with the Gadolinium contrast.

Whilst the presence of calcification does not affect the image quality, turbulent flow can result in an overestimation of the degree of a stenosis. Equally the presence of implants and stents may result in image artefacts which can render the images un-interpretable.

The association of PAOD with cardiovascular disease can mean that a relatively high proportion of patients have pacemakers or defibrillators which excludes them from MRI scanning(106).

1.9.6.2.4 Intra-arterial Angiography

Digital subtraction angiography (DSA) is still considered to be the gold-standard and it is against this that new imaging modalities are compared. Despite this it has its flaws, such as 2D imagery, unlike CTA and MRA, overestimation of the length of vessel occlusions, and difficulty imaging distal run off(94). DSA also involves use of contrast medium and as such has the associated risk of CIN unless CO_2 or gadolinium are used. Patients are exposed to ionising radiation during the investigation.

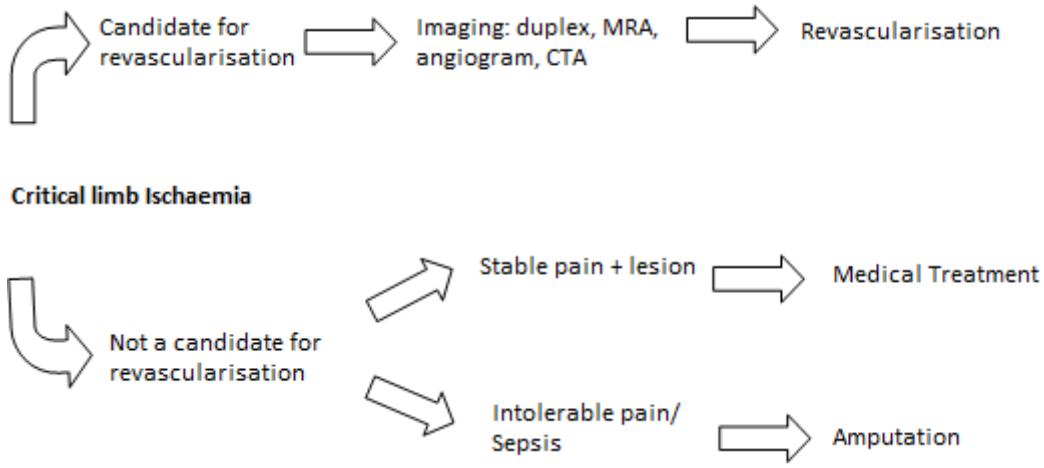
The technique is invasive and damage to the vessels such as dissection, rupture of atherosclerotic plaque and hence embolisation and damage to the access vessels can occur.

1.9.7 Treatment

The decision regarding intervention in patients with claudication is dependent on many factors such as smoking status, walking distance and the impact the disease is having on their quality of life. This must be weighed against the possible risks associated with intervention.

Patients with chronic critical limb ischaemia will require some form of intervention, in most instances, unless quality of life and survival odds dictate otherwise.

Figure 13: Treatment of critical limb ischaemia within this institution: adapted from Norgren L et al(107)



1.9.7.1 Medical management

The aims of treatment for peripheral arterial occlusive disease are two-fold:

1. Relieve the symptoms through improvement of blood supply and reduction in ischaemia
2. Reducing the risk of cardiovascular events also referred to as secondary prevention

Secondary prevention involves lifestyle modification and prevention/ control of the relevant risk factors.

1.9.7.1.1 Diet and exercise

Exercise is known to improve not only the functional capacity of patients but also reduce their overall cardiovascular risk (108). A systematic review by Parmenter *et al* investigated the role of exercise programmes in claudication and clearly demonstrated all forms of exercise to be beneficial for patients (109). This reflected the finding of the 2008 Cochrane review which concluded that exercise programmes improve both walking time and distance in patients with intermittent claudication (110). Supervision of the exercise is thought to be key to gaining benefit (111) and the Exercise Therapy in

Peripheral Arterial Disease (EXITPAD) study group demonstrated that absence of supervision leads to no benefit in terms of disease specific or generic quality of life from exercise therapy in this cohort of patients (112). The ACC/ AHA 2005 Guidelines have stated that the supervised exercise should be performed for a minimum of 30-45 minutes three times a week for a minimum of twelve weeks(98).

Weight loss is associated with an improvement in cardiovascular health and can improve walking distance in patients with intermittent claudication(113). Beneficial effects may also be seen with diets low in fat on plasma lipid levels and may aid weight reduction. Glucose tolerance is known to increase with weight loss. Low salt diets are also associated with improvements in blood pressure (BP) and it has been demonstrated that long term salt reduction can reduce not only BP but also the risk of coronary events(114).

1.9.7.1.2 Smoking cessation

Studies have shown that a combination of advice, and frequent follow up is more effective at achieving smoking cessation than no physician intervention (5% versus 0.1% at one year) (115). The use of nicotine replacement therapy or bupropion results in cessation rates of 16% and 30% respectively (116). There is evidence that in women, who stop smoking, their overall cardiovascular risk returns to the level of non-smokers within 2-4 years(117).

1.9.7.1.3 Antiplatelet/ antithrombotic agents

A meta-analysis analysing 31 randomised control trials demonstrated that that long-term aspirin therapy significantly reduced overall vascular mortality, as well as nonfatal stroke and MI (118). Studies have also demonstrated that treatment with aspirin improves the patency of infra-inguinal bypass grafts but this effect is more marked with artificial grafts(119). More recently studies have been carried out which appear to demonstrate that whilst the effect seen with aspirin is marginal clopidogrel may be more beneficial for the prevention of vascular events(120).

A Cochrane review to examine the role of antithrombotic agents in patients following infra-inguinal grafts concluded that following bypass with a venous

graft a vitamin K antagonist e.g. warfarin, may be beneficial whilst antiplatelet agents are more beneficial for those receiving an artificial graft (121).

1.9.7.1.4 Blood pressure lowering medications

Angiotensin converting enzyme inhibitors have been shown to have beneficial effects in patients with PAOD. The HOPE study showed a significant reduction in the number of cardiovascular events in patients being treated with ramipril versus placebo (122). The ABCD trial compared nisoldipine with enalapril and illustrated beneficial effects in diabetic patients with PAOD (123). Another trial investigating the role of perindopril demonstrated a marginal increase in claudicating distance but this was not indicative of an increase in ABPI or maximum walking distance when compared to placebo(124). The Cochrane Review in 2009 concluded that the evidence pertaining to anti-hypertensive drugs in peripheral arterial occlusive disease is inadequate to determine whether significant benefits result from their use (125). However sufficient evidence exists regarding the benefit of hypertension management to control blood pressure.

1.9.7.1.5 Heart rate lowering medications

Beta blockers have been shown to decrease the risk of adverse coronary events and death in patients known to have coronary atherosclerosis (126) and despite initial concerns do not have an adverse effect on walking capacity (127).

1.9.7.1.6 Lipid lowering medications

The 2009 Cochrane review to examine the role of lipid lowering drugs in peripheral arterial disease concluded that whilst the effect on all cause mortality was inconclusive that statin therapy results in a beneficial effect on the total number of cardiovascular events(128). Statins have also been shown to reduce claudication symptoms and increase walking distance (129, 130).

1.9.7.1.7 Diabetic control

Whilst the United Kingdom Prospective Diabetes Study involving patients with type II diabetes mellitus did demonstrate a 16% reduction in the risk of myocardial infarction this was only of borderline significance and no significant reduction in the mortality rate of risk of cerebro-vascular events or amputation was demonstrated (131). It is however known that improved control of diabetes reduces the risk of microangiopathy and therefore nephropathy and retinopathy. As such the recommendations state that patients should be managed aggressively to obtain an HbA1c level less than 7% (98).

1.9.7.1.8 Vasoactive drugs

The two main drugs in this category are Cilostozol and Naftidrofuryl. Cilostazol has been shown to increase maximal walking distance in claudicants by 38% and 51% at low and high doses respectively, when compared to placebo. Patients also reported an improvement in quality of life (132). However a subsequent review demonstrated only a small (15.7-41.3m) increase in walking distance and the cost effectiveness has yet to be established(133).

A recent meta-analysis demonstrated a 28% improvement in walking distance with naftidrofuryl when compared with placebo (134). The National Institute of Health and Care Excellence (NICE) have recommended natftidrofuryl as a treatment option for patients with intermittent claudication determining it to be the only cost effective treatment when compared with cilostazol, pentoxifylline and inositol nicotinate(135).

1.9.7.1.9 Prostanoids

These are powerful vasodilators, which also reduce platelet aggregation and improve endothelial function. Illoprost is the most commonly used in clinical practice due to its stability and relatively long half life. In patients with critical limb ischaemia treatment with illoprost improved limb salvage rates from 35% to 55%. Treatments are given intravenously and as such have to be carried out as an inpatient and side effects, such as headaches and nausea, are common (136).

1.9.7.2 Interventional Radiology

1.9.7.2.1 Angioplasty

The feasibility and likelihood of success of angioplasty is dependent on several factors:

- The length of the stenosed or occluded segment. It has been shown that patients with lesions greater than 10cm in length are less likely to benefit (137) and lesions less than 3cm show the best results (138).
- The nature of the lesion. Lesions due to the accumulation of atheroma may be treated by balloon angioplasty and in some cases a stent or may require a surgical intervention such as endarterectomy or bypass. Plaque morphology, as assessed with duplex ultrasound, can help predict the initial success and restenosis risk following angioplasty. Echolucent plaques with a lower greyscale median, due to higher cholesterol content, are more compressible and less likely to restenose that echogenic fibrous plaques with a higher greyscale median(139). Thrombotic lesions may benefit from thrombolysis whilst emboli require embolectomy.
- The site of the lesion. It is known that the more proximal a lesion the greater the restriction to flow(140) and equally that proximal lesions give better results, with reduced restenosis rates and greater symptomatic relief(138).

This led the TASC working group to develop a series of recommendations to facilitate decision making regarding the optimal treatment methodology for lesions in relation to operative versus endovascular treatment.

Table 5: TASC II Working Group recommendations on interventions(107)

Arterial segment	Type A (usually endovascular)	Type B (preferentially endovascular)	Type C (preferentially open surgery)	Type D (usually open surgery)
Infrarenal aorta		Stenoses ≤3cm		Occlusion
Iliac disease	Stenoses ≤ 3cm	Stenosis 3-10cm OR unilat occlusion	Bilat CIA occlusion OR unilat CIA + EIA occlusion	Bilat EIA occlusion OR extension into aorta +/- CFA
Femoral	SFA stenosis ≤10cm OR occlusion ≤ 5cm	SFA occlusion or stenosis ≤ 15 cm. Pop stenosis	SFA occlusion or stenosis >15 cm OR recurrent disease	Complete SFA or popliteal occlusion
Crural			Stenoses ≤ 4cm OR occlusions ≤ 2cm	Diffuse disease OR occlusions > 2cm

The role of angioplasty in infra-inguinal disease was investigated in the BASIL trial. It concluded that mortality in both groups was low, 5% versus 3% for surgery and angioplasty respectively. Surgery was associated with higher morbidity and longer hospital stays however at 6 months the amputation free survival rates and quality of life scores were comparable. This was also associated with comparable cost effectiveness at 3 years due to a significantly higher failure rate for angioplasty, 20% versus 3% at 12 months(141).

The continuing evolution of endovascular therapy has led to the development and routine usage of additional technologies to improve outcomes following angioplasty. Whilst initially stent usage was reserved for cases with demonstrable residual stenosis following angioplasty or dissection to improve primary patency (142), some studies have reported improved patency in TASC A lesions (143, 144).

A recent meta-analysis has demonstrated that drug coated balloons show significantly lower re-intervention rates when compared to standard angioplasty.

A significant advantage in restenosis rates was demonstrated when comparing self expanding stents, balloon expanding stents, endovascular brachytherapy and drug coated balloons when compared with standard

angioplasty. Drug-eluting stents also conferred an additional benefit compared to bare metal stents. However significant variations existed in the outcome measures reported in the studies and the sample sizes were small (145).

1.9.7.3 Surgery

The success of surgical bypass procedures is dependent on the quality of the inflow and run-off vessels.

1.9.7.3.1 Choice of bypass graft material

Autogenous vein grafts, in particular the saphenous vein are the conduits of choice for performing infra-inguinal bypass grafts. A systematic review examining the outcomes of randomised controlled trials comparing above knee PTFE with vein grafts determined that primary patency at 2 years was 80 and 69% and at 5 years 74 and 39% for vein and PTFE respectively(146). Infection rates are also significantly lower in vein versus prosthetic grafts (147) with reported rates of between 1 and 5%(148).

Pre-operative ultrasound assessment should always be carried out to determine the suitability of the vein, in terms of calibre and degree of varicosities. A long saphenous vein of diameter less than 3mm has a 2 fold increased risk of failure and as such should not be used (149). In patients with unsuitable long saphenous veins, harvesting bilateral short saphenous veins may be an option or the arm veins may be considered although outcomes are significantly poorer (150).

The calibre of the supra-inguinal vessels leaves little alternative save for the use of prosthetic material grafts, either Dacron or polytetrafluoroethylene (PTFE). A 2008 meta-analysis comparing Dacron and PTFE grafts found no significant differences in terms of patency or infection rates(151). This contradicted the findings of an earlier multicentre RCT (152) which showed significantly higher secondary patency rates for Dacron grafts. Surgeons are believed to prefer the handling and suturing characteristics of Dacron and as such this is currently the prosthetic graft of choice.

Aorto-iliac disease

For patients with aorto-occlusive disease there is currently no endovascular treatment option. Whilst laparoscopic aortic surgery has gained support in some European countries, with suggestions that it reduces morbidity and mortality as well as length of stay(153), there is no high level evidence to support these claims. Open surgery, in the form of an aorto-iliac or aorto femoral bypass, remains the most commonly practiced surgical technique. For those patients unfit for major abdominal surgery or with a hostile abdomen an axillo-bifemoral bypass graft may be contemplated, however patency rates are poorer and morbidity and mortality rates are higher, perhaps related to the higher incidence of significant co-morbidities(154).

For patients with either extensive external iliac disease or disease refractory to endovascular procedures an ileofemoral bypass is the procedure of choice. It is reliant on adequate inflow and in cases where the ipsilateral common iliac is extensively diseased or occluded an ileofemoral cross-over graft may be performed if the contralateral side is minimally diseased. An alternative is a femoro-femoral bypass graft if there is no inflow on the affected side but a relatively disease free contralateral side.

Femoral disease

To ensure adequate outflow/ inflow in patients undergoing supra-inguinal/ infra-inguinal bypass surgery respectively and in patients with isolated origin disease i.e. stenosis at the bifurcation, an endarterectomy may be performed. The repair typically involves patching of the vessel with vein, preferentially due to reduced infection rates, or a prosthetic material.

Infra-inguinal disease

The first vascular bypass graft reported in the literature was performed by Goyanes in 1906, using in situ popliteal vein to bypass a popliteal aneurysm. Current estimates are that approximately 3,400 femoro-popliteal bypass operations are performed annually in the UK (155). In cases where no suitable vein is available to perform the bypass the use of a small section of

vein to form a cuff at the distal prosthetic anastomosis has been shown to improve patency rates of bypasses below the knee (156).

Table 6: Patency rates of infrainguinal bypass grafts(157)

Year	1	2	3
Primary patency (%)			
Claudicants			
Above Knee Prosthetic	85.3	77.4	**70.9**
Above Knee Vein	87.5	84.1	**81.1**
Below Knee Vein	81.4	76.0	**73.8**
Critical Ischaemics			
Above Knee Prosthetic	76.3	64.7	**57.2**
Above Knee Vein	83.4	81.2	**76.6**
Below Knee Vein	**84.3**	**80.4**	**76.2**

1.9.7.4 Alternative therapies

For patients with critical ischaemia, who are not candidates for endovascular or surgical revascularisation or in whom these techniques have already failed, treatment options are limited. The outcome for these patients is bleak and the reality is that within 6 months, the mortality rate is 20%, the amputation rate is 35%, and amputation-free survival is 45% (158). Typically treatment comprises symptom control through the use of analgesics, wound care, including pressure relief, and optimisation of medical therapy.

1.9.7.4.1 Lumbar Sympathectomy

Chemical lumbar sympathectomy involves the injection of phenol and alcohol around the lumbar sympathetic chain at the level of the 2nd and 3rd lumbar vertebrae. It has been shown to increase blood flow by the abolition of sympathetic tone and as such the basal and reflex constriction of arterioles. The maximal result is seen immediately but the duration of any affect is unknown and variable.

It is known that it has limited use in patients with tissue loss when compared to those with ischaemic rest pain only. Reports of effectiveness in the

literature vary widely with long term pain relief ranging from 6% to 78% and early amputation rates from 11% to 70%.
Despite the lack of high level evidence pertaining to its use lumbar sympathectomy is still utilised (159).

1.9.7.4.2 Neurostimulation

Several techniques are encompassed by the term neurostimulation including transcutaneous nerve stimulation, spinal cord stimulation (SCS), peripheral nerve stimulation and cortical stimulation. The development of these techniques rapidly following the proposition of the Gate control theory by Melzack and Wall in 1965(160).

It is hypothesised that the transmission of a nerve impulse from the afferent fibres to the cells responsible for its transmission in the spinal cord was modulated by a 'gating mechanism' in the dorsal horn. As such higher levels of activity in large myelinated nerve fibres inhibit the transmission in the small unmyelinated nerve fibres. In this way epidural electrodes can stimulate the dorsal horn to inhibit or temper the input from the smaller fibres (161). SCS not only modulates the gating mechanism but also appears to increase the inhibitory effects of gamma-aminobutyric acid (162).
The effects of neurostimulation are not limited to pain control. Animal studies have shown that stimulation below the motor threshold can activate afferent fibres of the dorsal column resulting in the peripheral release of calcitonin gene-related peptide which causes vasodilatation within the skin (163). This has been shown to be associated with an increase in tissue partial pressure of oxygen(164).
In vivo endogenous electrical fields occur naturally and are fundamental for tissue development and repair. Disease processes can interrupt the generation of these electrical fields which can impede tissue repair (165). Application of exogenous electrical stimulation can stimulate the generation of these electrical fields and aid healing.
Electrical stimulation has many physiological effects such as an increase in the migration of macrophages, granulocytes, fibroblasts and epithelial cells

when applied to a wound (166-168). Exogenous stimulation can also increase the formation and distribution of collagen and as such increase the tensile strength of treated wounds(169).
Electrical stimulation stimulates the migration of endothelial cells, the release of VEGF and angiogenesis (170-172). Wounds treated with exogenous stimulation feature granulation tissue with a dense network of capillaries, however these vessels are unstable in nature and the effects are believed to be short lived.
Electrical stimulation, particularly with direct currents, has also been shown to have an antibacterial effect due to the inhibition of pathogens (173).

1.9.7.4.2.1 Spinal cord stimulation

This involves the placement of epidural electrodes to stimulate the dorsal columns and has been found to be beneficial in patients with intractable pain(174). The role of SCS in peripheral arterial occlusive disease was first reported by Cook *et al* in 1976 (175). A Cochrane Review investigating the role of spinal cord stimulation for non- reconstructable chronic critical limb ischaemia determined that limb salvage after 12 months of treatment with SCS was significantly higher than the control group. It also demonstrated improved pain relief although found no significant improvement in wound healing. The complication rate associated with the treatment was high with 9% of patients experiencing implantation problems, 15% of patients experiencing changes in stimulation requiring re-intervention, and 3% of patients suffering infections(176).

1.9.7.4.2.2 Transcutaneous electrical nerve stimulation
The first portable, wearable device was developed in 1974 (177) and it was determined that low currents with short wave lengths can be given to produce a painless motor current but as the current increases the less preferential the stimulation is for motor nerves(178). Whilst the term transcutaneous electrical nerve stimulation is generally restricted to pain relief devices it actually applies to all currents applied via surface electrodes for the purpose of nerve stimulation, however most studies relate to the stimulation of cutaneous

nerve fibres. TENS has been demonstrated to be advantageous in the treatment of pain secondary to diabetic peripheral neuropathy (179).
Few studies have been carried out to determine other non-analgesic effects of TENS in patients with peripheral arterial occlusive disease. Some circulatory improvements have been reported in those with arterial disease (180) and accelerated arterial ulcer healing (181). More recently Debreceni *et al* reported improved outcomes, with increased pain-free walking distances and ulcer healing, in 20 of 24 patients treated with transcutaneous electrical stimulation (182).

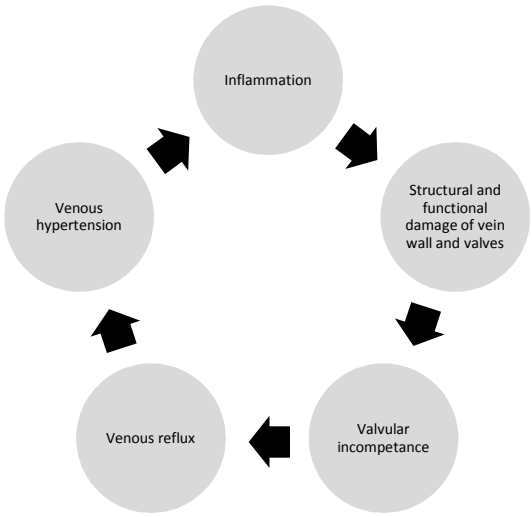
1.10 Venous disease

1.10.1 Pathophysiology

During normal ambulation the muscles of the calf contract; this “muscle pump” compresses the deep veins of the leg causing antegrade flow against gravity back towards the heart. The presence of the valves within the veins prevents the retrograde flow of blood with gravity. Venous hypertension results from a failure of this mechanism. The vast majority of cases are due to valvular insufficiency (venous reflux), commonly in the superficial venous system but occasionally in the deep or a combination of both systems. This occurs due to the initiation of a vicious cycle of events driven by inflammation.
The remainder of cases are due to a physical or functional obstruction of normal venous return. The most common case for a physical obstruction is thrombosis of a major part of the venous outflow (DVT), but other causes include trauma, surgery, cancer (groin or pelvic tumour) and May-Thurner syndrome. Perhaps a greater number of people have a functional obstruction secondary to a failure in the calf-muscle pump. This is commonly due to immobility, ankle pathology and trauma.
Rarely congenital disorders may be responsible, for example in Klippel-Trenaunay syndrome where patients present with varicose veins, limb

hypertrophy and cutaneous capillary haemangiomas. The venous abnormalities in this cohort include atresia, agenesis, valvular incompetence and rarely venous aneurysms (183, 184).
Several hypotheses exist regarding the pathogenesis of venous insufficiency. The most commonly quoted is that mechanical disruption of a proximal valve results in the propagation of increasing pressure down the vein axis. This results in failure of subsequent valves. Macroscopic and histological examination of veins show deformity and destruction of valves and inflammation (185-187).

Figure 14: Pathophysiologic cycle of venous disease



1.10.2 Epidemiology

Chronic Venous insufficiency (CVI) refers to a common disorder of the venous drainage of the lower limbs resulting in venous hypertension. This frequently leads to symptoms (pain, swelling, itching) and complications (eczema, phlebitis, bleeding, ulceration), which have a deleterious impact on patients quality of life. The Edinburgh Vein study estimated that 9.4% of men and 6.6% of women have evidence of chronic venous insufficiency (188).
In 1997 Ruckley reported that the annual venous disease expenditure in the UK was around £400 million which equated to approximately 1-3% of the health care budget (189, 190).

1.10.3 Symptomatology

Chronic venous insufficiency can present with a spectrum of symptoms. Patients may complain of aching, pain, itching and tightness whilst signs such as eczema, hyperpigmentation (haemosiderosis) and lipodermatosclerosis may be evident (191, 192). With increasing severity of the insufficiency patients can experience oedema and ulceration.
Venous symptoms are worse when the limbs are dependant and are frequently precipitated/ aggravated by prolonged standing and relieved by elevation.
CVI has a significant adverse impact on patients' health related quality of life, the severity of which correlates with the severity of CVI (193-195). Walters *et al*(196) demonstrated that a patient with simple, uncomplicated varicose veins will experience a loss of 0.7 (95% CI 0.3 to 1.2) quality adjusted life years over 10 years when compared with someone in full health, if no treatment is carried out.

1.10.4 Classification

Venous disease is classified using the CEAP classification, which includes the Clinical picture of the disease, the aEtiology, the Anatomy of the disease and the underlying Pathophysiology.

Table 7: CEAP Classification

Clinical	
C0	No visible or palpable signs of venous disease
C1	Telengectasia or reticular veins
C2	Varicose veins
C3	Oedema
C4a	Pigmentation or eczema
C4b	Lipodermatosclerosis
C5	Healed venous ulcer
C6	Active venous ulceration
Etiology	
Ec	Congenital
Ep	Primary
Es	Secondary (post thrombotic)
En	No cause identified
Anatomic	
As	Superficial
Ap	Perforator
Ad	Deep
An	No location identified
Pathophysiology	
Pr	Reflux
Po	Obstruction
Pr,o	Reflux and obstruction
Pn	No venous pathophysiology identifiable

The venous clinical severity score assesses both symptoms and their impact on normal daily activities. It is useful as an adjunct to the ‘C’ classification of the CEAP score.

Table 8: Venous Clinical Severity Score

Attribute	Absent (0)	Mild (1)	Moderate (2)	Severe (3)
Pain	None	Occasional, no impact on activity and no analgesia required	Daily, moderate restriction on activity, occasional analgesic use	Daily, severe restrictions on activity and regular analgesic use
Varicose veins	None	Few scattered VV's	Multiple VV's confined to calf or thigh	Extensive thigh and calf VV's
Venous oedema	None	Evening ankle	Afternoon oedema, above ankle	Morning oedema above ankle and requiring activity changes

1.10.5 Risk factors

A full history is necessary to determine the presence of risk factors for the development of varicose veins. Over one third of patients presenting with varicose veins will have a family history of venous disease (197, 198) although evidence of a genetic predisposition is lacking (199). However congenital disorders, for example Klippel-Trenaunay syndrome can lead to the development of varicose veins at an early age(197).

Studies have shown that the occurrence of venous disease increases with increasing age (188, 198, 200) and is more common in the female sex (Odds ratio 2.3 (95%-CI 1.9-2.7))(201). As the number of children a woman has borne increases so does her risk of developing venous disease (202, 203). Increasing weight in women has also been found to be a risk factor which is independent of parity (203).

A sedentary job, with a predominant seating position at work has been determined to be a risk factor for the development of venous disease (201) as have occupations involving long periods of standing (204, 205).

It is imperative to determine if patients have a prior history of deep vein thrombosis as in these instances the varicosities may be secondary to the thrombosis.

1.10.6 Diagnosis

A thorough examination of the lower limbs is carried out to determine the distribution of all significant varicosities. Any skin changes or areas of ulceration should be noted. Palpation of the limbs enables the determination of the presence of any oedema and may locate perforators.
The source of venous hypertension may be determined by some simple bedside tests. These tests (e.g. Tap test, the Brodie- Trendelenburg test) have been demonstrated to have poor sensitivity and specificity when compared with venous duplex and thus have been largely abandoned in routine clinical practice (206).

1.10.7 Investigations

Use of a hand held Doppler (HHD) to detect junctional reflux is commonly performed in the outpatient department. The probe is positioned over the SFJ/ SPJ while the calf is manually compressed. Reflux is audible on release of the calf. HHD examination has a sensitivity of 97% and 73% specificity for SFJ reflux. These figures are poorer for SPJ reflux (207).

Venography

Whilst it used to be the gold standard investigation for venous disease venography has now been replaced by duplex ultrasound because of the associated risks. Extravasation of contrast can result in cellulitis and in extreme cases necrosis and the development of ulcers. Some studies report the development of thrombosis up to 13% (208). In certain situations venography may still be used to assist in the planning of intervention.

Duplex ultrasound

Duplex is non-invasive, repeatable and reproducible and provides information pertaining to the anatomy and haemodynamics of the venous system.
In B mode information pertaining to the anatomy and relationships of the venous system can be established. The addition of Doppler supplies

information regarding the velocity and direction of blood flow, represented as colour on the screen. Spectral Doppler provides more information on the nature of flow, in the form of a graph.
Incompetence within a vein section is determined by the duration of reflux. Controversy exists surrounding the exact duration deemed significant however the Society of Vascular Surgery Guidelines clearly state that significant reflux is greater than or equal to one second in the great saphenous vein and popliteal vein but only 0.5 seconds in all other vein segments (209).

1.10.8 Treatment

1.10.8.1 Conservative management

Graduated compression hosiery provides external support which reduces the venous hypertension, promoting the antegrade flow of blood and augmenting the function of the muscle pump (210-213). Imaging has demonstrated that to obtain the haemodynamic effects requires sufficient pressure to narrow the vein lumens which has been found be to be between 30 and 40mmHg(214). However a meta-analysis investigating low grade compression, 10-20mmHg, determined that patients report improved symptoms and reduced swelling compared to no compression (215). Compression plays a significant role in the treatment of venous ulcers. Ulcer healing rates have been found to be improved (216) and recurrence rates reduced (217).
Some patients fail to benefit from compression but this may be due to poor patient selection or compliance. Venous symptoms can be non-specific and a trial of compression hosiery may help distinguish patients who will benefit from further intervention (218). Compliance with compression hosiery is known to be poor with recent estimates around 35-40% and up to one third of patients will not obtain any benefit despite apparent compliance (219, 220).

1.10.8.2 Intervention

Surgical intervention for those patients demonstrated to have superficial venous incompetence, in the form of open surgery, endovenous laser therapy, VNUS closure and foam sclerotherapy, improves generic and disease specific quality of life, reduce the recurrence rates of venous ulcers and increase 'ulcer free time'(221). Indeed studies have shown that treatment of superficial incompetence can eliminate or reduce deep venous incompetence (222, 223). The REACTIV trial also demonstrated a post operative improvement at one year in the EQ-5D scores of 0.1(224). Treatment of varicose veins with surgery or sclerotherapy has also been shown to be highly cost effective (224).

1.10.8.2.1. Endothermal techniques

These involve the introduction of a catheter at the lowest point of reflux into an incompetent, axial, superficial vein, under ultrasound guidance. Perivenous tumescent anaesthesia in addition to eliminating procedural pain, protects surrounding structures by hydro-dissection and heat dissipation. Thermal energy, laser (EVLA) or electrical current (RFA) is supplied via the catheter to obliterate the vein.

EVLA is not without complications with bruising occurring in up to 52.2% of cases and paraesthesia in up to 2.7%. The incidence of DVT is significantly lower than open surgery 0.3-0.6% (225, 226). Comparisons of EVLA and open surgery have determined that the incidence of complications, such as paraesthesia, infections and haematomas is less following EVLA (227). Recurrence rates at 1,2 (227) and 5 years are comparable as are reoperation rates and QoL scores (228).

Radiofrequency ablation has complication and recurrence rates which are similar to EVLA. A meta-analysis by Siribumrungwong *et al* determined that primary failure and recurrence rates of EVLA and RFA were non-significantly different to those with open surgery but the incidence of haematomas, wound infections and pain were lower and patients returned to normal activities faster (229).

Indeed the recent NICE guidelines stipulate that endothermal ablation should be first line treatment for varicose veins with truncal reflux. If the patient is deemed unsuitable for endothermal ablation sclerotherapy should be considered before open surgical repair (230). Interestingly these guidelines also recommend that compression hosiery should not be prescribed for symptomatic, uncomplicated superficial venous insufficiency.

1.10.8.2.2 Sclerotherapy

This entails the use of chemical agents which are injected into the necessary veins and cause occlusion by the initiation of chemical thrombophlebitis and fibrosis. Initially liquid chemicals were utilised but quickly lost favour due to high recurrence rates. The development of foam sclerosants has led to a resurgence in interest with success rates of up to 80% at 5 years being reported in the literature (231, 232).

The incidence of minor complications such as skin pigmentation are high (~17%) but serious complications (neuropathic problems, anaphylaxis) are rare (233).

1.10.8.2.3 Open surgery

This technique incorporates ligation at the site of junctional incompetence, stripping of the incompetent axial vein and phlebectomies, usually performed under general anaesthetic. In experienced hands open surgery is safe, cost effective (224) and beneficial in terms of improvements in quality of life (234). Unfortunately open surgery has significant recurrence rates, up to 30% at one year and 66% at 10 years although not all patients request re-intervention (235-237).

Complications following open surgery are not uncommon but are usually temporary. Superficial skin infections can occur in up to 18% of patients (238), whilst haematomas may occur in up to 33% of patients (239). Sensory cutaneous nerve damage is the most common cause of complaints following open surgery for varicose veins, the incidence of which has been shown to be related to the length of vein stripped (stripping to the knee 7% versus full length stripping 39%)(240). Surgery of the saphenopopliteal junction may also be complicated by damage to the sural nerve or common peroneal

nerve (241). Deep vein thromboses may occur in up to 5% of patients although the majority are asymptomatic (242).
For patients who have no surgically correctable cause or who are unable to tolerate or obtain no benefit from compression, treatment options are limited. Horse chestnut extract has been shown to have potential benefits in relation to the reduction of pain, oedema and itching in patients with chronic venous insufficiency (243). MPFF and pentoxyphylline have also been shown to be beneficial in the treatment of venous ulcers (244, 245).

1.11 Venous thromboembolism

Venous thromboembolism (VTE) refers to the formation of a blood clot within the lumen of a vein which impedes or occludes the normal flow of blood. Deep vein thromboses typically occur in the deep veins draining the calf muscles but may occasionally occur more proximally or in the upper limbs. Fragmentation of the clot may occur, if untreated, and result in embolisation to the arteries of the lungs, termed pulmonary embolism.
The true prevalence of venous thromboembolic disease is difficult to determine however several epidemiological studies have been carried out to attempt to quantify this. In the United States the incidence has been estimated to be approximately 150 per 100,000 of the population (246, 247). In Europe information on the incidence of VTE was derived from large French and Swedish studies which suggested higher results ranging from 160 per 100,000 (248) to 180 per 100,000 (249).
The VITAE trial was carried out in 6 European Union countries to determine the extent of the health burden of venous thromboembolic disease. The study reported an estimated annual incidence of 460,000 DVT and 300,000 pulmonary embolisms with an associated annual mortality rate of 370,000 from VTE (250). It is estimated that two thirds of VTE result from hospitalisation and VTE is the third most common cause of hospital related death (251, 252).

1.11.1 Aetiology and Risk factors

As previously mentioned the aetiology for the development of thrombosis is described by Virchow's triad. Risk factors therefore impact upon blood flow, constituents or the vessel wall. An understanding of the strength of risk factors and their cumulative effects is imperative when making decisions regarding prophylaxis.

Table 9: Risk factors for venous thromboembolism. Adapted from Anderson and Spencer 2003(253)

Strong risk factors (odd ratio > 10)	Hip or leg fracture
	Hip or knee replacement
	Major general surgery
	Major trauma
	Spinal cord injury
Moderate risk factors (odds ratio 2-9)	Arthroscopic knee surgery
	Central venous lines
	Chemotherapy
	Congestive heart or respiratory failure
	Hormone replacement therapy
	Malignancy
	Oral contraceptive therapy
	Paralytic stroke
	Pregnancy/ post partum
	Previous venous thromboembolism
	Thrombophilia
Weak risk factors (odds ratio <2)	Bed rest > 3 days
	Prolonged travel
	Increasing age
	Laparoscopic surgery
	Obesity
	Pregnancy/ antepartum
	Varicose veins

1.11.2 Signs and symptoms

Initially patients may experience pain, swelling and tenderness in the affected limb, and on examination the limb may be warm and erythematous. In extreme cases where complete occlusion of more proximal veins occurs there is extensive swelling associated with venous hypertension. If untreated venous gangrene may result.
In the absence of treatment approximately 25% of calf VTE propagate to involve the popliteal vein or more proximal veins. Of these 50% will develop a pulmonary embolism which has a 30 day mortality rate of 15-25% (254). Post-thrombotic syndrome (PTS) refers to the collection of symptoms, such as pain, swelling and varicose veins which can result in up to 60% of patients in the 2 years following VTE. Approximately 10% of patients will develop severe PTS including the development of venous ulcers (255).

1.11.3 VTE prophylaxis

The development of venous thromboembolism is preventable and yet the annual UK mortality is reported to be 25,000 patients (256). Indeed a recent survey revealed that approximately 71% of patients deemed to be of medium or high risk of developing VTE did not receive prophylaxis of any form (257). This knowledge led to the formulation of NICE guideline 92 in 2010 which highlighted priorities in terms of risk assessment and the use of prophylaxis. Preventative therapies can be divided into mechanical and pharmacological.

1.11.3.1 Mechanical

Graduated Compression stockings

Graduated compression stockings (GCS) are the most commonly used mechanical VTE prophylaxis. Graduated stockings apply varying degrees of pressure with the greatest being at the ankle and reducing proximally. These have been used as the stocking of choice since the study by Sigel *et al* (258) demonstrated that GCS result in gradually increasing flow velocity within the veins as they pass proximally. External compression reduces the cross

sectional area of the limb and hence the veins, reducing stasis and increasing flow rates (259). A meta-analysis published in 1994 reported a 68% risk reduction in the incidence of VTE following moderate risk surgery with the use of graduated compression stockings. More recently a Cochrane review reported an approximate relative risk reduction of 52% when compared to no prophylaxis in a combination of both medical and surgical patients (260). Data regarding the use of thigh length versus knee length stockings is insufficient to determine which is more effective in surgical patients (261). It is however known that patients are more likely to tolerate knee high stockings (94% versus 59%)(262).

For GCS to be effective in reducing the risk of DVT they must be correctly sized and fitted. A 2008 study assessed 142 post operative patients and determined that 25% of patients were wearing the wrong size of stocking and almost 1/3 (predominantly thigh high) were incorrectly placed (262). In addition the stockings are difficult to apply and as a result compliance may suffer (219).

As early as the 1950s it was emphasised that patients must be assessed for peripheral arterial occlusive disease prior to the application of GCS (263). Despite this several case reports are found in the literature of patients developing ischaemic ulceration (264, 265). Most recently the CLOTS study, investigating DVT prevention in stroke patients reported that the incidence of skin breaks, ulcers, blisters and necrosis was higher (64% versus 16%) in those wearing GCS than not (266).

Intermittent pneumatic compression (IPC) of the calf

IPC operate via cuffs which are placed around the lower limbs and periodically inflate and deflate. Blood is squeezed from the underlying deep veins and displaced proximally on inflation and refill on deflation. This reduces venous stasis, an important component of Virchow's triad (267).

A meta-analysis in 1996 reported that IPC devices cause a relative risk reduction for DVT of 62% compared to placebo, 47% compared to graduated compression stockings and 48% compared to prophylactic heparin (268). Several studies have shown that IPC may have a role in stimulating

fibrinolysis (269-271) and as such preventing hypercoagulable states, another component of Virchow's triad.

1.11.3.3 Electrical stimulation

Studies exploring a role for electrical stimulation in DVT prevention have demonstrated reduced venous stasis (272, 273) and increased flow (274) even when applied to patients immobilised in a cast (275). Electrical stimulation also appears as efficacious as intermittent pneumatic calf compression at increasing blood flow velocity (276, 277).

1.11.3.2 Pharmacological

Heparins

These are injectable forms of anticoagulation derived from the mucosal tissues of porcine intestine or bovine lung (278). Heparin binds to antithrombin III resulting in its activation which in turn inactivates thrombin and other pro-coagulant proteases. As such it prevents clot formation but does not play a role in clot lysis. The effects of heparin can be measured by establishing the time for blood plasma to clot i.e. aPTT (actual partial thromboplastin time). The anticoagulant effects of heparin are counteracted by Protamine sulphate.

Unfractionated heparin

The half life of unfractionated heparin is approximately one to two hours and as such is typically given as an infusion (279). The value of low dose unfractionated heparin for patients undergoing 'general' surgery was emphasised in the published meta-analyses by Collins *et al* (280). It demonstrated a DVT risk reduction of 13% compared to no prophylaxis and a reduced incidence of clinically overt PE (1.3% as opposed to 2.0%). Unfractionated heparin is associated with increased number of bleeding events however a further meta-analysis determined that this was predominantly in the form of wound haematomas and not more major bleeds (281). Platelet count must be monitored to prevent development of heparin induced thrombocytopaenia.

Low molecular weight heparin (LMWH)

LMWH has a half life between four and five hours and as such can be given once or twice daily removing the necessity for a continuous infusion (282). Studies have shown this to be as effective as low dose unfractionated heparin and with comparable risks (283). Studies have demonstrated however a lower incidence of heparin induced thrombocytopaenia with LMWH compared to unfractionated (284). Whilst more expensive than unfractionated heparin the benefits of easier administration and once-daily dose mean outpatient management is feasible and as such more economical (285).

Synthetic factor Xa inhibitor (Fondaparinux)

Fondaparinux is the only recommended drug in this class (286). It has a longer half life than heparin, approximately 17 hours and is more active against anti-X_a. This has led to the belief that is more stable and as such may have reduced risks (287). It does however still convey the risk of thrombocytopaenia (288).

Vitamin K antagonists

Warfarin is still considered the main drug within this class and is certainly the most widely used. It is most commonly used for long-term anticoagulation. It acts by reducing the levels of vitamin K which is necessary for the activation of clotting factors II, VII, IX and X and whilst it does not have a role in the breakdown of existing clots it prevents new clot development and propagation. Vitamin K antagonists are taken orally and loading can take between four and seven days. Side effects include bleeding and constant monitoring of the 'INR' (international normalised ratio) is required as it is known that many other medications may interact with warfarin. Options for warfarin reversal include simply stopping warfarin administration, or in instances of bleeding vitamin K, fresh frozen plasma and/ or prothrombin complex concentrate administration.

Direct thrombin inhibitors

As the name suggests these act as anticoagulants due their direct inhibition of thrombin. They are useful for patients who have previously suffered from heparin induced thrombocytopaenia. Similar to the other anticoagulants the side effects include significant bleeding and no effective monitoring exists (289).

1.12 Neuropathy

Patients with peripheral arterial occlusive disease often display signs consistent with peripheral neuropathy. This is likely to be multifactorial. Data from the Framingham Heart Study(290) revealed that 20% of symptomatic patients with PAOD had diabetes, but this probably greatly underestimates the prevalence, given that many more people with PAOD are asymptomatic. Conversely a study of 54 patients with diabetic neuropathy revealed that 48% of patients had asymptomatic peripheral vascular disease (291).

It is also known that chronic kidney disease (CKD) is independently associated with an increased prevalence of PAOD, and with the future risk for developing clinically significant peripheral arterial occlusive disease. Of patients with a creatinine clearance <60ml/min 24% have PAOD defined as an ABI<0.9 (versus 3.7% of persons with CRCL>/=60 ml/ minute (292)).

1.12.1 Diabetic neuropathy

Diabetic peripheral polyneuropathy is a progressive disorder resulting in both large and small fibre loss. The morphological changes correspond to loss of function (293, 294). Clinical scoring systems, assessing both signs and symptoms, such as the Toronto Neuropathy score (295) exist to establish the extent. Loss of vibration perception has been found to be an independent risk factor for the development of neuropathic ulcers (296, 297).

Nerve conduction studies can be carried out to determine the degree of neuropathy (298, 299). Autonomic damage impacts upon morbidity and

mortality and has been shown to be the most important prognostic indicator of poor outcome when compared to other diabetic complications (300).

1.12.2 Chronic renal failure and neuropathy

Autonomic and peripheral neuropathy in patients with chronic renal failure affect up to 65% of patients commencing dialysis (301). This incidence increases up to 80% of patients with diabetic nephropathy (302, 303). It has been shown to be related to not only the underlying pathology of the renal failure but also the degree. Indeed high levels of urea, creatinine and PTH levels have been shown to be correlated with lower conduction velocities and neuropathic symptoms (301). In advanced disease histological examination reveals degeneration of the axons and secondary demyelination of peripheral nerves (301).

Examination of the peripheral nervous system may reveal muscle atrophy, abnormal or absent reflexes and impaired sensation. However the most accurate test to detect neuropathy is reduced conduction velocity (304, 305). Dialysis has been shown to improve neuropathic symptoms, however the nerve conduction velocity remains reduced in between 60% to 80% of individuals (306). Transplantation improves both the symptoms and signs of peripheral neuropathy (301).

1.12.3 Ischaemic Neuropathy

Ischaemic neuropathy is a rare but underreported sensory neuropathy. It has been shown to be a predominantly distant axonopathy which affects nerves of all sizes. The degree of reduction in blood flow correlates with the patient's signs and symptoms and findings of electrophysiologic studies (307).

1.13 Previous research

A literature search was undertaken to assess the current evidence base for the use of nerve stimulation in individuals with lower limb vascular disease.

1.13.1 Peripheral arterial occlusive disease:

1.13.1.1 Phantom pain and stump pain

The role of transcutaneous electrical nerve stimulation(TENS) in the treatment of phantom and stump pain following lower limb amputation was evaluated by Mulvey *et al* in 2010 (308). It is evident from the literature that post procedural pain is a common problem following amputation affecting up to 80% of patients and interfering with quality of life and rehabilitation (309, 310). Many case reports and clinical studies have reported beneficial effects from the use of TENS (311-317). However no randomised controlled trials have been carried out to evaluate the effects.

1.13.1.2 Claudication

Several studies have been carried out to investigate the role of spinal cord stimulation in patients with claudication. *Sciacca et al* reported that the TcpO2 measurements in the limbs of 6 claudicants increased significantly with spinal cord stimulation which appeared to continue for at least 30 minutes after the stimulation was stopped (318).

Broseta *et al* evaluated the role of epidural spinal stimulation in patients with PAOD ranging from stage II to stage IV. Of 33 patients tested 15 demonstrated a significant improvement in claudication distance with no improvement in 8 patients. Doppler ultrasound assessment of the dorsalis pedis artery in 12 of these patients demonstrated an improvement in blood flow with normalisation of the pulse wave morphology and increase in velocity. The skin temperature during stimulation was also shown to rise in 13 patients (319).

However only a few studies have investigated the role of transcutaneous electrical neuromuscular stimulation in patients with claudication. Anderson

et al observed a significant increase in claudicating and maximum walking distance with chronic muscle stimulation, whilst those receiving control treatment demonstrated no significant change from baseline (320).
Tsang *et al* investigated the role of chronic electromyostimulation in patients with claudication. They demonstrated a significant increase, after 4 weeks of treatment, of claudication distance (88 to 111 metres) and maximum walking distance (118 to 158 metres). However these changes were not maintained following cessation of treatment (321).
Tallis *et al* evaluated the impact of transcutaneous (sham) electrical stimulation and spinal cord stimulation on claudication distance and reported a significant increase in claudication distance from 65 +/- 45 metres to 212 +/- 147 metres (P<0.025) with spinal cord stimulation and a 61% increase in exercise tolerance measured utilising a bicycle ergometer. There was no increase in claudication distance or exercise tolerance with transcutaneous stimulation (322).

1.13.1.3 Critical limb ischaemia

Spinal cord stimulation (SCS) as a treatment adjunct in patients with non-reconstructable chronic critical limb ischaemia has been the predominant focus of research within this area. The first study of this kind was reported in 1976 by Cook *et al* who reported significant pain relief and improved ulcer healing in up to 82% of patients. A Cochrane review by Ubbink and Vermeulen in 2013 evaluated the efficacy of SCS compared to conservative treatment alone from 6 published studies (323-333).
The review reported a significant improvement in limb salvage rates after 12 months of treatment (Relative risk of amputation 0.74, 95% CI 0.57 to 0.94). This equated to the number needed to treat to prevent one major amputation being 9 patients. Some beneficial effect was found relating to pain relief however the scores utilised and incomplete result reporting meant data pooling could not be performed.
There also appeared to be a beneficial effect on ulcer healing within this cohort with spinal cord stimulation.
The meta-analysis concluded that SCS is better than conservative management alone in the treatment of patients with non-reconstructable

chronic critical limb ischaemia in reducing the risk of amputation, providing pain relief and improving the clinical picture. However the risk associated with SCS must be borne in mind, in particular implantation failures and complications. A period of test stimulation should be utilised to establish those most likely to obtain benefit.

1.13.2 Wound healing

Endogenous electrical fields occur naturally *in vivo* and are vital for tissue development and repair. They occur due to the transport of ions across the polarised epi- and endothelium. The generation of natural electrical fields can be disrupted by disease processes. When compared to the surrounding skin wounds show a comparative positive electrical charge which results in a current which stimulates the biological repair mechanism(165).

The application of exogenous electrical stimulation may restart and stimulate these naturally occurring electrical fields promoting healing. Indeed there is an increasing volume of evidence that exogenous electrical currents enhance the body's natural bioelectrical signals.

Studies to examine the patho-physiological effects of electrical stimulation have revealed that the migration of macrophages, granulocytes, fibroblasts and epithelial cells is increased in size and velocity when electrical stimulation is applied to a wound (166-168). The formation of collagen has also been shown to increase, as does the distribution of collagen fibres around the periphery of the wound. This results in greater tensile strength (169).

The endothelial cells have been shown to release more vascular endothelial growth factor (VEGF) levels when under the influence of electrical stimulation, promoting angiogenesis (170-172). Thus the granulation tissue displays a denser network of capillaries, however these networks are unstable and hence the effects are short lived.

Some studies have also suggested that application of a direct current to a wound inhibits the activity of pathogens. The result is reduced colonisation and infection of the wounds (334).

The role of electrical stimulation in wound healing has been reported for many years but there has been a recent resurgence of interest in the past couple of decades (335-369). Despite this renewed interest electrical stimulation has failed to become a recognised adjunct to wound healing believed to be due to the small numbers recruited to the studies, the diversity of stimulation devices tested and the methodology of the studies (370).
A meta-analysis performed by Barnes *et al* determined that the quality of the studies reported in the literature varied from poor to good. There was also significant variance between studies in the current utilised and the outcome measures analysed.
The effect of electrical stimulation on percentage change in ulcer size over the total study periods was assessed in 6 RCTs (351, 357, 362-365). Exogenous electrical stimulation was found to significantly improve the percentage mean change in ulcer size by 24.62%, (95% C.I. 19.98 to 29.27, $p < 0.00001$) with no heterogeneity (I^2=0%, p=0.66) when compared to placebo or usual treatment.
3 RCTs evaluated the effect of electrical stimulation effect on percentage weekly change in ulcer size (366, 368, 369). Overall, electrical stimulation showed no significant difference in the percentage weekly change in ulcer size by 1.64%, 95% C.I. -3.81 to 7.09, p=0.56 with significant heterogeneity across trials (I^2=96%, p<0.00001) when compared to placebo or usual treatment.
One study reported that electrical stimulation insignificantly improved the percentage daily change in ulcer size by 0.63 %, 95% C.I. -0.12 to 1.37, p=0.10 when compared to placebo or usual treatment(347).
Electrical stimulation effect on ulcer size (cm^2) was assessed in 6 RCTs (350, 352, 355, 359, 361, 371). It was found to be superior when compared to placebo or usual treatment improving ulcer size by 2.42 cm^2, 95% C.I. 1.66 to 3.17, p<0.00001 compared to placebo or usual treatment. However, there was significant heterogeneity across trials (I^2=94%, P < 0.00001).
The meta-analysis concluded that use of electrical stimulation as an adjunct to ulcer treatment accelerates healing when compared to placebo or usual treatment. This reflected the findings of a previous meta-analysis by Gardner

et al (372). Further studies to compare the relative merits of the differing treatment modalities, i.e. the effect of differing current types would be beneficial and studies to establish whether the efficacy is equal for ulcers of all aetiologies are required.
Few studies have examined the cost effectiveness of electrical stimulation when compared to standard treatment for the healing of chronic ulcers. One trial which examined electrical stimulation in the treatment of pressure ulcers in spinal cord injured patients revealed that the treatment is cost effective within this patient cohort (373). Similarly a study by Taylor and colleagues (374) established that electrical stimulation is a cost effective treatment adjunct in the treatment of chronic venous leg ulcers, however, it stated that this was dependant on the number of required treatment units, the costs of these units and the degree of required nurse input. It is clear that these vary greatly between the devices tested. To be recognised as a treatment adjunct cost-effectiveness analysis would be required.

1.13.3 Venous disease

Calf and foot electrical stimulation has been proposed as a treatment for the prevention of deep venous thrombosis. Kaplan *et al* determined that it was a safe, tolerable and efficacious method of increasing flow in the popliteal and femoral veins and thus has the potential to be utilised as a method for reducing venous stasis (274). This reflected the findings of a previous study by Faghri *et al* (375).

Clarke Maloney *et al* explored the role of calf muscular stimulation and compression to improve venous blood flow and demonstrated a significant increase in flow within the popliteal vein in patients with venous ulceration. This increase was only a fraction of that seen with voluntary calf contraction. Compression resulted in no increase in venous blood flow (376). In a similar study two patients, treated with electrical stimulation and compression hosiery simultaneously, demonstrated increased venous velocity flow with reduced pain scores after one week (377).

A study investigating the role of an electrical stimulation device determined that treatment with Veinoplus reduces oedema, pain and improved quality of life in patients with venous disease (378).

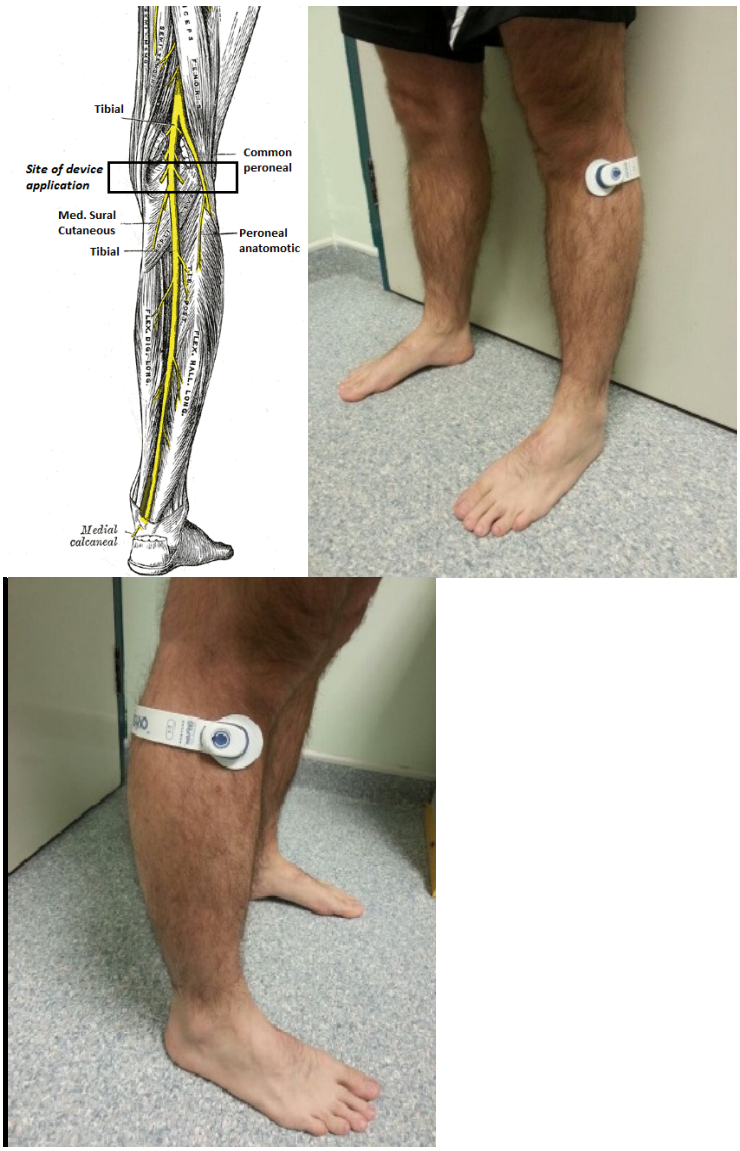
The increase in venous flow velocity, with electrical stimulation, is reported to be equivalent to that achieved with intermittent pneumatic calf compression, suggesting a role in deep venous thrombosis prevention (276).

1.14 The Geko™ Device

The geko™ device, (CE 558928; British Standards Institute notified body 0086) is a portable, discrete, self-contained stimulation unit which adheres to the skin. It delivers a pulsed electrical current at 27mA at a repetition rate of 1Hz. There are 7 pulse width settings ranging from 70- 560μs allowing adjustments for patient comfort.

It is applied to the postero-lateral aspect of the knee in order that the electrical impulses activate the common peroneal nerve within the popliteal fossa. This nerve, in turn activates the venous muscle pumps of the calf and foot responsible for returning blood to the heart. It was initially developed to reduce the incidence of venous thromboembolism by emulating the effects seen by normal mobilisation namely reduced venous stasis through the activation of the muscle pumps.

Figure 15: Application of geko^{TM} device (adapted from (379))



The device does not affect the normal movements of the limb nor patient mobility. It has charge-balanced waveforms that yield no build-up of charge in the subject and therefore adverse events e.g. electrical burns, can't occur within controlled use. It is battery powered and as such not attached to mains current.

Figure 16: The geko™ device

1.14.1 Evidence relating to geko™

In controlled clinical studies of normal, healthy subjects, at St Bartholomew's Hospital, London, the technique has been shown to be effective in stimulating lower limb musculature contraction and to enhance both blood flow velocity and flow volume in the lower limb as measured in dorsal foot veins by photoplethysmography and in the Femoral vein using duplex ultrasound. Further, the technique has been recently demonstrated to substantively increase arterial, venous and microcirculatory blood flow; together with a significant decrease in tissue Plasminogen Activator (t-PA) antigen which indicates increased fibrinolytic activity (380).

A study by Tucker *et al* 2010 examined the role of the geko™ device in 30 healthy volunteers and demonstrated that it resulted in an up to 25 fold increase in blood flow velocity and a significant increase in venous volume flow ($P<0.01$) at all stimulation levels, measured with ultrasound Doppler. This also corresponded to a four- fold increase in microcirculatory flow when measured with the laser Doppler (381). Another study by Jawad *et al* demonstrated that not only did the geko™ device increase venous blood volume flow by 33% from baseline on high stimulation and 14% on low stimulation, but on high stimulation settings also increased arterial blood volume flow by 30% (382).

The device potentially represents a significant enhancement on current attempts to develop clinically effective prophylaxis with high user compliance.

In particular, the approaches to enhancing lower limb blood flow are reported as painless/minimal sensation. The system facilitates isometric contraction of the anterior and posterior calf muscles, which practically eliminate the spasmodic leg movements intrinsic to other reported approaches. Furthermore, in combination with simultaneous activation of the foot musculature, this technique facilitates efficient muscle contraction leading to mobilisation of venous blood flow in response to a significantly lower applied current compared to other systems.

The THRIVE-II trial has now commenced as a multi-centre evaluation of the geko™ device compared to Intermittent Compression devices in patients prior to elective hip or knee replacement. The Sponsor of the study and lead site is Stanmore Orthopaedic Hospital NHS Trust.

To date no research has examined the role of geko™ device in lower limb vascular disease.

1.15 Justification for research

The above summary of the literature highlights the lower limb vascular disease processes and the potential role for electrical nerve stimulation as a therapy or therapeutic adjunct for such patients. The pathological and physiological processes involved in the success seen with other methods of electrical stimulation are multiple. The geko™ device has been demonstrated to be effective at flow augmentation in healthy volunteers and it was deemed necessary to investigate device efficacy in individuals with circulatory disorders. We, therefore, devised a programme of research to evaluate specific aspects of electrical stimulation in patients with lower limb vascular disease.

Research question 1
Aims: to assess the ease of application of the geko™ device by trained health care professionals and the tolerability and acceptability of the device to vascular patients

Research question 2
Aims: to assess the patient related factors associated with geko™ device failure- inability to initiate muscle twitch

Research question 3
Aims: to assess the effect of the geko™ electrical stimulation device on arterial, venous and microcirculatory flow in patients with claudication (Following amendment to replace patients with critical limb ischaemia).

Research question 4
Aims: to assess the effect of the geko™ electrical stimulation device on arterial, venous and microcirculatory flow in patients with infra-inguinal bypass for PAOD

Research question 5
Aims: to assess the effect of the geko™ electrical stimulation device on arterial, venous and microcirculatory flow in patients with superficial venous insufficiency.

Research question 6
Aims: to assess the impact of the geko™ electrical stimulation device on plasma levels of vascular endothelial growth factor, plasminogen activator inhibitor and tissue plasminogen activator

These studies were carried out under the auspices of two research protocols which evolved and underwent amendments as new data, derived from the studies became available.

Chapter Two: Methods

2.1 Study Approvals

The research and development department of Hull and East Yorkshire NHS Trust acted as the sponsor in the studies. Funding was provided by the Academic Department of Vascular Surgery at Hull Royal Infirmary.

The protocols, patient information leaflets, consent forms, and all other documents pertaining to these research studies were submitted via IRAS (Integrated Research Application System) to the National Research Ethics Service (NRES) approved by the NRES committee Yorkshire and the Humber- Sheffield; reference number 12/YH/0480 on the 5th December 2012 and the East of England- Cambridge East committee; reference number: 12/EE/0314 on the 9th April 2013.

A major amendment was submitted and approved following the determination that peroneal nerve stimulation was not effective in one of the initial study groups leading to the inclusion of claudicants and removal of critical limb ischaemics (see Chapter 4 and discussion).

A further amendment was submitted and approved to include a control arm to the studies following the observation of changes in flow in the contralateral limb i.e. the limb not receiving active stimulation to determine whether there was a systemic effect of peroneal nerve stimulation.

2.2 Ethical conduct of the studies

The conduct of the studies, dissemination of findings and thesis completion was performed in line with the principles outlined in the Declaration of Helsinki (383). The health and wellbeing of the research participants was the prime concern of all the researchers.
The investigator has undergone formal training in Good Clinical Practice and is appropriately qualified and experienced in performing all interventions and investigations.
All eligible patients identified were counselled regarding the opportunity to participate in the trials. If willing a written informed consent form was completed. No patient deemed to lack capacity was included in the studies. Patients were clearly informed that the research being undertaken was unlikely to offer them any benefit but may aid in establishing whether the geko^{TM} may be considered a useful treatment adjunct in the future. They were made aware of the additional burden of the assessments involved in the study and were aware that they were free to withdraw at any stage in the process, without any prejudice to their on-going or future care.

The studies were prospective in nature and approval was sought and obtained from independent and institutional ethics boards, before commencement of recruitment. The studies were registered on clinicaltrials.gov as per recommendations (384). The nature of the device led to limitations in relation to blinding.

2.3 Quality Assurance

The Chief Investigator was responsible for the day-to-day monitoring and management of the studies. The study was monitored in accordance with the Department of Health Research Governance Framework for Health & Social Care (385), and in accordance with the Sponsor's monitoring and audit policies and procedures.

The organisation, monitoring, and quality assurance of the studies was the responsibility of the Sponsor, and Principal Investigator. In order to ensure the accuracy of data, direct access to source documents by the representatives of both the Sponsor and regulatory authorities was ensured at all times. Anonymity of the subjects was maintained at all times.
The investigator permitted study-related monitoring, audits, REC review, and regulatory inspections, providing direct access to source data / documents. Patient consent to this was specifically sought in the Consent Form.

2.4 Data handling and storage

Participants were informed that their data would be held on file, and that this data may be viewed by the Sponsor and by external auditors on behalf of either the sponsor or regulatory agencies. They were similarly informed that this data and a report of the study would be submitted to the Sponsor and may also be submitted to government agencies and perhaps for publication, but that they would only be identified in such reports by their study identification number, initials and perhaps their gender and age.
The investigators undertook to hold all personal information in confidence and in compliance with the Data Protection Act 1998 (386) and Caldicott committee (387). Data was collected and collated using a specifically designed database. This was kept on hospital central servers on a limited access hard drive. Access was via password protected log-in on hospital servers only and was limited to members of the Academic Vascular Surgery Unit. The file itself had password protected opening.

2.5 Indemnity

This was an NHS sponsored research study. Indemnity was provided by the site in accordance with local policy and NHS guidance.
The Sponsor holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants were also able to claim compensation if they could prove that the hospital has been negligent. However, since this clinical study was carried out in a hospital, the hospital continued to have a duty of care to the participant of the clinical study. Hull Royal Infirmary did not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees.

2.6 Sample size calculations

A formal sample size calculation was not initially applied as this was a preliminary study in these patient populations and aimed to inform the design of future studies within these cohorts. Whilst studies had been previously carried out in healthy volunteers insufficient data was obtainable for calculations to be possible. As such the number of participants was a sample of convenience for this exploratory study and was expected to demonstrate significant differences between experimental conditions. A review of the literature determined that similar physiological studies commonly found significant results with n=6-10 (318)
As such the intention was to recruit 15 patients in the following groups:

- Claudicants (following amendment see Chapter 4 and discussion)
- Post-op infrainguinal bypass grafts
- Varicose veins

During the trial period it was established that there were changes in the flow measurements obtained in the passive limbs (i.e. the contralateral limb to that receiving direct stimulation). The data from the proof of concept studies was utilised to perform a sample size calculation for further study

randomising patients to either a control group or a stimulation group. This established that based on standard normality assumptions, to achieve 90% power of detecting a 10% increase in arterial flow with peroneal nerve stimulation with 5% significance 30 patients would be required.

The addition of the third group i.e. the control group who received no active stimulation, to the study would enable further analysis to determine if the changes seen in the passive limb were due to systemic effects of the stimulation.

2.7 Participant screening and recruitment

The potential participants for the studies were identified from outpatient clinics, multidisciplinary team meetings and from hospital inpatients, by their clinical team. All patients were assessed for eligibility and if eligible the potential for inclusion was discussed with the patient by the Chief Investigator and the patient information leaflet was issued.

Written consent was obtained only after a verbal explanation of the aims, methods, anticipated benefits and potential hazards of the trial and the provision of patient information sheet. Patients were clearly informed that participation in the study was voluntary and that refusal to participate would in no way disadvantage them. No patients were recruited if they lacked capacity and if it was deemed that capacity was lost during the trial period then the patient was removed from the trial.

Table 10: Study inclusion and exclusion criteria

	Claudicants	Infra-inguinal bypass	Varicose veins
Inclusion Criteria	Age > 18 years	Age > 18 years	Age > 18 years
	Intermittent claudication	Post op infra-inguinal vein bypass graft	Bilateral superficial venous insufficiency
	Absence of haematological disorder or DVT	Absence of haematological disorder or DVT	Absence of haematological disorder or DVT
	Intact cutaneous sensations to nocioception	Intact cutaneous sensations to nocioception	Intact cutaneous sensations to nocioception
	Intact, healthy skin at application site	Intact, healthy skin at application site	Intact, healthy skin at application site
	Effective contraception if sexually active	Effective contraception if sexually active	Effective contraception if sexually active
	Able to give informed consent	Able to give informed consent	Able to give informed consent
	Able to follow protocol requirements	Able to follow protocol requirements	Able to follow protocol requirements
Exclusion criteria	Haematological disorder/ DVT in past 6 months	Haematological disorder/ DVT in past 6 months	Haematological disorder/ DVT in past 6 months
	Pacemaker or implantable defib(388)	Pacemaker or implantable defib	Pacemaker or implantable defib(388)
	Use of TENS or neuromodulator	Use of TENS or neuromodulator	Use of TENS or neuromodulator
	Recent surgery/ trauma to limbs	Recent surgery/ trauma to limbs	Recent surgery/ trauma to limbs
	Recent significant illness	Recent significant illness	Recent significant illness
	Obesity, BMI >34	Obesity, BMI >34	Obesity, BMI >34
	Recent use of investigational drug or device	Recent use of investigational drug or device	Recent use of investigational drug or device
	Pregnant or planning pregnancy	Pregnant or planning pregnancy	Pregnant or planning pregnancy
	Use of significant medications	Use of significant medications	Use of significant medications

2.8 Outcome measures

Research question 1

The ease of application, tolerability and acceptability of the geko™ device to vascular patients

1. Ease of application questionnaire to be completed by health care professional following device application
2. Patient completed questionnaire, following intervention, including a visual analogue scale.

Research question 2

Patient related factors associated with geko™ device failure

1. Presence or absence of muscle twitch
2. Stimulation level required to produce twitch
3. Age
4. Smoking status
5. Neuropathy score
6. Calf circumference
7. ABPI
8. Presence of oedema
9. Concomitant medications
10. Co-morbidities

Research question 3

The effect of the geko™ electrical stimulation device on arterial, venous and microcirculatory flow in patients with intermittent claudication

1. *Arterial flow in the superficial femoral artery* (Duplex ultrasound bilaterally at baseline and after 30 minutes of stimulation):

   Mean and maximal arterial velocity (measured in triplicate and mean taken as result)

   Artery diameter (measured in triplicate and mean taken as result)

Arterial volume flow (measured in triplicate and mean taken as result)

2. *Venous flow in the superficial femoral vein* (Duplex ultrasound bilaterally at baseline and following 40 minutes of stimulation):

   Mean and maximal venous velocity (measured in triplicate and mean taken as result)

   Vein diameter (measured in triplicate and mean taken as result)

   Venous volume flow (measured in triplicate and mean taken as result)

3. *Microcirculatory flow* (Laser Doppler flowmetry and skin temperature- dorsum of the first web space bilaterally)

   Recorded continuously throughout study period (mean reading over 5 minutes taken at 15 minute intervals)

4. *Blood pressure and heart rate*- baseline, 30 minutes and following device removal

5. *Calf circumference*- baseline and following device removal

6. *ABPI*

N.B. the final 10-12 patients recruited to each study, who were randomised to active or control stimulation were analysed separately.

7. *Tolerability questionnaire*

Research question 4

The effect of the geko^{TM} electrical stimulation device on arterial, venous and microcirculatory flow in patients with infra-inguinal bypass for PAOD

1. *Arterial flow in the infrainguinal bypass graft* [see study 3]
2. *Venous flow in the superficial femoral vein* [see study 3]
3. *Microcirculatory flow* [see study 3]
4. *Blood pressure and heart rate*- baseline, 30 minutes and following device removal
5. *Calf circumference*- baseline and following device removal
6. *ABPI*
7. *Pulse wave velocity*
8. *Augmentation index*
9. *Cardiac output*
10. *Peripheral vascular resistance*

N.B. the final 10 patients recruited, who were randomised to active or control stimulation were analysed separately.

11. *Tolerability questionnaire*

Research question 5

The effect of the geko^{TM} electrical stimulation device on arterial, venous and microcirculatory flow in patients with superficial venous insufficiency.

1. *Arterial flow in the superficial femoral artery* [see study 3]
2. *Venous flow in the superficial femoral vein* [see study 3]
3. *Microcirculatory flow* [see study 3]
4. *Blood pressure and heart rate*- baseline, 30 minutes and following device removal
5. *Calf circumference*- baseline and following device removal

N.B. the final 10 patients recruited, who were randomised to active or control stimulation were analysed separately.

6. *Tolerability questionnaire*

Research question 6
The impact of the geko™ electrical stimulation device on plasma levels of vascular endothelial growth factor, plasminogen activator inhibitor and tissue activator

1. *Baseline levels of t-PA, PAI-1 and VEGF* (bilateral femoral puncture under ultrasound guidance)
2. *Plasma t-PA, PAI-1 and VEGF following 45 minutes of peroneal nerve stimulation* (bilateral femoral puncture under ultrasound guidance)
3. *Arterial flow in the superficial femoral artery* [see study 3]
4. *Venous flow in the superficial femoral vein* [see study 3]

2.9 Study Interventions

2.9.1 Screening evaluation

Having obtained informed consent the participants were invited to a screening assessment. In this meeting the baseline data was collected including demographics, medical, smoking and drug history. Patients also underwent a physical examination including vital signs. The geko™ device was trialled on the individuals to establish the appropriate setting and position and a neurological examination was completed for the first 100 participants on whom the device was trialled. For those individuals in whom the device was ineffective their participation in the study was discontinued *[Research question 2]*.

Following the determination that the device failed to produce visible muscle twitch in a significant proportion of the patients screened, analysis was undertaken to determine possible responsible factors. This led to the

amendment of the protocol to exclude patients with critical limb ischaemia and include patients with claudication.

2.9.2 Study day methods

On the study day the examinations were carried out in a designated temperature controlled clinical room in the Vascular Lab at Hull Royal Infirmary. Participants were required to abstain from vigorous exercise, nicotine, caffeine and fatty foods prior to their examination and were required to wear light garments with their legs exposed.
Only after ensuring adherence to the protocol inclusion and exclusion criteria did the study commence.

The geko™ device was unilaterally applied to outer/ posterior aspect of the knee whilst the subjects were semi-recumbent on a padded examination couch, with their knees slightly flexed. The participants were given 30 minutes in the quiet, environmentally controlled room prior to commencement of measurements to enable equilibration.

The final 10-12 patients recruited to each study were randomised, using a sealed envelope technique, to either a control group (device applied but inactive) or a stimulation group (device applied unilaterally and activated). Both groups followed the protocol with identical measurements taken.

Baseline measurements were recorded following this acclimatization. These included:

- Blood pressure
- Heart rate
- Respiratory rate
- Calf circumference
- ABPI

- Mean and maximal arterial velocity (measured in triplicate and mean taken as result)
- Artery diameter (measured in triplicate and mean taken as result)
- Arterial volume flow (measured in triplicate and mean taken as result)
- Mean and maximal venous velocity (measured in triplicate and mean taken as result)
- Vein diameter (measured in triplicate and mean taken as result)
- Venous volume flow (measured in triplicate and mean taken as result)
- Laser Doppler (mean reading over 5 minutes)

[Research questions 3, 4 and 5]

Blood was taken from one femoral vein for:

- VEGF
- t-PA
- PAI-1

[Research question 6]

Following the collection of baseline recordings all participants for active stimulation had the stimulation intensity of the geko^{TM} device increased until visible muscle stimulation was evident (concentric isotonic contractions) or until patient tolerance was reached as per Cramp *et al* (389). Patients who were not for active stimulation had the device applied as above but not turned on *[Research question 3, 4 and 5]*.

The infra-inguinal bypass participants also underwent Vicorder analysis at baseline comprising recording of augmentation index, peripheral vascular resistance, cardiac output and pulse wave velocity *[Research study 4]*.

After 30 minutes of stimulation the following measurements were repeated:

- heart rate
- blood pressure
- Maximal arterial velocity (measured in triplicate and mean taken as result)
- Artery diameter (measured in triplicate and mean taken as result)

- Arterial volume flow (measured in triplicate and mean taken as result)

[Research studies 3, 4 and 5]

At 40 minutes:

- Maximal venous velocity (measured in triplicate and mean taken as result)
- Vein diameter (measured in triplicate and mean taken as result)
- Venous volume flow (measured in triplicate and mean taken as result)

[Research studies 3, 4 and 5]

At 45 minutes blood was taken from both femoral veins for VEGF, PAI-1, t-PA antigen and full blood count *[Research study 6]*.

The device was then deactivated. The individuals with infra-inguinal bypass grafts had the Vicorder recordings repeated immediately following device deactivation *[Research study 4]*.

The participants then remained at rest in the same position for a further 30 minutes. Following this repeated measurements were made of:

- Blood pressure
- Heart rate
- Respiratory rate
- Calf circumference
- ABPI

[Research studies 3, 4 and 5]

The participants in whom the device was active were asked to complete a Patient tolerance/ acceptability Questionnaire using Visual Analogue Scores and Verbal Rating Scores following removal of geko™ device (see appendix) *[Research study 1]*.

2.9.3 Neuropathy assessment

As aforementioned the neuropathy seen in patients with vascular disease may be multifactorial. There is no validated score to determine the degree of neuropathy in patients with vascular disease. As such the decision was made to utilize the Toronto Neuropathy Scoring System to determine the extent of the patient's neuropathy. This is well validated for use in patients with diabetic peripheral neuropathy with results corresponding well with the presence of diabetic sensorimotor polyneuropathy as measured with sural nerve morphology and electrophysiology. It has also been shown to have good intra and inter-rater reliability (295, 390).

Table 11: Toronto Clinical Neuropathy Scoring System

Symptom scores	Reflex scores	Sensory test scores
Foot: Pain **Numbness** **Tingling** **Weakness**	Knee reflexes	Pinprick
Ataxia	Ankle reflexes	Temperature
Upper limb symptoms		Light touch
		Vibration
		Position

Sensory testing was performed on the first toe.

Symptom scores: present _ 1; absent _ 0.

Reflex scores: absent_2; reduced_1, normal_0.

Sensory test score: abnormal_1. normal_0.

Total scores range from normal _ 0 to maximum of 19.

2.10 Equipment

2.10.1 Laser Doppler

The laser Doppler provides a non-invasive, measurement of the local microcirculatory flow in capillaries, arterioles and venules (391, 392). It can

therefore be used to measure changes with provocations such as the application of the geko™. Laser Doppler has been established as a good method for distinguishing patients with peripheral arterial occlusive disease from healthy individuals (393, 394). The technique has been validated against other techniques utilized to assess skin blood flow (395, 396).

The technique utilizes a laser beam which is emitted from a fibre optic probe. The light is both scattered and absorbed by the tissues and upon hitting moving objects i.e. blood cells, a change in wavelength occurs known as Doppler shift. The number and velocity of the blood cells is directly proportional to the change in wavelength which is detected by fibres within the probe. The resultant perfusion values are reported as perfusion units, which are arbitrary units and as such comparisons must be made with baseline values to determine change.
Calibration of the machine is imperative to ensure accuracy and is performed utilizing a fluid known as Motility standard. At a given temperature the Doppler perfusion for this fluid is constant due to Brownian motion i.e. the random moving of suspended particles in fluid (397).
For the purpose of this study the instruments utilized were the Laser Doppler Perfusion and Temperature Monitor DRT4; Moor Instruments Ltd, UK. The probes were attached to the dorsum of both feet, in the first web space or closest ulcer free area. The signals were recorded throughout the study period.

2.10.2 Duplex Ultrasound

All ultrasound assessments were undertaken by the author who has a Postgraduate Certificate in diagnostic vascular imaging and has over 300 hours of practical scanning experience.

The Toshiba Aplio XG scanner with a 7.5MHz linear array transducer was used for the duration of the study. The equipment was compliant with all the

necessary technical standards (398), regularly maintained (399) and calibrated (400).

Image optimization is imperative to ensure that measurements are accurate and reproducible, reducing inter and intra-operator discrepancies. Every patient is an individual with differing body habitus and subtle anatomical variances and therefore modifications were made to the fundamental ultrasound control settings, thereby altering the beam characteristics, to ensure the best image was obtained (401).

B-mode settings

In order to clearly and accurately demonstrate the anatomy of the arteries and veins in transverse and longitudinal views the focal zone was set to the depth of the vessels to be imaged.

The depth of the image was adjusted in order to focus in on the vessel required. It is known that greater field of views than necessary results in the display of irrelevant deeper structures and as such reduces spatial resolution secondary to a reduced frame rate. Images appear darker due to increased tissue attenuation. As such the accuracy of measurements will decline with increasing depth (402, 403). The callipers utilized for measurements have limited increment sizes, usually one pixel. Thus to ensure measurement accuracy related to calliper placement and increments the appropriate depth was selected (404).

The gain and TGC were tailored to optimize the imaging for each vessel. Excessive gain results in increased image 'noise' and increased beam width and therefore reduced lateral and contrast resolution (405). Increased beam width is known to reduce measurement accuracy (406).

Doppler settings:

Colour gain (CDG) was optimized as it is known that too much reduces image quality, obscuring the true Doppler signal, due to colour in non-flow areas, termed oversaturation or excessive 'noise', distorting flow continuity. This is known to result in potentially missing or incorrectly characterizing intra-luminal thrombus (407). Failure to optimize CDG can also result in

overestimation of vessel dimensions due to increased spatial distribution of colour signals (408). If too low the system is unable to detect small Doppler shifts i.e. slow flow (409). This can result in a diagnosis of no flow i.e. occlusion not stenosis. The gain is responsible for 'the spatial representation of the limits of an abnormal flow jet' (410).
The setting of CDG involved increasing until 'noise' was encountered before lowering until it cleared (411, 412). This ensured no colour in non-flow areas and no zero-velocity sections in areas of actual blood flow (413). Frequent adjustments to the gain were performed whilst scanning.

The colour Doppler scale (CDS) was optimized for the vessel being imaged. Correct CDS setting is imperative to successfully display signal echoes (409). If too high no flow may be detected, as low flows may be lost in noise and wall filter (411). A patent vessel with slow flow, may therefore appear occluded or thrombosed (409). Rescanning utilizing low velocity settings may enhance trickle flow (414). If the scale is too low aliasing occurs i.e. the production of ambiguous signals when the frequency of returning echo signals is greater than the Nyquist frequency/sampling rate. When the flow velocity is greater than half the PRF, the direction and speed are inaccurately displayed, as the signals are 'folded round' into lower frequencies (409, 414), as seen in the centre of vessels due to higher velocity laminar flow (409). Aliasing can result in a high velocity post stenotic jet being missed (414).

Sonographic protocol

The vessels were identified in B mode transversely before the appropriate longitudinal section was selected. The position of the probe was marked in permanent marker on the patient to ensure correct positioning for subsequent measurements.
The spectral Doppler function was activated. The sample volume (gate size) was adjusted. A spectral trace of the flow within the vessel was obtained, ensuring appropriate gain and scale settings were utilized. The trace was then frozen prior to activating the Volume flow function. The spectral callipers were positioned to include a full screen width of good quality full waveforms.

The B-mode callipers were positioned on the vessel walls to obtain the diameter. Volume flow in ml/min was automatically displayed.
Whilst the ability to obtain volume flow measurements was first reported in 1981(415) it is only with the improvements in hardware and software than such measurements have become more accurate (416). Studies have previously shown that if the sampling volume covers the entire vessel lumen, the incident angle is less than 60^{o} and the sample time is maximized that the measurements are reproducible and comparable to thermal dilution techniques(417). However despite this many publications have documented errors associated with volume flow measurements (418-420), as such three measurements were made at each time point for each vessel and the mean calculated.

Ultrasound Doppler measurements of arterial flow velocity and blood volume flow within the superficial femoral artery (5 cm distal to bifurcation), or within the infra-inguinal bypass graft, were taken bilaterally at baseline (rest) and 30 minutes while the device was active. In cases of superficial femoral artery occlusion measurements of the most distal section of the common femoral artery were recorded.
Ultrasound Doppler measurements of flow velocity and blood volume flow within the superficial femoral vein were taken bilaterally at baseline (rest) and 40 minutes while the geko^{TM} was active.

2.10.3 Vicorder

The Vicorder system utilizes two cuffs which are placed over arterial segments and inflated to 65mmHg. The oscillometric signal is digitally analyzed to determine the pulse transit time and hence the pulse wave velocity. The velocity of the pulse wave is calculated by dividing the distance between the transducers (measured in metres) by the time taken for the wave to travel that distance (measured in seconds). The system can also be used to measure peripheral resistance, MAP(421), SEVR, stroke volume and

cardiac output with a single easy to use technique requiring no skilled users (422) or expensive consumables.

A study to compare the assessment of pulse wave velocity with the Vicorder device and the SphygmoCor device, which has been previously demonstrated to have excellent reproducibility, was undertaken. Following obtainment of ethical approval patients with symptomatic peripheral arterial occlusive disease i.e. intermittent claudication were approached for inclusion into the study. Consenting participants underwent initial assessment which included history, including smoking status and medications and clinical examination. Baseline measurements of heart rate, blood pressure and BMI were recorded. The diagnosis of peripheral arterial occlusive disease was confirmed with ankle brachial pressure measurements (included if ABPI < 0.9) and arterial duplex.

Each participant underwent two measurements of carotid femoral pulse wave velocity utilising both the SphygmoCor and Vicorder devices, on a single visit. Manufacturers guidelines were followed when performing the measurements which were undertaken following participants resting for 10 minutes in a supine position in a climate controlled environment.

SPSS was utilised for statistical analysis. Intra-rate reproducibility was assessed utilising the Bland-Altman method, intraclass correlation coefficient and the coefficient of variation. The pulse wave velocity measurements obtained with each device were compared utilising Bland- Altman plot and Students paired T-test. The correlation between the values obtained with the two devices was assessed utilising Pearson's correlation coefficient (r) and linear regression. A P value < 0.05 was considered to be statistically significant.

Pulse wave analysis

At baseline, pre-device activation a pulse wave analysis was performed by placing the brachial cuff at the level of the brachial artery. The cuff was connected to the Vicorder device. Data pertaining to the patient's age, height

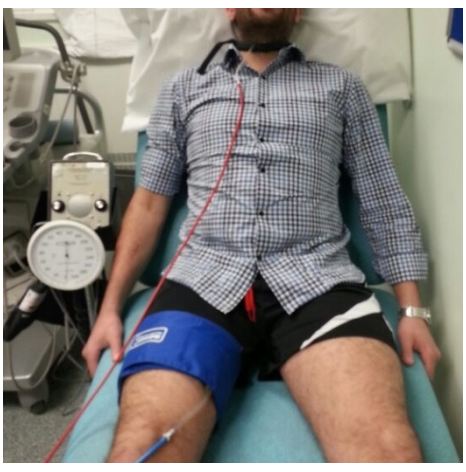
and weight was inputted. The cuff auto-inflated and waveforms displayed on the screen. The amplitude of the waveforms was adjusted as required. Upon freezing the screen measurements of cardiac output, stroke volume, peripheral vascular resistance etc. were automatically displayed.

Pulse wave velocity

Pulse wave velocity is accepted as the gold standard measurement of arterial stiffness as it is non-invasive and has been shown to be reproducible. It is influenced by the thickness of the vessel wall i.e. degree of hypertrophy, its radius, the blood pressure and the density of the blood i.e. its constituents. It is measured between the carotid and femoral artery as this is the gold standard technique(60).

After performing pulse wave analysis the aortic pulse wave velocity was measured. A neck pad was placed around the participant's neck with the pressure pad over the right Carotid area and secured with the Velcro fixing. A second cuff was placed around the participant's upper thigh on the treatment limb. The cuffs were connected to the Vicorder and the distance between Supra-Sterna Notch and thigh cuff in centimetres measured and entered into the computer. The cuffs were inflated to 65mmHg and displayed waveforms adjusted for amplitude if required. After acquiring several steady pulses of data the screen was frozen. Pulse Wave Velocity and Transit Time were automatically computed and displayed.

Figure 5: Cuff placement for the Vicorder



These recordings were repeated immediately following deactivation of the geko^{TM} device.

2.11 Sample collection, processing and storage

2.11.1 Sample collection

In both studies 15 to 20 ml samples were drawn from the femoral vein unilaterally using a 21 gauge needle, under ultrasound visualisation, at baseline i.e. pre-stimulation period and bilaterally at 45 minutes into the stimulation period.

2.11.2 Storage

Approximately 3mls of blood was decanted into a purple EDTA bottle and sent immediately to the Haematology lab at Hull Royal Infirmary for Full blood count analysis, to include platelet count.
The remainder of the participant's samples were decanted into citrate bottles and inverted a minimum of 5 times within the bottles to ensure complete mixing and then centrifuged at 1500 x g for 15 minutes (after 30 minutes). Plasma was then decanted into 1ml aliquots and labelled for freezing at -80^{0}C. Samples were immediately placed in a -20^{o} freezer and transfer to the -80^{o} freezer occurred within 4 hours.
Prior to assay the frozen sample was brought to room temperature slowly and mixed gently.
Samples were labelled with:

- Participant's initials
- Participant number
- Laterality and timing of the sample

Sample stability

eBioscience have demonstrated in studies that there is no significant loss of t-PA immunoreactivity detected by freezing and thawing or during storage. eBioscience have demonstrated in studies that if samples were thawed and refrozen repeatedly VEGF immuno-reactivity significantly decreased. As such samples were thawed only one. They also demonstrated that VEGF immuno-reactivity was not affected by storage at -80^{o}c.

PAI-1 immuno-reactivity was found to be stable despite repeated freeze and thaw in studies by eBioscience and no loss was detected during storage.

2.11.3 ELISA assay methods

The ELISA analysis was undertaken at the University of Hull in the Centre for Biomedical Research. Commercially available functional assays were utilized (eBioscience Platinum ELISA kits). All samples were processed in duplicate and if the results were within 5% the mean utilized. If the variability between sample duplicates was greater than 5% the samples were rerun.

2.11.3.1 Tissue Plasminogen Activator ELISA

A commercially available functional assay for the quantitative detection of human t-PA was utilized to process samples for t-PA. The kits were stored at 2-8^{o}C and were brought to room temperature (8-25^{o}C) prior to use.

Reproducibility

Independent experiments by eBioscience determined the overall intra-assay coefficient of variation to be 3.6%. The inter-assay coefficient of variation (CV) was calculated to be 6.5%. The use of commercial tests kits negated the need for further inter and intra plate CV calculation in this small study.

2.11.3.1.1 Assay principle

The anti-human t-PA coating antibody is coated on the microwells. The biological fluid samples and standard solutions are added and the Human t-

PA present binds to the adsorbed antibodies. The HRP- conjugated anti-human t-PA antibody is then added and also binds to the prebound human t-PA. After the incubation period the unbound HRP- conjugated anti-human t-PA is washed out with a substrate solution, reactive to HRP. The product formed is coloured in direct proportion to the human t-PA concentration present in the well. The reaction is stopped by adding phosphoric acid and the absorbance at 450nm measured.

2.11.3.1.2 Reagents and chemicals

In addition to the microtitre plate with 96 wells, several reagents are required.

Table 12: Reagents and chemicals for t-PA ELISA

Reagent	Description	Preparation
HRP- Conjugate anti-human t-PA polyclonal antibody	Detecting antibody	1:1000 dilution of concentrate with assay buffer
Human t-PA standard	Standard solution	Reconstitute as per vial instructions
Assay Buffer Concentrate	PBS with 1% Tween 20 and 10% BSA	Diluted to final volume of 100ml with distilled water
Wash buffer Concentrate	PBS with 1% Tween 20	Diluted to final volume of 1000ml with dionized water
Substrate solution	Tetramethyl- benzidine	
Stop solution	1M Phosphoric acid	

2.11.3.1.3 t-PA Assay procedure

Each well was washed with approximately 400µL of the wash buffer, thoroughly aspirating the contents between washes. 100µL of the sample diluents in duplicate was added to each standard well. To the first 2 wells (A1 and A2) 100µL of the prepared standard was added. The contents were mixed by aspiration and ejection of the well contents. 100µL from each of these wells was then transferred to B1 and B2 and mixed. This procedure was repeated 5 times to create the standard dilutions of concentrations 1000.0 to 15.6 pg/ml. N.B. 100µL of the contents of the last two microwells were discarded.

100µL of the sample diluent was added in duplicate to the blank wells and 90µL to the sample wells. 10µL of each biosample was added in duplicate to the sample wells.
50µL of the prepared HRP- Conjugate was added to all wells. The microwells were then covered with an adhesive film and incubated at room temperature (18-25^{0}C) for 2 hours.
The adhesive film was then removed and the wells emptied and washed 6 times. Immediately following this100µL of the TMB substrate solution was added to each well and incubated at room temperature for around 10 minutes. 100µL of the stop solution was added when the highest standard had developed a dark blue colour and/or had reached an OD of 0.9- 0.95 by ELISA reader.
The absorbance of each microwell was read by spectro-photometer, using 450NM as the primary wave length.

2.11.3.1.4 Calibration of results

The plate reader absorbance values were recorded for each well, standard and sample, subtracting the absorbance measured in the blank wells. A standard curve was created by plotting the mean absorbance of each standard concentration against the human t-PA concentration. A best fit curve was then drawn through the points on the graph. The concentration of circulating t-PA was then determined by utilizing the mean absorbance value for each sample.

2.11.3.2 Plasminogen Activator Inhibitor-1 ELISA Method

A commercially available functional assay for the quantitative detection of human PAI-1 was utilized to process samples for PAI-1. The kits were stored at 2-8^{o}C and were brought to room temperature (8-25^{o}C) prior to use.

Reproducibility

Independent experiments by eBioscience determined the overall intra-assay coefficient of variation to be 4.7%. The inter-assay coefficient of variation was calculated to be 5.0%. The use of commercial tests kits negated the need for further inter and intra plate CV calculation in this small study.

2.11.3.2.1 Assay Principle

The anti-human PAI-1 antibody was coated onto each microwell. This binds any Human PAI-1 present in either the sample or standard. The addition of a biotin-conjugated anti-human PAI-1 antibody leads to further binding of the captured PAI-1. Following incubation any biotin-conjugated anti-human PAI-1 antibody which is unbound was removed by washing. The streptavidin-HRP was added and bound the biotin-conjugated anti-human PAI-1 antibody. Any streptavidin- HRP unbound following incubation was removed by washing. The substrate solution, reactive with HRP wass added resulting in the formation of a coloured product. The amount of this is proportional to the amount of human PAI-1 in the sample or standard. The addition of acid terminated the reaction. The absorbance was measured at 450nm and the values for the standard dilutions used to prepare a standard calibration curve.

2.11.3.2.2 Reagents and chemicals

In addition to the microtitre plate with 96 wells, several reagents were required.

Table 13: Reagents and chemicals for PAI-1 ELISA

Reagent	Description	Preparation
Biotin- Conjugate anti-human PAI-1 polyclonal antibody	Detecting antibody	1:100 dilution with assay buffer
Streptavidin-HRP		1:200 dilution with assay buffer
PAI-1 standard	10000 pg/ml upon reconstitution	Reconstitute as per vial instructions
Assay buffer concentrate	PBS with 1% Tween 20 and 10% BSA	Dilute to final volume 100ml with distilled water
Wash buffer concentrate	PBS with 1% Tween 20	Dilute to final volume 1000ml with deionised water
Substrate solution	Tetramethyl-benzidine	
Stop solution	1M phosphoric acid	

2.11.3.2.3 PAI-1Assay procedure

The samples were prediluted 1:50 with the Assay buffer i.e. 10µL sample to 490µL Assay buffer. The microwells were washed twice with 400µL per well, thoroughly aspirating the contents between washes. The wash buffer was left for 10-15 seconds in the wells before aspiration. The wells were then emptied and used immediately.

100µL of the Assay buffer was added in duplicate to all the standard wells. To the first 2 wells (A1 and A2) 100µL of the prepared standard was added. The contents were mixed by aspiration and ejection of the well contents. 100µL from each of these wells was then transferred to B1 and B2 and mixed. This procedure was repeated 5 times to create the standard dilutions of concentrations 5000 to 78pg/ml. 100µL of the contents of the last 2 microwells was discarded.

100µL of the Assay buffer was added in duplicate to the blank wells and 50µL to the sample wells. 50µL of the prediluted samples was added in duplicate to the sample wells. 50µL of Biotin- Conjugate was added to all wells. The trays were then covered with an adhesive film and incubated at room temperature (18 to 25oC) for 2 hours. The adhesive film was then removed and the wells emptied and washed three times.

100µL of diluted Streptavidin-HRP was added to all wells and the trays recovered and incubated at room temperature for one hour. The adhesive film was then removed and the wells emptied and washed three times.

100µL of the TMB substrate solution was added to all wells and the trays incubated at room temperature for 10 minutes. 100µL of the stop solution was added when the highest standard had developed a dark blue colour and/or had reached an OD of 0.9- 0.95 by ELISA reader. The absorbance of each microwell was read immediately after by spectro-photometer, using 450NM as the primary wave length.

2.11.3.2.4 Calibration of results

The plate reader absorbance values were recorded for each well, standard and sample, subtracting the absorbance measured in the blank wells. A standard curve was created by plotting the mean absorbance of each standard concentration against the human PAI-1 concentration. A best fit

curve was then drawn through the points on the graph. The concentration of circulating PAI-1 was then determined by utilizing the mean absorbance value for each sample.

2.11.3.3 Vascular Endothelial Growth Factor ELISA Method

A commercially available functional assay for the quantitative detection of human VEGF-A was utilized to process samples for VEGF-A. The kits were stored at 2-8^{o}C and were brought to room temperature (8-25^{o}C) prior to use.

Reproducibility

Independent experiments by eBioscience determined the overall intra-assay coefficient of variation to be 6.2%. The inter-assay CV was calculated to be 4.3%. The use of commercial tests kits negated the need for inter and intra plate CV calculation in this small study.

2.11.3.3.1 Assay principle

The anti-human VEGF-A antibody was coated onto each microwell. The human VEGF-A present in the standard or sample binds to these antibodies. The wells were incubated and following this washed to remove any unbound biological components. The biotin-conjugated anti-human VEGF-A antibody was added and bound to the captured human VEGF-A. A further incubation was carried out before the unbound biotin-conjugated anti-human VEGF-A antibody was removed by washing. The Streptavidin-HRP was added and bound to the biotin-conjugated anti-human VEGF-A antibody. The unbound Streptavidin HRP was washed out following incubation. The product formed was coloured in proportion to the amount of human VEGF-A in the sample or standard. The reaction was stopped by the addition of acid solution prior to the absorbance being measured at 450nm. The values for the standard dilutions were used to prepare a standard calibration curve.

2.11.3.3.2 VEGF ELISA Reagents and Chemicals

In addition to the microtitre plate with 96 wells, several reagents were required.

Table 14: Reagents and chemicals for VEGF-A ELISA

Reagent	Description	Preparation
Biotin- Conjugate anti-human VEGF-A polyclonal antibody	Detecting antibody	1:100 dilution with assay buffer
Streptavidin-HRP		1:100 dilution with assay buffer
VEGF standard	2 ng/ml upon reconstitution	Reconstitute as per vial instructions
Assay buffer concentrate	PBS with 1% Tween 20 and 10% BSA	Dilute to final volume 100ml with distilled water
Wash buffer concentrate	PBS with 1% Tween 20	Dilute to final volume 1000ml with deionised water
Substrate solution	Tetramethyl-benzidine	
Stop solution	1M phosphoric acid	

2.11.3.3.3 Assay procedure

The microwells were washed with 400µL of the wash buffer, allowing the buffer to sit in the well for 15 seconds before thorough aspiration of the microwell contents between washes, and finally emptied. 100µL of the Assay buffer was added in duplicate to all the standard wells. To the first 2 wells (A1 and A2) 100µL of the prepared standard was added. The contents were mixed by aspiration and ejection of the well contents. 100µL from each of these wells was then transferred to B1 and B2 and mixed. This procedure was repeated 5 times to create the standard dilutions of concentrations 1000 to 15.6pg/ml. 100µL of the contents of the last 2 microwells was discarded. 100µL of the sample diluents was added in duplicate to the blank wells and 50µL to the sample wells. 50µL of each sample was added in duplicate to the sample wells. The plates were then covered with an adhesive film and incubated for 2 hours at room temperature (18 -25^{o}C) on a microplate shaker at 100rpm.

The film was then removed and the wells emptied and washed 6 times. 100µL of the biotin-conjugate was then added to each well before incubating at room temperature on the shaker for a further hour. The film was then removed and the wells emptied and washed 6 times. 100µL of the diluted Streptavidin-HRP was added to all wells, including the blanks. Incubation was undertaken for an hour on the microplate shaker and the wells emptied and washed 6 times. 100µL of the TMB Substrate solution was added to all wells. The strips were incubated for approximately 30 minutes.
100µL of the stop solution was added when the highest standard had developed a dark blue colour and/or had reached an OD of 0.9- 0.95 by ELISA reader. The absorbance of each microwell was read immediately after by spectro-photometer, using 450NM as the primary wave length.

2.11.3.3.4 Calibration of results

The plate reader absorbance values were recorded for each well, standard and sample, subtracting the absorbance measured in the blank wells. A standard curve was created by plotting the mean absorbance of each standard concentration against the human VEGF-A concentration. A best fit curve was then drawn through the points on the graph. The concentration of circulating VEGF-A was then determined by utilizing the mean absorbance value for each sample.

2.12 Statistical analysis

All data was transcribed into a secure dedicated database (Microsoft Excel). All analyses were performed utilising SPSS (Chicago, Il, USA). No assumptions were made prior to analysis regarding the direction of relationships and no imputation of missing data was attempted.

For the purpose of statistical analysis the studies were divided into the original ‘Proof of concept’ study results comprising 2 groups:

Active; limb on which the device was active

Passive; contralateral limb to the active limb

The final 10-12 patients recruited who were randomised to active or control stimulation was analysed according to:

Active; limb on which the device was active

Passive; contralateral limb to the active limb

Control; limb on which the device was applied but not turned on i.e. trial participants, with the vascular condition concerned, who did not receive active stimulation.

This was to enable us to ascertain whether:

a. The device has any haemodynamic effects
b. The haemodynamic effects were local (i.e. confined to the limb on which the device was active) or systemic
c. Similar effects are seen by participants remaining in the trial position with an inactive device applied.

2.12.1 Continuous data

Prior to any analysis of continuous data histogram analysis was performed to establish the distribution. If the data appeared normally distributed the Kolmogorov statistic or Shapiro Wilk statistic was utilized to confirm this, with a P value > 0.05 indicating normality.

Normally distributed data was described as mean (95% confidence interval) or mean (standard deviation). For data not normally distributed it was described as median (interquartile range).

Hypothesis testing was performed comparing groups as per distribution and whether it was paired or unpaired. N.B. paired data is that which is before and after in the same patient, whilst unpaired data is that from different patients.

The P value represents the probability of the null hypothesis being true (423) (i.e. no difference between the data). P values are quoted to 3 decimal places with values of less than 0.05 being considered significant i.e. suggesting rejection of the null hypothesis.

The comparison of baseline characteristics between the control and active groups i.e. intergroup analysis was performed using the unpaired student T test (424) for normally distributed data and Mann Whitney U test (425) for non-normally distributed data.

The ANOVA test was utilized to compare the changes in mean values seen in the 3 groups. If statistical significance was demonstrated post hoc Bonferroni testing was employed to characterize the difference.

2.12.2 Categorical data

Simple categorical data is presented as percentages. The primary test utilized was Pearson's Chi squared test (426). If more than 20% of the expected frequencies were <5 or if any were <1 then the Fisher's exact test was utilized (427).

2.12.3 Linear Regression analysis

Secondary analysis of covariates determined to be significant predictor of device failure on univariable analysis was carried out using linear regression analysis.

2.12.4 Intraclass correlation coefficient

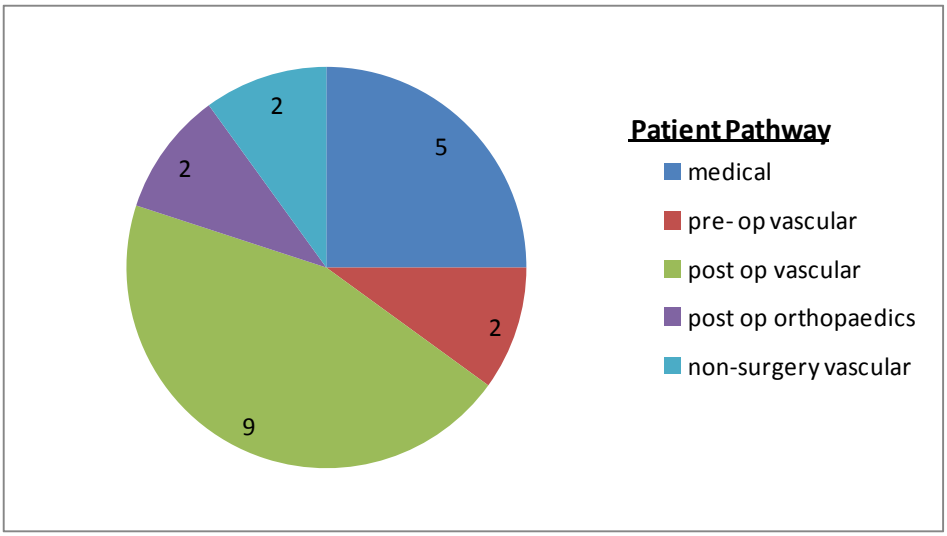
To determine the reliability/ reproducibility of the ultrasonographic measurements taken as part of the study the intraclass correlation coefficient was performed for each type of measurement taken. A P value of < 0.05 was considered to be statistically significant.

Chapter Three: Results- Research Question 1: Tolerability and ease of application

3.1 Patients

20 questionnaires (see appendix) were completed over a 2 week period by 3 members of the research team applying the device to inpatients on the vascular ward. The patients' clinical pathways varied as demonstrated in Figure 6.

Figure 6: Patient pathway



The indication for use were: DVT prevention 50%, DVT prevention and reduction of oedema 5%, DVT prevention and promotion of wound healing 20%, oedema reduction10%, promotion of wound healing 5% and treatment of venous stasis and/or ischaemia 10%.

3.1.1 Ease of application of the geko™ device

15% of patients were lying flat in bed, 10% of patients were mobile and ambulatory, 40% sitting in a chair and 35% sitting up in bed. In 25% of events the device was reported to be very easy to fit, 50% easy and 5% difficult. The operator was unable to obtain visible muscle twitch in 20% of patients. In those where visible twitch was obtainable the device positioning took between 1 and 5 minutes in 56% of cases. The ease of application was not affected by patient position (P = 0.276).

The device was reported to be easy/ very easy to start and stop in 80% of cases and to change settings in 95% of cases. The mean setting required to produce a visible twitch was 5.3 (SD 2.18).

The mean number of hours that the devices were worn was 8.19 (SD 8.41). Partial or complete detachment occurred in 30% of individuals, in 3 cases the head became detached, 2 the tail and in one case complete detachment occurred. The mean time of wear before detachment was 8.17 hours (SD 8.68). In all cases the device was reapplied without difficulty.

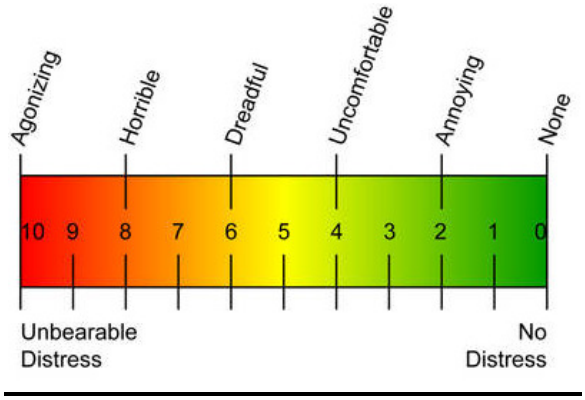
3.1.2 Functionality of the geko™ device in vascular patients

During initial trials it was determined that the geko™ device was unable to stimulate a visible twitch in 20% of the patients trialled. To determine the cause of this and attempt to establish a cohort of patients in whom the device would not be beneficial data was collected to enable the analysis of possible factors which may be associated with failure.

3.2 Tolerability of the geko™ device

The sixty five trial participants, for whom the device was active, completed a questionnaire regarding the tolerability of the device (see appendix). 38 participants (58.5%) reported that the device was very comfortable to wear whilst the remainder found it comfortable (41.5%). The participants were also given a visual analogue scale, from 0-10, to mark according to the level of distress they experienced whilst wearing the device. The mean score was 0.34 (SD 0.47).

Figure 7: Visual analogue scale



52 participants (80%) recorded that the device stuck very well to their leg with only 3 patients noting that the device became partially detached during the trial. In all cases the device was easily reapplied.

The patients were also asked how their leg felt after wearing the device. 10 patients (15.4%) reported a mild improvement in their symptoms, 1 patient (1.5%) reported a positive improvement, 5 patients (7.7%) reported a reduction in the heavy feeling or swelling of the limb and the remainder reported no difference (75.4%).

Of the patients who demonstrated visible muscle twitch the device was reported to be comfortable in 44% and very comfortable in 56%. Three patients wore the device overnight and were asked to report on the quality of their sleep, two reported that it was unchanged from normal whilst the third

reported an improvement in sleep. The patients were also asked to comment on the overall effect of the device: 50% reported no change in their symptoms, 31% a mild improvement and 19% a moderate improvement.

No post study complications were experienced by any patient involved in the studies.

Chapter Four: Research Question 2: Factors determining the efficacy of the geko™ device to produce visible muscle twitch

4.1 Patient factors affecting device functionality

Following the discovery that the device fails to produce visible muscle stimulation in a proportion of patients it was deemed imperative to establish contributing factors for non-responders. Data pertaining to age, disease, co-morbidities, body mass index, calf circumference, presence of oedema, concomitant medications and Toronto neuropathy score was collected for 100 patients trialled with the device.

The patients suffered from a range of vascular disorders: AAA (13%), claudication (57%), critical limb ischaemia (4%), post-op femoro-popliteal bypass graft (7%), post-angioplasty (1%), diabetic ulcers (8%), varicose veins (5%) and healthy volunteers (5%). The subjects were divided into two groups as per response to the geko™ device, namely visible twitch and no twitch.

Table 15: The impact of patient characteristics on efficacy of geko™

	Twitch N = 59	No Twitch N = 41	P value
Age in years (median (IQR))	67 (IQR 10)	73 (IQR 11)	0.002*
Sex: M	35	31	0.094~
F	24	10	
Hypertension: Y	36	24	0.803~
N	23	17	
Hypercholesterolaemia: Y	47	31	0.631~
N	12	10	
MI/ angina: Y	18	13	0.899~
N	41	28	
CVA/ TIA: Y	6	9	0.112~
N	53	32	
Diabetes: Y	6	19	<0.001~
N	53	22	
Current smoker: Y	17	4	0.028~
N	42	37	

* Independant samples median test

~Chi squared

Increasing age and diabetes had a statistically significant negative impact on device function whilst smoking appeared to confer a positive benefit for device function on univariate analysis.

Table 16: The impact of concomitant medications on geko™ efficacy

	Twitch N = 59	No twitch N = 41	P value
Aspirin: Y	46	29	0.412~
N	13	12	
Statin: Y	45	30	0.725~
N	14	11	
ACEI: Y	20	12	0.626~
N	39	29	
Codeine/ Tramadol: Y	10	6	0.756~
N	49	35	
Gabapentin/ pregabalin: Y	1	6	**0.037~**
N	58	35	
Oxycontin/ morphine: Y	2	5	0.111~
N	57	36	

~Chi squared

Use of gabapentin or pregabalin had a negative impact on device function.

Table 17: The impact of examination findings on geko™ efficacy

	Twitch	No twitch	P value
Obese:**			
BMI >30	9	8	0.093~
BMI ≤ 30	44	15	
Oedema:			
None or mild	56	15	**<0.001~**
Moderate or severe*	3	26	
Calf circumference:**			
<35cm	31	10	0.097~
≥35cm	22	16	
ABPI:			
<0.8	6	4	0.295
≥0.8	53	36	
Neuropathy score:			
<5	55	13	**<0.001~**
≥5	4	28	

~Chi squared

*Oedema was classified as mild (foot and ankle only), moderate (extending > 5cm proximal to the medial malleolus) and severe (extending proximal to the fibular head).

** Parameter unavailable for certain subjects

The presence of oedema and higher neuropathy score had a detrimental impact on the ability of the device to produce visible muscle twitch on univariable analysis.

Only the presence of moderate/ severe oedema (Odds ratio (OR) 161.245, 95% confidence interval (CI) 18.978-1370.007; $P < 0.001$) and a neuropathy score of greater than 5 (OR: 168.368, 95% CI 18.096- 1566.532; $P < 0.001$) retained prognostic impact in a multivariate analysis.

Following the determination that the device failed to produce visible muscle twitch in patients with the most significant arterial disease and as such greater degrees of neuropathy and oedema the protocol was amended to include patients with claudication and exclude those with critical limb ischaemia.

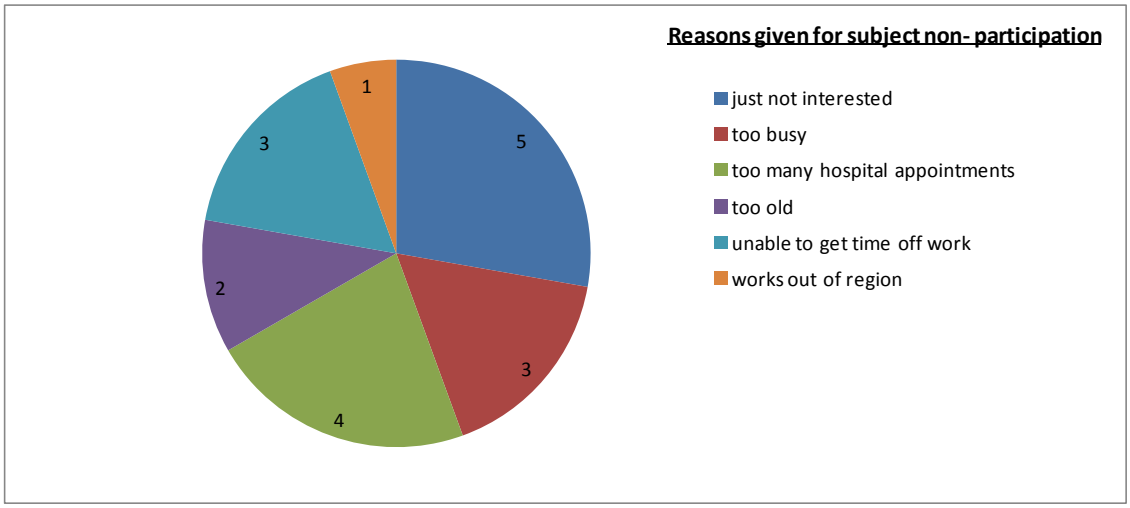
Chapter Five: Research question 3: The haemodynamic efficacy of peroneal nerve electrical neuromuscular stimulation in claudicants

5.1 Claudication Study population

A total of 54 potential participants were identified from out-patient clinics and the NHS Trust Supervised exercise programme. All were contacted by letter with an attached patient information sheet.

30 participated in the study. 6 patients were unable to participate in the study as there was no effect from the device. 18 patients were not willing to participate in the study *(Figure 8)*.

Figure 8: Reasons for subject non participation



Of those who participated the mean age was 67.73 years, 19 males and 11 females. 11 participants suffered from bilateral claudication and 19 unilateral,

affecting the calf only in all but 2 cases where the patients also experienced a degree of thigh claudication. The mean claudicating distance was 173 yards (SD 170).

5.2 Proof of concept study

5.2.1 Examination findings: ABPI

The mean ABPI at baseline in the active limbs was 0.69 (SD 0.21) and passive limbs 0.97 (SD 0.17). This was statistically significant on unpaired T test (P < 0.001). The mean change in ABPI from baseline in the active limbs was 0.12 (SD 0.11) [P < 0.001 Paired T test] and passive limbs was -0.04 (SD 0.12) [P = 0.141 Paired T test]. A comparison of the difference between changes seen in the two groups was statistically significant (P = 0.034 Unpaired T test) *(Figures 9, 10)*.

Figure 9: Scatter graph: Active limbs (limb on which device was active); change in ABPI in study period (n = 20)

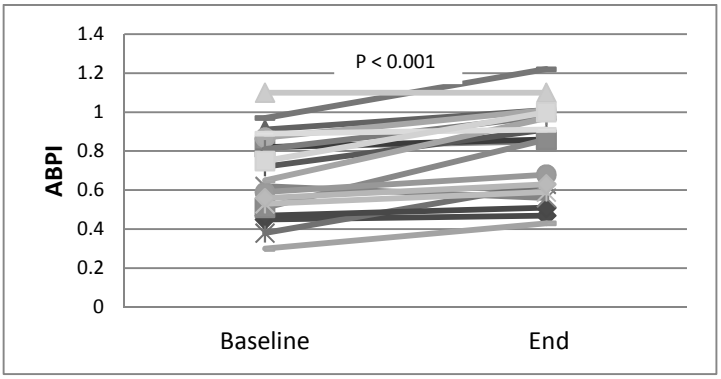
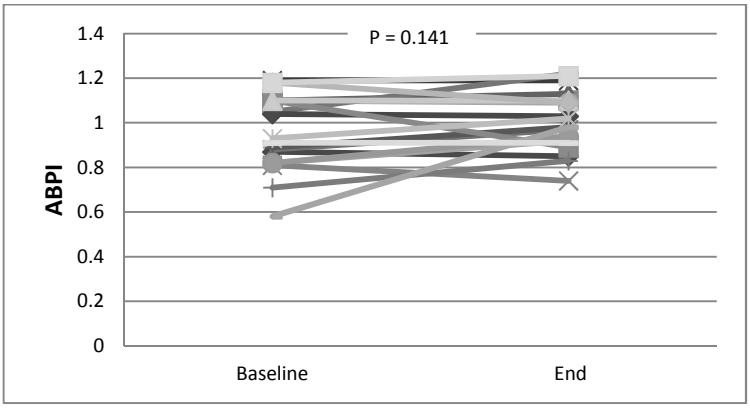


Figure 10: Scatter graph: Passive limbs (contralateral limb to active limb); Change in ABPI in the study period (n = 20)



Calf circumference

The mean calf circumference at baseline in the active limbs was 34.5cm (SD 3.4) and passive limbs 34.6cm (SD 3.2).The difference was not statistically significant on Unpaired T test (P = 0.890).
The mean change in calf circumference from baseline was -0.56cm (SD 0.73) [P = 0.003 Paired T test] in the active limbs and -0.32cm (SD 0.43) [P = 0.003 Paired T test] in the passive limbs. A comparison of the difference in the change seen was not statistically significant (P = 0.223 Unpaired T test).

5.2.2 Haemodynamic efficacy of geko^{TM} in claudicants: Proof of concept study

5.2.2.1 Assessment of reliability of haemodynamic measurements

The intra-class correlation coefficient was utilised to determine the intra-rater reliability of the ultrasound measurements collected in the study(428). The intra-observer reliability assessed the reproducibility of the observer for each measurement technique *(Table 18)*. In this study, each measurement was made in triplicate and the mean utilised for all subsequent analysis.

Table 18: Intraclass correlation coefficient analysis of ultrasound measurements

Measurement	Intraclass correlation coefficient (P)
Arterial maximum flow velocity.	0.949
Arterial mean flow velocity	0.960
Arterial vessel diameter	0.990
Arterial volume flow	0.971
Venous maximum flow velocity	0.828
Venous mean flow velocity	0.937
Venous vessel diameter	0.985
Venous volume flow	0.954

Thus it is possible to see that there was high intra-rater reliability for all ultrasound measurements performed as part of this study within the Claudicant population.

5.2.2.2 Arterial duplex

5.2.2.2.1 Maximum arterial velocity

The mean baseline maximal arterial velocity in the active limbs was 69.73 (SD 51.32) and passive limbs 81.44 (SD 27.69) cm/sec. Comparison of the groups demonstrated no statistical significance (P = 0.375) in the baseline values.

The change from baseline in the groups were 7.12 (SD 33.21) [P = 0.350 Paired T test] and -2.58 (SD 12.66) [P = 0.374 Paired T test] cm/sec in the active and passive groups respectively equating to percentage changes of 33.48% (SD 59.00) and -1.03% (SD 16.11). The differences in the change seen between the active and passive limbs were not statistically significant (P = 0.230 Unpaired T test) *(Figures 11,12,13)*.

Figure 11: Maximal arterial velocity change from baseline

Error bar chart demonstrating the maximal arterial velocity and at baseline at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI) (n= 20)

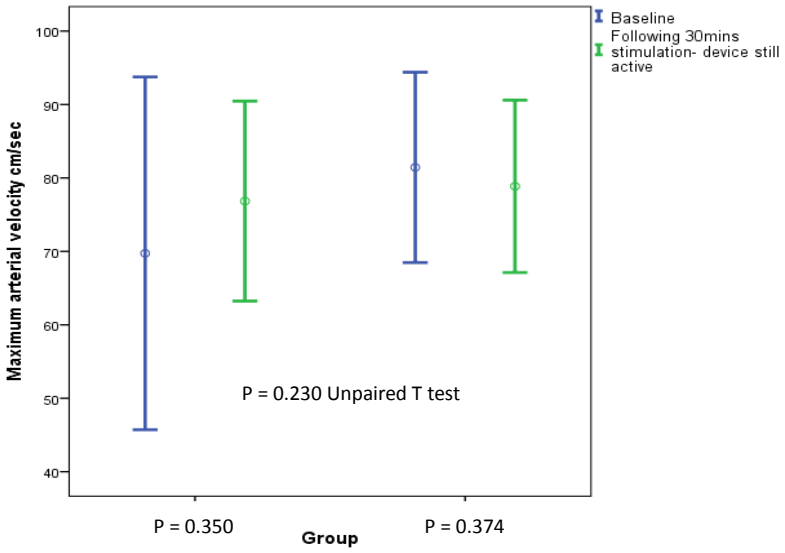


Figure 12: Active stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 20)

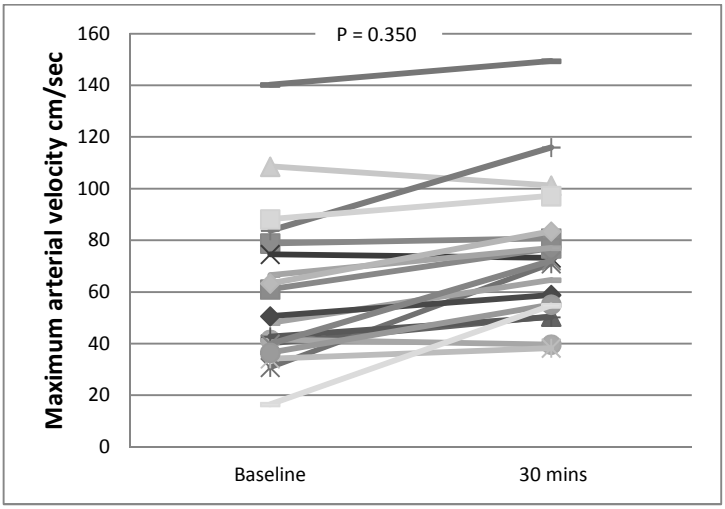
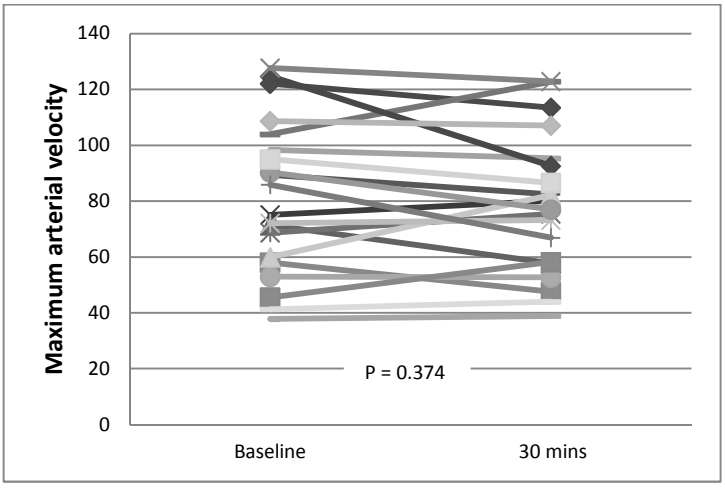


Figure 13: Passive stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 20)



5.2.2.2.2 Mean arterial velocity

The baseline mean arterial velocity in the active limbs was 9.10 (SD 4.25) and passive limbs 10.21 (SD 5.96) cm/sec. The difference between the limbs was not statistically significant (P = 0.502 Unpaired T test).

The change from baseline with the device was 2.21 (SD 2.23) [P <0.001 Paired T test] and passive limbs -0.12 (SD 3.56) cm/sec [P = 0.884 Paired T test] in the active and passive limbs respectively equating to percentage changes of 31.39% (SD 35.76) and 21.9% (SD 79.92) respectively. The difference in the changes seen between the groups was statistically significant (P = 0.018 Unpaired T test) *(Figures 14, 15, 16)*.

Figure 14: Mean arterial velocity change from baseline

Error bar chart demonstrating the mean arterial velocity at baseline and at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI) (n = 20)

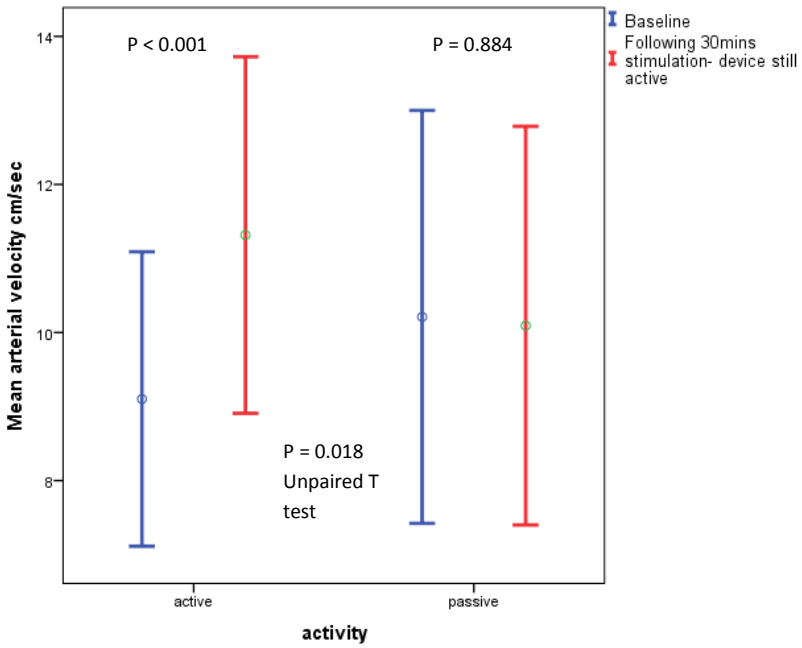


Figure 15: Active stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 20)

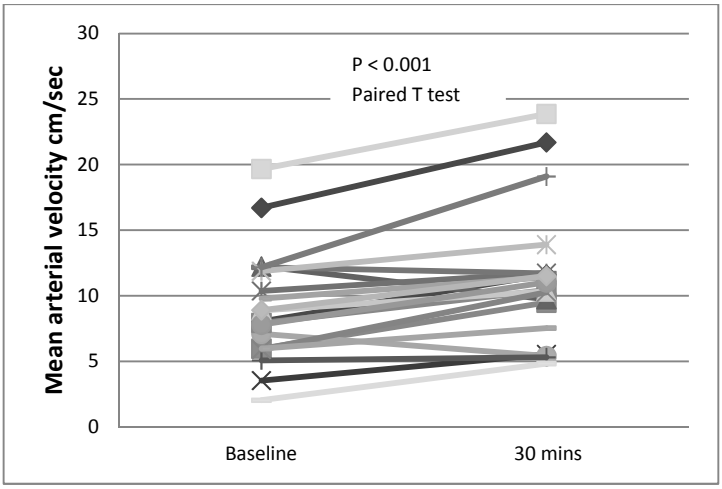
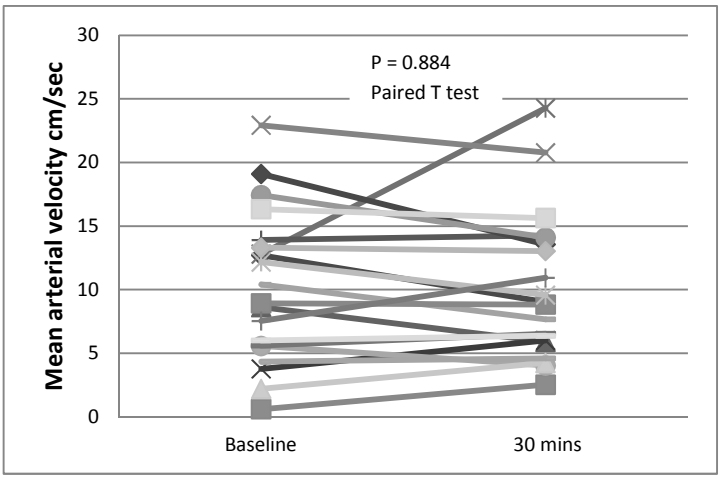


Figure 16: Passive stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 20)



5.2.2.2.3 Arterial diameter

The mean arterial diameter at baseline in the active limbs was 5.60 (SD 0.75) and passive limbs was 6.18 (SD 1.11) millimetres (mm). The difference between the baseline value in the groups was not statistically significant (P = 0.064 Unpaired T test).

The change from baseline with stimulation was 0.15 (SD 0.35) [P = 0.072 Paired T test] and -0.02 (SD 0.28) mm [P = 0.765 Paired T test] in the active and passive limbs respectively equating to percentage changes of 3.15% (SD 8.04) and -0.34% (SD 5.12). Unpaired T test analysis demonstrated this difference in the change between the limbs was not statistically significant (P = 0.101).

5.2.2.2.4 Arterial volume flow

The mean arterial flow at baseline in the active limbs was 0.22 (D 0.12) and passive limbs 0.29 (SD 0.17) litres/min (L/min). Flow at baseline was comparable between the two groups (P = 0.176 Unpaired T test).

The change from baseline with stimulation was 0.06 (SD 0.05) [P<0.001 Paired T test] and -0.01 (SD 0.08) L/min [P = 0.742 Paired T test] in the active and passive limbs respectively equating to percentage changes of 38.9% (SD 47.8) and 1.91% (SD 26.7). Unpaired T test analysis demonstrated that the difference in the change

seen between the active and passive limbs was statistically significant (P = 0.004) *(Figure 17,18,19)*.

Figure 17: Arterial volume flow change from baseline

Error bar chart demonstrating the arterial volume flow at baseline and at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI) (n = 20)

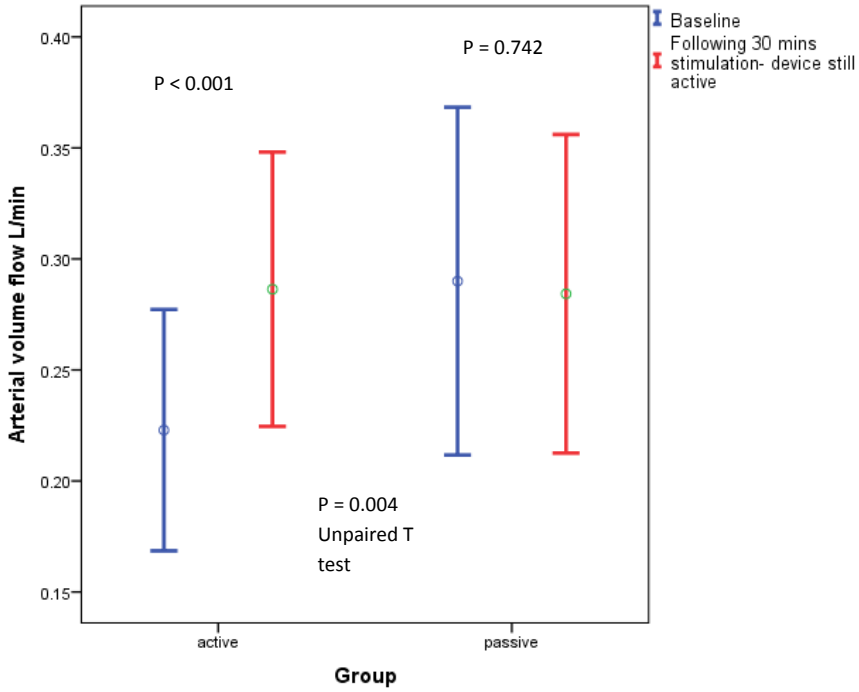


Figure 18: Active stimulation limbs: Change arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 20)

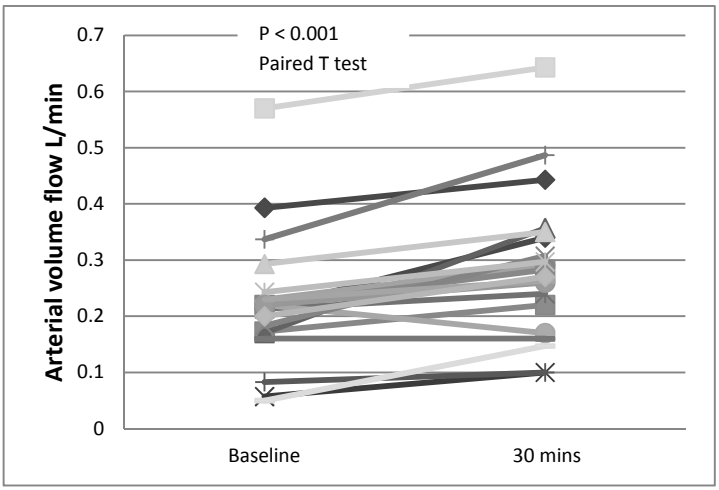
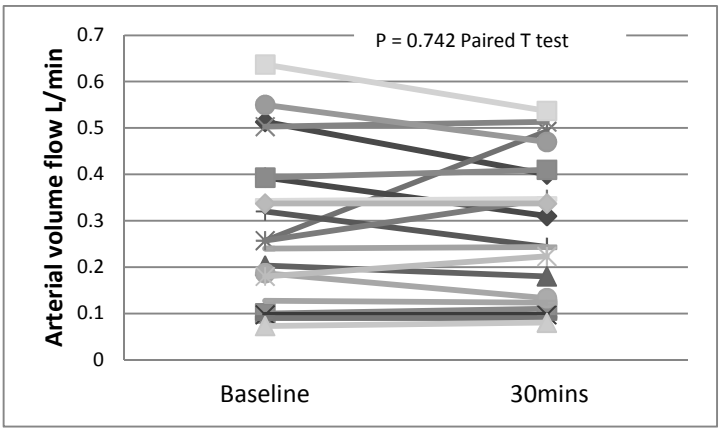


Figure 19: Passive stimulation limbs: Change arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 20)



5.2.2.3 Venous duplex

5.2.2.3.1 Venous maximum velocity

The baseline mean maximum venous velocity in the active limbs was 15.40 (SD 3.20) and the passive limbs 17.54 (SD 5.98) cm/sec. Comparison of the baseline values demonstrated the differences between the groups to be insignificant (P = 0.166 Unpaired T test).

The change from baseline with stimulation in the active limbs was 4.96 (SD 7.14) [P = 0.006 Paired T test] and passive limbs-1.83 (SD 3.69) cm/sec [P = 0.039 Paired T test]. Comparison of the changes from baseline in the 2 groups demonstrated that this was statistically significant (P = 0.001 Unpaired T test) *(Figures 20,21,22)*.

Figure 20: Maximal venous velocity change from baseline

Error bar chart demonstrating the maximal venous velocity at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI) (n = 20)

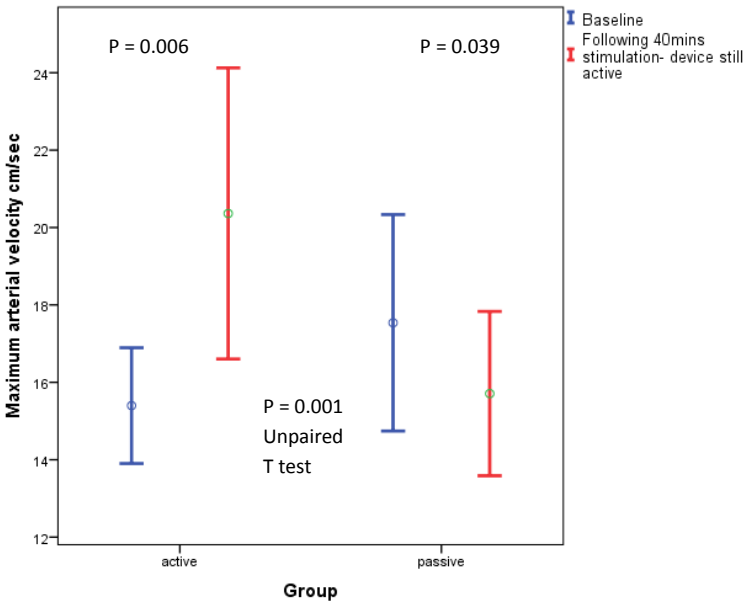


Figure 21: Active stimulation limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 20)

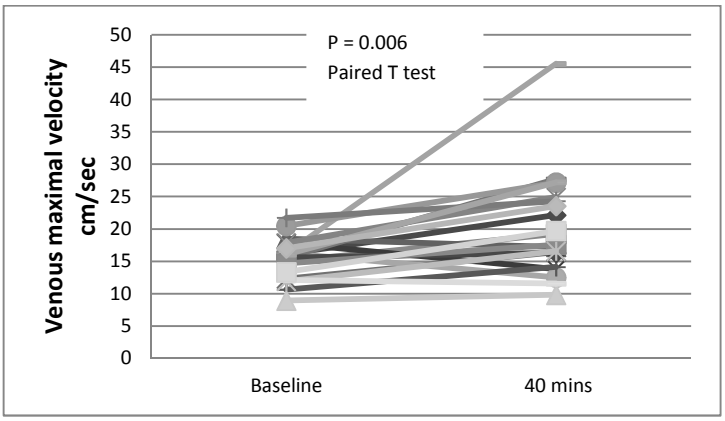
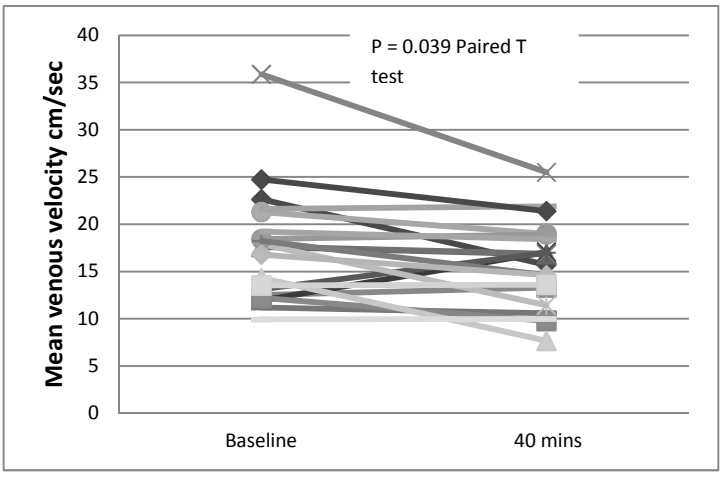


Figure 22: Passive stimulation limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 20)



5.2.2.3.2 Venous mean velocity

The average mean velocity in the active limbs at baseline was 5.65 (SD 1.71) and the passive limbs 6.14 (SD 3.20) cm/sec. Comparison of the baseline values demonstrated the difference to be insignificant (P = 0.552 Unpaired T test).
The change from baseline with stimulation in the active limbs was 1.35 (SD 1.90) [P = 0.005 Paired T test] and passive 0.156 (SD 1.07) cm/sec [P = 0.525 Paired T test]. Comparison of the changes from baseline in the active and passive groups demonstrated that that the differences between groups were statistically significant (P = 0.019 Unpaired T test) *(Figures 23,24,25)*.

Figure 23: Mean venous velocity change from baseline

Error bar chart demonstrating the mean venous velocity at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI) (n = 20)

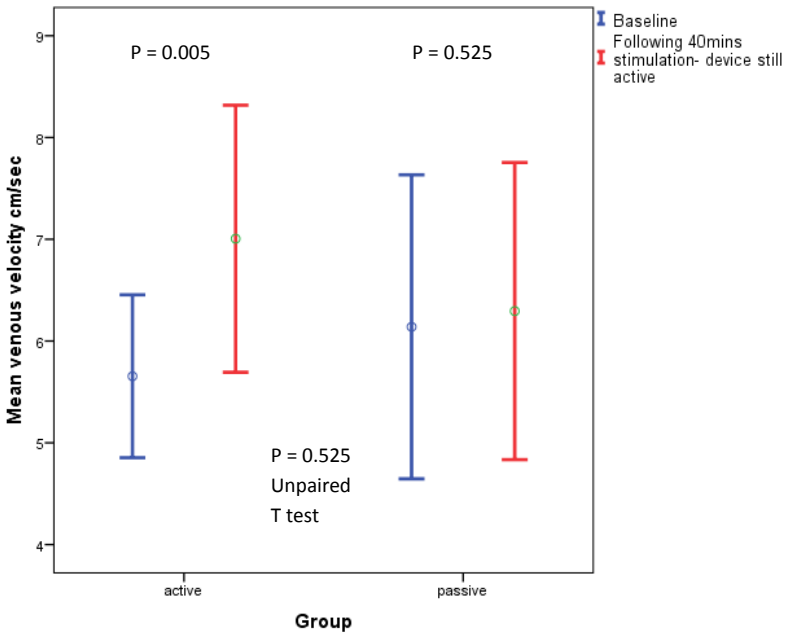


Figure 24: Active stimulation limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 20)

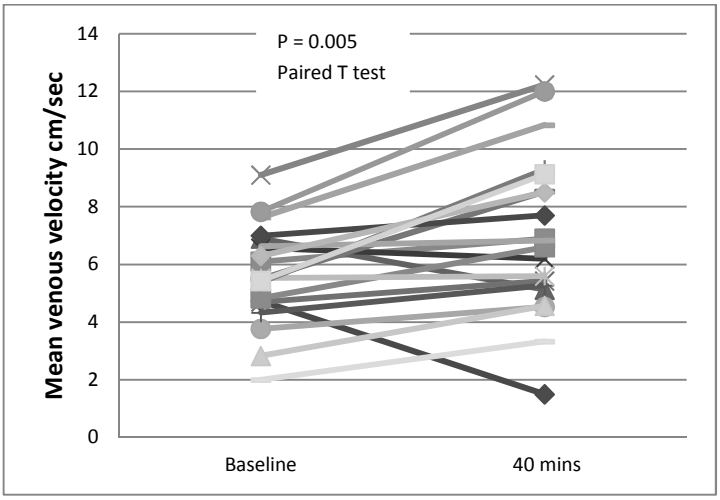
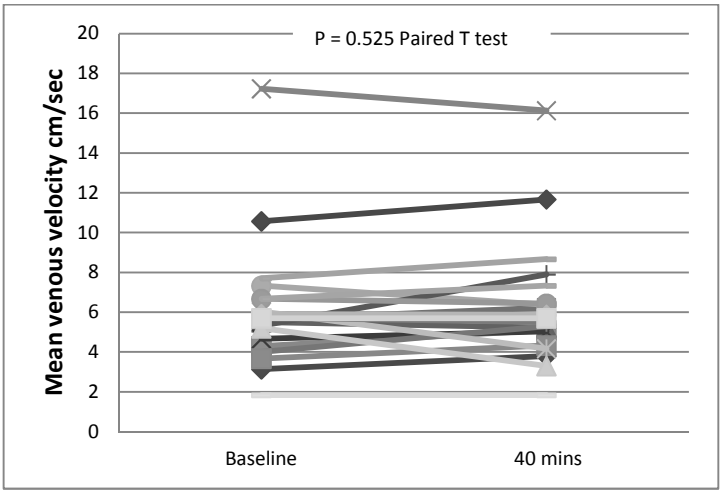


Figure 25: Passive stimulation limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 20)



5.2.2.3.3 Venous diameter

The average venous diameter at baseline in the active limbs was 5.88 (SD 1.05) and the passive limbs 6.03 (SD 1.05) mm. Comparison of the baseline value between the groups demonstrated an insignificant difference (P = 0.653 Unpaired T test).
The change from baseline with stimulation in the active limbs was 0.21 (SD 0.90) [P = 0.298 Paired T test] and passive 0.03 (SD 0.34) mm [P = 0.727 Paired T test]. The difference in change from baseline in the 2 groups was not statistically significant (P = 0.387 Unpaired T test).

5.2.2.3.4 Venous volume flow

The mean volume flow at baseline in the active limbs was 0.16 (SD 0.06) and the passive limbs 0.165 (SD 0.07) L/min. Comparison of the baseline values demonstrated an insignificant difference (P = 0.643 Unpaired T Test),
The change from baseline with stimulation in the active limbs was 0.03 (SD 0.04) [P = 0.001 Paired T test] and passive 0.00 (SD 0.04) L/min [P = 0.935 Paired T test]. Comparison of the difference in change from baseline in the 2 groups was statistically significant (P = 0.010 Unpaired T test) *(Figures 26,27,28)*.

Figure 26: Venous volume flow change from baseline

Error bar chart demonstrating the venous volume flow at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI) (n = 20)

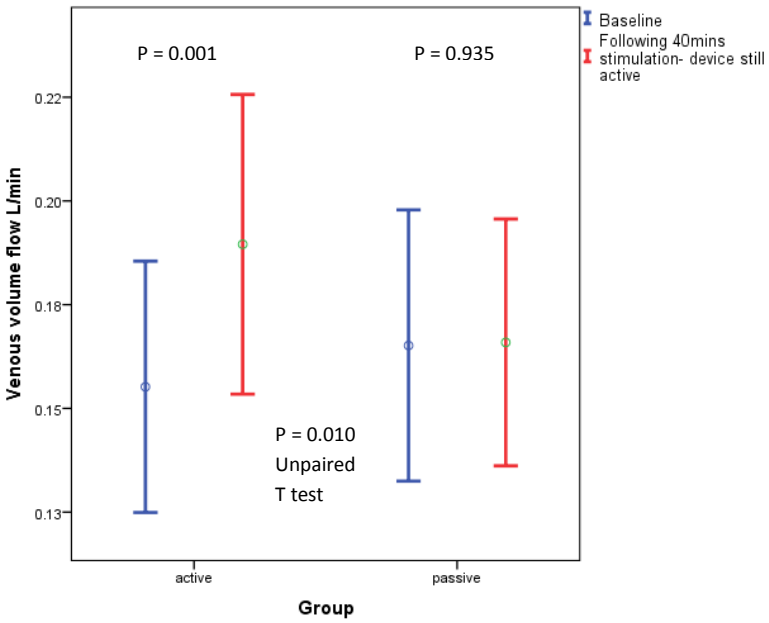


Figure 27: Active stimulation limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 20)

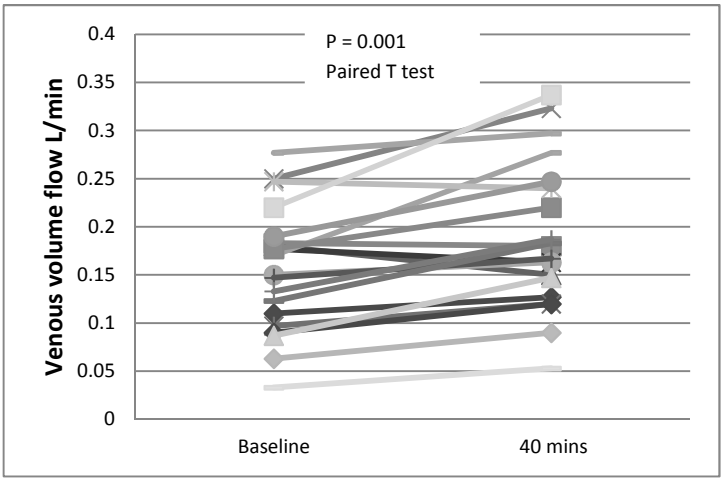
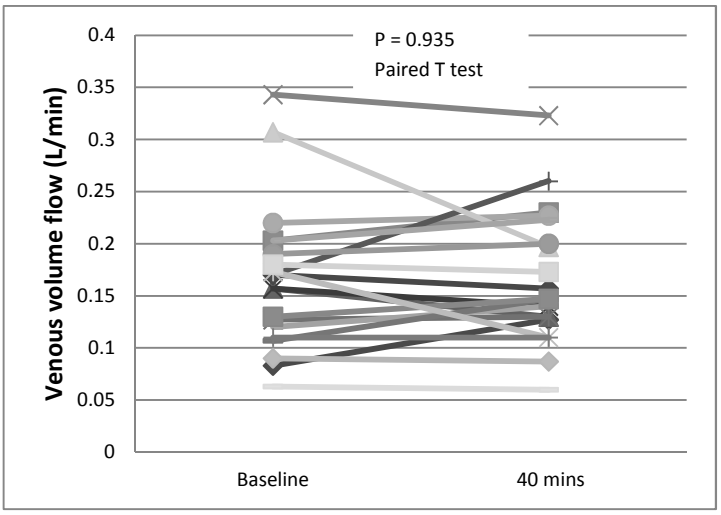


Figure 28: Passive stimulation limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 20)



5.2.2.4 Skin perfusion/ Laser Doppler flowmetry

There was no appreciable change in skin temperature during the study period. Baseline temperature in the active group was 29.24 (SD 2.05) and passive group 29.42°C (SD 1.84). The difference in baseline values was not statistically significant (P = 0.772 Unpaired T test).
The change in temperature from baseline was 0.14 (SD 0.96)[P = 0.536 Paired T test], passive group 0.03°C (SD 0.87) [P = 0.899 Paired T test]. The differences in change from baseline between the two groups was not statistically significant (P = 0.706 Unpaired T test).

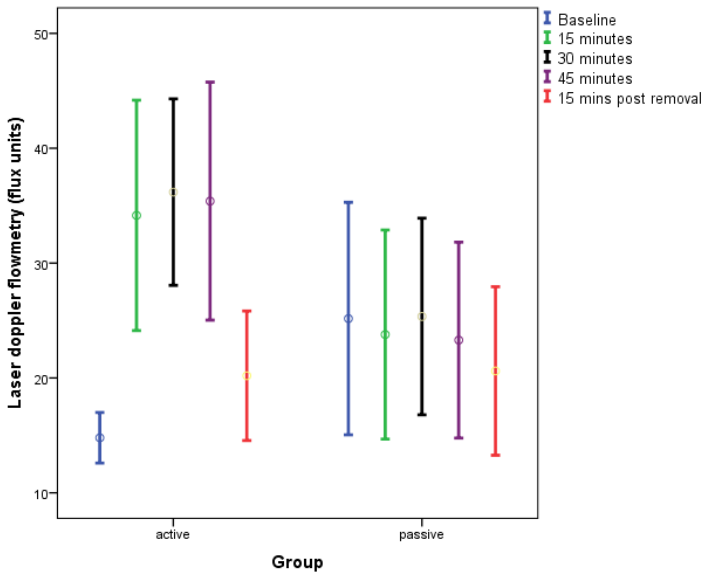
Baseline microcirculatory flow in the active groups was 14.79 (SD 4.71) and the passive group 25.17 (SD 4.84) flux units. The difference in baseline values seen in the groups was statistically significant (P = 0.043).
The change at 15 minutes from baseline in the active group was 19.36 (SD 19.52) [P < 0.001 Paired T test], in the passive group was -1.39 (SD 11.03) flux units [P = 0.580 Paired T test]. Analysis of the differences seen between the active and passive groups demonstrated statistical significance (P < 0.001 Unpaired T test).
The change at 30 minutes from baseline in the active groups was 21.39 (SD 15.02) [P < 0.001 Paired T test] and passive 0.18 (SD 8.97) flux units [P = 0.929 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance (P <0.001 Unpaired T test).

The change at 45 minutes from baseline in the active group was 20.61 (SD 19.2) [P < 0.001 Paired T test] and passive -1.88 (SD 7.36) flux units [P = 0.269 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance (P <0.001 Unpaired T test).

The change at 15 minutes following device deactivation in the active groups was 5.40 (SD 9.41) [P = 0.019 Paired T test] and passive -4.57 (SD 8.83) flux units [P = 0.032 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance (P = 0.001 Unpaired T test) *(Figure 29)*.

Figure 29: Laser Doppler flowmetry change from baseline

Error bar chart demonstrating the laser Doppler flowmetry at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI) (n = 20)



5.2.2.5 Cardiovascular effects

The mean heart rate at baseline was 69.25 (SD 11.83) and decreased to 64.50 (SD 9.89) beats per minute (bpm) 30 minutes after initiation of stimulation (P = 0.001 Paired T test). The heart rate increased to 65.20 bpm (SD 11.19) following deactivation of the device (P = 0.374 Paired T test).
There were no significant changes in systolic blood pressure with device activation (P = 0.691 Paired T test) or deactivation (P = 0.085 Paired T test).

There were no significant changes in mean diastolic blood pressure on device activation (P = 0.880 Paired T test) or deactivation (P = 0.123 Paired T test).

5.3 Randomised patients

5.3.1 Baseline Characteristics

The final 10 participants were randomised to either active stimulation (i.e. one limb received active stimulation and other limb deemed passive) or a control group (i.e. stimulation device applied but not activated). Statistical analysis of the patients in the active and control groups was carried out to establish if they were comparable *(Tables 19, 20, 21)*.

Table 19: Comparison of the baseline characteristics of participants in the active and control groups

	Active **N = 5**	**Control** **N = 5**	**P value**
Age (median)	65 (IQR 11)	78 (IQR 16)	0.206*
Sex	3F, 2M	3F. 2M	1.000~
Current smoker	1	2	0.500~
Pack years	31.4 (SD 14.9)	20.6 (SD 19.3)	0.351^
EtOH	Yes 3	Yes 4	0.500~
EtOH units/ week	6.8 (SD 12.0)	4.6 (SD 4.8)	0.713^
BMI	27.4 (SD 4.7)	28.6 (SD 4.1)	0.678^
Symptoms location	Calf only 5	Calf only 5	1.000~
Patient reported claudication distance (yards)	88.0 (SD 21.7)	120 (SD 44.7)	0.188^
Claudication duration (months)	27.8 (SD 24.3)	13.2 (SD 13.1)	0.272^
ABPI	0.78 (IQR 0.18)	0.78 (IQR 0.39)	1.000*
Previous procedures	Yes 2, No 3	Yes 0, No 5	0.222~

* Independent samples median test

^ Unpaired T test (NB Shapiro Wilk test utilised to establish normality of distribution for data. P> 0.05 in all cases)

~ Fishers exact test

Table 20: Comparison of participant co-morbidities between active and control groups

	Active N = 5	Control N = 5	P value
Diabetes	Yes 0, No 5	Yes 2, No 3	0.222*
Cerebrovascular disease	Yes 0, No 5	Yes 1, No 4	0.500*
Hypertension	Yes 4, No 1	Yes 3, No 2	0.500*
Cardiovascular disease	Yes 1, No 4	Yes 1, No 4	0.778*
Respiratory	Yes 1, No 4	Yes 1, No 4	0.778*

* Fishers exact test

Table 21: Comparison of concomitant medications in the active and control groups

	Active N = 5	Control N = 5	P value
Antiplatelet	Yes 4, No 1	Yes 4, No 1	0.778*
Aspirin	Yes 3, No 2	Yes 4, No 1	0.500*
Clopidogrel	Yes 1, No 4	Yes 0, No 5	0.500*
Beta blocker	Yes 0, No 5	Yes 0, No 5	0.778*
ACE inhibitor	Yes 2, No 3	Yes 2, No 3	0.778*
Statin	Yes 4, No 1	Yes 2, No 3	0.262*

* Fishers Exact Test

It is possible to see that the groups of patients, active and control, were similar in all regards.

5.3.2 Randomised patients: Haemodynamic Efficacy of geko™ in claudicants

5.3.2.1 Arterial duplex

5.3.2.1.1 Maximum arterial velocity

The baseline values for mean maximal arterial velocity were active limbs (limb on which the device was active) 76.62 (SD 17.23), passive (contralateral limb to active) 60.63 (SD 11.79) and control (limb on which device was applied but not activated) 90.38 (SD 36.89) cm/sec. The difference in baseline values was not significant [P = 0.191 ANOVA]. The mean change in maximum arterial velocity from baseline in the active limbs was 19.06 (SD 8.43) (Paired T test P = 0.007); passive 2.73 (SD 5.18) (Paired T test P = 0.304) and control limbs 0.62 (SD 15.67) cm/sec (Paired T test P = 0.904) cm/ second equating to percentage changes of 24.43%, 3.79% and 0.38% respectively *(Figures 30-33)*.

Figure 30: Maximal arterial velocity change from baseline

Error bar chart demonstrating the maximal arterial velocity at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 5)

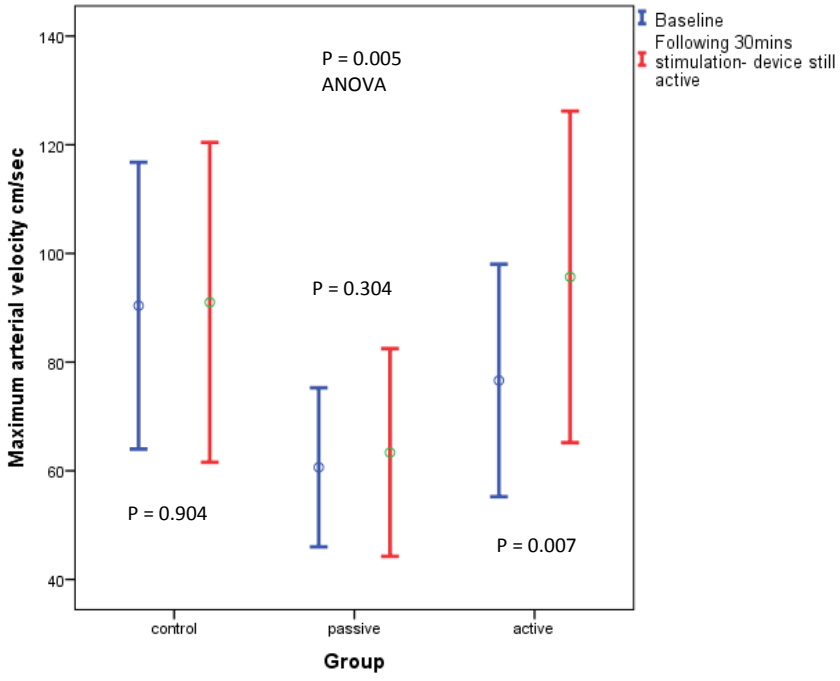


Figure 31: Active stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 5)

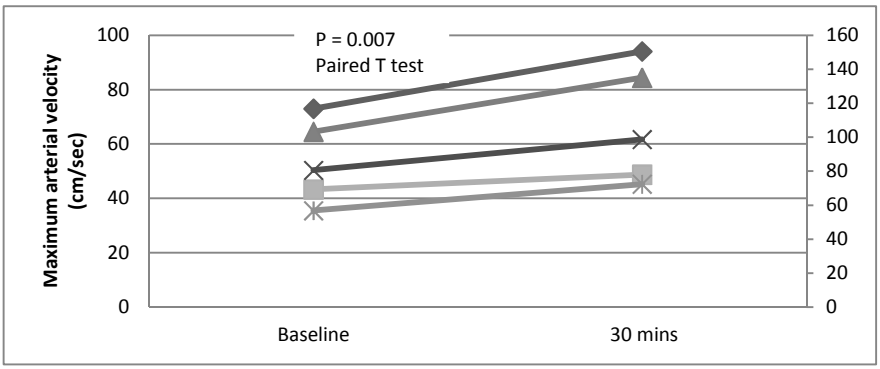


Figure 32: Passive limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation of contralateral limb (n = 5)

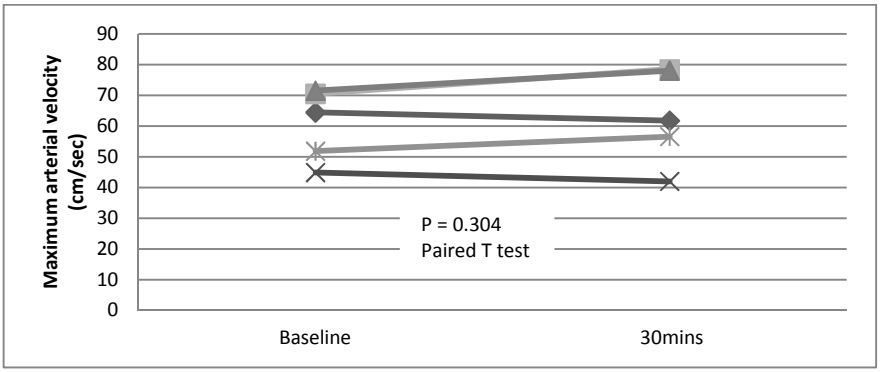
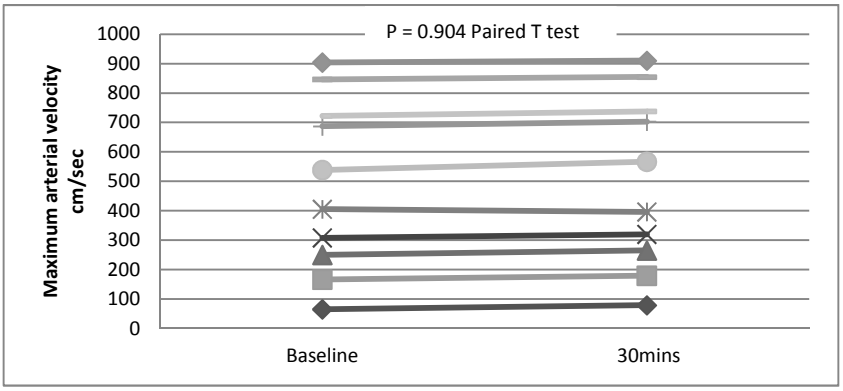


Figure 33: Control limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of device applied to limbs but not activated (n = 10)



The difference in change from baseline between the active and passive groups was statistically significant (Unpaired T Test: P = 0.003) whilst not significant when comparing the passive and control limbs, P = 0.633 (Unpaired T Test). ANOVA analysis demonstrated statistical significance P = 0.005, which was further

investigated with post hoc Bonferonni analysis: active versus passive limbs P = 0.037, active versus control limbs P = 0.005 and control versus passive limbs: P = 1.000

5.3.2.1.2 Mean arterial velocity

The baseline values for mean arterial velocity in the groups was: active 14.66 (SD 6.30); passive 11.89 (SD 5.43) and control 14.83 (SD 9.57) cm/sec. The baseline values were not statistically significantly different (P = 0.788 ANOVA). The change in mean arterial velocity from baseline in the active limbs was 4.60 (SD 4.02)[Paired T Test P = 0.063], passive limbs - 0.73 (SD 1.70)[Paired T test P = 0.393] and control limbs was -0.89 (SD 0.98) cm/ sec [Paired T Test P = 0.019] equating to percentage changes of 29.79%, -3.76% and -7.59% respectively. The difference in change from baseline between the active and passive groups was statistically significant (Unpaired T Test: P = 0.014). Passive vs control P = 0.485 (Unpaired T test). ANOVA analysis of the change from baseline for the 3 groups was statistically significant (P<0.001) with post hoc Bonferonni demonstrating: active vs control P = < 0.001, active vs passive P = 0.002 but passive vs control P = 1.000 *(Figures 34-37)*.

Figure 34: Mean arterial velocity change from baseline

Error bar chart demonstrating the mean arterial velocity at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 5)

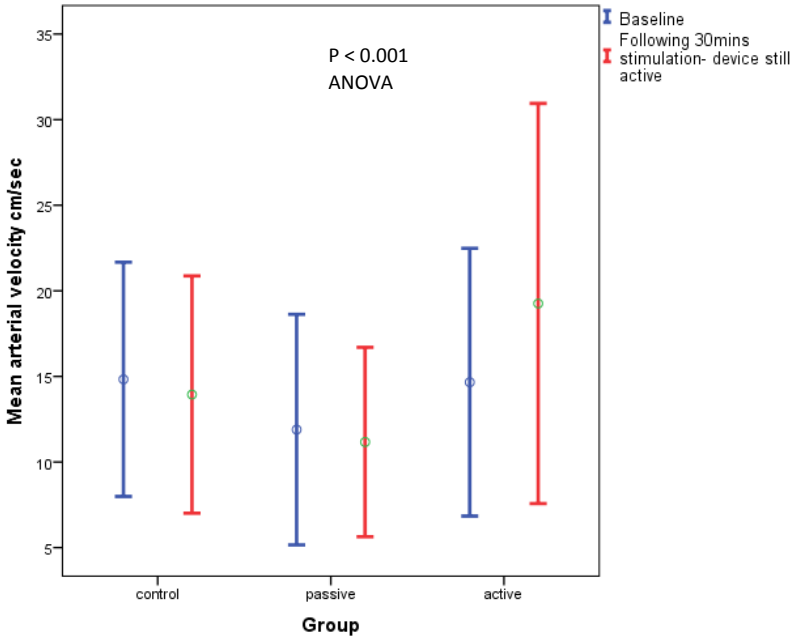


Figure 35: Active stimulation limbs; Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 5)

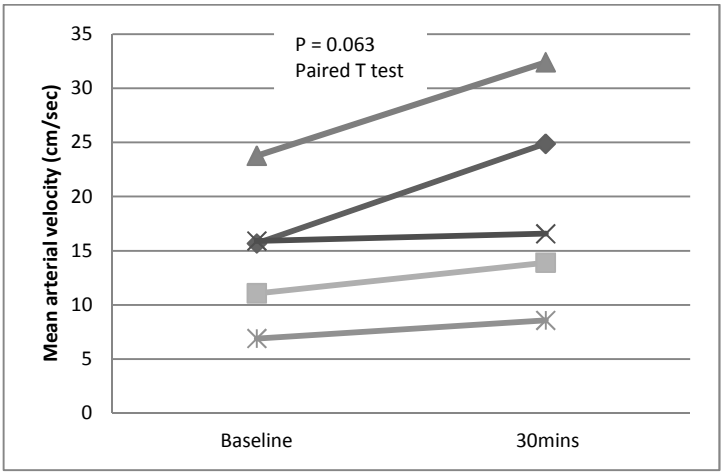


Figure 36: Passive limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation of contralateral limb (n = 5)

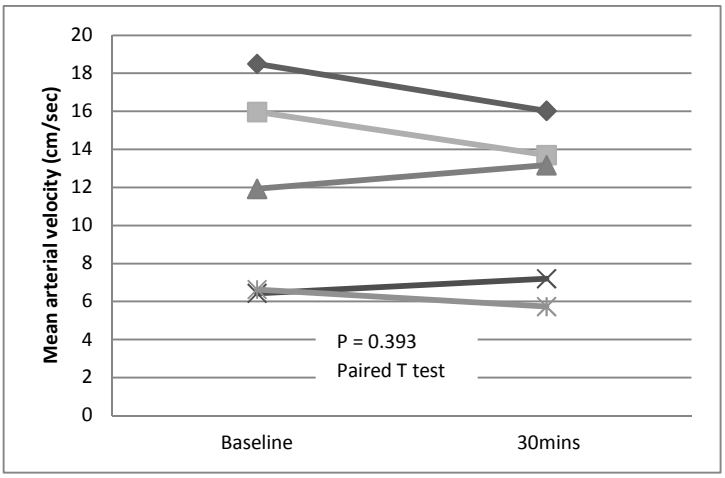
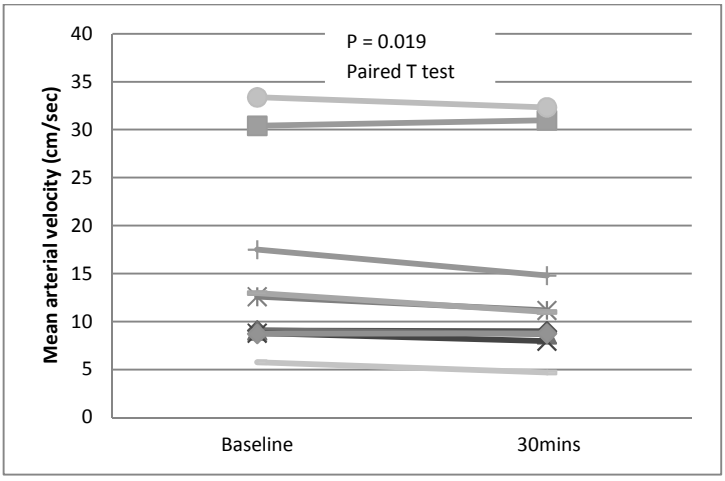


Figure 37: Control limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of device applied to limbs but not activated (n = 10)



5.3.2.1.3 Arterial diameter

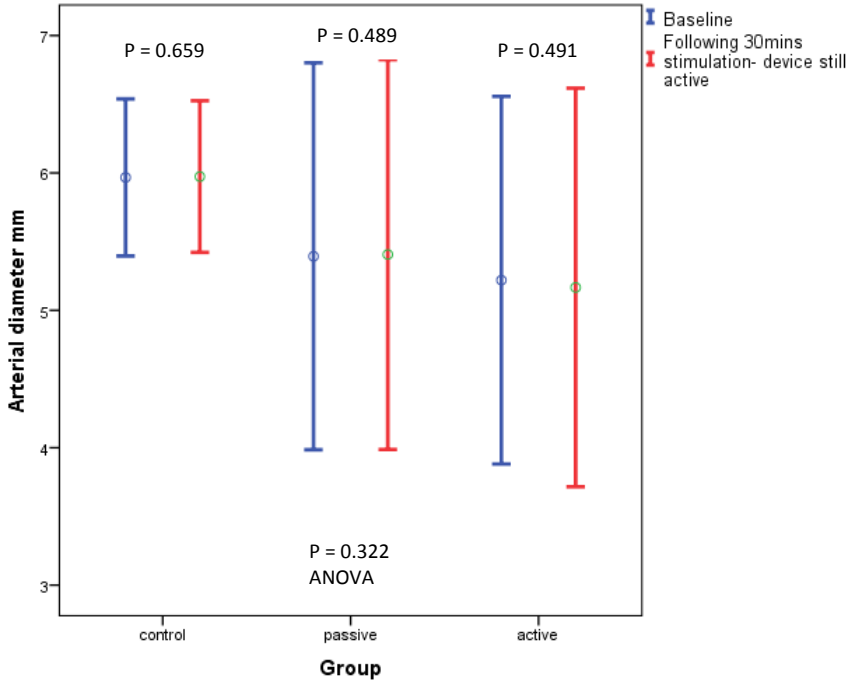
The baseline values for arterial diameter were active 5.21 (SD 1.08), passive 5.39 (SD 1.13) and control limbs 5.97 (SD 0.80) mm. The difference in baseline values was not statistically significant [P = 0.314 ANOVA]. The mean change in arterial diameter in the active group was -0.05 (SD 0.156)[Paired T test P = 0.491], passive group 0.013 (SD 0.038)[Paired T test P = 0.489] and control group 0.006 (SD 0.437) mm [Paired T test P = 0.659], equating to percentage changes of -1.34%, 0.23% and 0.16% respectively.

The difference in change from baseline between the active and passive groups was not statistically significant (Unpaired T Test: P = 0.378), passive vs control P = 0.834 (Unpaired). ANOVA analysis to investigate the change in diameter from baseline demonstrated the differences in change seen in each group was statistically insignificant (P = 0.322) *(Figure 38)*.

Figure 38: Arterial diameter change from baseline

Error bar chart demonstrating the arterial diameter at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 5)



5.3.2.1.4 Arterial volume flow

The baseline arterial volume flow in the active limbs was 0.287 (SD 0.10), passive 0.240 (SD 0.06) and control 0.417 (SD 0.27) L/min. The difference in baseline values was not statistically significant [P = 0.257 ANOVA]. The mean change in mean arterial volume flow in the active limbs was 63.6 (SD 21.94) (Paired T test P = 0.003), passive limbs -9.4 (SD 13.67) (Paired T test P = 0.199) and control limbs -12.0 (SD 17.02) (Paired T Test P = 0.053) equating to percentage changes of 23.44%, -3.82% and -3.85% respectively.
The difference in change from baseline between the active and passive groups was statistically significant (Unpaired T Test: P < 0.001) but not when comparing the passive and control groups (Unpaired T test P = 0.992). This was confirmed on ANOVA analysis (P < 0.001) with post hoc Bonferonni: active versus passive P < 0.001; active versus control P < 0.001 and passive versus control P = 1.000 *(Figures 39-42)*.

Figure 39: Arterial volume flow change from baseline

Error bar chart demonstrating the arterial volume flow at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

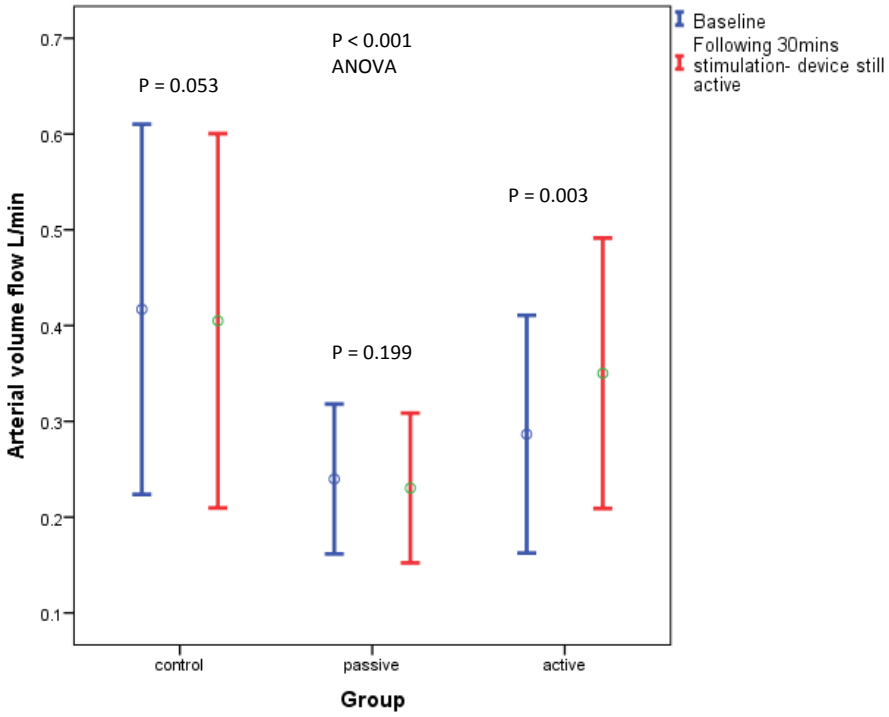


Figure 40: Active stimulation limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 5)

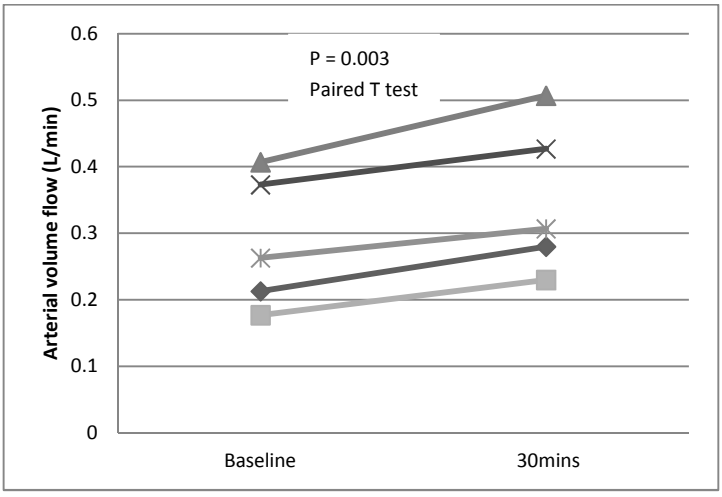


Figure 41: Passive limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of stimulation of contralateral limb (n = 5)

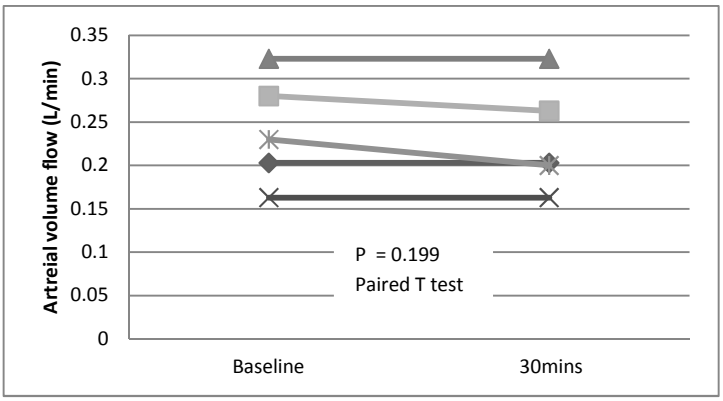
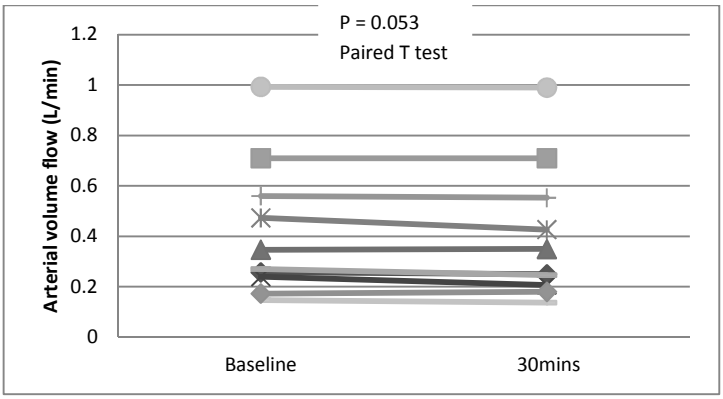


Figure 42: Control limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of device applied to limbs but not activated (n = 10)



5.3.2.2 Venous duplex

5.3.2.2.1 Max venous velocity:
Baseline maximum venous velocity in the active limbs was 14.29 (SD 1.37), passive limbs 13.17 (SD 2.14) and control limbs 15.51 (SD 1.85) cm/sec. ANOVA analysis determined the difference in baseline values was not statistically significant (P = 0.695). The mean change in maximum venous velocity in the active limbs was 6.86 (SD 5.71) (Paired T test P = 0.055), passive limbs 1.04 (SD 1.41) (Paired T test P = 0.174) and control limbs 0.219 (SD 1.76) cm/sec [Paired T test P = 0.702] equating to percentage changes of 52.64%, 9.70% and 1.22% respectively.

The difference in change from baseline between the active and passive groups was not statistically significant [Unpaired T Test: P = 0.063] whilst not for passive and control [Unpaired T test P = 0.383]. ANOVA analysis determined that the differences in the changes from baseline were significant (P = 0.004) and post hoc Bonferonni demonstrated: active versus passive P = 0.027; active versus control P = 0.004 and control versus passive P = 1.000 *(Figures 43-46)*.

Figure 43: Maximal venous velocity change from baseline

Error bar chart demonstrating the maximum venous velocity at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

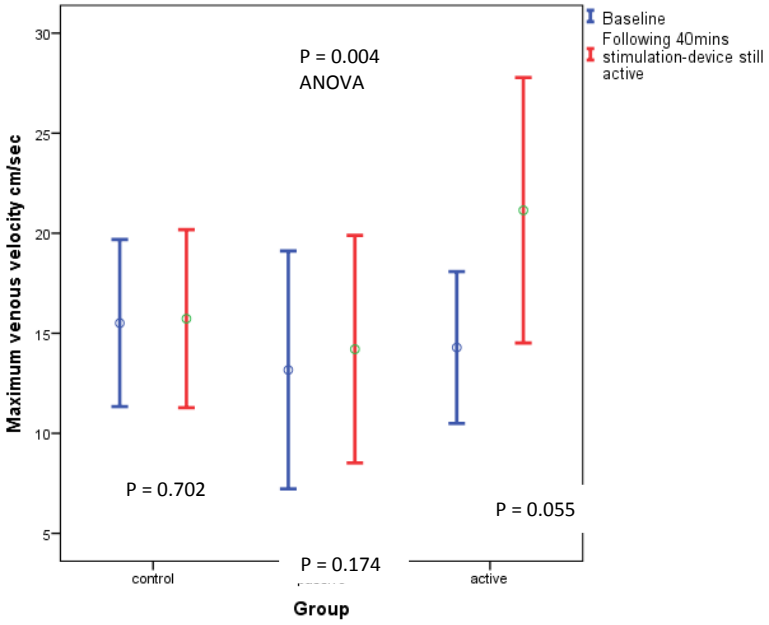


Figure 44: Active limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 5)

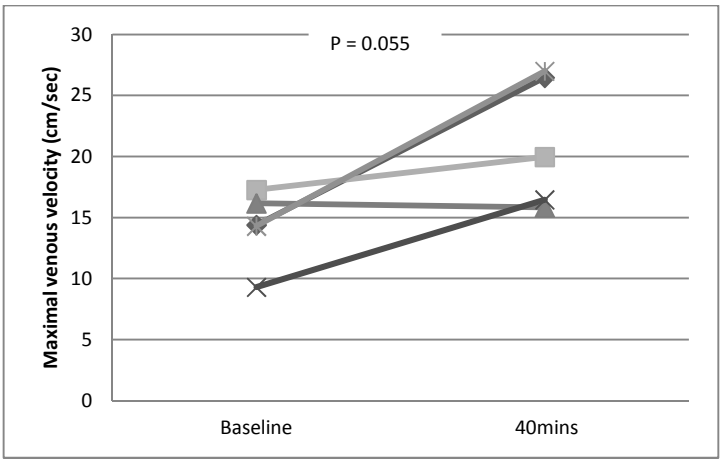


Figure 45: Passive limbs: Change in maximal venous velocity (cm/sec) from baseline with 40 minutes of stimulation of contralateral limb (n = 5)

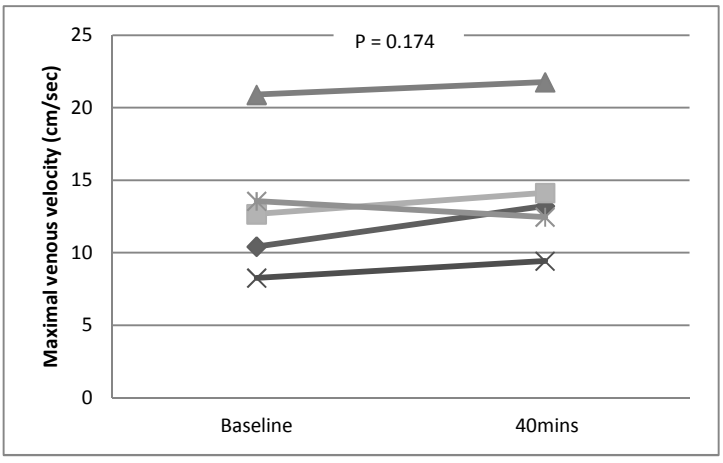
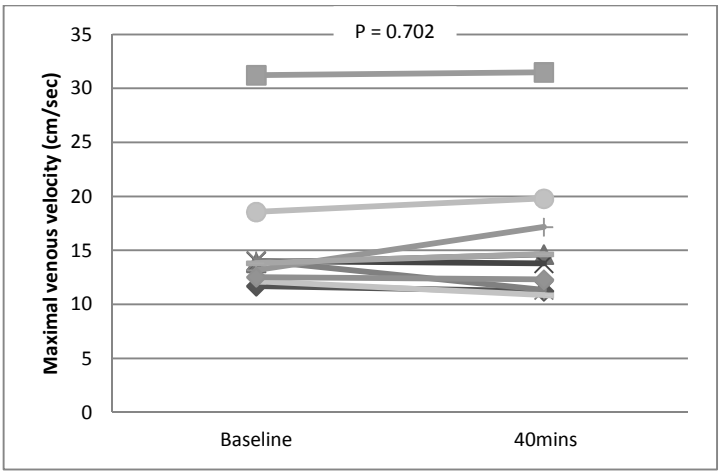


Figure 46: Control limbs: Change in maximal venous velocity (cm/sec) from baseline with 40 minutes of device applied to limbs but not activated (n = 10)



5.3.2.2.2 Mean venous velocity

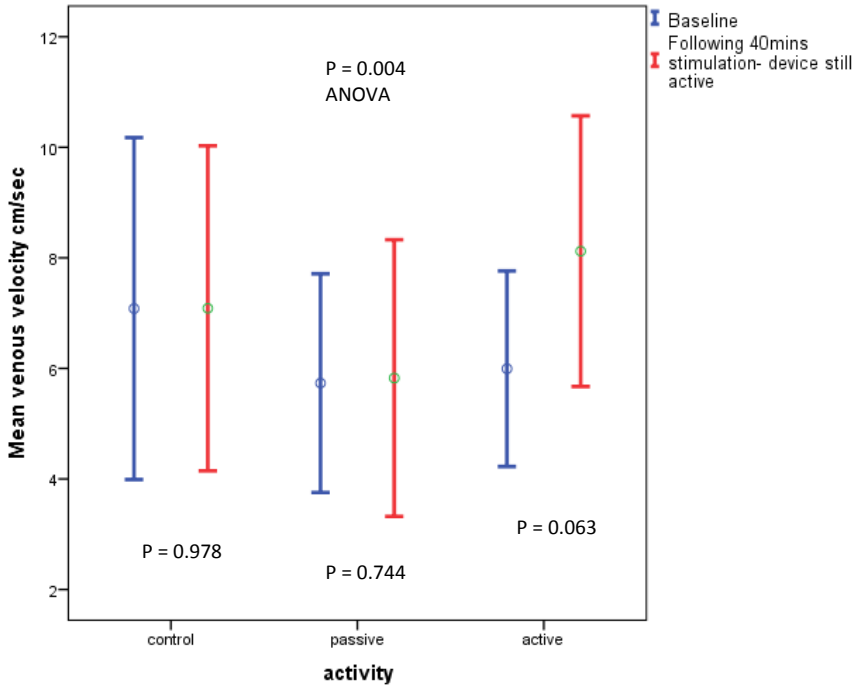
The baseline values for mean venous velocity in the active limbs was 5.99 (SD 1.43), passive limbs 5.73 (SD 1.59) and control limbs 7.08 (SD 4.32) cm/sec. ANOVA analysis determined that the difference in baseline values was not statistically significant (P = 0.712).
The change in mean venous velocity in the active limbs was 2.13 (SD 1.86) [Paired T test P = 0.063], passive 0.09 (SD 0.59) [Paired T test P = 0.744] and control limbs 0.00 (SD 0.56) cm/sec [Paired T test P = 0.978] equating to percentage changes of 38.38%, 0.53% and 1.12% respectively.

The difference in change from baseline between the groups was statistically significant (ANOVA P = 0.004). Post hoc Bonferonni demonstrated that: active versus passive P = 0.018; active versus control P = 0.005 and control versus passive P = 1.000 *(Figure 47)*.

Figure 47: Mean venous velocity change from baseline

Error bar chart demonstrating the mean venous velocity at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)



5.3.2.2.3 Venous diameter

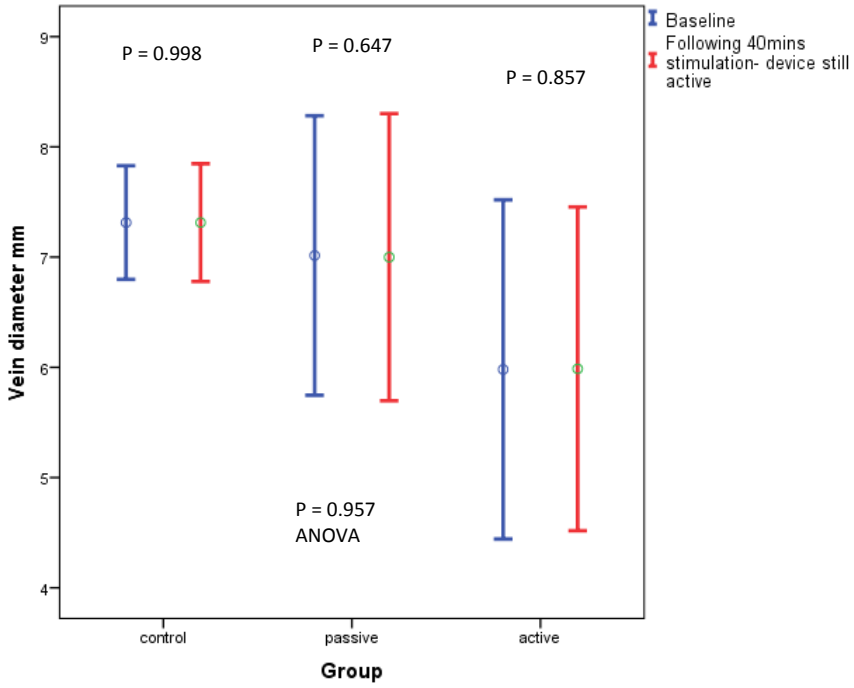
The baseline values for mean venous diameter were active 5.98 (SD 1.24), passive 7.01 (SD 1.02) and control 7.31 (SD 0.72) mm. ANOVA analysis determined that the difference in baseline values was not statistically significant [P = 0.057].The mean change in mean venous diameter was active limbs 0.006 (SD 0.07) [Paired T test P = 0.857], passive -0.15 (SD 0.07) [Paired T test P = 0.647] and control 0.000 (SD 0.15) mm [Paired T test P = 0.998] equating to percentage changes of 0.27%, 0.26% and -0.01% respectively.

The difference in change from baseline between the groups was not statistically significant (ANOVA P = 0.957) *(Figure 48)*.

Figure 48: Venous diameter change from baseline

Error bar chart demonstrating the venous diameter at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)



5.3.2.2.4 Venous volume flow

The baseline venous volume flow in the active limbs was 0.17 (SD 0.09), passive limbs 0.20 (SD 0.06) and control limbs 0.27 (SD 0.10) L/min. The difference in baseline values between the groups was not significant (ANOVA P = 0.147). The change in mean venous volume flow from baseline in the active limbs was 0.053 (SD 0.049) [P = 0.076 Paired T test], passive limbs -0.003 (SD 0.007) [P = 0.307 Paired T test] and control limbs 0.002 (SD 0.011) L/min [P = 0.559 Paired T test] equating to percentage changes of 41.87%, -1.20% and 1.04% respectively.

The difference in venous volume flow from baseline between the groups was statistically significant (ANOVA P = 0.004). Post hoc Bonferonni demonstrated: active versus passive P = 0.009; active versus control P = 0.007 and control versus passive P = 1.000 *(Figures 49- 52)*.

Figure 49: Venous volume flow change from baseline

Error bar chart demonstrating the venous volume flow at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

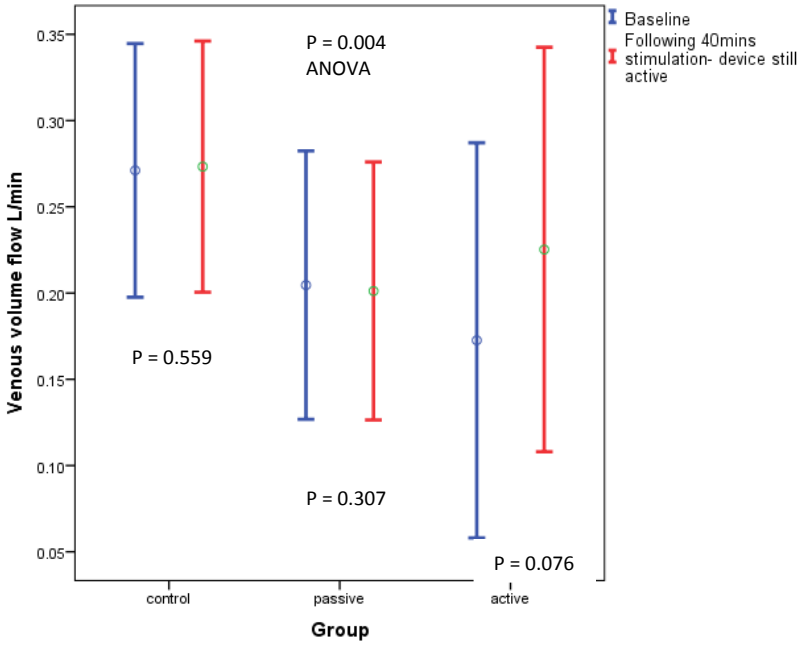


Figure 50: Active limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 5)

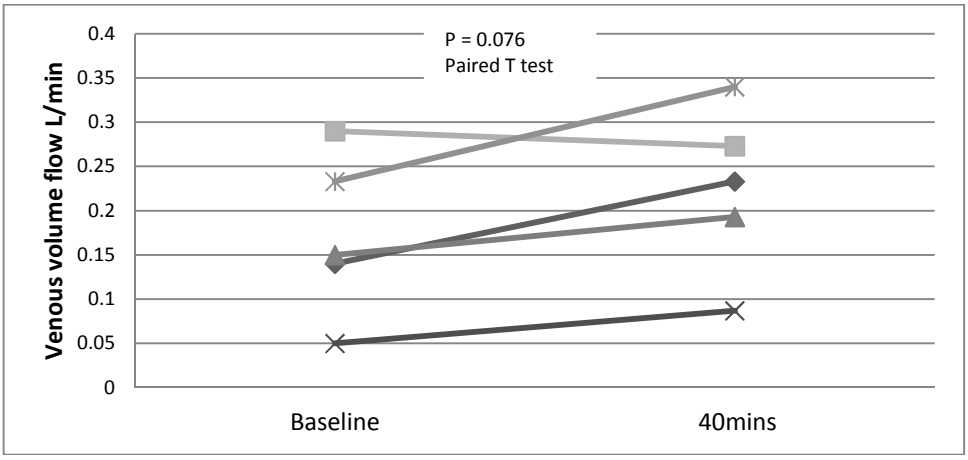


Figure 51: Passive limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation of contralateral limb (n = 5)

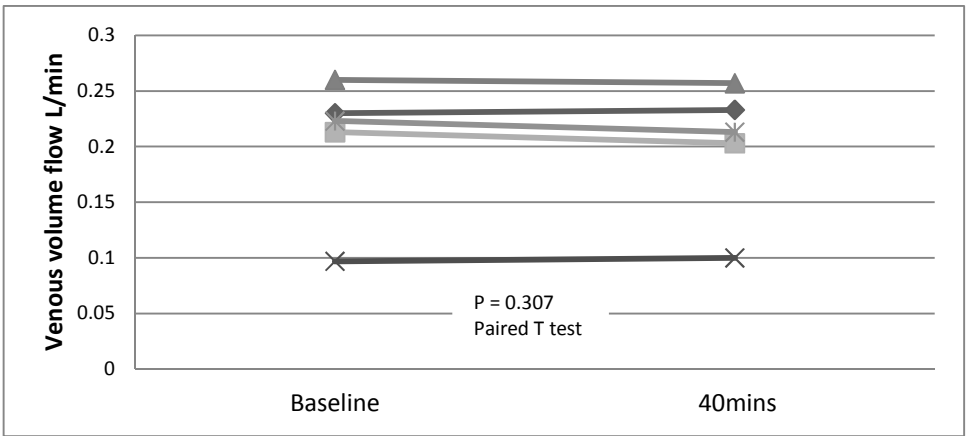
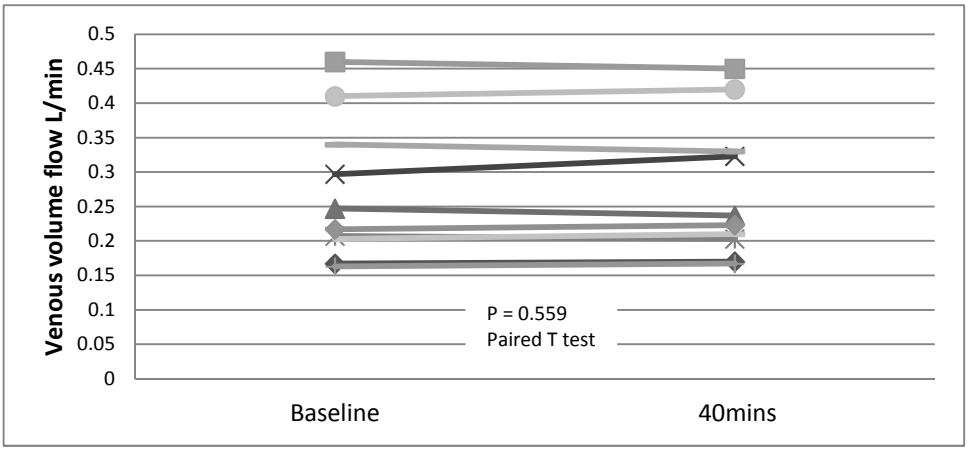


Figure 52: Control limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of device applied to limbs but not activated (n = 10)



5.3.2.3 Skin perfusion/ Laser Doppler flowmetry

There was no appreciable change in skin temperature during the study period. Baseline temperature in the active group was 29.16 (SD 2.10), passive group 29.38 (SD 1.79) and control group 27.62 (SD 1.48) ^{o}C. The difference in baseline values was not statistically significant [P = 0.127 ANOVA].

The change in temperature from baseline was 0.28 (SD 0.68)[P = 0.409 Paired T test], passive group 0.28 (SD 0.66) [P = 0.397 Paired T test] and control group -0.23 (SD 0.31) ^{o}C [P = 0.045 Paired T test]. The differences in change from baseline between the two groups was not statistically significant [P = 0.114 ANOVA].

Baseline microcirculatory flow in the active groups was 14.38 (SD 11.12), the passive group 18.76 (SD 8.44) and control group 16.68 (SD 8.10) flux units. The difference in baseline values seen in the groups was not statistically significant [P = 0.746 ANOVA]. The change at 15 minutes from baseline in the active group was 21.52 (SD 20.95) [P = 0.083 Paired T test], the passive group 3.36 (SD 12.20) [P = 0.571 Paired T test] and control group -3.94 (SD 8.08) [P = 0.157 Paired T test]. Analysis of the differences seen between the groups demonstrated statistical significance [P = 0.009 ANOVA].

The change at 30 minutes from baseline in the active groups was 25.88 (SD 19.23) [P = 0.040 Paired T test], passive 1.14 (SD 7.89) [P = 0.763 Paired T test] and control group -4.22 (SD 7.28) [P = 0.100 Paired T test]. Analysis of the differences in the changes seen was demonstrated to be statistically significant [P = 0.001 ANOVA].

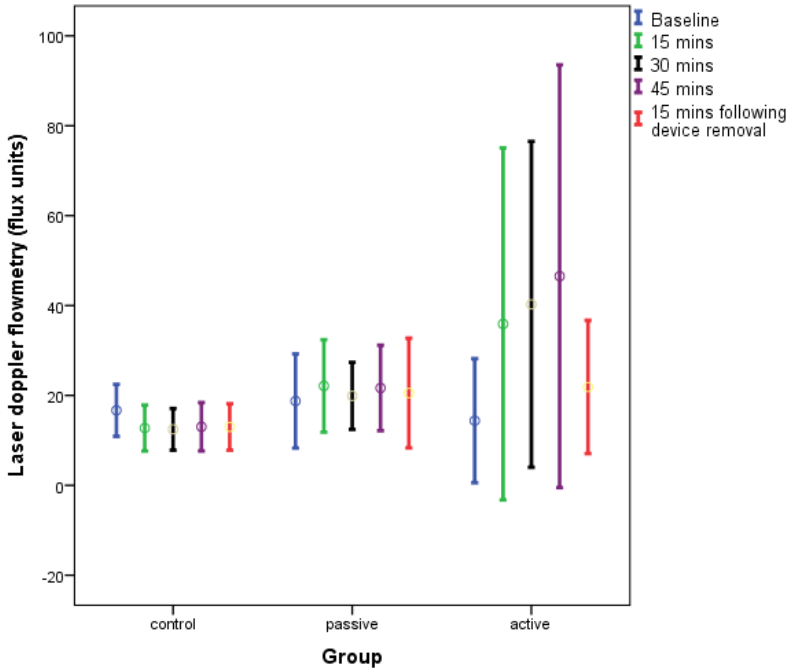
The change at 45 minutes from baseline in the active group was 32.14 (SD 27.42) [P = 0.059 Paired T test], passive 2.90 (SD 10.52) [P = 0.571 Paired T test] and control group -3.65 (SD 8.45) [P = 0.205 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance [P = 0.002 ANOVA].

The change at 15 minutes following device deactivation in the active groups was 7.50 (SD 6.00) [P = 0.049 Paired T test], passive 1.76 (SD 14.33) [P = 0.797 Paired T test] and control group -3.69 (SD 7.79) [P = 0.168 Paired T test]. Analysis of the differences in the changes seen demonstrated non-significance [P = 0.120 ANOVA] *(Figure 53)*.

Figure 53: Laser Doppler flowmetry change from baseline

Error bar graph demonstrating mean laser Doppler flowmetry readings through the study period in each group. [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)



5.3.2.4 Cardiovascular effects

The mean heart rate at baseline in the active group was 79.40 (SD 6.02) and 72.20 (SD 3.77) beats per minute in the passive group. Comparison of the baseline values demonstrated statistical significance [P = 0.101 Unpaired T test].
After 30 minutes of stimulation the heart rate decreased by 0.80 (SD 3.70) in the active group [P = 0.784 Paired T test] and in the control group 7.00 (SD 6.48) [P = 0.073 Paired T test]. Comparison of the changes demonstrated non-significance [P = 0.778 Unpaired T test].
The heart rate decreased by 1.80 (SD 3.70) [P = 0.338 Paired T test] in the active group and 1.20 (SD 1.92) in the control group [P = 0.235 Paired T test] following deactivation of the device. Unpaired T test analysis of the changes demonstrated non-significance [P = 0.122].

Systolic blood pressure at baseline in the active group was 138.20 (SD 13.33) and control 136.60 (SD 24.28) mmHg. Comparison of baseline values determined them to be comparable [P = 0.397 Unpaired T test]. Systolic BP increased at 30 minutes

by 5.90 (SD 8.92) in the active group [P = 0.213 Paired T test] and decreased by 0.50 (SD 16.16) in the control group [P = 0.948 Paired T test]. Comparison of the differences seen demonstrated insignificance [P = 0.371 Unpaired T test]. Systolic BP decreased from 30 minutes to the end of the study period in the active group by 4.90 (SD 13.79) [P = 0.472 Paired T test] and increased by 6.40 (SD 14.53) [P = 0.380 Paired T test] mmHg in the control group. Comparison of the differences seen demonstrated insignificance [P = 0.799 Unpaired T test].

Diastolic blood pressure at baseline in the active group was 80.40 (SD 7.70) and control 72.40 (SD 14.24) mmHg. Comparison of baseline values determined them to be comparable [P = 0.400 Unpaired T test]. Diastolic BP increased at 30 minutes by 0.10 (SD 4.14) in the active group [P = 0.960 Paired T test] and increased by 5.70 (SD 6.82) in the control group [P = 0.135 Paired T test]. Comparison of the differences seen demonstrated insignificance [P = 0.333 Unpaired T test]. Diastolic BP increased from 30minutes to the end of the study period in the active group by 1.20 (SD 2.86) [P = 0.402 Paired T test] and 2.30 (SD 8.07) [P = 0.558 Paired T test] mmHg in the control group. Comparison of the differences seen demonstrated insignificance [P = 0.058 Unpaired T test].

Chapter Six: Research question 4:

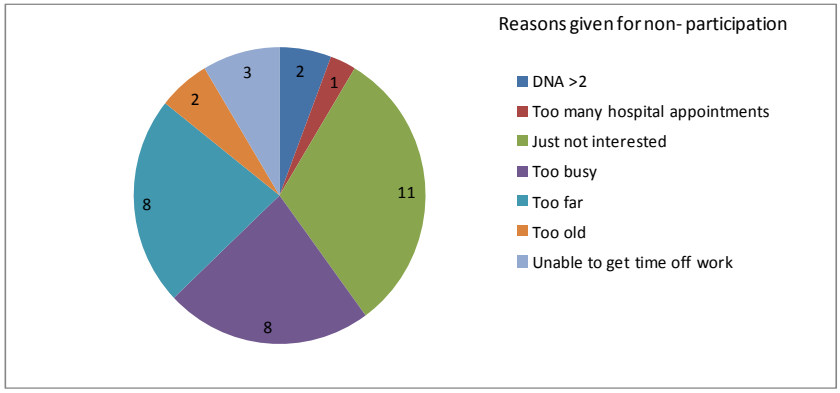
The haemodynamic efficacy of peroneal nerve electrical neuromuscular stimulation in patients following infra-inguinal bypass

6.1 Infra-inguinal bypass grafts Study population

A total of 71 potential participants were identified from out-patient clinics and the graft surveillance register. All were contacted by letter with an attached patient information sheet.

25 participated in the study. 11 patients were unable to participate in the study: 3 patients had no effect from the device, 3 patients' grafts were determined to have occluded when they attended for the study, 2 patients were participating in another study, 2 had undergone amputation at another institution, 3 patients suffered from severe dementia, 2 had ongoing issues with their wound, 1 had a recent MI and 1 patient had a permanent pacemaker. 35 patients were not willing to participate in the study.

Figure 54: Reason given for subject non-participation



6.2 Proof of concept study

6.2.1 Examination findings: ABPI

The mean ABPI at baseline in the active limbs was 0.99 (SD 0.24) and passive limbs 0.95 (SD 0.39). This was not statistically significant on unpaired T test (P = 0.896).

The mean change in ABPI from baseline in the active limbs was 0.05 (SD 0.13) [P = 0.074 Paired T test] and passive limbs was 0.05 (SD 0.07) [P = 0.017 Paired T test]. The difference between changes seen in the two groups was not statistically significant (P = 0.185 Unpaired T test) *(Figures 55,56)*.

Figure 55: Scatter graph: Active limbs; change in ABPI in study period (n = 19)

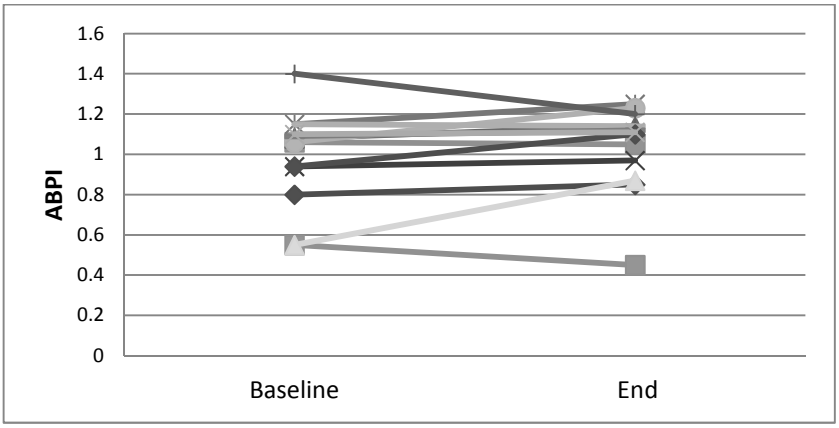
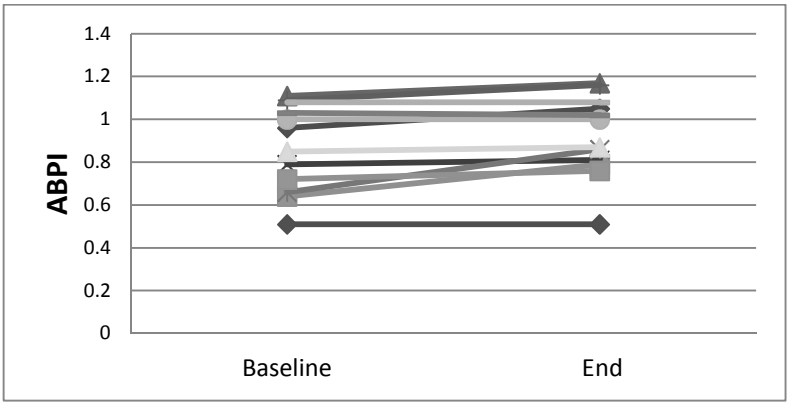


Figure 56: Scatter graph: Passive limbs; Change in ABPI in the study period (n = 19)



Calf circumference

The mean calf circumference at baseline in the active limbs was 35.51cm (SD 3.13) and passive limbs 34.56cm (SD 3.36).The difference was not statistically significant on Unpaired T test (P =0.892). The mean change in calf circumference from baseline was -0.73cm (SD 0.61) [P = 0.001 Paired T test] in the active limbs and -0.36cm (SD 0.64) [P = 0.077 Paired T test] in the passive limbs. The difference in the change seen was not statistically significant (P = 0.856 Unpaired T test).

6.2.2 Haemodynamic efficacy of geko^{TM} in infra-inguinal bypass grafts: Proof of concept study

6.2.2.1 Assessment of reliability of haemodynamic measurements

The intra-class correlation coefficient was utilised to determine the intra-rater reliability of the ultrasound measurements collected in the study(428). The intra-observer reliability assessed the reproducibility of the observer for each measurement technique *(Table 22)*. In this study, each measurement was made in triplicate and the mean utilised for all subsequent analysis.

Table 22: Intraclass correlation coefficient analysis of ultrasound measurements

Measurement	Intraclass correlation coefficient (P)
Arterial maximum flow velocity.	0.977
Arterial mean flow velocity	0.977
Arterial vessel diameter	0.998
Arterial volume flow	0.991
Venous maximum flow velocity	0.915
Venous mean flow velocity	0.975
Venous vessel diameter	0.999
Venous volume flow	0.992

Thus it is possible to see that there was high intra-rater reliability for all ultrasound measurements performed as part of this study within the infra-inguinal bypass population.

6.2.2.2 Arterial duplex

6.2.2.2.1 Maximum arterial velocity

The mean baseline maximal arterial velocity in the active limbs was 76.78 (SD 27.71) and passive limbs 67.61 (SD 17.65) cm/sec. Comparison of the groups demonstrated no statistical significance (P = 0.324 Unpaired T test) in the baseline values. The change from baseline in the groups were 10.78 (SD 11.37) [P = 0.005 Paired T test] and 1.43 (SD 3.93) cm/sec [P = 0.212 Paired T test] in the active and passive groups respectively equating to percentage changes of 15.60% (SD 15.65) and 1.31% (SD 5.77). The differences in the change seen between the active and passive limbs were statistically significant (P = 0.010 Unpaired T test) *(Figures 57, 58, 58)*.

Figure 57: Maximal arterial velocity change from baseline

Error bar chart demonstrating the maximal arterial velocity and at baseline at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)

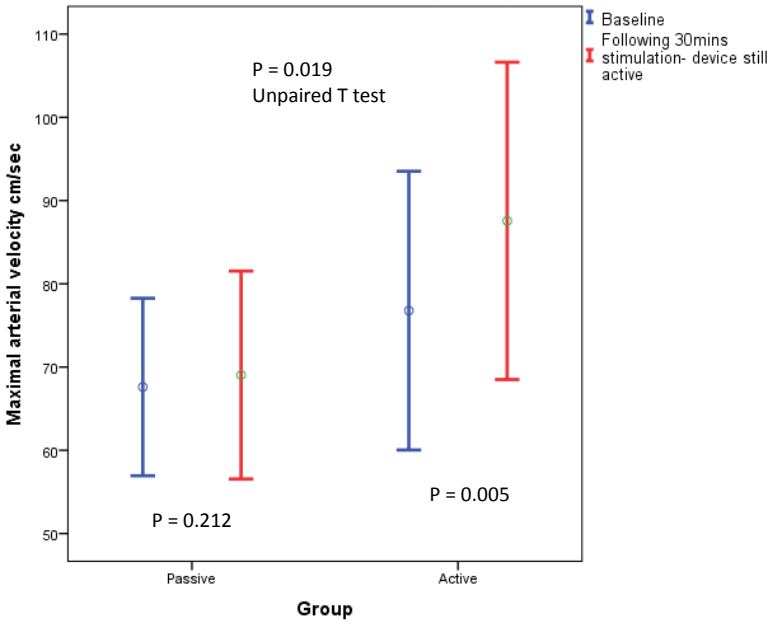


Figure 58: Active stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 19)

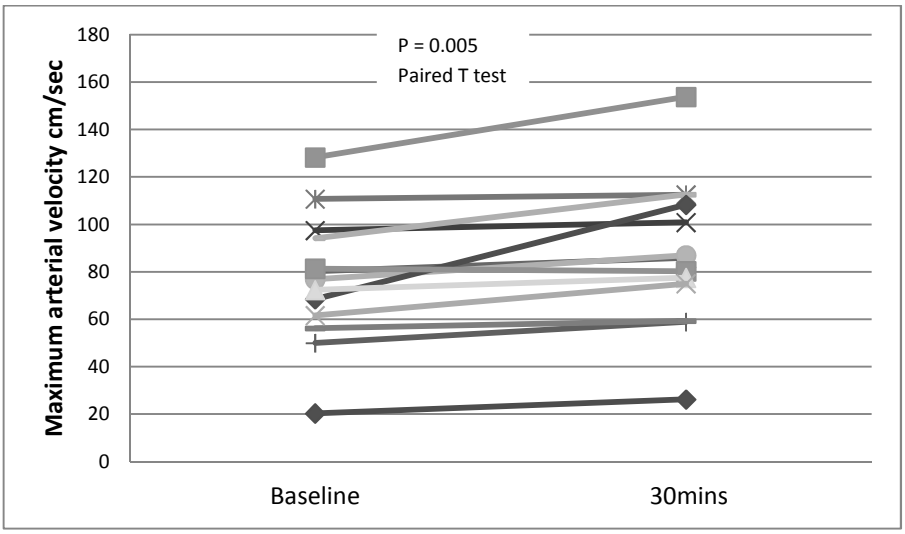
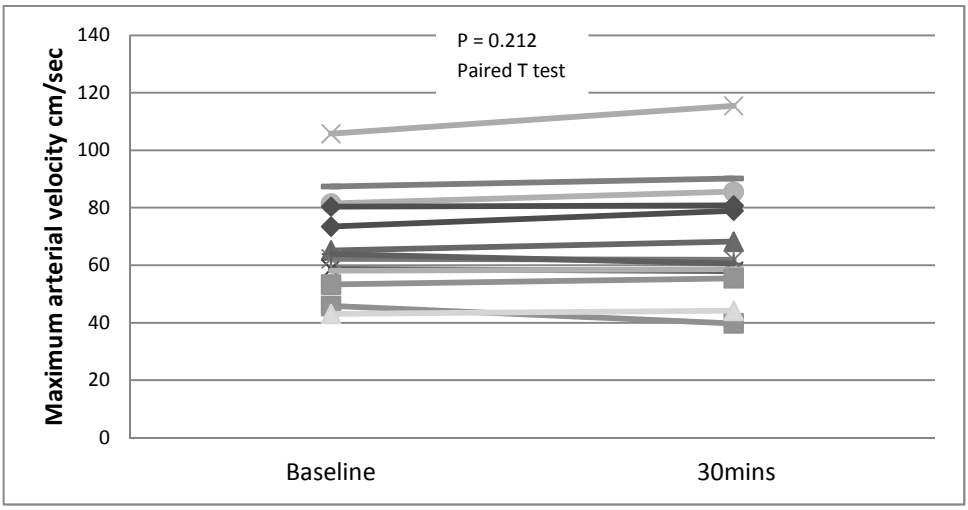


Figure 59: Passive stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 19)



6.2.2.2.2 Mean arterial velocity

The baseline mean arterial velocity in the active limbs was 10.88 (SD 5.36) and passive limbs 10.44 (SD 3.35) cm/sec. The difference between the limbs was not statistically significant (P = 0.805 Unpaired T test).
The change from baseline with the device was 2.68 (SD 3.34) [P= 0.014 Paired T test] and passive limbs -0.10 (SD 1.28) cm/sec [P = 0.774 Paired T test] in the active and passive limbs respectively equating to percentage changes of 29.93% (SD 33.38) and -0.88% (SD 11.26) respectively. The difference in the changes seen between the groups was statistically significant (P = 0.013 Unpaired T test) *(Figure 60, 61, 62)*.

Figure 60: Mean arterial velocity change from baseline

Error bar chart demonstrating the mean arterial velocity at baseline and at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)

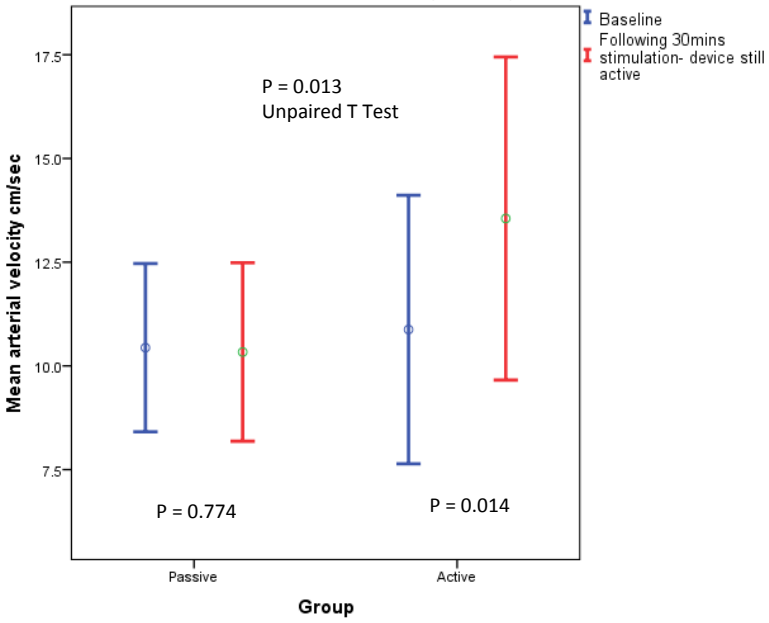


Figure 61: Active stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 19)

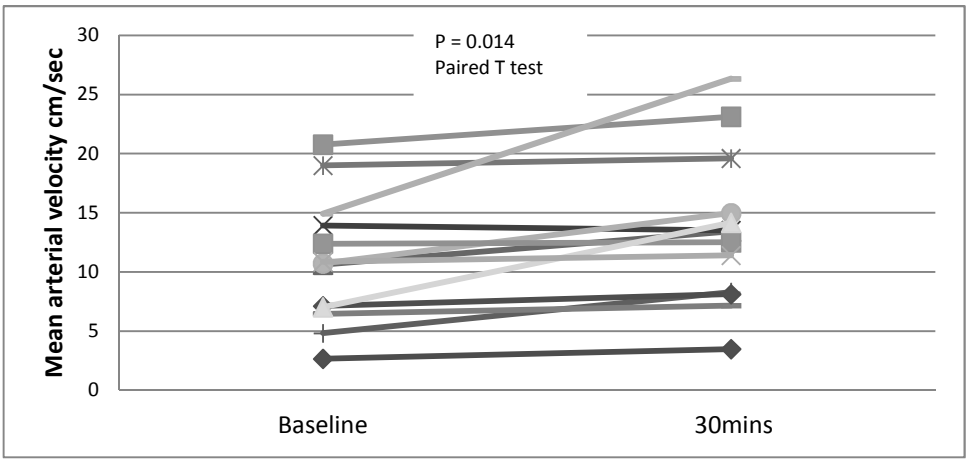
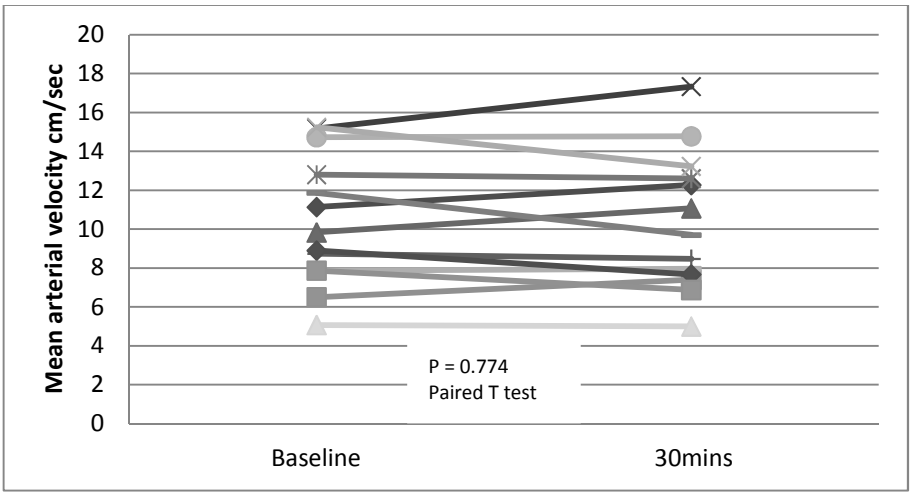


Figure 62: Passive stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 19)



6.2.2.2.3 Arterial diameter

The mean arterial diameter at baseline in the active limbs 6.08 (SD 1.48) and passive limbs 5.86 (SD 0.87) mm. The difference between the baseline value in the groups was not statistically significant (P = 0.654 Unpaired T test). The change from baseline with stimulation was 0.019 (SD 0.06) [P = 0.291 Paired T test] and -0.009 (SD 0.03) mm [P = 0.273 Paired T test] in the active and passive limbs respectively equating to percentage changes of 0.40% (SD 1.13) and -0.16% (SD 0.52). Unpaired T test analysis demonstrated this difference in the change between the limbs was not statistically significant (P = 0.151).

5.2.2.2.4 Arterial volume flow

The mean arterial flow at baseline in the active limbs was 0.29 (SD 0.17) and passive limbs 0.27 (SD 0.14) L/min. Flow at baseline was comparable between the two groups (P = 0.777 Unpaired T test). The change from baseline with stimulation was 0.07 (SD 0.06) [P = 0.001 Paired T test] and 0.00 (SD 0.01) L/min [P = 0.485 Paired T test] in the active and passive limbs respectively equating to percentage changes of 29.8% (SD 24.21) and -0.22% (SD 2.81). Unpaired T test analysis demonstrated that the difference in the change seen between the active and passive limbs was statistically significant (P < 0.001) *(Figure 63-65)*.

Figure 63: Arterial volume flow change from baseline

Error bar chart demonstrating the arterial volume flow at baseline and at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)

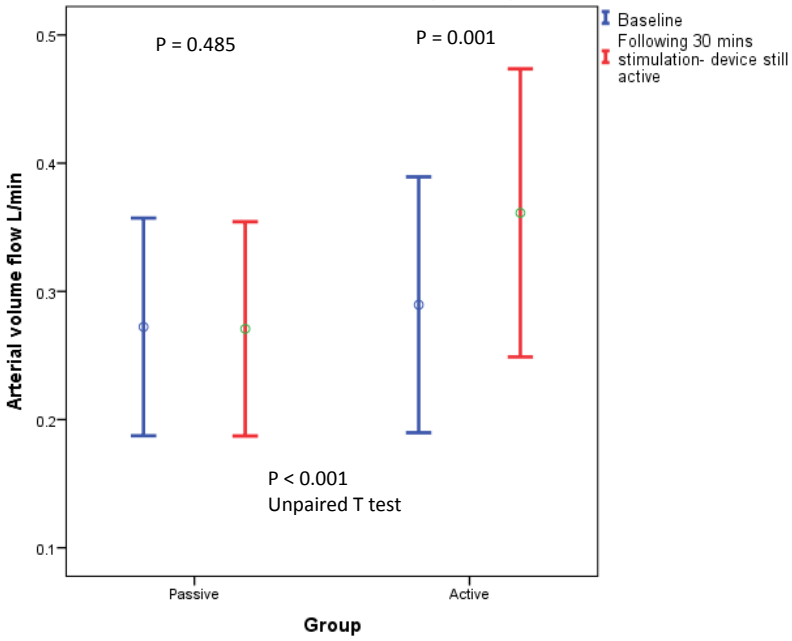


Figure 64: Active stimulation limbs: Change arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 19)

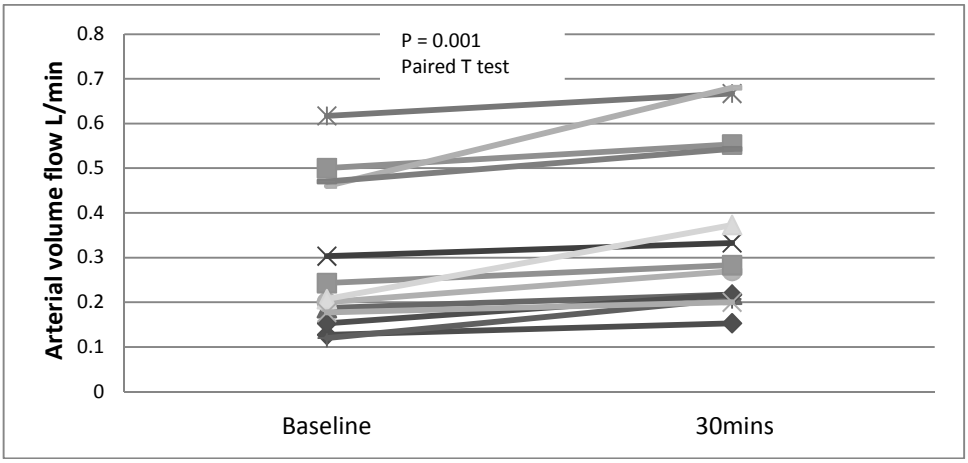
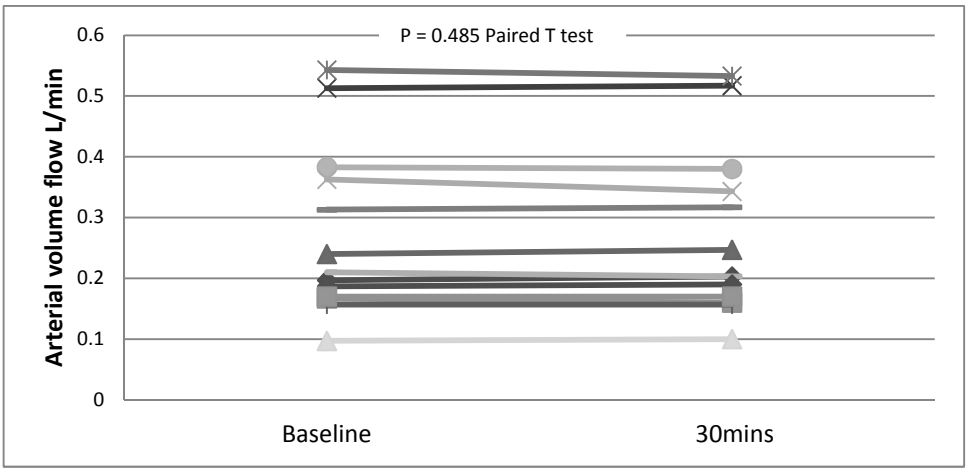


Figure 65: Passive stimulation limbs: Change arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 19)



6.2.2.3 Venous duplex

6.2.2.3.1 Venous maximum velocity

The baseline mean maximum venous velocity in the active limbs was 16.14 (SD 7.04) and the passive limbs 15.98 (SD 6.94) cm/sec. Comparison of the baseline values demonstrated the differences between the groups to be insignificant (P = 0.953 Unpaired T test).

The change from baseline with stimulation in the active limbs was 3.49 (SD 6.20) [P = 0.065 Paired T test] and passive limbs -1.09 (SD 2.57) cm/sec [P = 0.153 Paired T test], equating to percentage changes of 28.42% (SD 48.20) and -5.83% (SD 15.64). Comparison of the changes from baseline in the 2 groups demonstrated that this was statistically significant (P = 0.021 Unpaired T test) *(Figures 66-68)*.

Figure 66: Maximal venous velocity change from baseline

Error bar chart demonstrating the maximal venous velocity at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)

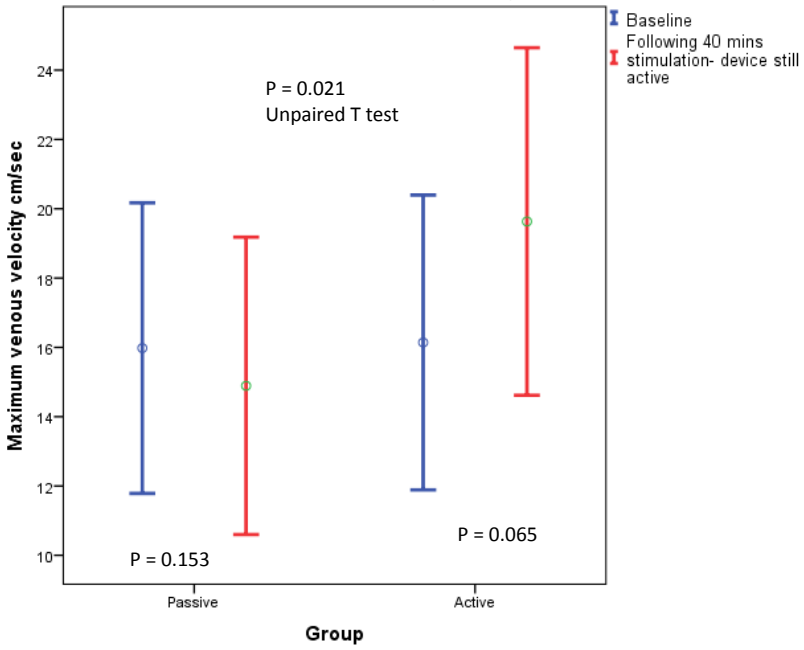


Figure 67: Active stimulation limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 19)

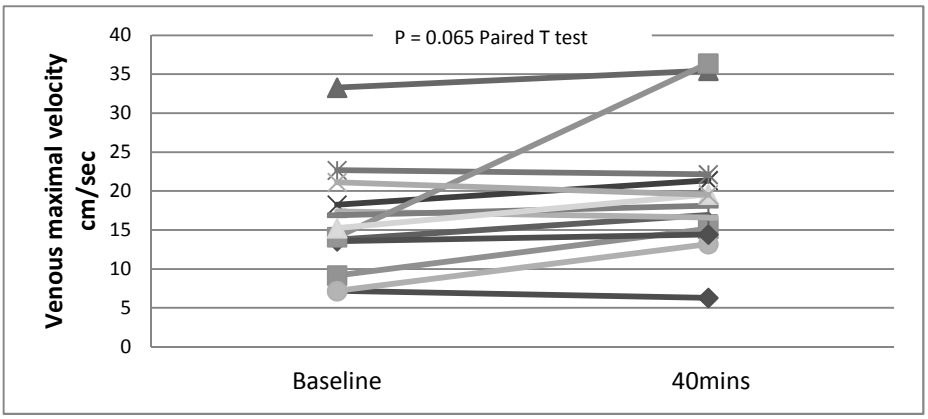
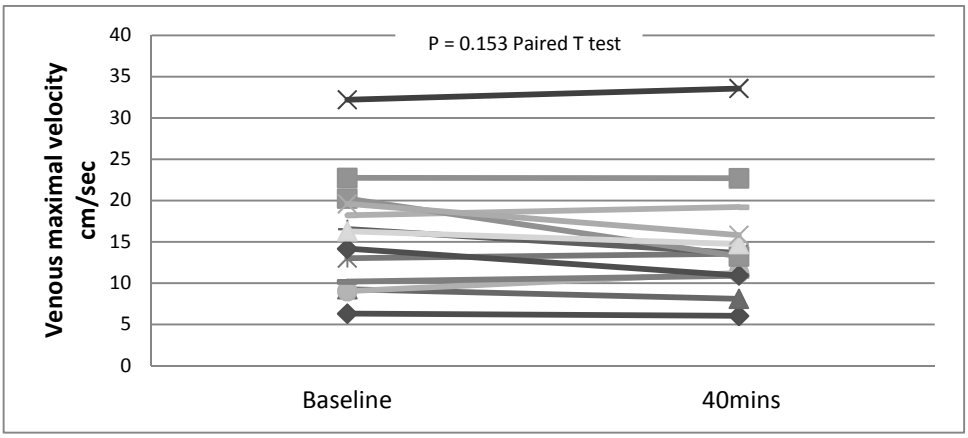


Figure 68: Passive stimulation limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 19)



6.2.2.3.2 Venous mean velocity

The average mean velocity in the active limbs at baseline was 6.57 (SD 3.54) and the passive limbs 5.45 (SD 2.52) cm/sec. Comparison of the baseline values demonstrated the difference to be insignificant (P = 0.364 Unpaired T test). The change from baseline with stimulation in the active limbs was 0.95 (SD 0.86) [P = 0.002 Paired T test] and passive -0.01 (SD 0.70) cm/sec [P = 0.943 Paired T test] equating to percentage changes of 19.63% (SD 21.74) and 0.79% (SD 15.28). Comparison of the changes from baseline in the active and passive groups demonstrated that that the differences were statistically significant (P = 0.005 Unpaired T test) *(Figures 69-71)*.

Figure 69: Mean venous velocity change from baseline

Error bar chart demonstrating the mean venous velocity at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)

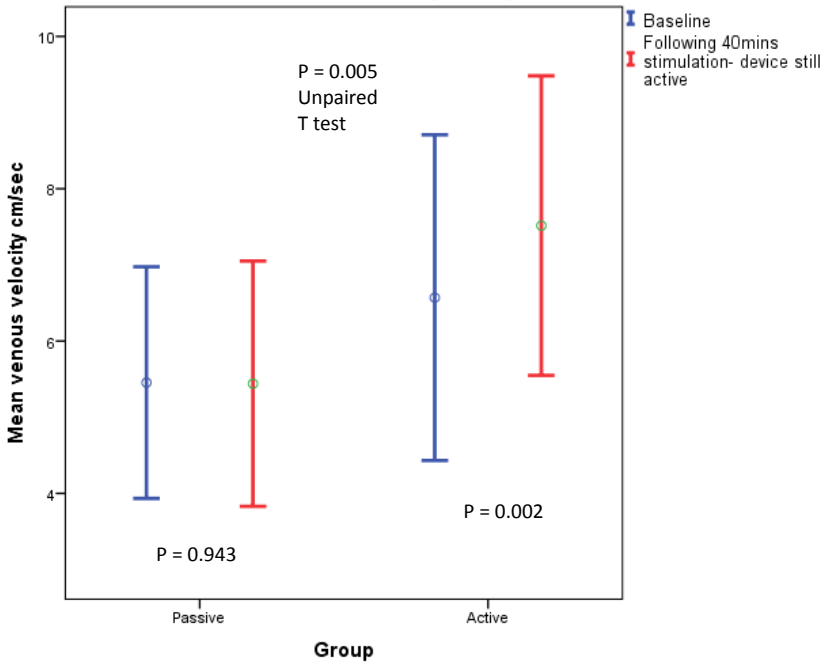


Figure 70: Active stimulation limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 19)

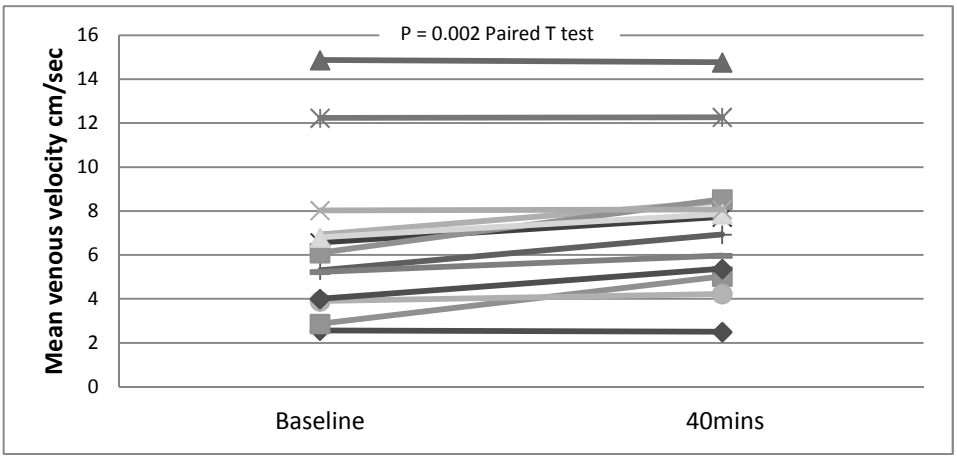
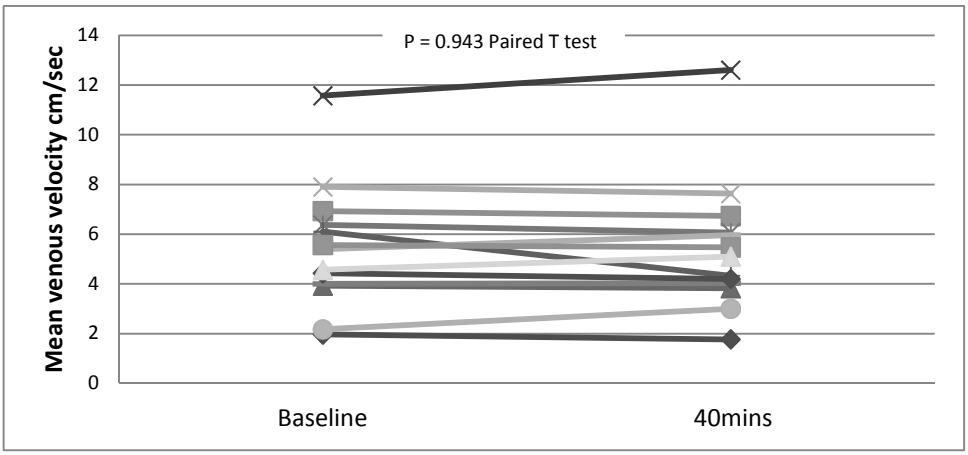


Figure 71: Passive stimulation limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 19)



6.2.2.3.3 Venous diameter

The average venous diameter at baseline in the active limbs was 6.36 (SD 1.46) and the passive limbs 5.57 (SD 1.49) mm. Comparison of the baseline value between the groups demonstrated an insignificant difference (P = 0.183 Unpaired T test). The change from baseline with stimulation in the active limbs was 0.04 (SD 0.05) [P = 0.011 Paired T test] and passive -0.02 (SD 0.07) mm [P = 0.397 Paired T test] equating to percentage changes of 0.67% (SD 0.86) and -0.19% (SD 1.11). The difference in change from baseline in the 2 groups was not statistically significant (P = 0.019 Unpaired T test)(*Figure 72-74*).

Figure 72: Venous diameter change from baseline

Error bar chart demonstrating the venous diameter at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)

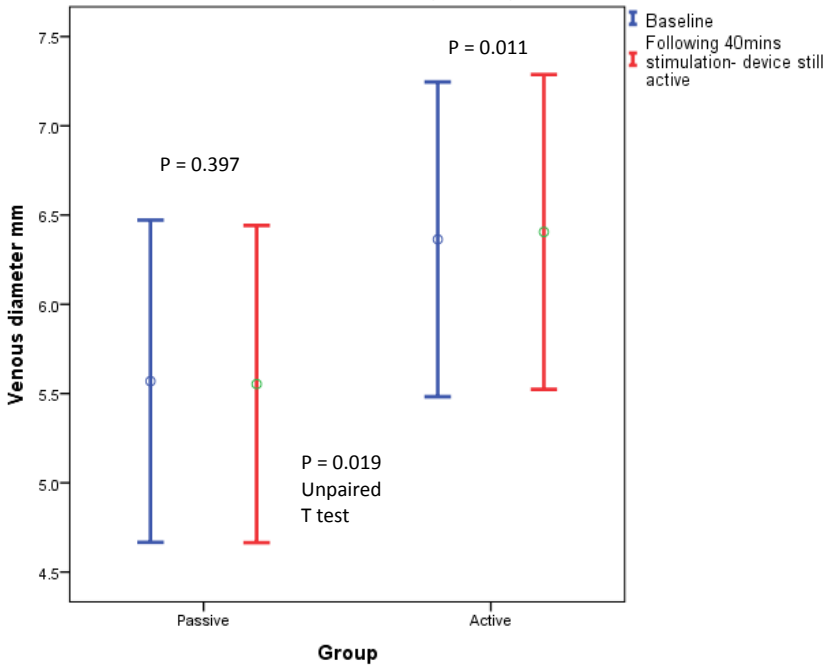


Figure 73: Active stimulation limbs: Change in mean venous diameter (mm) from baseline with 40 minutes of stimulation (n = 19)

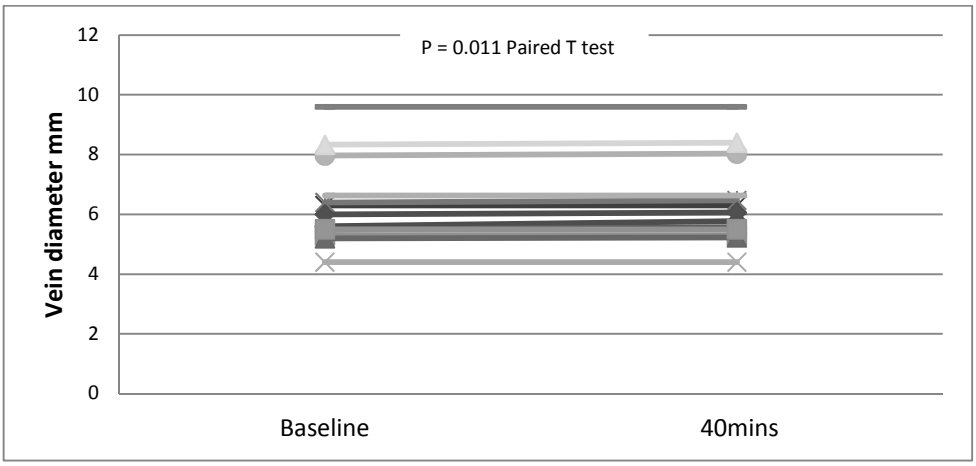
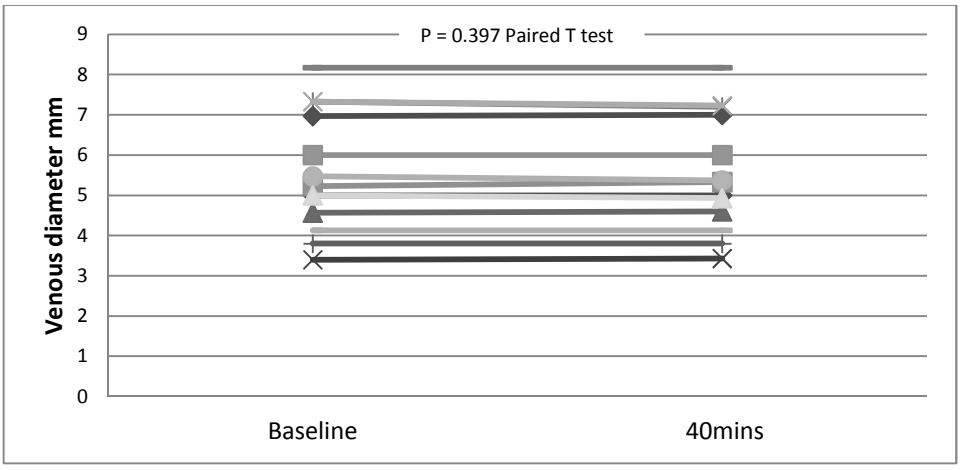


Figure 74: Passive stimulation limbs: Change in mean venous diameter (mm) from baseline with 40 minutes of stimulation (n = 19)



6.2.2.3.4 Venous volume flow

The mean volume flow at baseline in the active limbs was 0.19 (SD 0.10) and the passive limbs 0.13 (SD 0.08) L/min. Comparison of the baseline values demonstrated an insignificant difference (P = 0.087 Unpaired T Test). The change from baseline with stimulation in the active limbs was 0.03 (SD 0.02) [P < 0.001 Paired T test] and passive 0.00 (SD 0.01) L/min [P = 0.736 Paired T test] equating to percentage changes of 21.77% (SD 21.15) and 0.36% (SD 9.48). Comparison of the difference in change from baseline in the 2 groups was statistically significant [P < 0.001 Unpaired T test] *(Figures 75-77)*.

Figure 75: Venous volume flow change from baseline

Error bar chart demonstrating the venous volume flow at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)

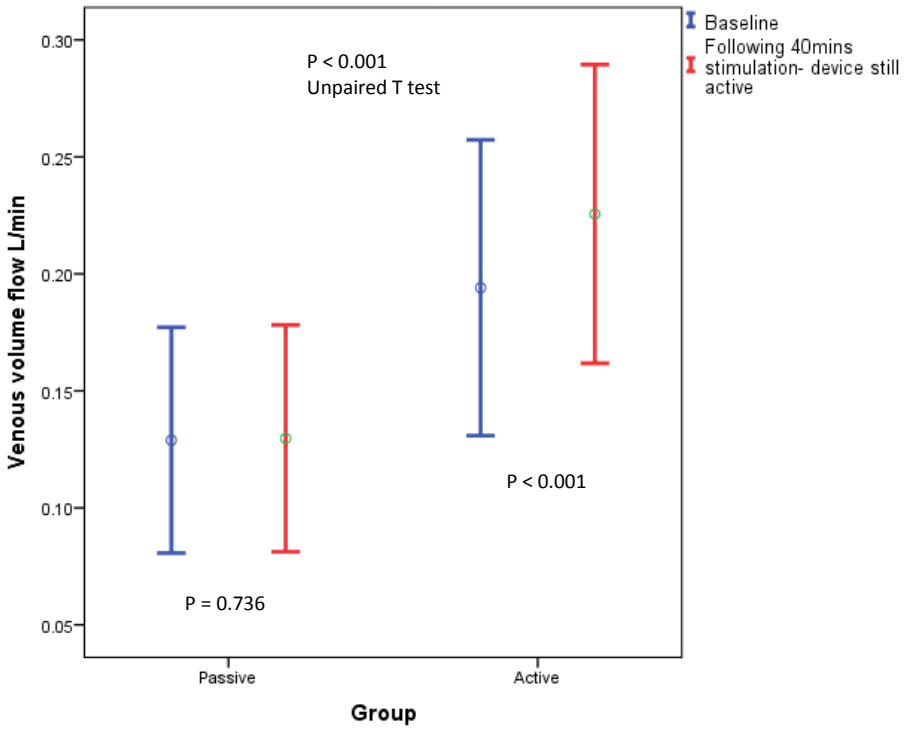


Figure 76: Active stimulation limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 19)

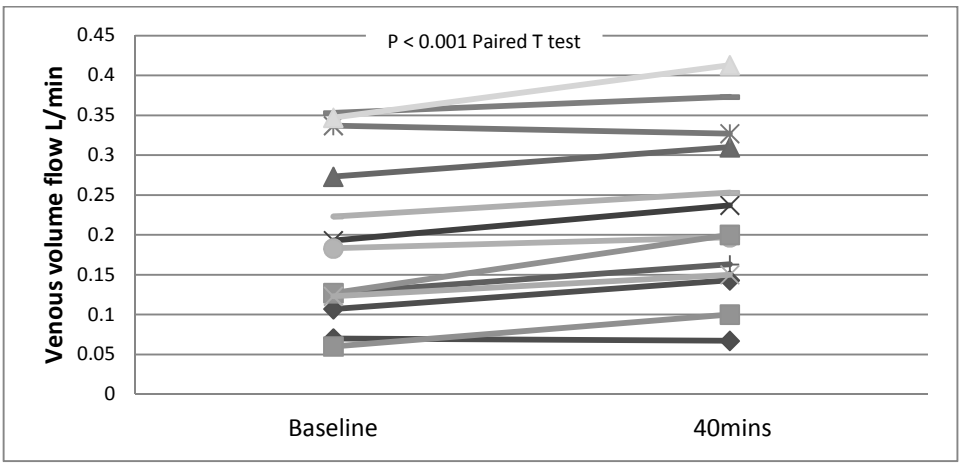
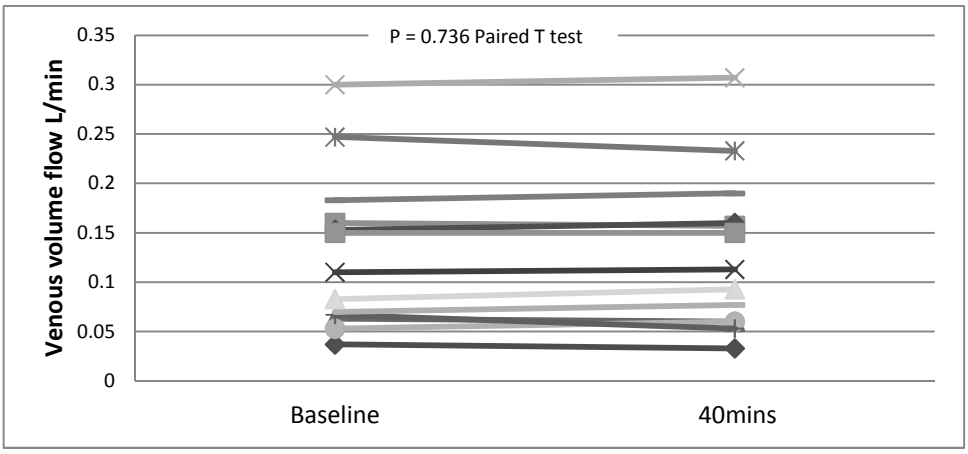


Figure 77: Passive stimulation limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 19)



6.2.2.4 Skin perfusion/ Laser Doppler flowmetry

There was no appreciable change in skin temperature during the study period. Baseline temperature in the active group was 29.66 (SD 1.63) and passive group 29.28 (SD 1.76)^{o}C. The difference in baseline values was not statistically significant (P = 0.569 Unpaired T test).
The change in temperature from baseline was 0.39 (SD 1.45)[P = 0.347 Paired T test], passive group 0.11 (SD 0.91) ^{o}C [P = 0.677 Paired T test]. The differences in change from baseline between the two groups was not statistically significant (P = 0.554 Unpaired T test).

Baseline microcirculatory flow in the active groups was 24.13 (SD 10.78) and the passive group 17.38 (SD 5.81). The difference in baseline values seen in the groups was statistically significant (P = 0.059). The change at 15 minutes from baseline in the active group was 18.85 (SD 12.42) [P < 0.001 Paired T test], in the passive group was 0.05 (SD 3.02) [P = 0.950 Paired T test]. Analysis of the differences seen between the active and passive groups demonstrated statistical significance (P < 0.001 Unpaired T test).
The change at 30 minutes from baseline in the active groups was 18.87 (SD 13.44) [P < 0.001 Paired T test] and passive -0.42 (SD 4.58) [P = 0.745 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance (P < 0.001 Unpaired T test).

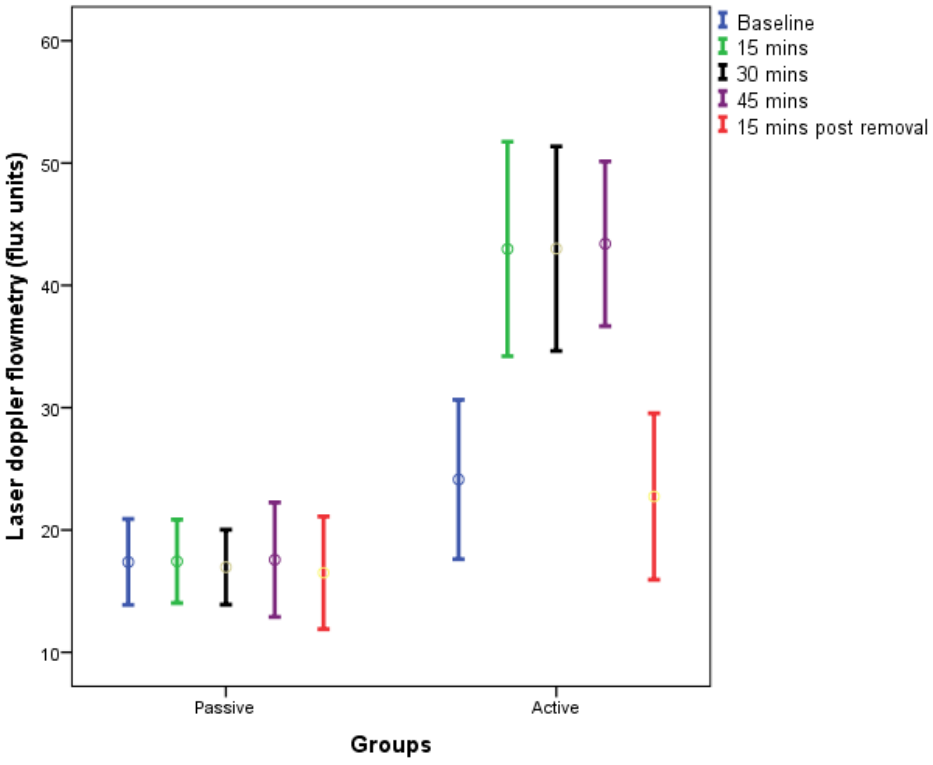
The change at 45 minutes from baseline in the active group was 19.27 (SD 10.19) [P < 0.001 Paired T test] and passive 0.18 (SD 6.39) [P = 0.919 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance (P < 0.001 Unpaired T test).

The change at 15 minutes following device deactivation in the active groups was -1.39 (SD .38) [P = 0.163 Paired T test] and passive -0.88 (SD 3.51) [P = 0.381 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance (P = 0.710 Unpaired T test) *(Figure 78)*.

Figure 78: Laser Doppler flow change from baseline

Error bar chart demonstrating laser Doppler flowmetry readings throughout the study period in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 19)
Passive: contralateral limb to active limbs (n = 19)



6.2.2.5 Cardiovascular effects

The mean heart rate at baseline was 75.77 (SD 11.88) and decreased to 74.00 (SD 12.99) 30 minutes after initiation of stimulation [P = 0.128 Paired T test]. The heart

rate decreased to 72.62 (SD 10.75) following deactivation of the device [P = 0.246 Paired T test].
There was no significant change in systolic blood pressure on device activation [P = 0.660 Paired T test] or deactivation [P = 0.007 Paired T test]. There was no significant change in diastolic blood pressure on device activation [P = 0.329 Paired T test] or deactivation [P = 0.119 Paired T test].

6.3 Randomised patients

6.3.1 Baseline Characteristics

The final 12 participants were randomised to either active stimulation (i.e. one limb received active stimulation and other limb deemed passive) or a control group (i.e. stimulation device applied but not activated). Statistical analysis of the patients in the active and control groups was carried out to establish if they were comparable *(Tables 23, 24, 25)*.

Table 23: Comparison of the baseline characteristics of participants in the active and control groups

	Active n=6	Control n=6	p value
Age (mean in yrs)	63.5 (IQR 18.75)	68.5 (IQR 28.25)	0.567*
Sex	6M	6M	1.000^
Smoking status	3 current, ex	1 current, 5 ex	0.545~
Pack years (mean)	33.29 (SD 22.26)	28.83 (SD 7.70)	0.653^
EtOH	5 current, 1 never	3 current, 1 never	0.545~
EtOH units/ week (mean)	16.67 (SD 14.02)	6.33 (SD 7.23)	0.140^
BMI (mean)	26.30 (SD 3.26)	25.78 (SD 2.45)	0.763^
Time since surgery (mean in months)	20.33 (SD 5.89)	13.67 (10.82)	0.214^
Laterality	3 right, 3 left	4 left, 2 right	0.500~
Indication	5 claud, 1 CLI	5 claud, 1 CLI	1.000~

* Independent samples median test

^ Unpaired T test (NB Shapiro Wilk test utilised to establish normality of distribution for data. P> 0.05 in all cases)

~ Fishers exact test

Table 24: Comparison of comorbidities

	Active	Control	P value
Diabetes	5 No, 1 Yes	5 No, 1 Yes	1.000~
Cerebrovascular disease	6 No	4 No, 2 Yes	0.227~
Hypertension	2 Yes, 4 No	5 Yes, 1 No	0.121~
Cardiovascular disease	1 Yes, 5 No	4 Yes, 2 No	0.121~
Respiratory	6 No	6 No	1.000~

~Fisher's exact test

Table 25: Comparison of concomitant medications between active and control groups

	Active	Control	P value
Antiplatelet	6 Yes	5 Yes, 1 No	0.500~
Aspirin	5 Yes, 1 No	4 Yes, 2 No	0.500~
Clopidogrel	1 Yes, 5 No	1 Yes, 5 No	0.773~
Beta blocker	1 Yes, 5 No	4 Yes, 2 No	0.121~
ACE inhibitor	6 No	4 Yes, 2 No	0.061~
Statin	6 Yes	5 Yes, 1 No	0.500~

~Fisher's exact test

It is possible to see that the groups of patients, active and control, were similar in all regards.

6.3.2 Randomised patients: Haemodynamic Efficacy of geko^{TM} in infrainguinal bypass

6.3.2.1 Arterial duplex

6.3.1.1.1 Maximum arterial velocity

The baseline maximum arterial velocity was 74.40 (SD 31.43) in the active limbs, 72.60 (SD 11.11) in the passive limbs and 71.20 (SD 26.59) cm/sec in the control limbs [P = 0.968 ANOVA]. The mean change in maximum arterial velocity from baseline in the active limbs was 15.39 (SD 7.44) (Paired T test P = 0.004); passive -1.69 (SD 8.81) [Paired T test P = 0.659] and control limbs -0.28 (SD 4.24) cm/sec [Paired T test P = 0.825] equating to percentage changes of 23.54% (SD 13.86), -1.84% (SD 12.47) and -1.28% (SD 6.22) respectively *(Figures 23-26)*.

The difference in change from baseline between the active and passive groups was statistically significant [Unpaired T Test: P = 0.005] whilst not significant when comparing the passive and control limbs, P = 0.647 [Unpaired T Test]. ANOVA

analysis demonstrated statistical significance P < 0.001, which was further investigated with post hoc Bonferonni analysis: active versus passive limbs P < 0.001, active versus control limbs P < 0.001 and control versus passive limbs P = 1.000. *(Figures 79-81)*

Figure 79: Maximum arterial velocity change from baseline

Error bar chart demonstrating the maximum arterial velocity and at baseline at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)

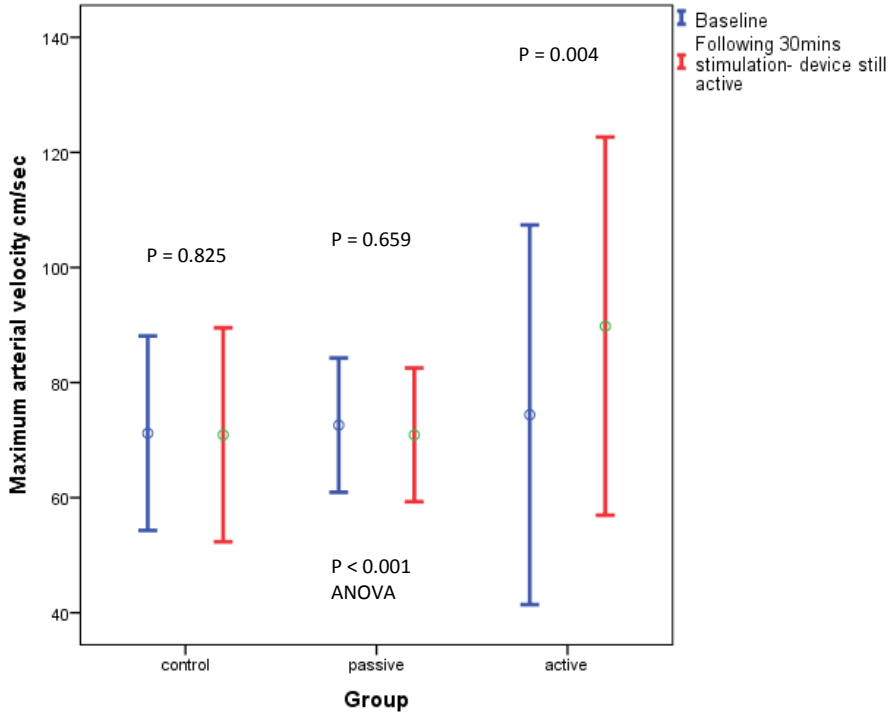


Figure 80: Active stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 6)

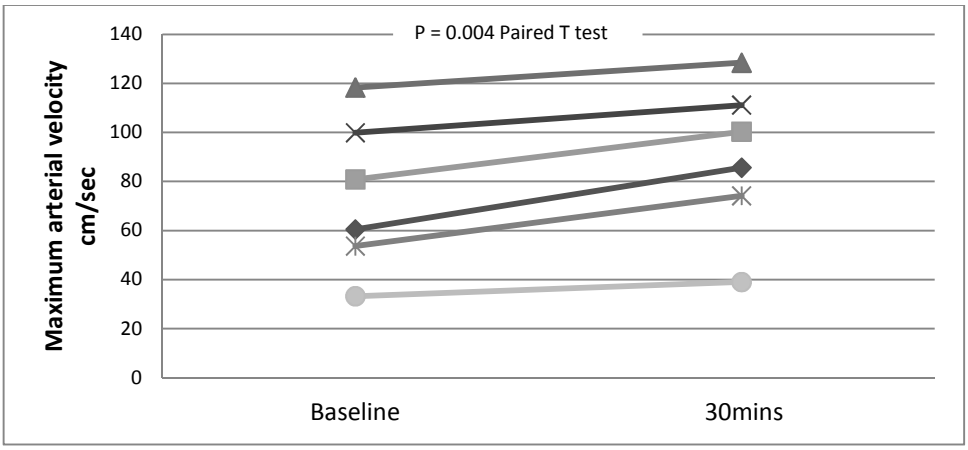


Figure 81: Passive limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation of contralateral limb (n = 6)

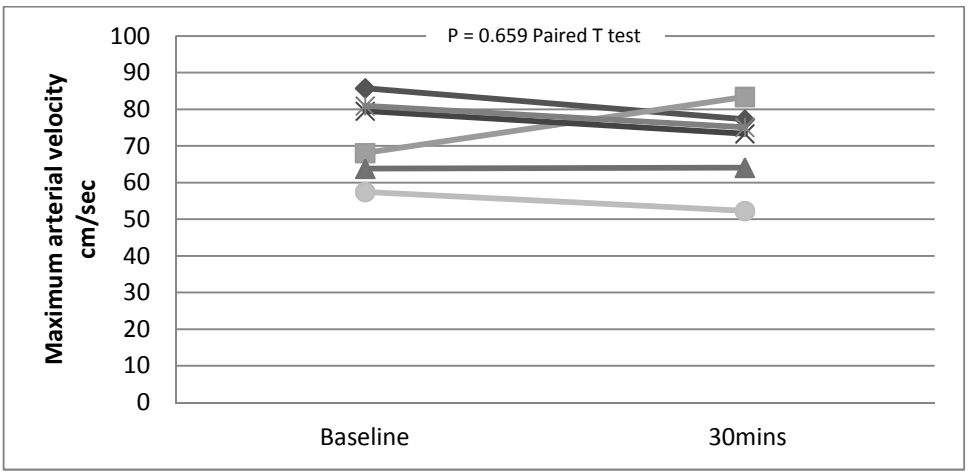
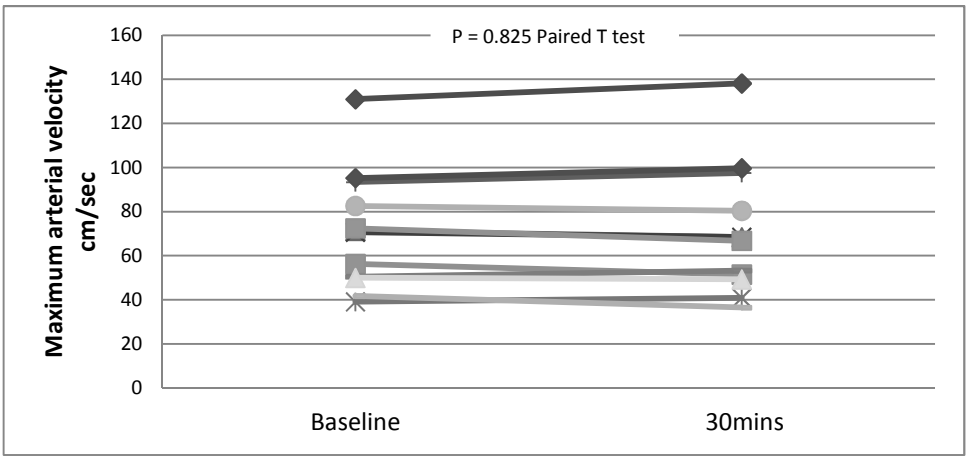


Figure 82: Control limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of device applied to limbs but not activated (n = 12)



6.3.2.1.2 Mean arterial velocity

The mean baseline mean arterial velocity in the active limbs was 11.60 (SD 6.27), passive limbs 11.42 (SD 3.68) and control limbs was 9.50 (SD 4.88) cm/sec. There was no statistically significant difference in baseline values on ANOVA analysis [P = 0.623].

The change in mean arterial velocity from baseline in the active limbs was 2.85 (SD 1.94) [Paired T Test P = 0.016], passive limbs -0.21(SD 1.16)[Paired T test P = 0.675] and control limbs was -0.24 (SD 0.88) cm/sec [Paired T Test P = 0.354] equating to percentage changes of 33.74% (SD 25.43), -3.26% (SD 10.48) and -2.26% (SD 9.35) respectively. The difference in change from baseline between the active and passive groups was statistically significant (Unpaired T Test: P = 0.008). Passive versus control P = 0.946 (Unpaired T test). ANOVA analysis of the change from baseline for the 3 groups was statistically significant (P < 0.001) with post hoc Bonferonni demonstrating: active versus control P < 0.001, active versus passive P = 0.001 but passive versus control P = 1.000 *(Figures 83-86)*.

Figure 83: Mean arterial velocity change from baseline

Error bar chart demonstrating the mean arterial velocity at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)

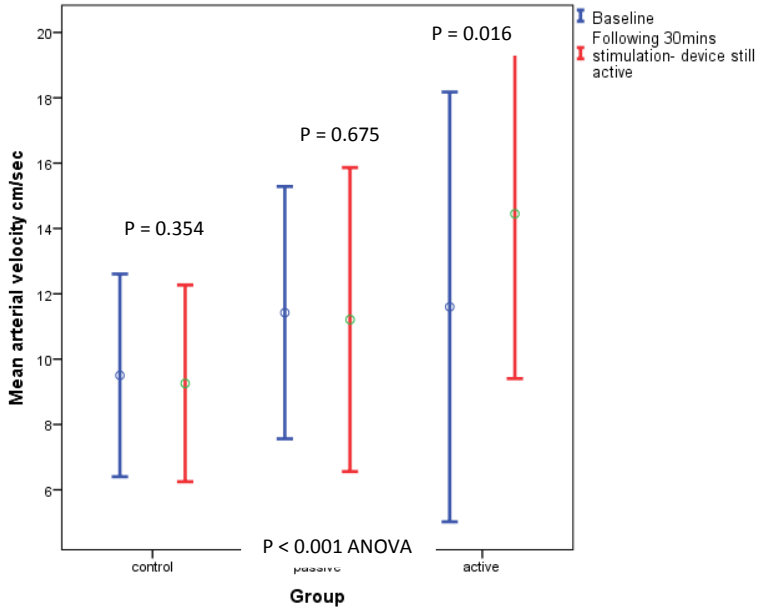


Figure 84: Active stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 6)

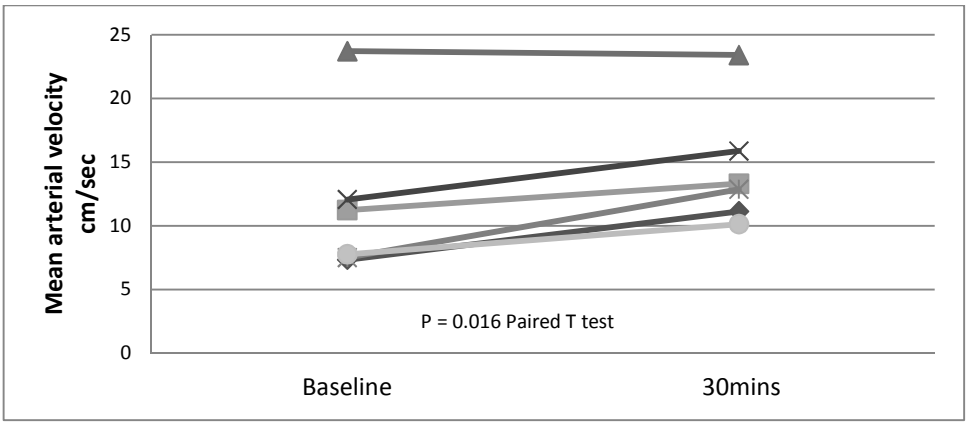


Figure 85: Passive limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation of contralateral limb (n = 6)

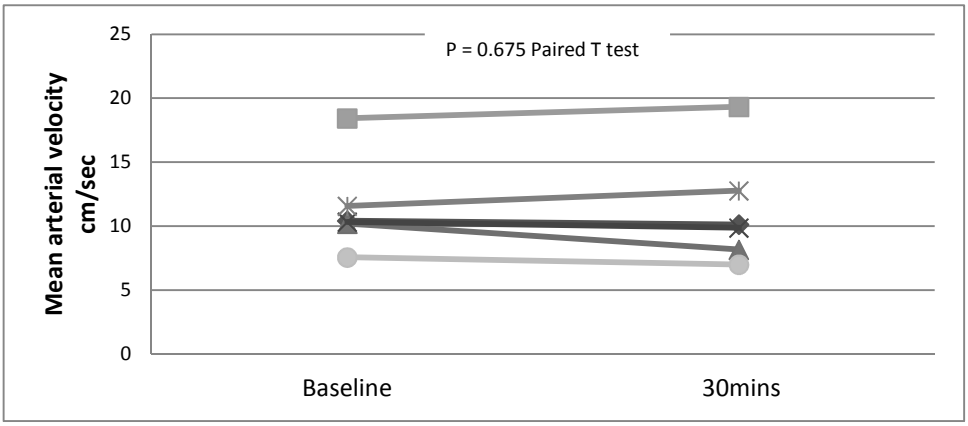
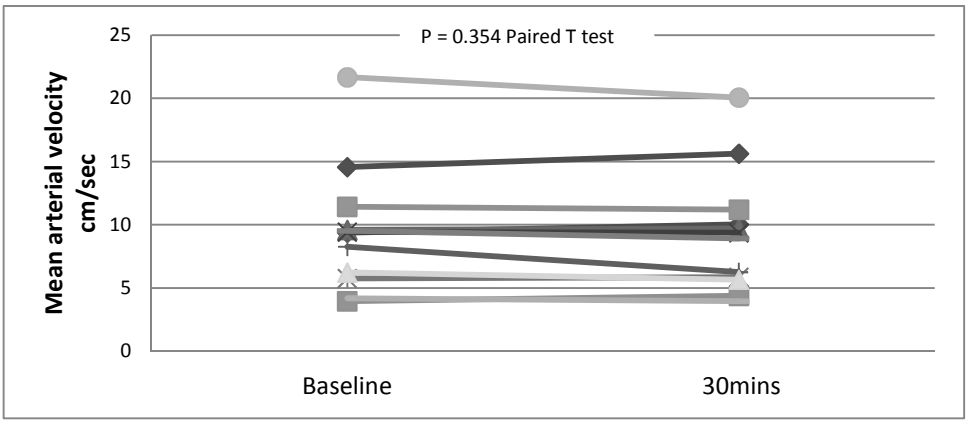


Figure 86: Control limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of device applied to limbs but not activated (n = 12)



6.3.2.1.3 Arterial diameter

The mean baseline arterial diameter in the active group was 5.57 (SD 1.12), passive group 5.84 (SD 1.90) and control group 6.23 (SD 1.20) mm. The difference in baseline values was not statistically significant (P = 0.616 ANOVA). The mean change in arterial diameter in the active group was -0.02 (SD 0.06)(Paired T test P = 0.538), passive group 0.01 (SD 0.03)(Paired T test P = 0.363) and control group -0.00 (SD 0.04) mm (Paired T test P = 0.782), equating to percentage changes of -0.25% (SD 1.14), 0.23% (SD 0.42) and 0.00% (SD 0.58) respectively.

The difference in change from baseline between the active and passive groups was not statistically significant (Unpaired T Test: P = 0.335), passive vs control P = 0.429 (Unpaired T Test). ANOVA analysis to investigate the change in diameter from baseline was not statistically insignificant (P = 0.545).

6.3.2.1.4 Arterial volume flow

The baseline values for arterial volume flow for the active limbs was 0.25 (SD 0.11), passive group 0.28 (SD 0.11) and control group 0.27 (SD 0.12) L/min. The difference in baseline values was not statistically significant (P = 0.897 ANOVA). The mean change in mean arterial volume flow in the active limbs was 0.08 (SD 0.04) (Paired T test P = 0.004), passive limbs -0.01 (SD 0.02) (Paired T test P = 0.103) and control limbs -0.01 (SD 0.01) (Paired T Test P = 0.082) equating to

percentage changes of 39.54% (SD 29.16), -6.89% (SD 7.49) and -1.84% (SD 3.82) respectively.

The difference in change from baseline between the active and passive groups was statistically significant (Unpaired T Test: P < 0.001) but not when comparing the passive and control groups (Unpaired T test P = 0.235). This was confirmed on ANOVA analysis (P < 0.001) with post hoc Bonferonni: active versus passive P < 0.001; active versus control P < 0.001 and passive versus control P = 1.000 *(Figures 87-90)*.

Figure 87: Arterial volume flow change from baseline

Error bar chart demonstrating the arterial volume flow at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)

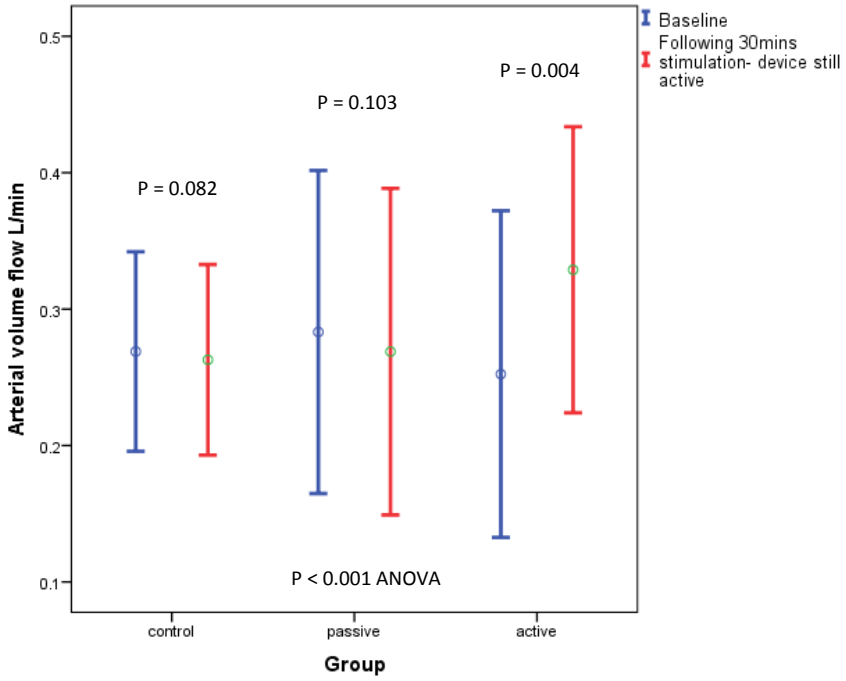


Figure 88: Active stimulation limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 6)

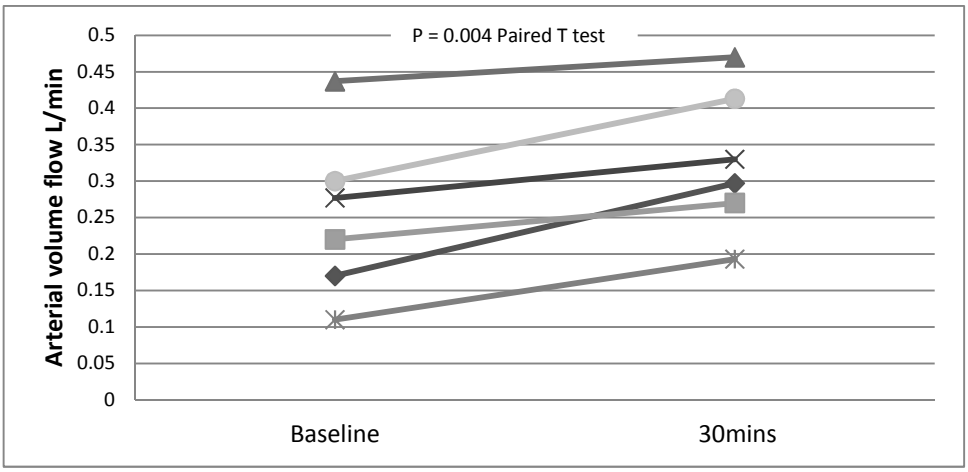


Figure 89: Passive limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of stimulation of contralateral limb (n = 6)

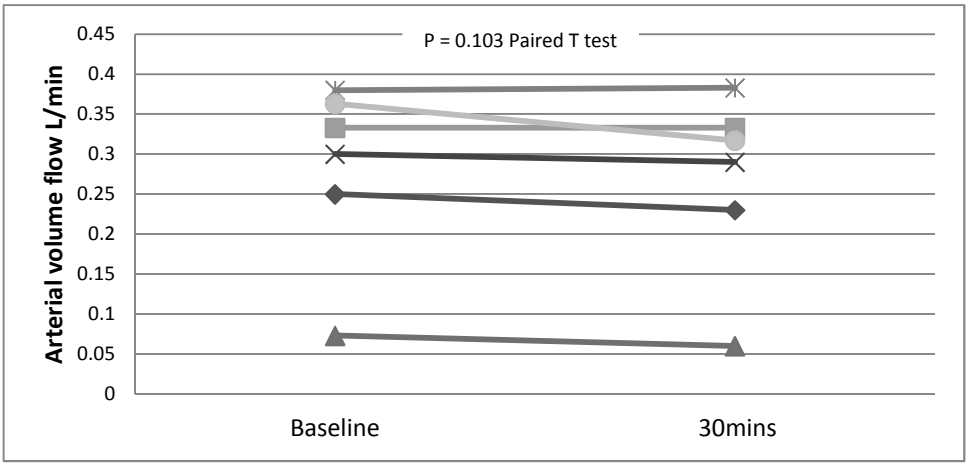
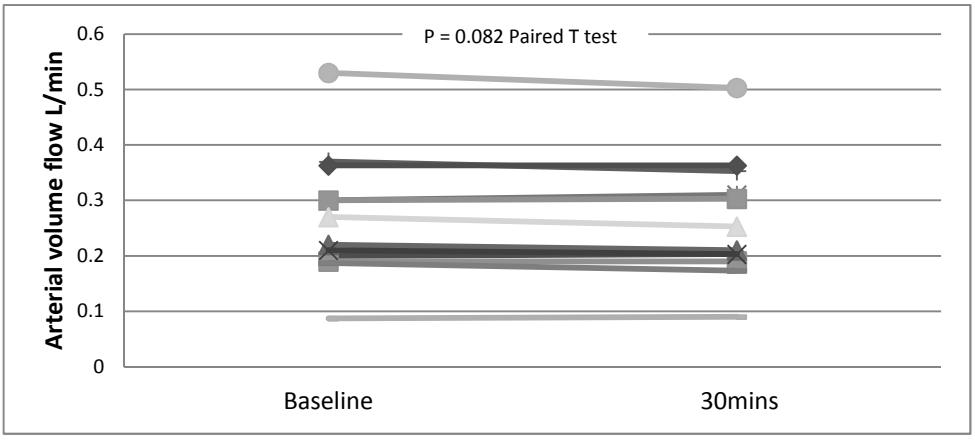


Figure 90: Control limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of device applied to limbs but not activated (n = 12)



6.3.2.2 Venous duplex

6.3.2.2.1 Max venous velocity:

Baseline maximum venous velocity in the active limbs was 19.48 (SD 9.65), passive limbs 24.79 (SD 21.22) and control limbs 15.26 (SD 3.40) cm/sec. ANOVA analysis determined the difference in baseline values was not statistically significant [P = 0.279]. The mean change in maximum venous velocity in the active limbs was 7.28 (SD 8.24) [Paired T test P = 0.083], passive limbs -6.52 (SD 20.76) [Paired T test P = 0.477] and control limbs 0.17 (SD 1.87) cm/sec [Paired T test P = 0.756] equating to percentage changes of 46.79% (SD 54.53), 1.88% (SD 43.48) and 2.25% (SD 13.46) respectively.

The difference in change from baseline between the active and passive groups was statistically significant [Unpaired T Test: P = 0.161] whilst not for passive and control [Unpaired T test P = 0.270]. ANOVA analysis determined that the differences in the changes from baseline were significant [P = 0.046] however post hoc Bonferonni demonstrated: active versus passive P = 0.118; active versus control P = 0.060 and control versus passive P = 1.000 *(Figures 91-94)*.

Figure 91: Maximum venous velocity change from baseline

Error bar chart demonstrating the maximum venous velocity at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)

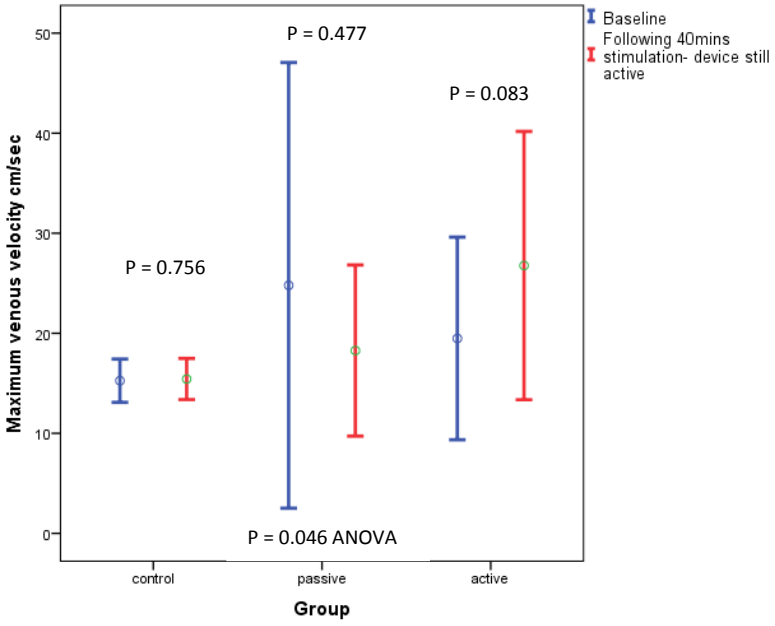


Figure 92: Active limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 6)

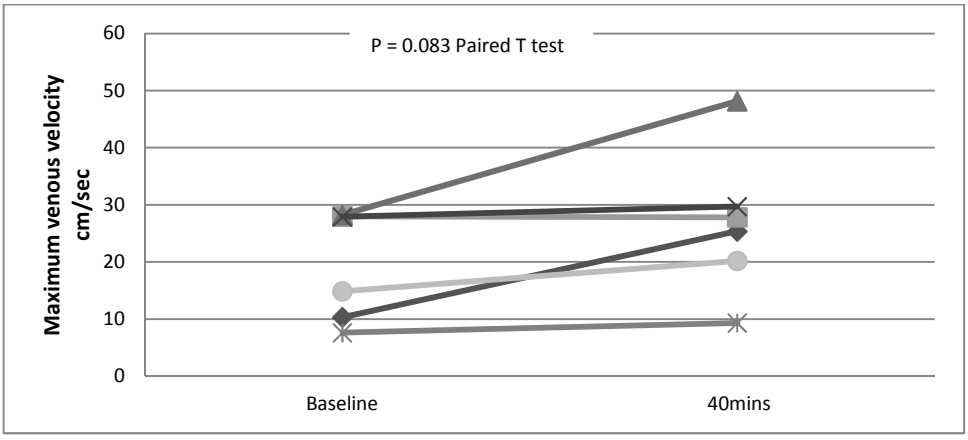


Figure 93: Passive limbs: Change in maximal venous velocity (cm/sec) from baseline with 40 minutes of stimulation of contralateral limb (n = 5)

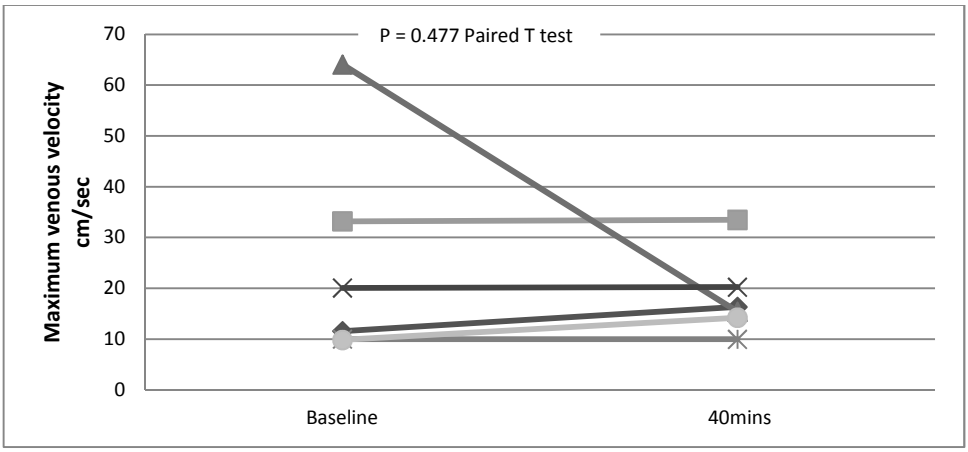
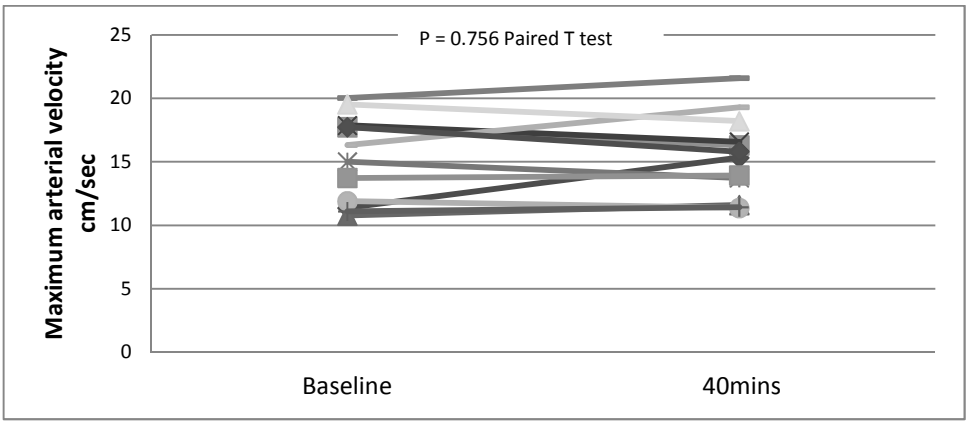


Figure 94: Control limbs: Change in maximal venous velocity (cm/sec) from baseline with 40 minutes of device applied to limbs but not activated (n = 12)



6.3.2.2.2 Mean venous velocity

The baseline values for mean venous velocity in the active limbs was 7.82 (SD 5.05), passive limbs 6.85 (SD 4.06) and control limbs 5.56 (SD 1.77) cm/sec. ANOVA analysis determined that the difference in baseline values was not statistically significant (P = 0.410).

The change in mean venous velocity in the active limbs was 1.10 (SD 1.04) [Paired T test P = 0.049], passive 0.20 (SD 0.77) [Paired T test P = 0.555] and control limbs -0.02 (SD 0.41) cm/sec [Paired T test P = 0.856] equating to percentage changes of 23.63% (SD 20.76), 5.18% (SD 14.16) and -0.09% (SD 7.47) respectively.

The difference in change from baseline between the active and passive groups was not statistically significant [Unpaired T Test: P = 0.120] whilst not for passive and control [Unpaired T test P = 0.431]. The difference in change from baseline between the groups was statistically significant (ANOVA P = 0.014). Post hoc Bonferonni demonstrated that: active versus passive P = 0.110; active versus control P = 0.013 and control versus passive P = 1.000*(Figure 95-98)*.

Figure 95: Mean venous velocity change from baseline

Error bar chart demonstrating the mean venous velocity at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)

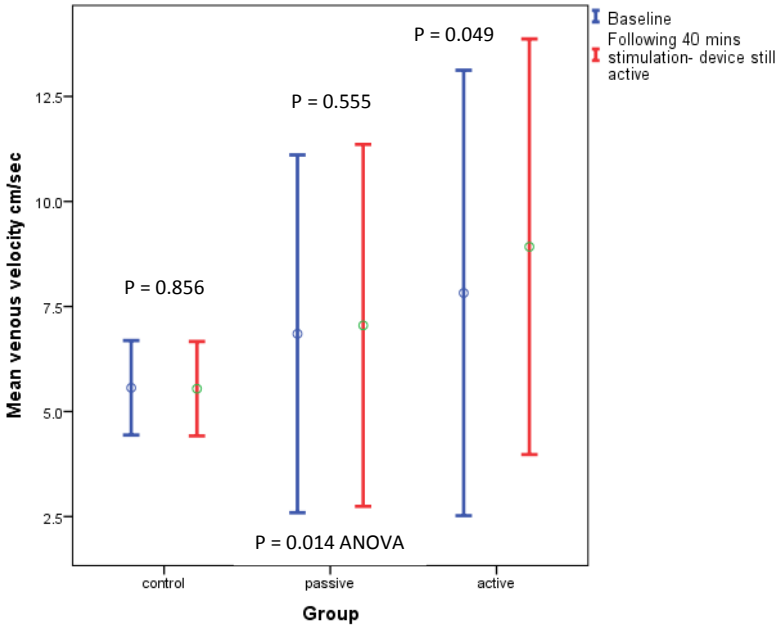


Figure 96: Active limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 6)

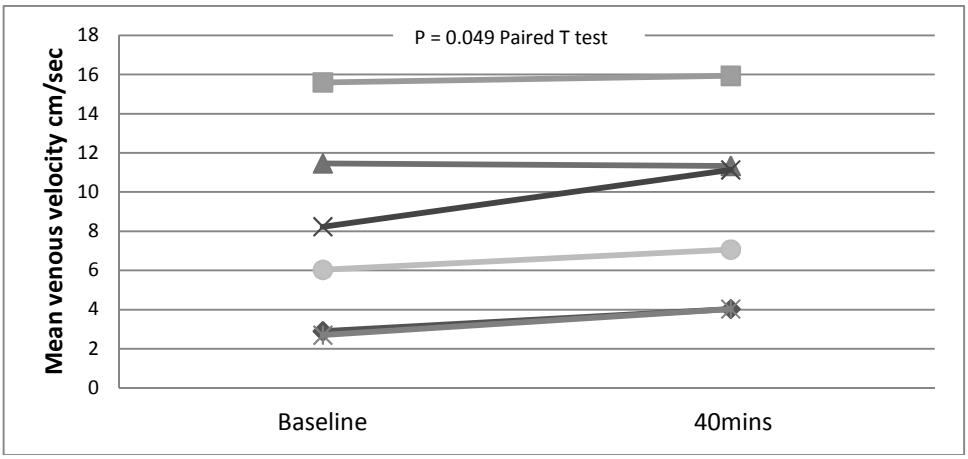


Figure 97: Passive limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 6)

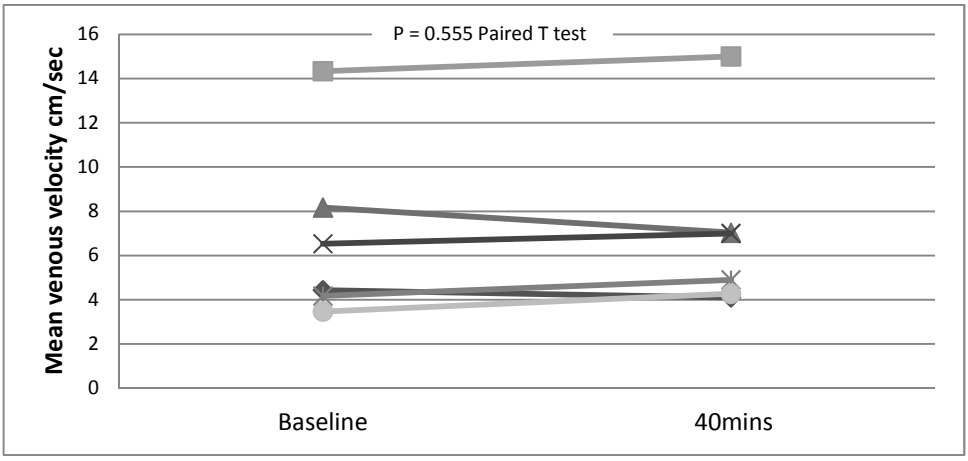
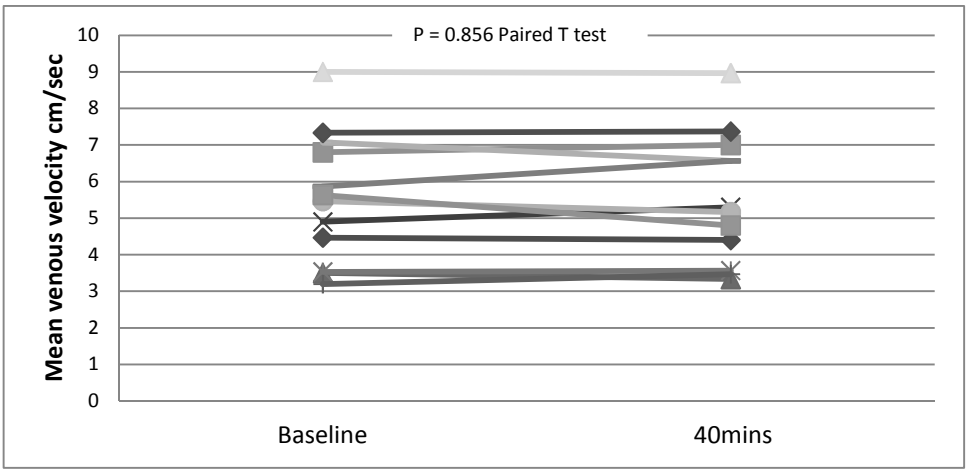


Figure 98: Control limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 12)



6.3.2.2.3 Venous diameter

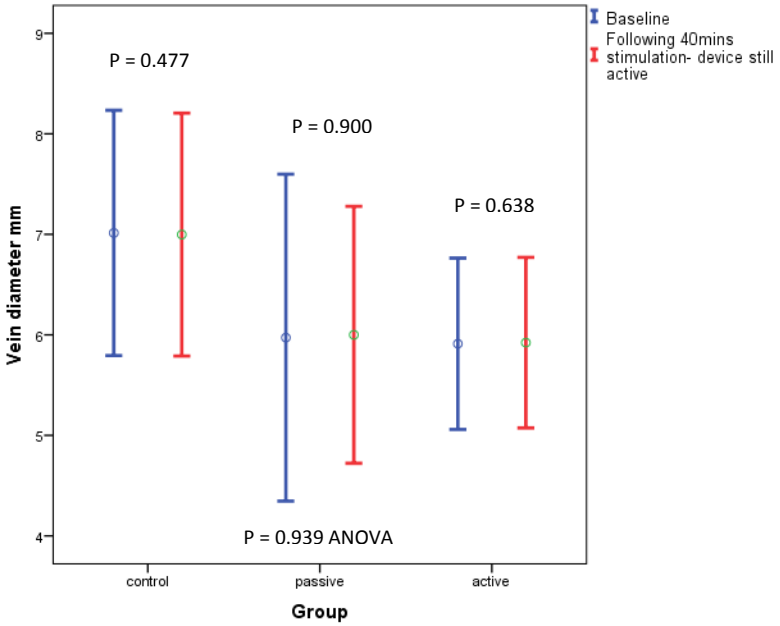
The baseline values for mean venous diameter were active 5.91 (SD 0.81), passive 5.97 (SD 1.55) and control 7.01 (SD 1.92) mm. ANOVA analysis determined that the difference in baseline values was not statistically significant [P = 0.294]. The mean change in mean venous diameter was active limbs 0.01 (SD 0.05) [Paired T test P = 0.638], passive 0.03 (SD 0.52) (Paired T test P = 0.900) and control -0.02 (SD 0.08) mm [Paired T test P =0.477] equating to percentage changes of 0.21% (SD 1.04), 2.30% (SD 11.75) and -0.13% (SD 1.33) respectively.

The difference in change from baseline between the groups was not statistically significant [ANOVA P = 0.939] *(Figure 99)*.

Figure 99: Venous diameter change from baseline

Error bar chart demonstrating the venous diameter at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)



6.3.2.2.4 Venous volume flow

The baseline venous volume flow in the active limbs was 0.20 (SD 0.14), passive limbs 0.16 (SD 0.09) and control limbs 0.23 (SD 0.15) L/min. The difference in baseline values between the groups was not significant [ANOVA P = 0.600]. The change in mean venous volume flow from baseline in the active limbs was 0.03 (SD 0.01) [P < 0.001], passive limbs 0.01 (SD 0.02) [P = 0.211] and control limbs 0.00 (SD 0.02) L/min [P = 0.459] equating to percentage changes of 28.62% (SD 23.06), 16.67% (SD 35.39) and -0.29% (SD 5.86) respectively.

The difference in venous volume flow from baseline between the groups was statistically significant [ANOVA P < 0.001]. Post hoc Bonferonni demonstrated: active versus passive P = 0.061; active versus control P < 0.001 and control versus passive P = 0.170 *(Figures 100-103)*.

Figure 100: Venous volume flow change from baseline

Error bar chart demonstrating the venous volume flow at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)

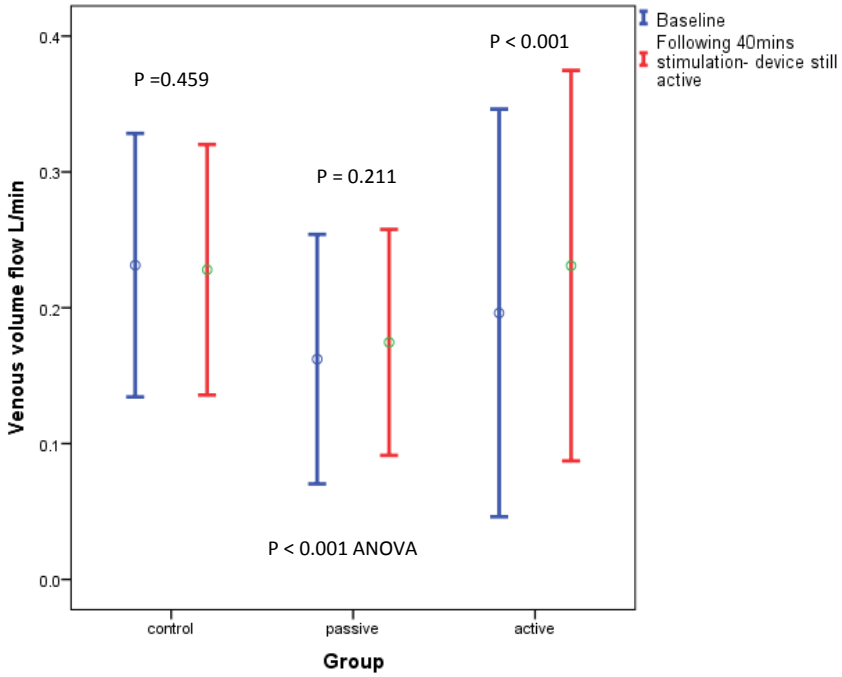


Figure 101: Active limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 6)

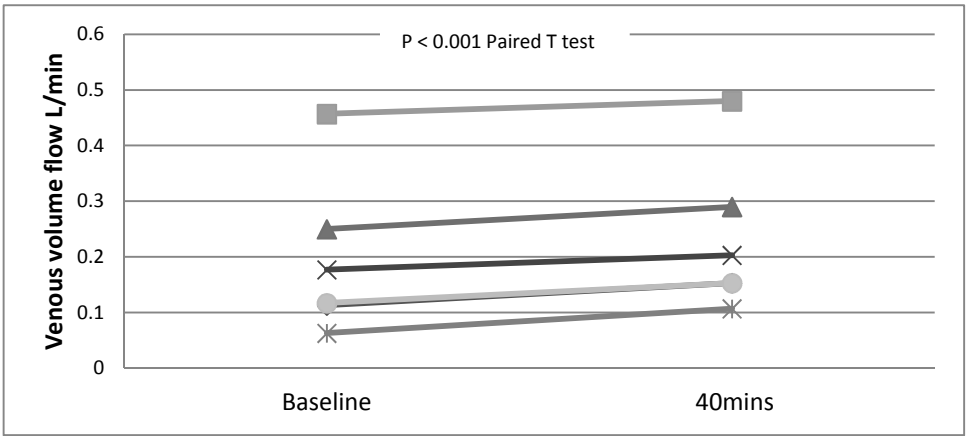


Figure 102: Passive limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 6)

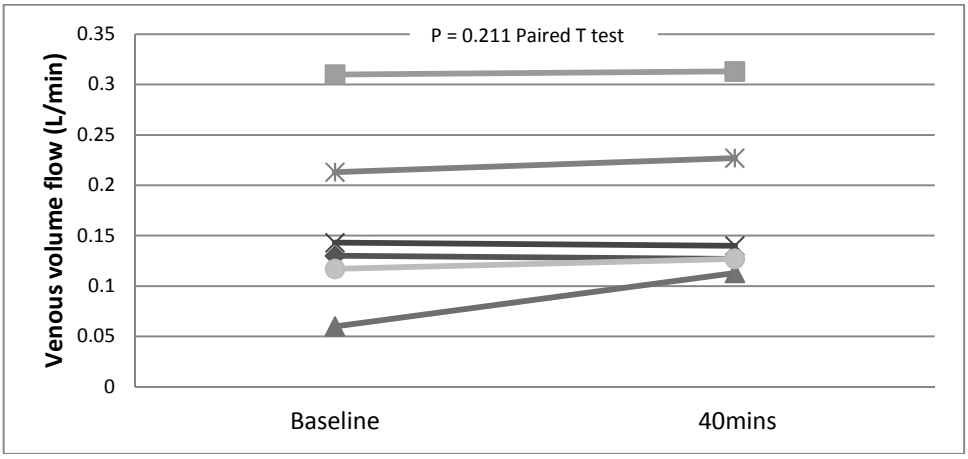
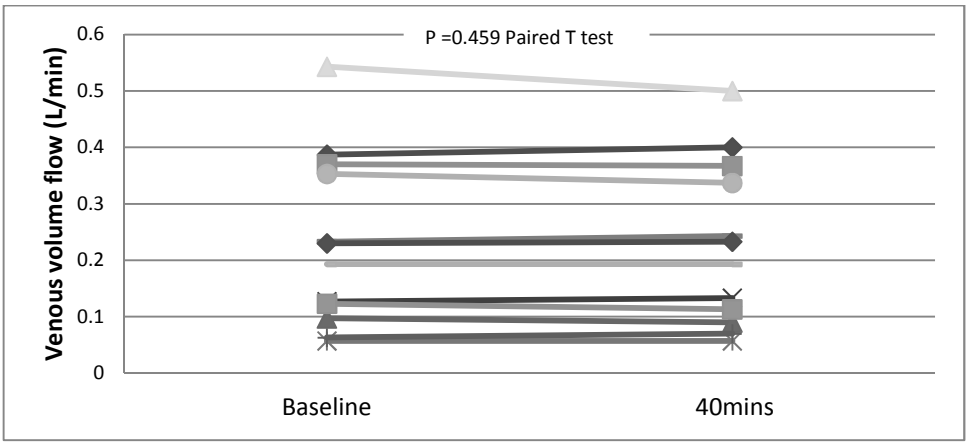


Figure 103: Control limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 12)



6.3.2.3 Skin perfusion/ Laser Doppler flowmetry

There was no appreciable change in skin temperature during the study period. Baseline temperature in the active group was 29.13 (SD 2.04), passive group 29.40 (SD 1.50) and control group 29.84 (SD 1.87) ^{o}C. The difference in baseline values was not statistically significant [P = 0.199 ANOVA].

The change in temperature from baseline was 0.13 (SD 0.44)[P = 0.488 Paired T test], passive group 0.17 (SD 0.26) ^{o}C [P = 0.175 Paired T test] and control group -0.26 (SD 0.28) [P = 0.009 Paired T test]. The differences in change from baseline between the two groups was not statistically significant [P = 0.018 ANOVA: Post hoc

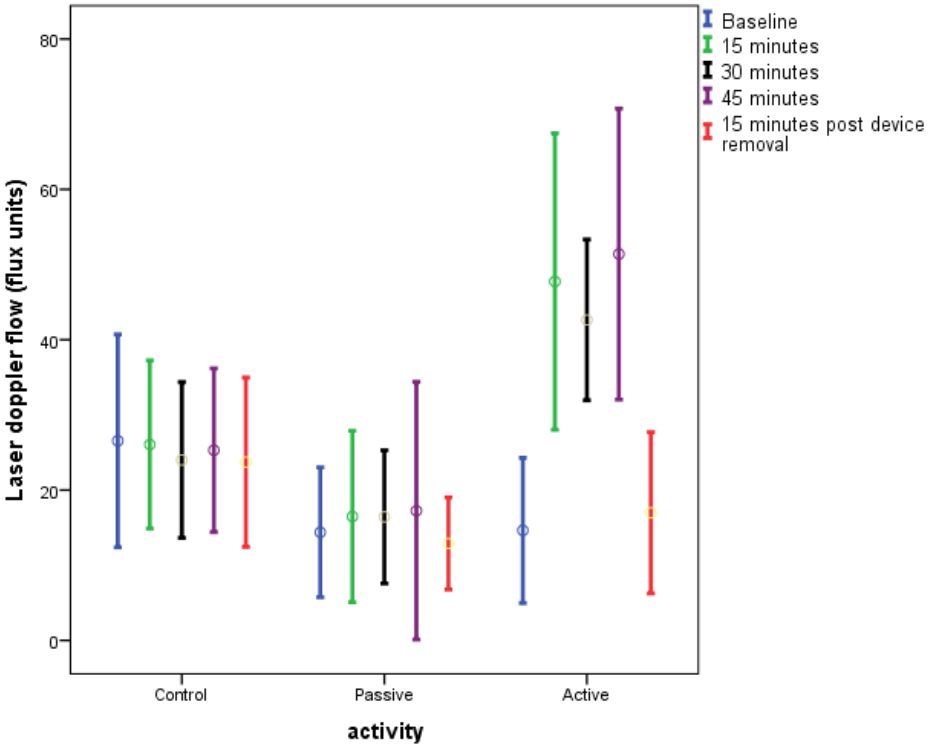
Bonferonni; active versus passive P = 1.000; active versus control P = 0.070; passive versus control P = 0.045].

Baseline microcirculatory flow in the active groups was 14.63 (SD 9.21), the passive group 14.38 (SD 8.23) and control group 26.55 (SD 22.28). The difference in baseline values seen in the groups was not statistically significant [P = 0.253 ANOVA]. The change at 15 minutes from baseline in the active group was 33.12 (SD 15.72) [P = 0.004 Paired T test], passive group 2.10 (SD 2.97) [P = 0.144 Paired T test] and control group -0.49 (SD 6.09) [P = 0.785 Paired T test]. Analysis of the differences seen between the active and passive groups demonstrated statistical significance [P < 0.001 ANOVA: Bonferonni- Control versus passive P = 1.000; Active versus passive P < 0.001; Active versus passive P < 0.001].
The change at 30 minutes from baseline in the active group was 28.00 (SD 13.41) [P = 0.004 Paired T test], passive 2.05 (SD 3.00) [P = 0.155 Paired T test] and control group -2.55 (SD 9.12) [P = 0.353 Paired T test]. Analysis of the differences in the changes seen was demonstrated to be statistically significant [P < 0.001 ANOVA: Bonferonni- Control versus passive P = 1.000; Active versus passive P < 0.001; Active versus control P < 0.001].
The change at 45 minutes from baseline in the active group was 36.77 (SD 19.66) [P = 0.006 Paired T test], passive 2.87 (SD 9.29) [P = 0.484 Paired T test] and control group -1.25 (SD 6.85) [P = 0.541 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance [P < 0.001 ANOVA: Bonferonni- Passive versus control P = 1.000; Active versus passive P < 0.001; Active versus control P < 0.001].
The change at 15 minutes following device deactivation in the active groups was 2.35 (SD 3.30) [P = 0.142 Paired T test], passive -1.48 (SD 3.10) [P = 0.294 Paired T test] and control group -2.84 (SD 6.62) [P = 0.159 Paired T test]. Analysis of the differences in the changes seen demonstrated non-significance [P = 0.161 ANOVA] *(Figure 104)*.

Figure 104: Laser Doppler flow change from baseline

Error bar graph demonstrating mean laser Doppler flowmetry readings through the study period in each group. [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 6)
Passive: contralateral limb to active limbs (n = 6)
Control: limbs on which device was applied but not activated (n = 12)



6.3.2.4 Cardiovascular effects

The mean heart rate at baseline in the active group was 79.67 (SD 14.39) and control group 66.67 (SD 14.12) beats per minute in the passive group. Comparison of the baseline values demonstrated non significance [P = 0.330 Unpaired T test]. After 30 minutes of stimulation the heart rate decreased by -3.17 (SD 11.77) in the active group [P = 0.539 Paired T test] and in the control group decreased by 2.67 (SD 3.67) [P = 0.135 Paired T test]. Comparison of the changes demonstrated non-significance [P = 0.054 Unpaired T test].

The heart rate increased by 0.50 (SD 4.18) [P = 0.781 Paired T test] in the active group and 0.67 (SD 6.15) in the control group [P = 0.801 Paired T test] following deactivation of the device. Unpaired T test analysis of the changes demonstrated non-significance [P = 0.640].

Systolic blood pressure at baseline in the active group was 151.50 (SD 26.06) and control 121.67 (SD 22.70) mmHg. Comparison of baseline values determined them

to be comparable [P = 0.453 Unpaired T test]. Systolic BP increased at 30 minutes by 2.00 (SD 16.54) in the active group [P = 0.779 Paired T test] and 9.00 (SD 11.51) in the control group [P = 0.114 Paired T test]. Comparison of the differences seen demonstrated insignificance [P = 0.330 Unpaired T test]. Systolic BP decreased from 30minutes to the end of the study period in the active group by 1.83 (SD 10.72) [P = 0.693 Paired T test] and increased by 8.83 (SD 11.69) [P = 0.123 Paired T test] mmHg in the control group. Comparison of the differences seen demonstrated insignificance [P = 0.493 Unpaired T test].

Diastolic blood pressure at baseline in the active group was 89.50 (SD 6.16) and control 66.50 (SD 10.50) mmHg. Comparison of baseline values determined them to be comparable [P = 0.158 Unpaired T test]. Diastolic BP decreased at 30 minutes by 3.00 (SD 10.94) in the active group [P = 0.531 Paired T test] and increased by 12.00 (SD 18.56) in the control group [P = 0.174 Paired T test]. Comparison of the differences seen demonstrated insignificance [P = 0.074 Unpaired T test]. Diastolic BP increased from 30minutes to the end of the study period in the active group by 6.50 (SD 9.65) [P = 0.160 Paired T test] and decreased by 8.00 (SD 17.40) [P = 0.505 Paired T test] mmHg in the control group. Comparison of the differences seen demonstrated insignificance [P = 0.084 Unpaired T test].

6.3.3 Vicorder

6.3.3.1 Comparison of the Vicorder and SphygmoCor devices

The mean difference in pulse wave velocity measurements with the SphygmoCor and Vicorder devices were 0.03 (S.D 0.92) m/ sec (P = 0.85) and 0.01 (S.D. 0.54) m/sec (P = 0.54) respectively. Both devices demonstrated high reproducibility; intraclass correlation co-efficients 0.92 and 0.94 for SphygoCor and Vicorder. The SphygmoCor had a lower coefficient of variation than the Vicorder; 5% versus 6%. The limits of agreement between the Vicorder measurements were 1.07 to 1.09m/sec and for the SphygmoCor 1.79 and 1.85 m/sec. 90% of the PWV measurements using the Vicorder and 93% of the measurements using the SphygmoCor fell within

2 standard deviations of the mean demonstrated on Bland-Altman plots. *(Figures 105-106)*

Figure 105: Bland-Altman plot illustrating intra-rater reproducibility of the cfPWV measurements by the Vicorder device.

The upper and lower dotted lines represent the LoA between repeated cfPWV measurements (mean difference +/- 2 SDs). The middle dotted line represents the mean of the difference between all repeated cfPWV measurements generated by the device.

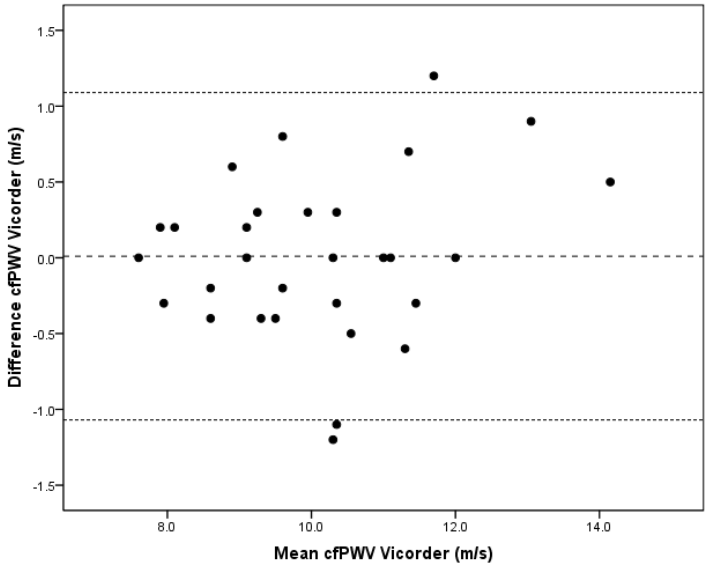
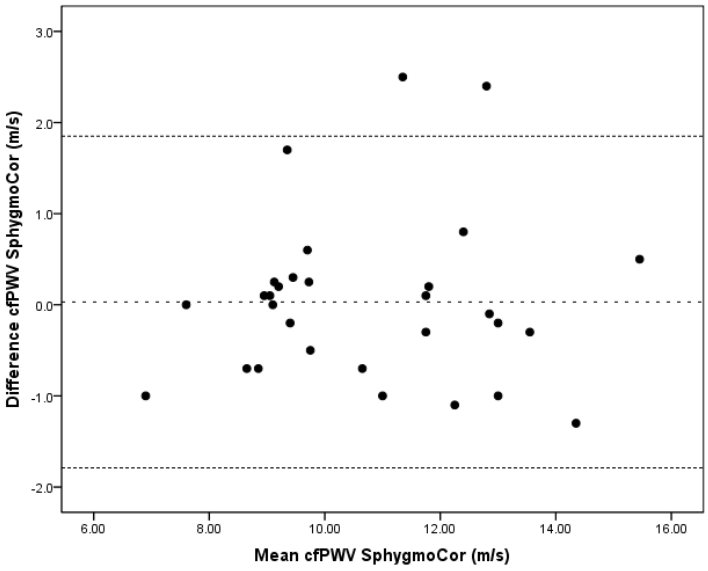


Figure 106: Bland-Altman plot illustrating intra-rater reproducibility of the cfPWV measurements by the SphygmoCor device.



The mean difference in the PWV measurements obtained by the two devices was statistically significant 0.69 (S.D. 1.6) m/sec (P = 0.02), with higher values recorded with the SphygmoCor than the Vicorder (10.77 (SD 1.6) m/s versus 10.08 (SD 2.1)m/sec respectively). Despite this the PWV measurements demonstrated a linear relationship with strong correlation (r = 0.67, P < 0.001). The limits of agreement between the two devices' measurements were 2.53 to -3.91 m/sec with 97% of measurements falling within 2 standard deviations *(Figures 107-108)*.

Figure 107: Scatter plot illustrating the relationship between cfPWV values obtained by the SphygmoCor and those obtained by the Vicorder.

The black line is the regression line.

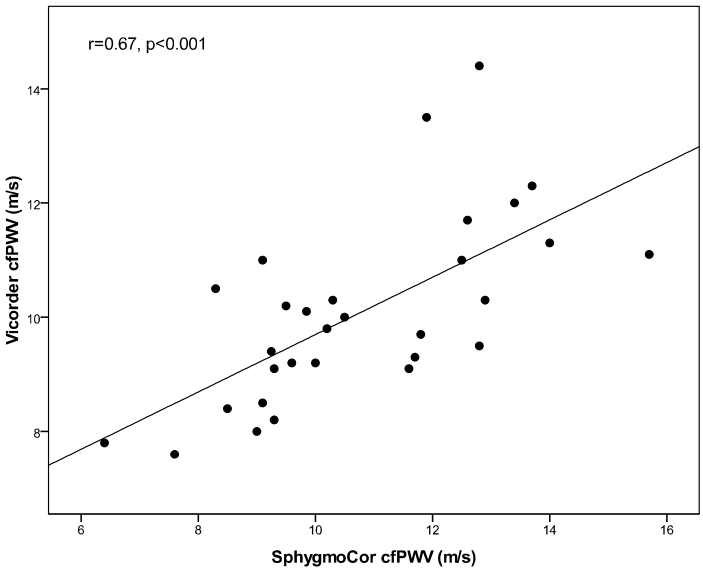
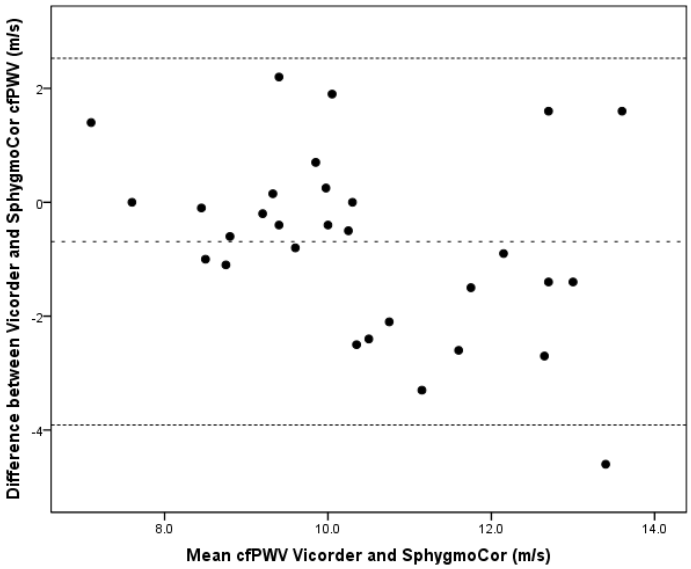


Figure 108: Bland-Altman plot illustrating the agreement between cfPWV measurements obtained by the SphygmoCor and those obtained by the Vicorder.



6.3.3.2 Pulse wave velocity

Pulse wave velocity increased from baseline in the active group by a mean of 4.35 metres/ second whilst it decreased in the control group by 2.05 metres/ second. This was not statistically significant (P = 0.190 Unpaired T test).

6.3.3.3 Augmentation index

Augmentation index increased by 1.00% in the active group compared to an increase of 2.00% in the control group (P = 0.834 Unpaired T test).

6.3.3.4 Cardiac output

Cardiac output increased by 0.05 in the active group compared to an increase of 0.18 in the control group. This was not statistically significant (P = 0.932 Unpaired T test).

6.3.3.5 Peripheral vascular resistance

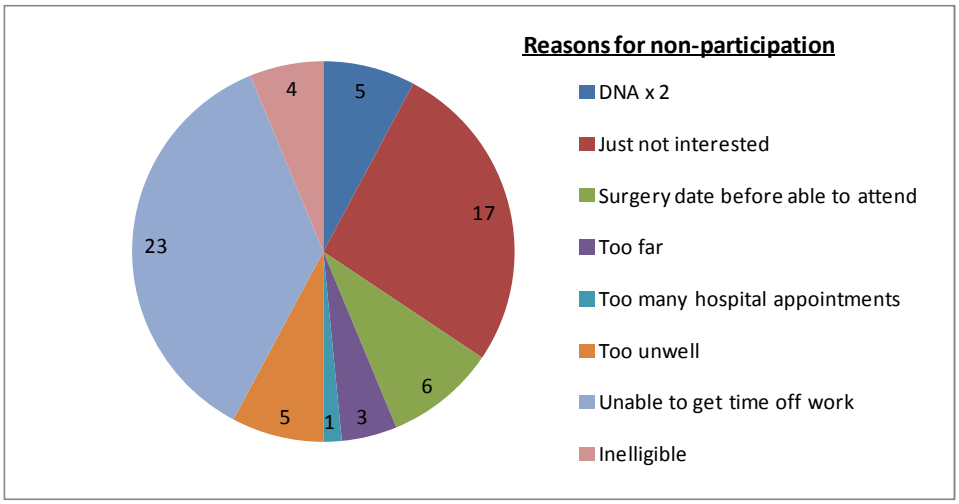
Peripheral vascular resistance increased by 0.06 in the active group compared to an increase of 0.21 in the control group (P = 0.334 Unpaired T test).

Chapter Seven: Research question 5: The haemodynamic efficacy of peroneal nerve electrical neuromuscular stimulation in patients with varicose veins

7.1 Varicose Vein Patients Study population

A total of 86 potential participants were identified from out-patient clinics, the waiting list and from patients attending for venous duplex. All were contacted by letter with an attached patient information sheet. The participation status of those contacted is listed below with the reasons for non participation as stated by the patient.

Figure 109: Reasons given for subject non-participation



22 patients participated in the study whilst 64 were unwilling or unable to take part.

7.2 Proof of concept study

7.2.1 Examination findings: ABPI

The mean ABPI at baseline in the active limbs was 1.16 (SD 0.17) and passive limbs 1.12 (SD 0.11). This was statistically significant on unpaired T test [P = 0.530]. The mean change in ABPI from baseline in the active limbs was -0.04 (SD 0.10) [P = 0.171 Paired T test] and passive limbs was -0.06 (SD 0.12) [P = 0.966 Paired T test]. A comparison of the difference between changes seen in the two groups was statistically significant [P = 0.784 Unpaired T test] *(Figures 110-111)*.

Figure 110: Scatter graph: Active limbs; change in ABPI in study period (n = 12)

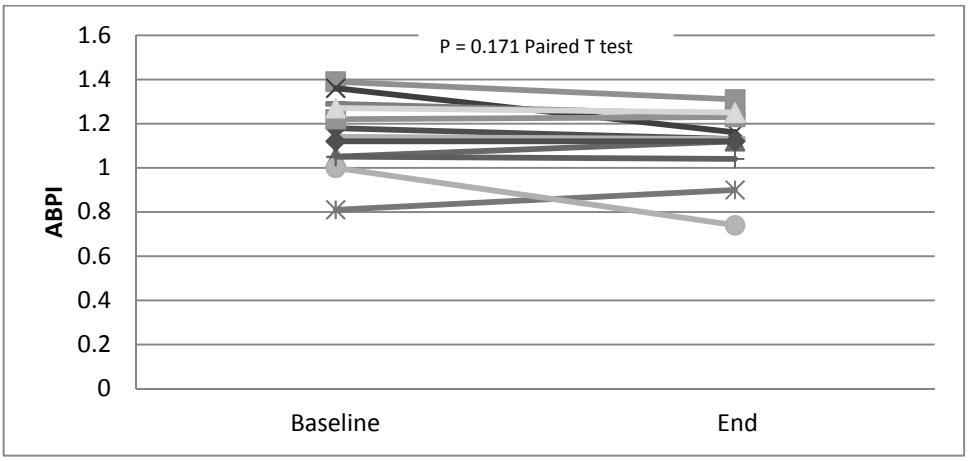
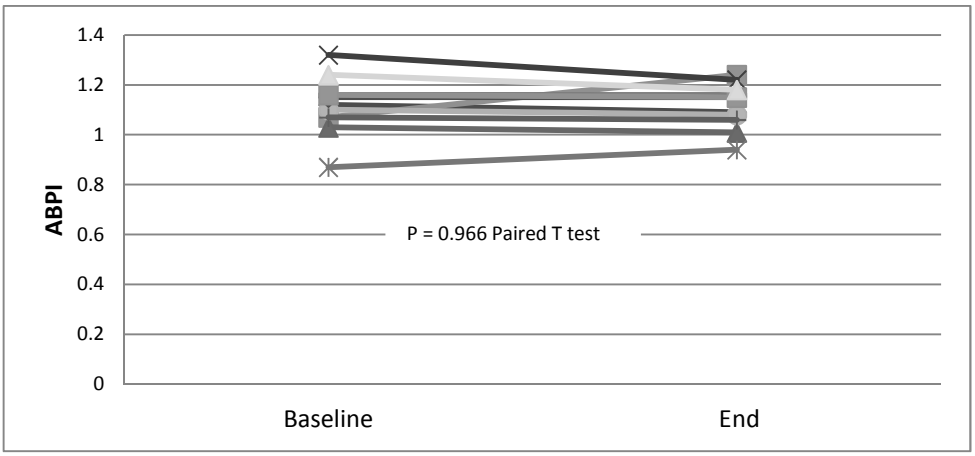


Figure 111: Scatter graph: Passive limbs; Change in ABPI in the study period (n = 12)



Calf circumference

The mean calf circumference at baseline in the active limbs was 37.96 (SD 4.61) and passive limbs 37.9 (SD 4.56) mm. The difference in baseline values was not statistically significant on Unpaired T test [P = 0.975].
The mean change in calf circumference from baseline was -0.54 (SD 0.62) [P = 0.011 Paired T test] in the active limbs and -0.30 (SD 0.37) mm [P = 0.017 Paired T test] in the passive limbs. A comparison of the difference in the change seen was not statistically significant [P = 0.368 Unpaired T test].

7.2.2 Haemodynamic efficacy of geko^{TM} in varicose vein patients: Proof of concept study

7.2.2.1 Assessment of reliability of haemodynamic measurements

The intra-class correlation coefficient was utilised to determine the intra-rater reliability of the ultrasound measurements collected in the study(428). The intra-observer reliability assessed the reproducibility of the observer for each measurement technique *(Table 26)*. In this study, each measurement was made in triplicate and the mean utilised for all subsequent analysis.

Table 26: Intraclass correlation coefficient analysis of ultrasound measurements

Measurement	Intraclass correlation coefficient (P)
Arterial maximum flow velocity.	0.976
Arterial mean flow velocity	0.983
Arterial vessel diameter	0.999
Arterial volume flow	0.791
Venous maximum flow velocity	0.961
Venous mean flow velocity	0.990
Venous vessel diameter	1.000
Venous volume flow	0.773

Thus it is possible to see that there was high intra-rater reliability for all ultrasound measurements performed as part of this study within the varicose vein population.

7.2.2.2 Arterial duplex

7.2.2.2.1 Maximum arterial velocity

The mean baseline maximal arterial velocity in the active limbs was 70.89 (SD 15.56) and passive limbs 69.19 (SD 13.07) cm/sec. Comparison of the groups demonstrated no statistical significance [P = 0.774] in the difference between baseline values. The change from baseline in the groups were 7.81 (SD 7.65) [P = 0.005 Paired T test] and 0.02 (SD 5.88) [P = 0.991 Paired T test] cm/sec in the active and passive groups respectively equating to percentage changes of 11.49% (SD 10.92) and 0.05% (SD 8.53). The differences in the change seen between the active and passive limbs were statistically significant [P = 0.011 Unpaired T test] *(Figures 112-114)*.

Figure 112: Maximal arterial velocity change from baseline

Error bar chart demonstrating the maximal arterial velocity and at baseline at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)

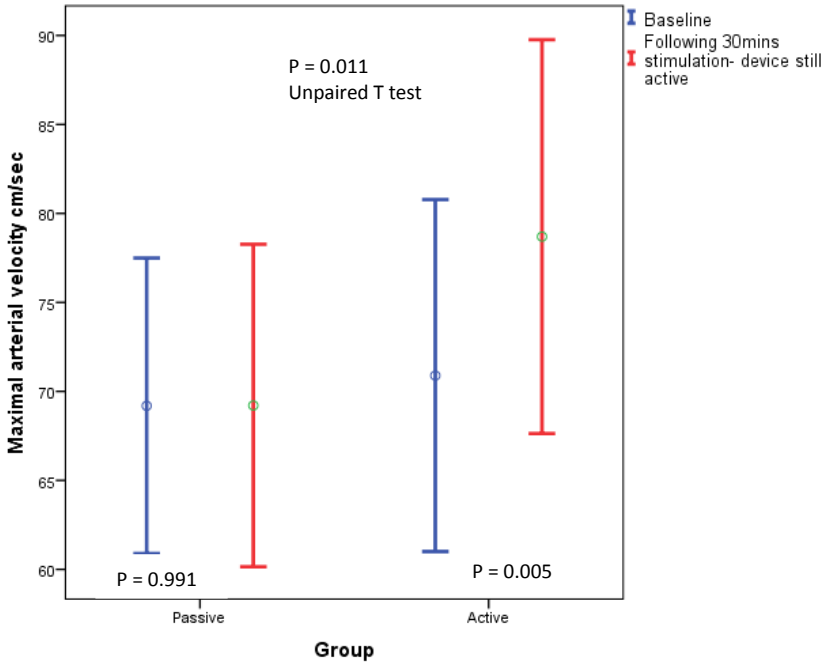


Figure 113: Active stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 12)

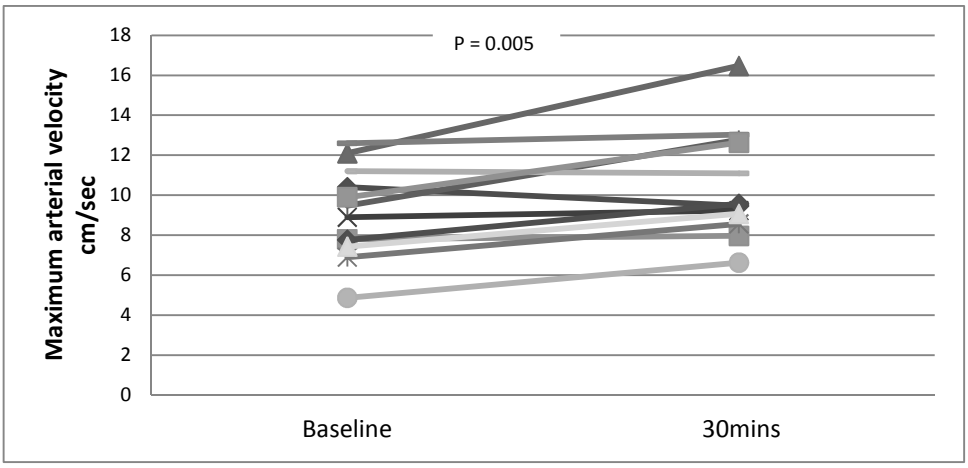
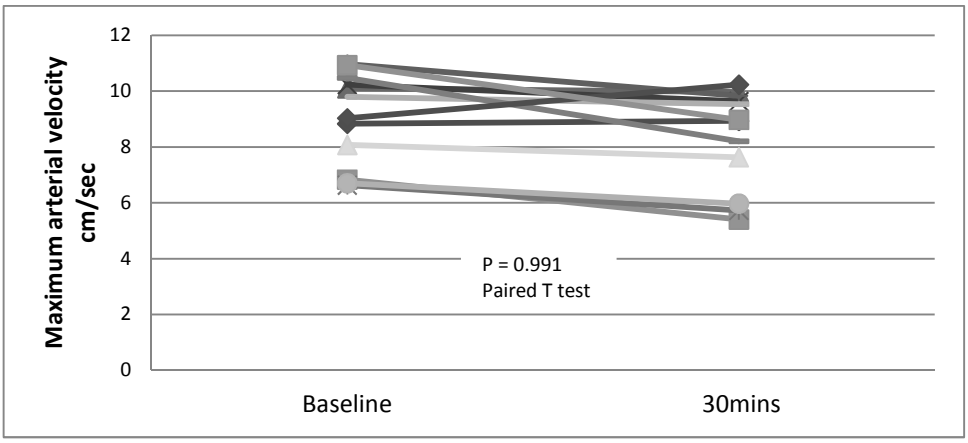


Figure 114: Passive stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 12)



7.2.2.2.2 Mean arterial velocity

The baseline mean arterial velocity in the active limbs was 9.11 (SD 2.28) and passive limbs 9.05 (SD 1.64) cm/sec. The difference between the limbs was not statistically significant (P = 0.774 Unpaired T test).

The change from baseline with the device was 1.43 (SD 1.53) [P = 0.008 Paired T test] and passive limbs -0.71 (SD 0.94) cm/sec [P = 0.023 Paired T test] in the active and passive limbs respectively equating to percentage changes of 16.97% (SD 16.11) and -8.02% (SD 10.09) respectively. The difference in the changes seen between the groups was statistically significant (P = 0.011 Unpaired T test) *(Figures 115-117)*.

Figure 115: Mean arterial velocity change from baseline

Error bar chart demonstrating the mean arterial velocity at baseline and at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)

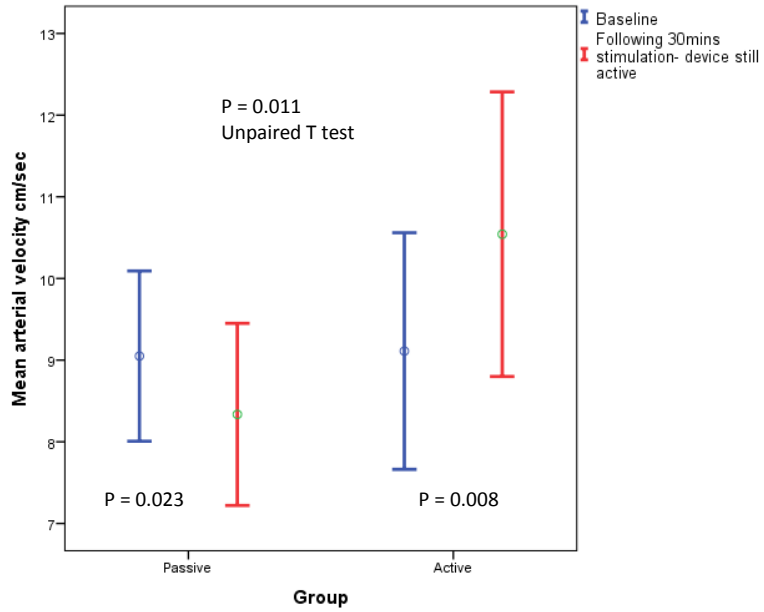


Figure 116: Active stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 12)

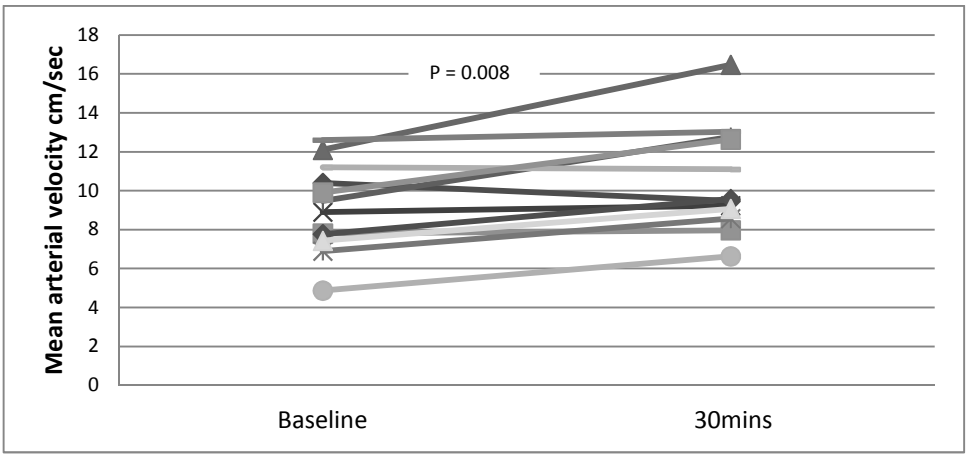
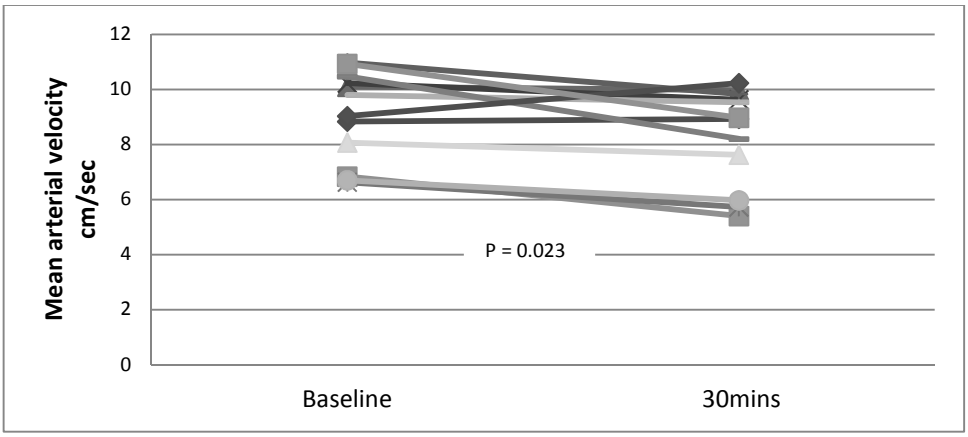


Figure 117: Passive stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 12)



7.2.2.2.3 Arterial diameter

The mean arterial diameter at baseline in the active limbs 7.80 (SD 1.27) and passive limbs 7.75 (SD 1.27) mm. The difference between the baseline value in the groups was not statistically significant (P = 0.924 Unpaired T test). The change from baseline with stimulation was 0.01 (SD 0.08) [P = 0.727 Paired T test] and 0.04 (SD 0.04) mm [P = 0.003 Paired T test] in the active and passive limbs respectively equating to percentage changes of 0.18% (SD 0.96) and 0.53% (SD 0.44). Unpaired T test analysis demonstrated this difference in the change between the limbs was not statistically significant (P = 0.076).

7.2.2.2.4 Arterial volume flow

The mean arterial flow at baseline in the active limbs was 0.38 (SD 0.14) and passive limbs 0.38 (SD 0.14) L/min. Flow at baseline was comparable between the two groups (P = 0.986 Unpaired T test). The change from baseline with stimulation was 0.08 (SD 0.04) [P < 0.001 Paired T test] and -0.02 (SD 0.03) L/min [P = 0.012 Paired T test] in the active and passive limbs respectively equating to percentage changes of 21.72% (SD 10.41) and -5.90% (SD 6.69). Unpaired T test analysis demonstrated that the difference in the change seen between the active and passive limbs was statistically significant (P < 0.001) *(Figures 118-120)*.

Figure 118: Arterial volume flow change from baseline

Error bar chart demonstrating the arterial volume flow at baseline and at 30 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)

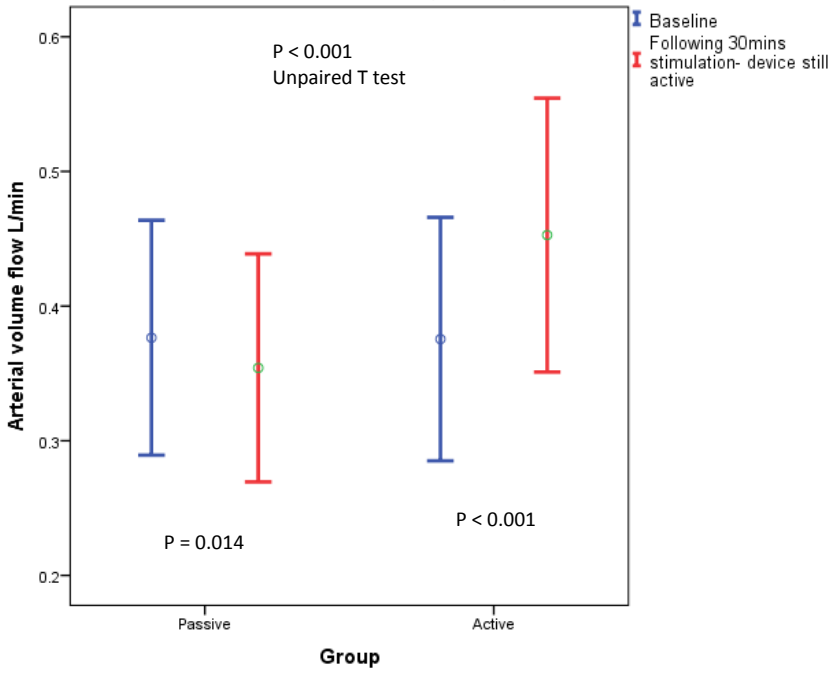


Figure 119: Active stimulation limbs: Change arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 12)

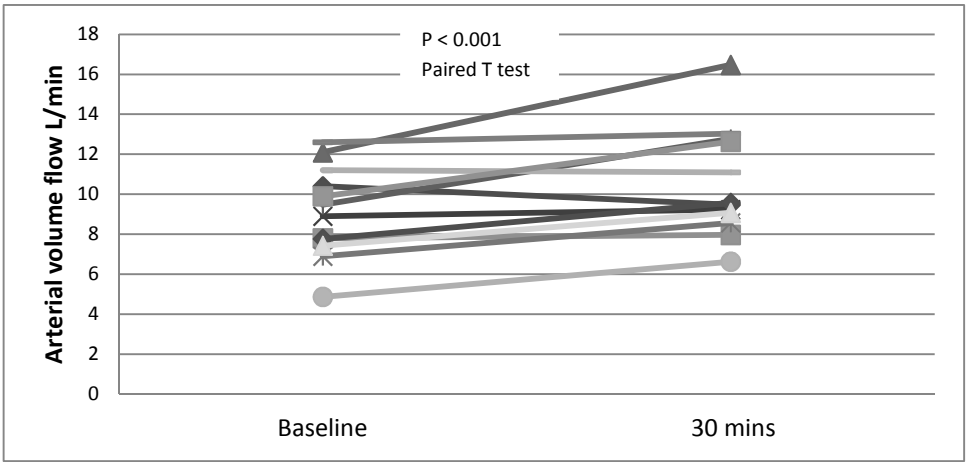
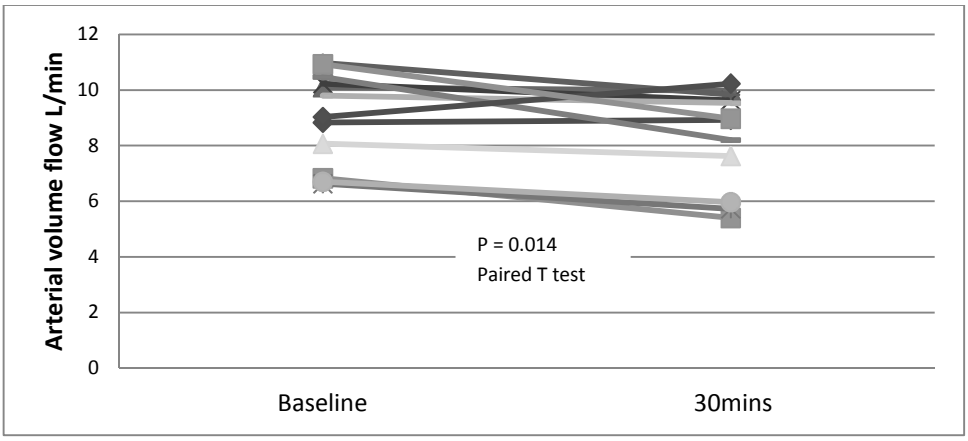


Figure 120: Passive stimulation limbs: Change arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 12)



7.2.2.3 Venous duplex

7.2.2.3.1 Venous maximum velocity

The baseline mean maximum venous velocity in the active limbs was 15.09 (SD 5.57) and the passive limbs 15.45 (SD 4.88) cm/sec. Comparison of the baseline values demonstrated the differences between the groups to be insignificant (P = 0.868 Unpaired T test).

The change from baseline with stimulation in the active limbs was 4.30 (SD 4.15) [P = 0.004 Paired T test] and passive limbs -0.71 (SD 2.75) cm/sec [P = 0.390 Paired T test], equating to percentage changes of 33.65% (SD 33.03) and -4.90% (SD 15.23). Comparison of the changes from baseline in the 2 groups demonstrated that this was statistically significant (P = 0.002 Unpaired T test) *(Figures 121-123)*.

Figure 121: Maximal venous velocity change from baseline

Error bar chart demonstrating the maximal venous velocity at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)

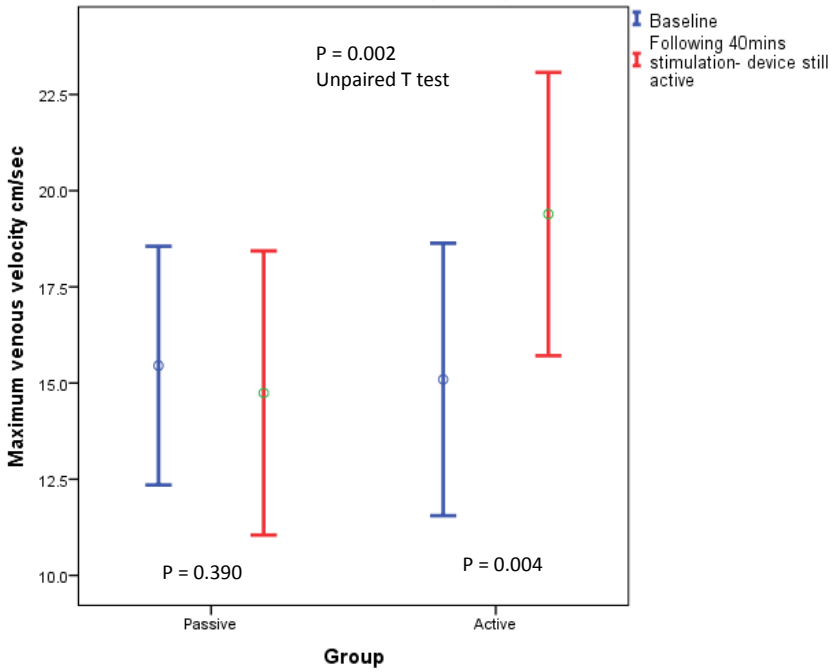


Figure 122: Active stimulation limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 12)

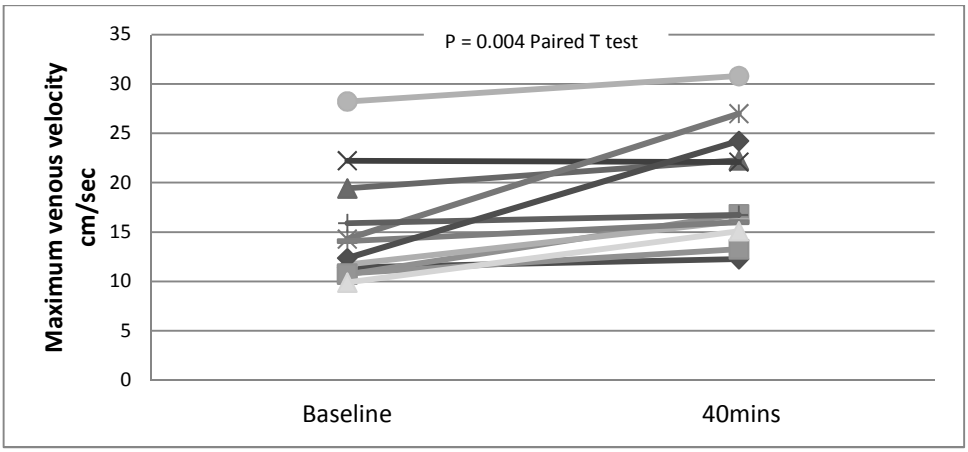
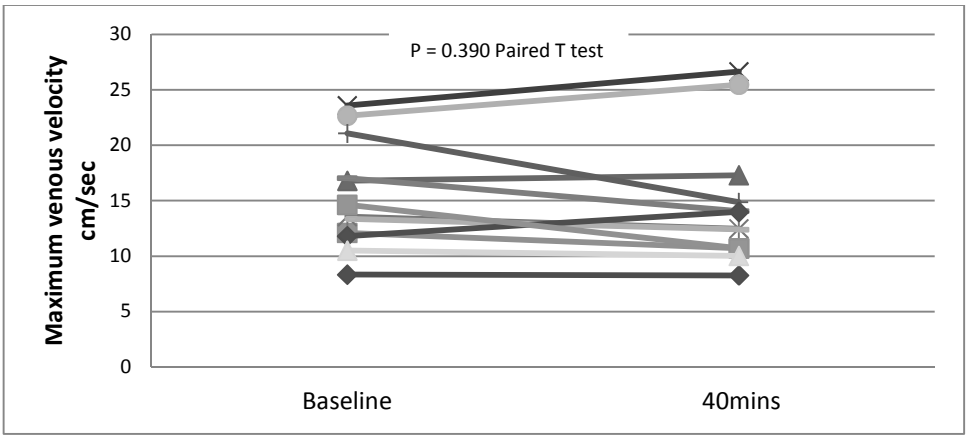


Figure 123: Passive stimulation limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 12)



7.2.2.3.2 Venous mean velocity

The average mean velocity in the active limbs at baseline was 5.50 (SD 1.78) and the passive limbs 5.68 (SD 2.20) cm/sec. Comparison of the baseline values demonstrated the difference to be insignificant (P = 0.825 Unpaired T test). The change from baseline with stimulation in the active limbs was 1.24 (SD 1.06) [P = 0.002 Paired T test] and passive -0.35 (SD 0.49) cm/sec [P = 0.031 Paired T test] equating to percentage changes of 24.97% (SD 20.92) and -6.30% (SD 10.67). Comparison of the changes from baseline in the active and passive groups demonstrated that that the differences were statistically significant (P < 0.001 Unpaired T test) *(Figures 124-126)*.

Figure 124: Mean venous velocity change from baseline

Error bar chart demonstrating the mean venous velocity at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)

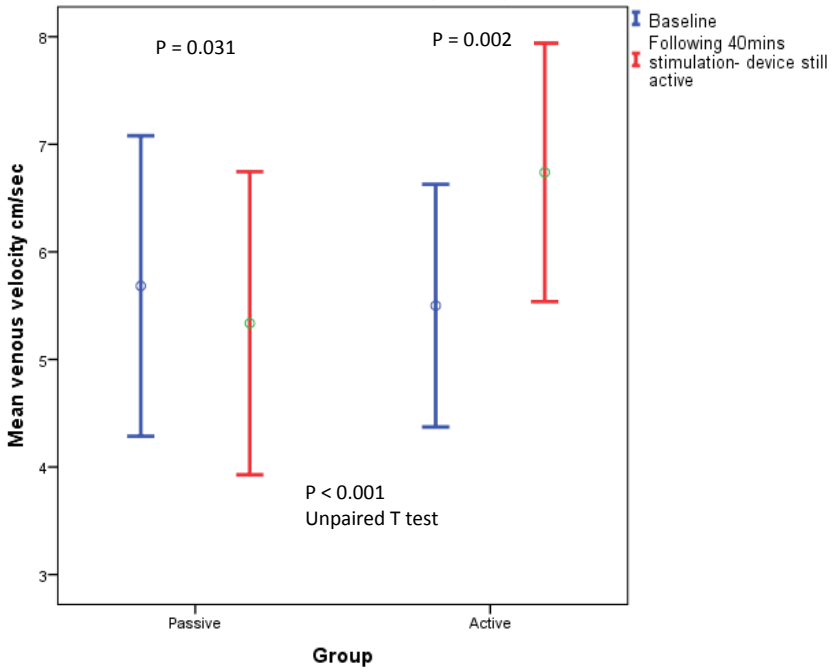


Figure 125: Active stimulation limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 12)

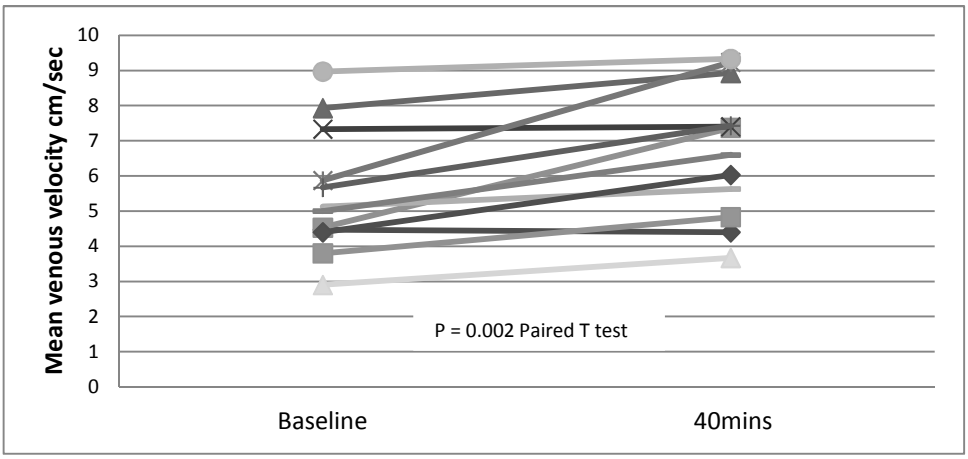
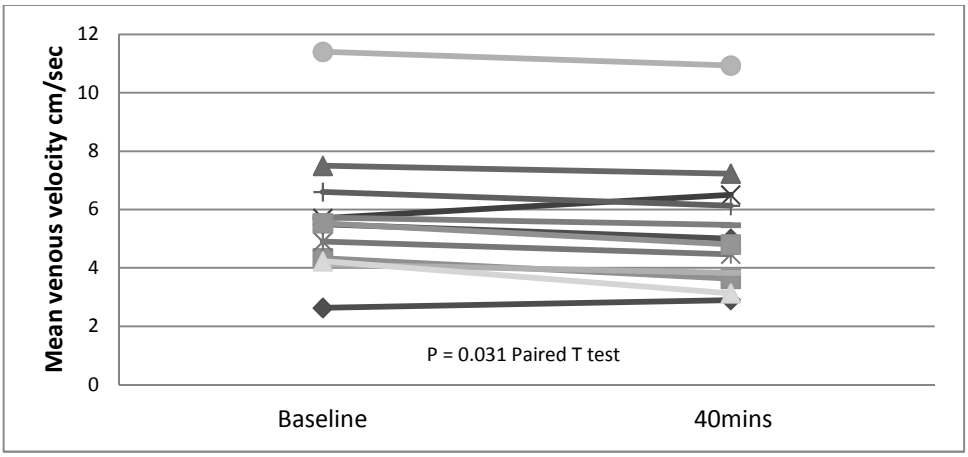


Figure 126: Passive stimulation limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 12)



7.2.2.3.3 Venous diameter

The average venous diameter at baseline in the active limbs was 8.33 (SD 2.00) and the passive limbs 8.32 (SD 2.04) mm. Comparison of the baseline value between the groups demonstrated an insignificant difference (P = 0.825 Unpaired T test). The change from baseline with stimulation in the active limbs was -0.01 (SD 0.05) [P = 0.734 Paired T test] and passive -0.06 (SD 0.10) mm [P = 0.050 Paired T test] equating to percentage changes of -0.06% (SD 0.71) and -0.72% (SD 1.10). The difference in change from baseline in the 2 groups was not statistically significant (P < 0.001 Unpaired T test). *(Figures 127-129)*

Figure 127: Venous diameter change from baseline

Error bar chart demonstrating the venous diameter at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)

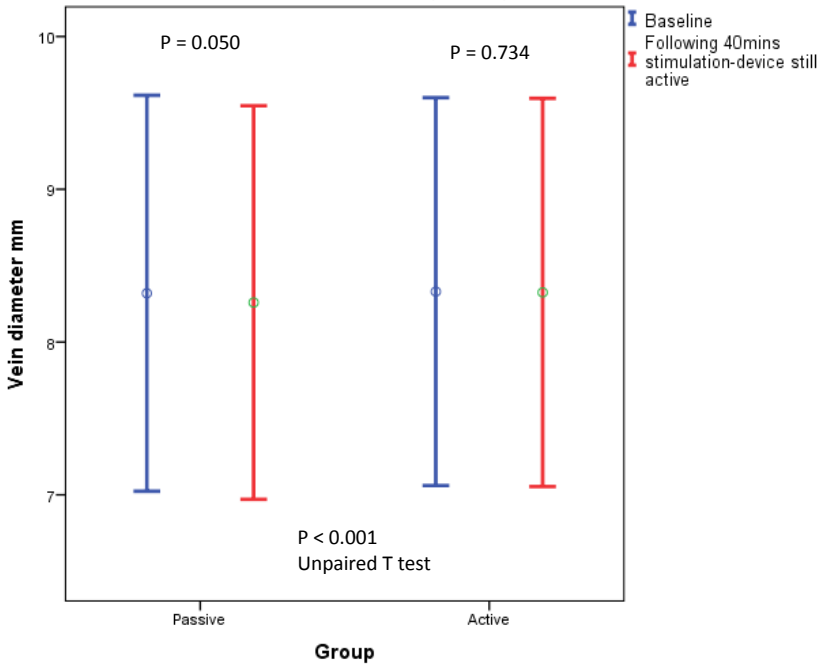


Figure 128: Active stimulation limbs: Change in mean venous diameter (mm) from baseline with 40 minutes of stimulation (n = 12)

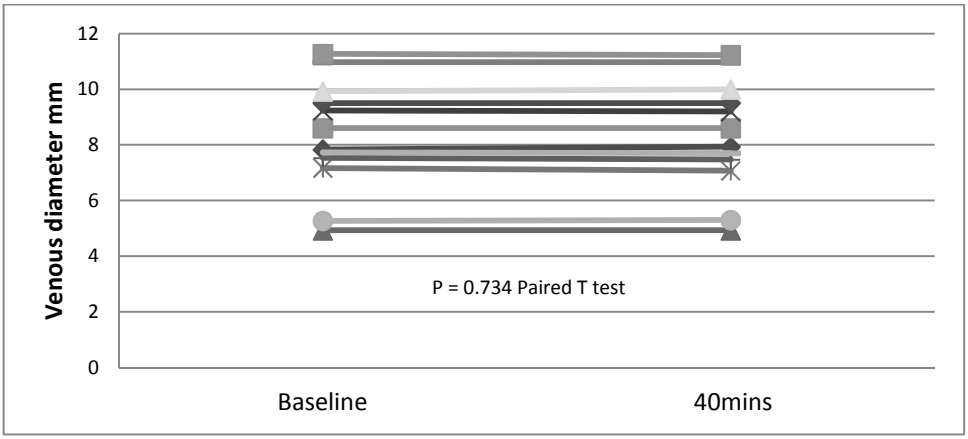
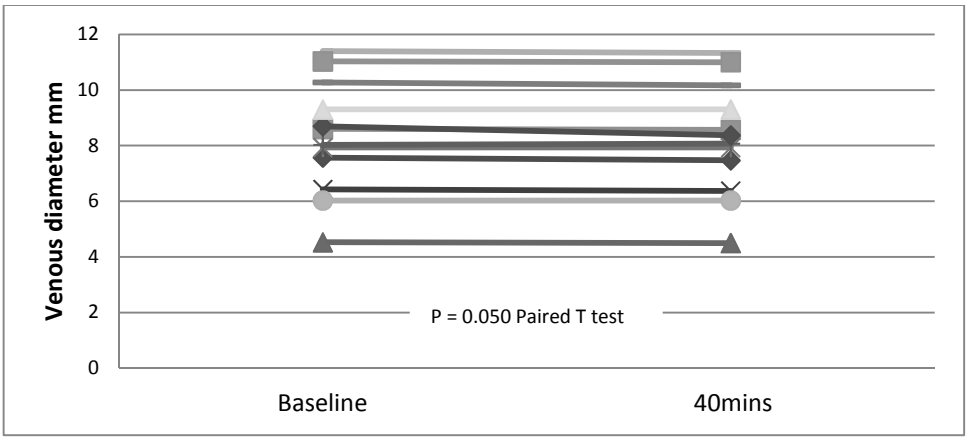


Figure 129: Passive stimulation limbs: Change in mean venous diameter (mm) from baseline with 40 minutes of stimulation (n = 12)



7.2.2.3.4 Venous volume flow

The mean volume flow at baseline in the active limbs was 0.27 (SD 0.10) and the passive limbs 0.28 (SD 0.12) L/min. Comparison of the baseline values demonstrated an insignificant difference (P = 0.707 Unpaired T Test). The change from baseline with stimulation in the active limbs was 0.08 (SD 0.06) [P = 0.001 Paired T test] and passive -0.02 (SD 0.02) L/min [P = 0.024 Paired T test] equating to percentage changes of 78.50% (SD 57.12) and -18.50% (SD 24.41). Comparison of the difference in change from baseline in the 2 groups was statistically significant [P < 0.001 Unpaired T test] *(Figures 130-132)*.

Figure 130: Venous volume flow change from baseline

Error bar chart demonstrating the venous volume flow at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)

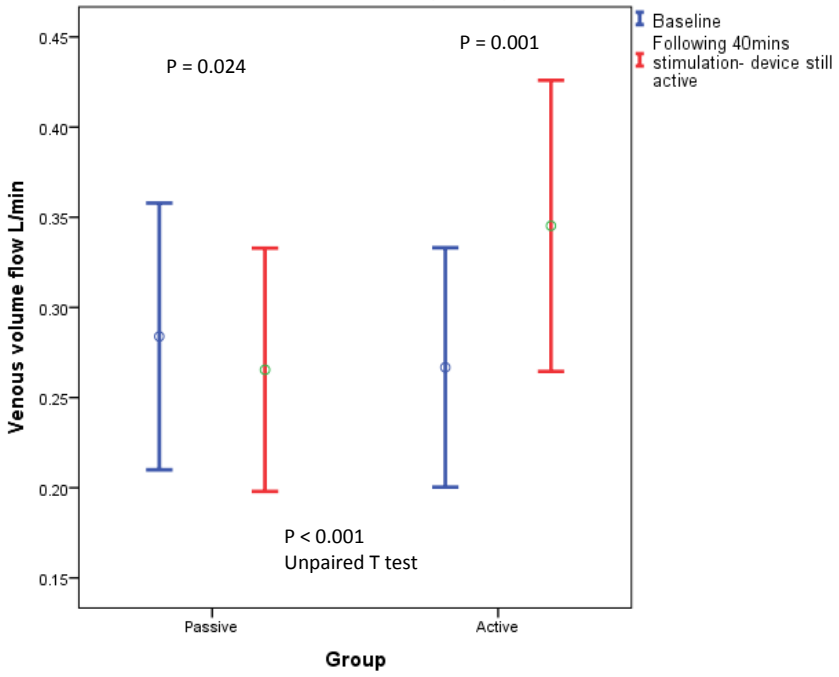


Figure 131: Active stimulation limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 12)

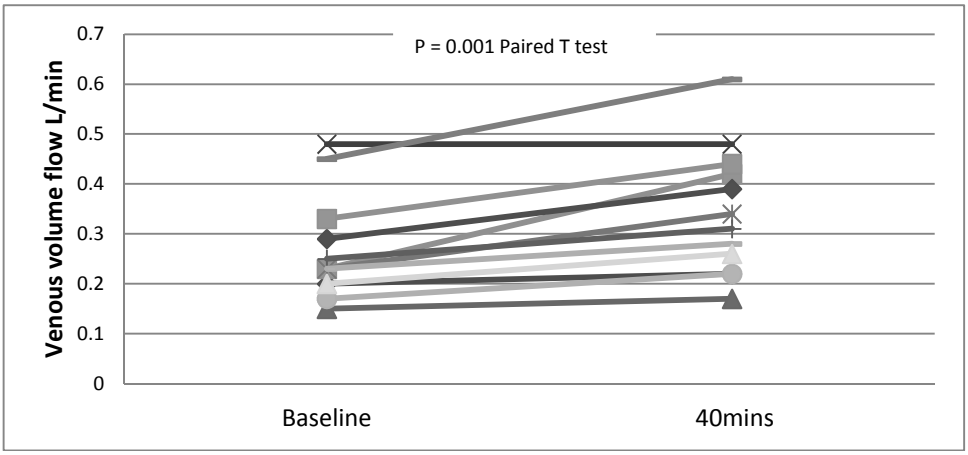
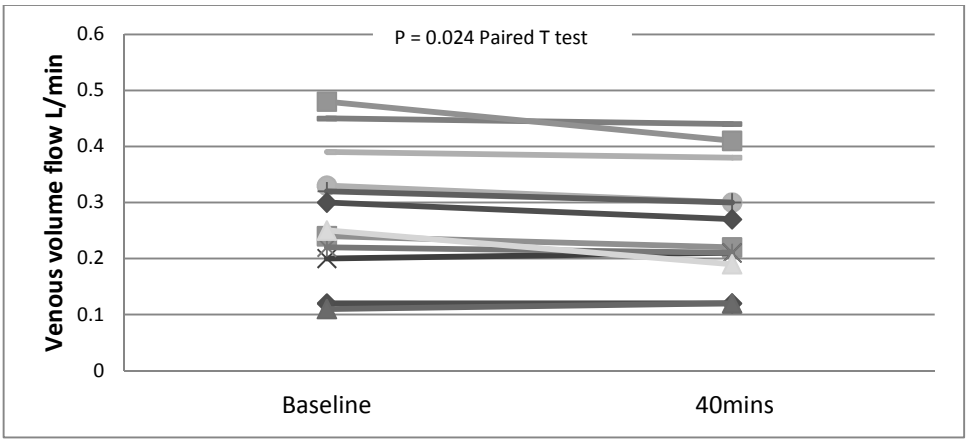


Figure 132: Passive stimulation limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 12)



7.2.2.4 Skin perfusion/ Laser Doppler flowmetry

There was no appreciable change in skin temperature during the study period. Baseline temperature in the active group was 29.84 (SD 2.32) and passive group 29.38 (SD 2.04) ^{o}C. The difference in baseline values was not statistically significant [P = 0.630 Unpaired T test].
The change in temperature from baseline was 0.33 (SD 0.94)[P = 0.257 Paired T test], passive group 0.52 (SD 1.14) ^{o}C [P = 0.258 Paired T test]. The differences in change from baseline between the two groups was not statistically significant [P = 0.657 Unpaired T test].

Baseline microcirculatory flow in the active groups was 12.37 (SD 7.24) and the passive group 35.30 (SD 36.70). The difference in baseline values seen in the groups was not statistically significant [P = 0.056 Unpaired T test]. The change at 15 minutes from baseline in the active group was 18.69 (SD 23.28) [P = 0.024 Paired T test], in the passive group was 1.16 (SD 6.74) [P = 0.579 Paired T test]. Analysis of the differences seen between the active and passive groups demonstrated statistical significance [P = 0.026 Unpaired T test].
The change at 30 minutes from baseline in the active groups was 25.29 (SD 27.07) [P = 0.011 Paired T test] and passive -1.81 (SD 9.42) [P = 0.538 Paired T test]. Analysis of the differences in the changes seen was demonstrated to be statistically significant [P = 0.005 Unpaired T test].
The change at 45 minutes from baseline in the active group was 22.31 (SD 17.37) [P = 0.002 Paired T test] and passive -3.38 (SD 4.68) [P = 0.038 Paired T test].

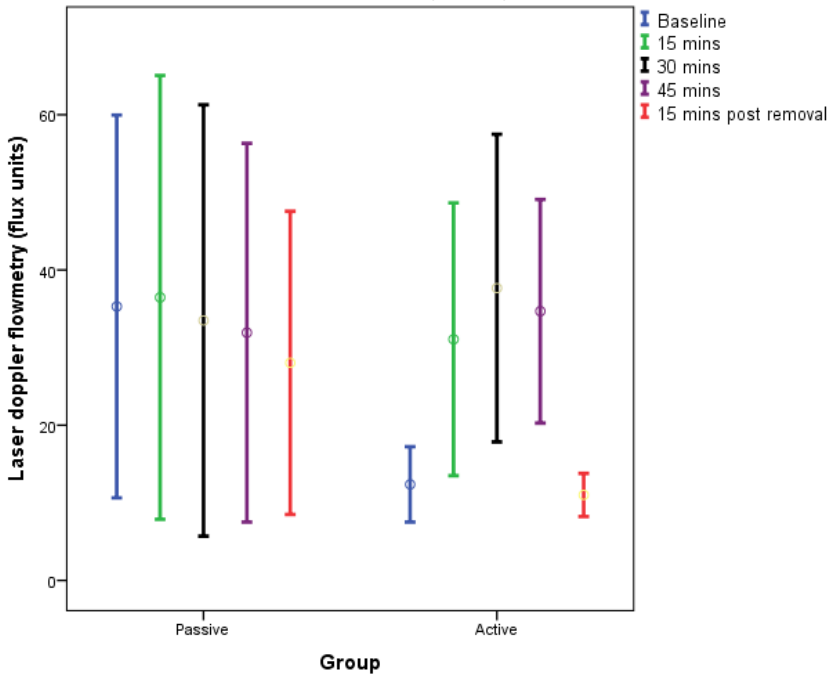
Analysis of the differences in the changes seen demonstrated statistical significance (P < 0.001 Unpaired T test).

The change at 15 minutes following device deactivation in the active groups was -1.36 (SD 4.27) [P = 0.315 Paired T test] and passive -7.27 (SD 8.61) [P = 0.019 Paired T test]. Analysis of the differences in the changes seen demonstrated non-significance (P = 0.055 Unpaired T test) *(Figure 133)*.

Figure 133: Laser Doppler flowmetry change from baseline

Error bar chart demonstrating laser Doppler flowmetry at baseline and at 40 minutes in the active and passive groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 12)
Passive: contralateral limb to active limbs (n = 12)



7.2.2.5 Cardiovascular effects

The mean heart rate at baseline was 73.25 (SD 11.32) and decreased to 69.50 (SD 11.16) 30 minutes after initiation of stimulation [P = 0.078 Paired T test]. The heart rate was 71.41 (SD 9.50) following deactivation of the device [P = 0.298 Paired T test].

There was no significant change in systolic blood pressure following device activation [P = 0.593 Paired T test] or deactivation [P = 0.246 Paired T test]. There

was no significant change in diastolic blood pressure following device activation [P = 0.734 Paired T test] or deactivation [P = 0.297 Paired T test].

7.3 Randomised patients

7.3.1 Baseline Characteristics

The final 10 participants were randomised to either active stimulation (i.e. one limb received active stimulation and other limb deemed passive) or a control group (i.e. stimulation device applied but not activated). Statistical analysis of the patients in the active and control groups was carried out to establish if they were comparable *(Tables 27-29)*.

Table 27: Comparison of the baseline characteristics of participants in the active and control groups

	Active n=5	Control n=5	P value
Age (mean in yrs)	61.40 (SD 4.28)	58.00 (SD 8.00)	0.426^
Sex	3 F, 2 M	3 F, 2 M	1.000~
Smoking status	1 current, 4 never	2 current, 3 never	0.500~
Pack years (mean)	3.00 (SD 6.71)	12.00 (17.89)	0.323^
EtOH	4 current, 1 never	4 current, 1 never	1.000~
EtOH units/ week (mean)	7.20 (SD 5.76)	8.20 (SD 8.67)	0.835^
BMI (mean)	27.84 (SD 4.26)	27.80 (SD 3.62)	0.988^

^ Unpaired T test (NB Shapiro Wilk test utilised to establish normality of distribution for data. P> 0.05 in all cases)

~ Fishers exact test

Table 28: Comparison of comorbidities

	Active	Control	P value
Diabetes	4 No, 1 Yes	5 No	0.500~
Cerebrovascular disease	4 No, 1 Yes	5 No	0.500~
Hypertension	3 No, 2 Yes	4 No, 1 Yes	0.500~
Cardiovascular disease	4 No, 1 Yes	5 No, 1 Yes	0.500~
Respiratory	5 No	4 No, 1 Yes	0.500~

~Fisher's exact test

Table 29: Comparison of concomitant medications between active and control groups

	Active	Control	P value
Antiplatelet	5 Yes	5 Yes	1.000~
Beta blocker	3 No, 2 Yes	5 No	0.222~
ACE inhibitor	4 No, 1 Yes	4 No, 1 Yes	0.778~
Statin	3 No, 2 Yes	5 No	0.222~

~Fisher's exact test

It is possible to see that the groups of patients, active and control, were similar in all regards.

7.3.2 Randomised patients: Haemodynamic Efficacy of geko^{TM} in patients with varicose veins

7.3.2.1 *Arterial duplex*

7.3.2.1.1 Maximum arterial velocity

The baseline maximum arterial velocity was 74.13 (SD 13.99) in the active limbs, 71.47 (SD 15.58) in the passive limbs and 67.51 (SD 11.70) cm/sec in the control limbs [P = 0.646 ANOVA]. The mean change in maximum arterial velocity from baseline in the active limbs was 3.33 (SD 6.26) [Paired T test P = 0.300]; passive -

6.32 (SD 6.31) [Paired T test P = 0.089] and control limbs -1.29 (SD 5.12) cm/sec [Paired T test P = 0.446] equating to percentage changes of 5.30% (SD 8.79), -8.29% (SD 7.51) and -1.12% (SD 7.26) respectively *(Figures 134-137)*.

The difference in change from baseline between the active and passive groups was statistically significant [Unpaired T Test: P = 0.041] whilst not significant when comparing the passive and control limbs, P = 0.119 [Unpaired T Test]. ANOVA analysis demonstrated statistical significance P = 0.050, which was further investigated with post hoc Bonferonni analysis: active versus passive limbs P = 0.048, active versus control limbs P = 0.471 and control versus passive limbs P =0.376.

Figure 134: Maximum arterial velocity change from baseline

Error bar chart demonstrating the maximum arterial velocity and at baseline at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

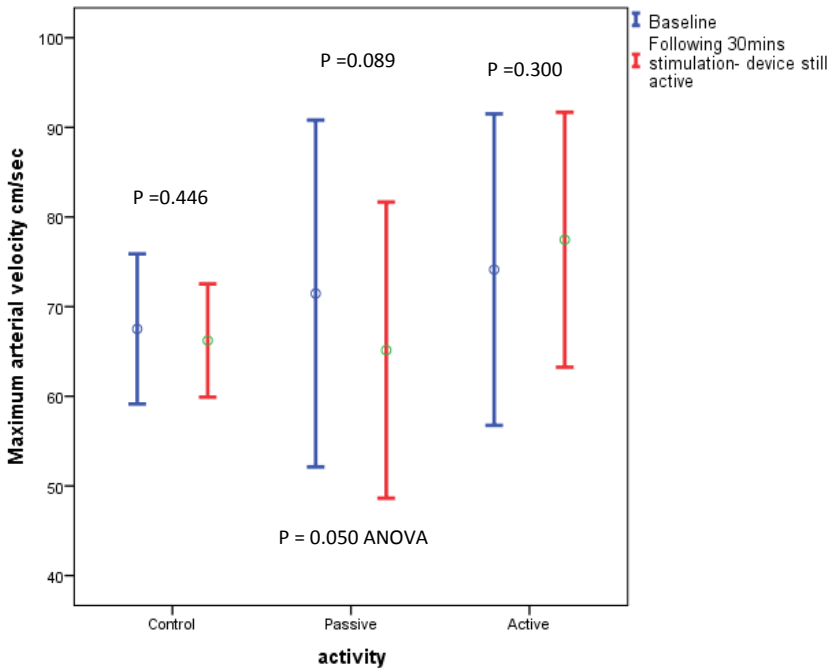


Figure 135: Active stimulation limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 5)

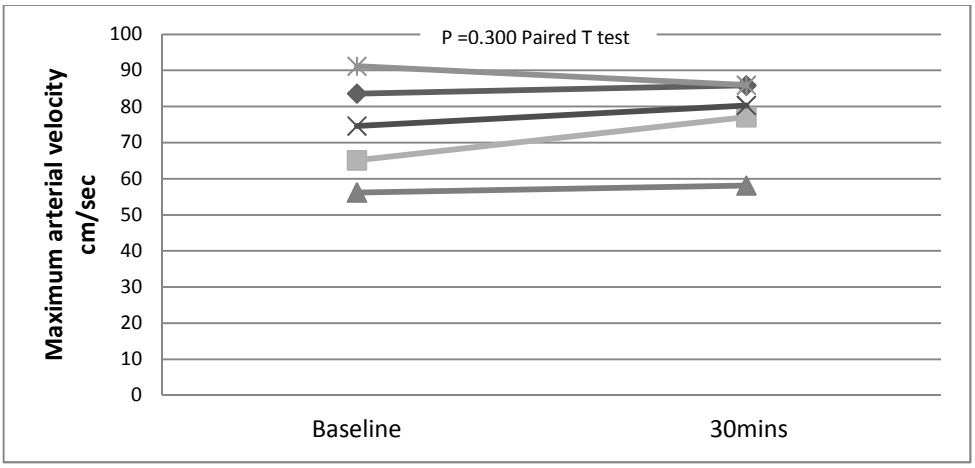


Figure 136: Passive limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of stimulation of contralateral limb (n = 5)

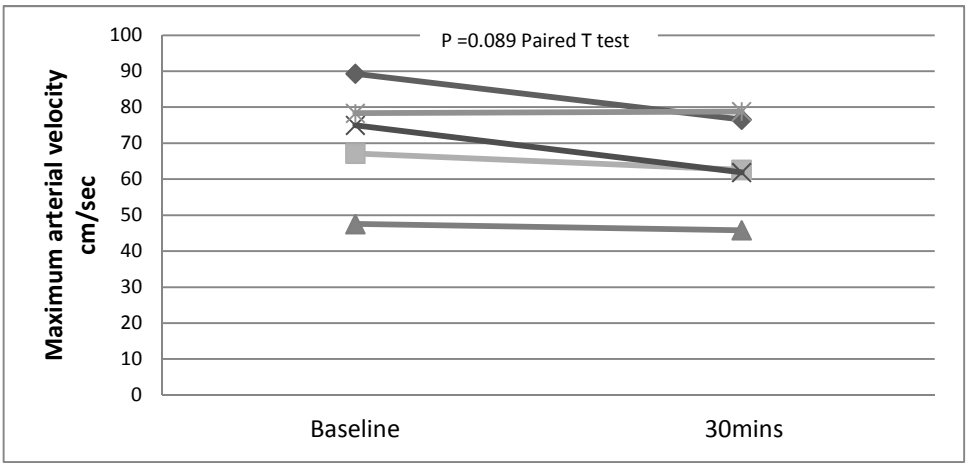
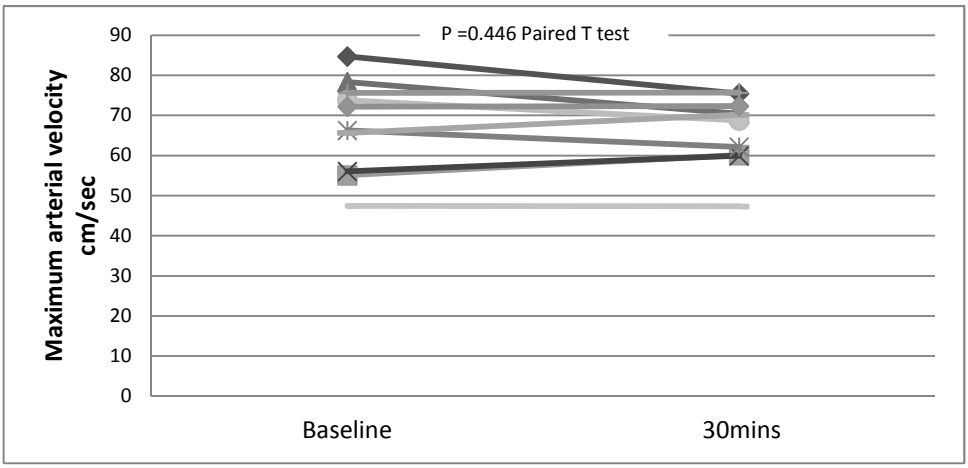


Figure 137: Control limbs: Change in maximum arterial velocity (cm/sec) from baseline with 30 minutes of device applied to limbs but not activated (n = 10)



7.3.2.1.2 Mean arterial velocity

The mean baseline mean arterial velocity in the active limbs was 9.45 (SD 4.24), passive limbs 8.95 (SD 3.98) and control limbs was 9.95 (SD 2.15) cm/sec. There was no statistically significant difference in baseline values on ANOVA analysis [P = 0.848].

The change in mean arterial velocity from baseline in the active limbs was 1.11 (SD 1.07) [Paired T Test P = 0.083], passive limbs -1.23 (SD 1.93)[Paired T test P = 0.227] and control limbs was -0.09 (SD 0.34) cm/sec [Paired T Test P = 0.422] equating to percentage changes of 17.40% (SD 23.29), -9.98% (SD 14.67) and -0.81% (SD 3.61) respectively. The difference in change from baseline between the active and passive groups was statistically significant (Unpaired T Test: P = 0.046). Passive versus control P = 0.083 (Unpaired T test). ANOVA analysis of the change from baseline for the 3 groups was statistically significant (P = 0.013) with post hoc Bonferonni demonstrating: active versus control P = 0.191, active versus passive P = 0.011 but passive versus control P = 0.226 *(Figures 138-141)*.

Figure 138: Mean arterial velocity change from baseline

Error bar chart demonstrating the mean arterial velocity at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

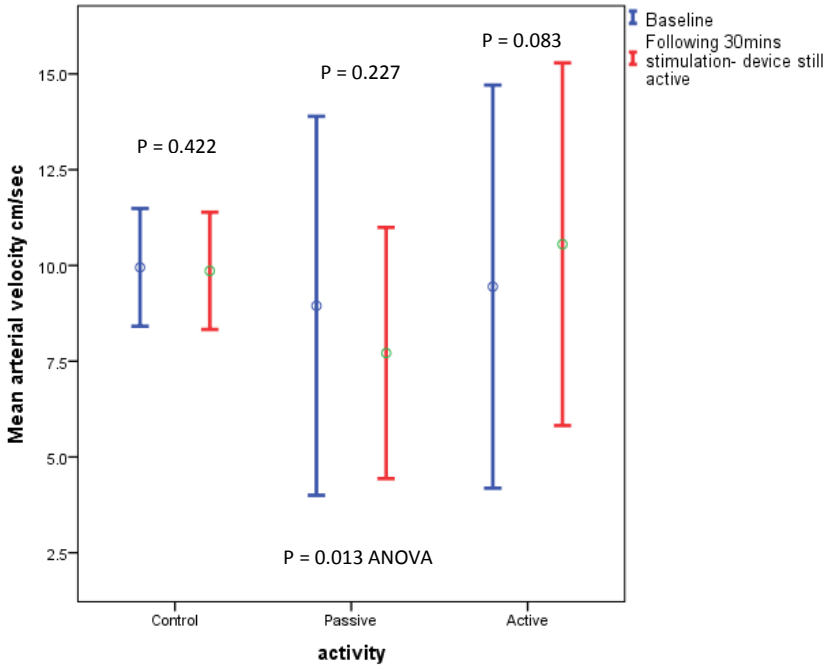


Figure 139: Active stimulation limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation (n = 5)

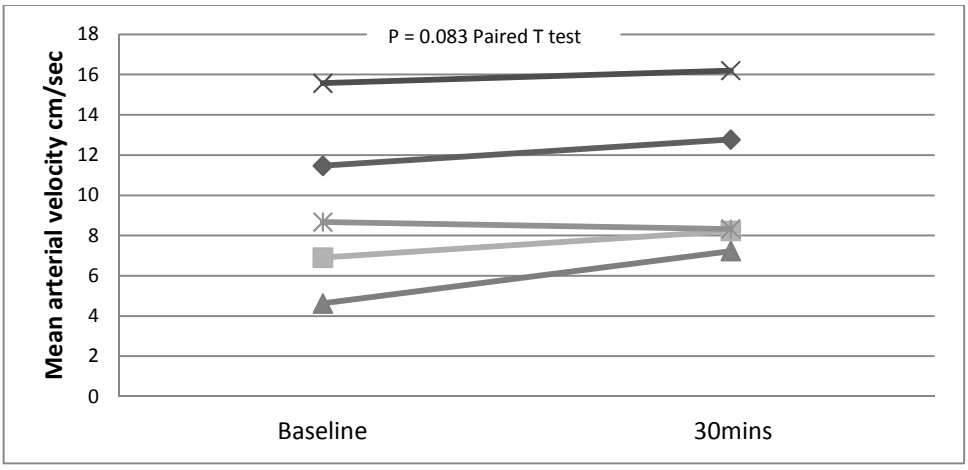


Figure 140: Passive limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of stimulation of contralateral limb (n = 5)

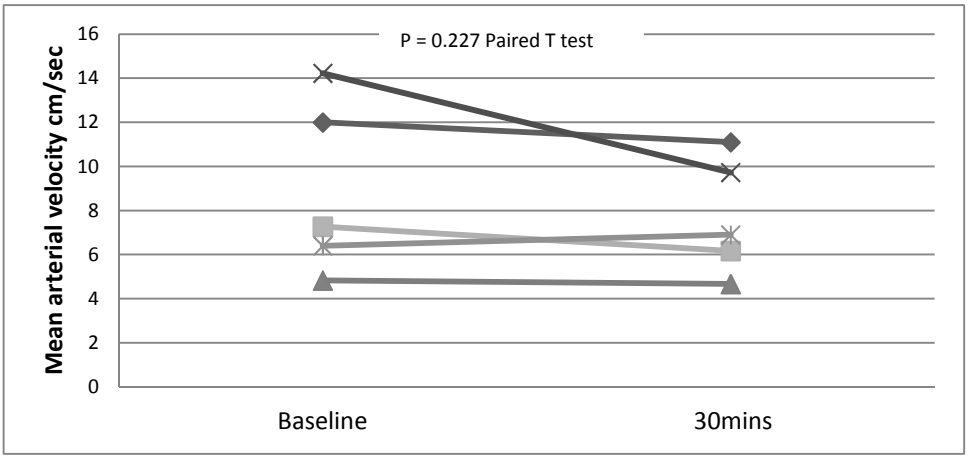
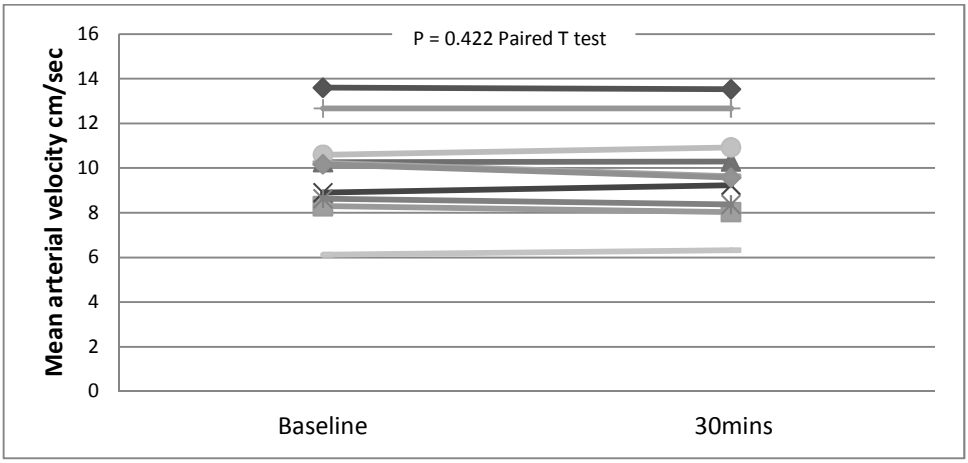


Figure 141: Control limbs: Change in mean arterial velocity (cm/sec) from baseline with 30 minutes of device applied to limbs but not activated (n = 10)



7.3.2.1.3 Arterial diameter

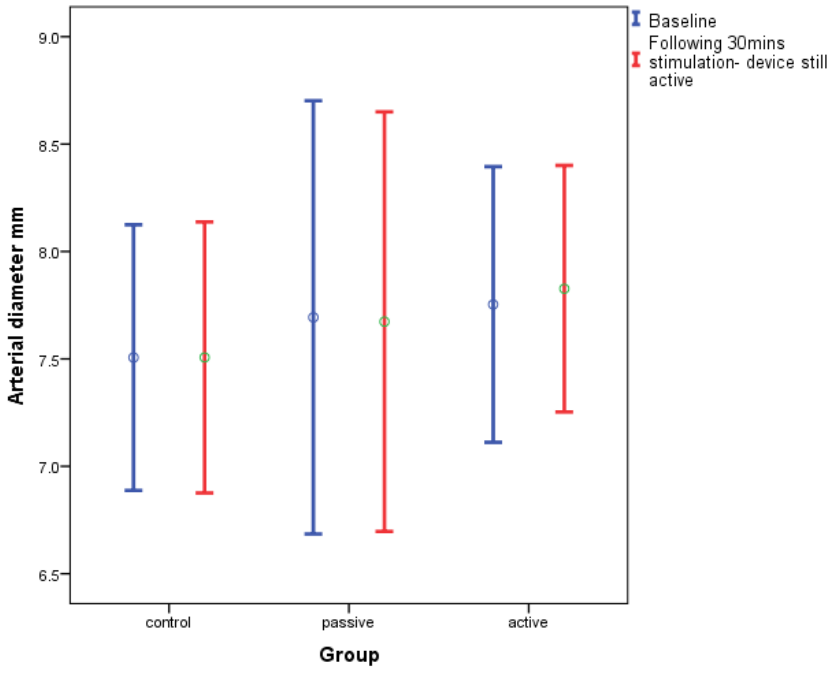
The mean baseline arterial diameter in the active group was 7.75 (SD 0.52), passive group 7.69 (SD 0.81) and control group 7.51 (SD 0.86) mm. The difference in baseline values was not statistically significant [P = 0.821 ANOVA]. The mean change in arterial diameter in the active group was 0.07 (SD 0.07)[Paired T test P = 0.074], passive group -0.02 (SD 0.08)[Paired T test P = 0.622] and control group 0.00 (SD) mm [Paired T test P = 0.957], equating to percentage changes of 0.99% (SD 0.92), -0.23% (SD 1.01) and -0.01% (SD 0.52) respectively.

The difference in change from baseline between the active and passive groups was not statistically significant [Unpaired T Test: P = 0.090], passive versus control P = 0.521 [Unpaired T Test]. ANOVA analysis to investigate the change in diameter from baseline was statistically insignificant [P = 0.054] *(Figure 142)*.

Figure 142: Arterial diameter change from baseline

Error bar chart demonstrating the arterial diameter at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)



7.3.2.1.4 Arterial volume flow

The baseline values for arterial volume flow for the active limbs was 0.38 (SD 0.16), passive group 0.35 (SD 0.14) and control group 0.38 (SD 0.11) L/min. The difference in baseline values was not statistically significant [P = 0.893 ANOVA]. The mean change in arterial volume flow in the active limbs was 0.07 (SD 0.03) [Paired T test P = 0.010], passive limbs -0.05 (SD 0.08) [Paired T test P = 0.255] and control limbs -0.01 (SD 0.01) [Paired T Test P = 0.035] equating to percentage changes of 21.07% (SD 16.11), -9.57% (SD 12.62) and -2.79% (SD 3.75) respectively.

The difference in change from baseline between the active and passive groups was statistically significant [Unpaired T Test: P = 0.016] but not when comparing the passive and control groups [Unpaired T test P = 0.178]. This was confirmed on ANOVA analysis [P = 0.001] with post hoc Bonferonni: active versus passive P = 0.001; active versus control P = 0.008 and passive versus control P = 0.454 *(Figures 143-146)*.

Figure 143: Arterial volume flow change from baseline

Error bar chart demonstrating the arterial volume flow at baseline and at 30 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

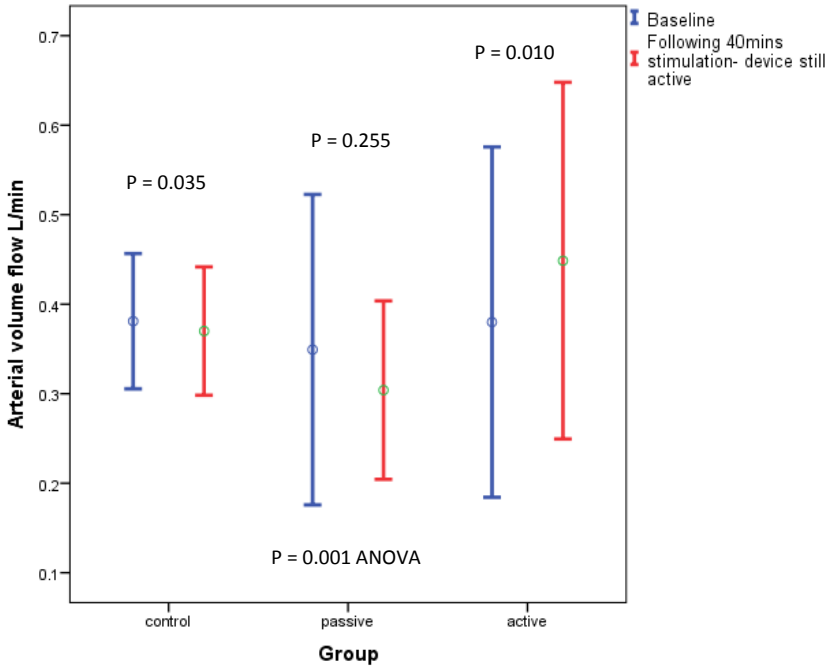


Figure 144: Active stimulation limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of stimulation (n = 5)

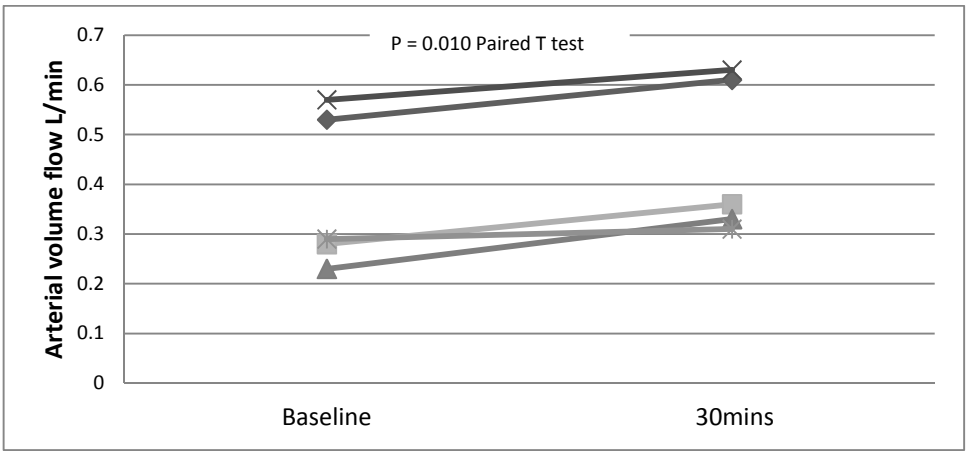


Figure 145: Passive limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of stimulation of contralateral limb (n = 5)

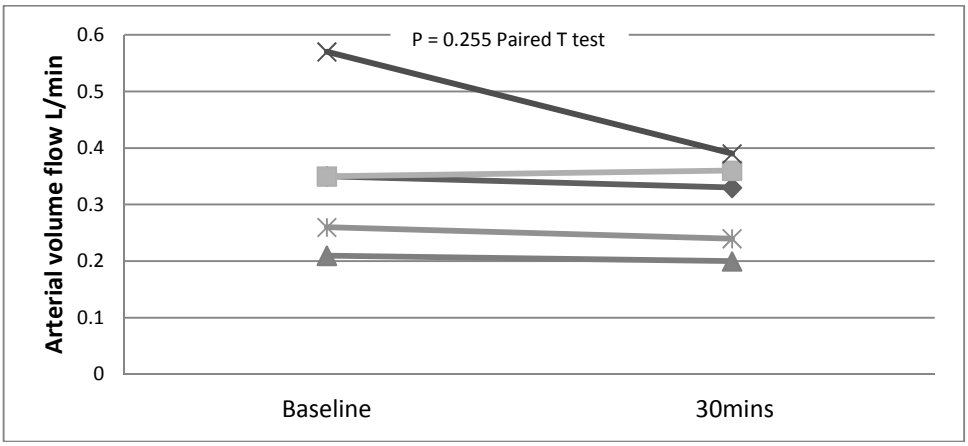
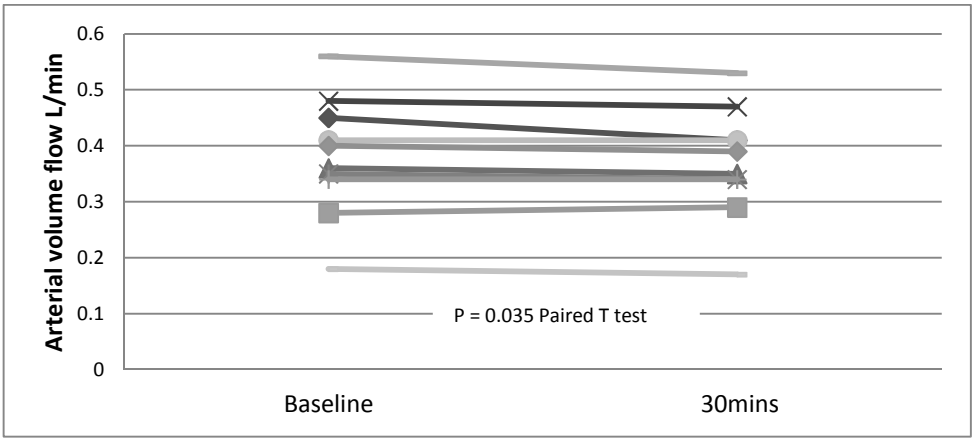


Figure 146: Control limbs: Change in arterial volume flow (L/min) from baseline with 30 minutes of device applied to limbs but not activated (n = 10)



7.3.2.2 Venous duplex

7.3.2.2.1 Maximum venous velocity

Baseline maximum venous velocity in the active limbs was 15.55 (SD 3.24), passive limbs 13.93 (SD 2.40) and control limbs 17.23 (SD 5.49) cm/sec. ANOVA analysis determined the difference in baseline values was not statistically significant [P = 0.407]. The mean change in maximum venous velocity in the active limbs was 6.33 (SD 2.87) [Paired T test P = 0.008], passive limbs 0.89 (SD 1.91) [Paired T test P = 0.356] and control limbs 0.86 (SD 1.82) cm/sec [Paired T test P = 0.170] equating to percentage changes of 44.08% (SD 21.62), 6.27% (SD 11.92) and 6.33% (SD 10.10) respectively.

The difference in change from baseline between the active and passive groups was statistically significant [Unpaired T Test: P = 0.008] whilst not for passive and control [Unpaired T test P = 0.972]. ANOVA analysis determined that the differences in the changes from baseline were significant [P = 0.001] however post hoc Bonferonni demonstrated: active versus passive P = 0.003; active versus control P = 0.001 and control versus passive P = 1.000 *(Figures 147-150)*.

Figure 147: Maximum venous velocity change from baseline

Error bar chart demonstrating the maximum venous velocity at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

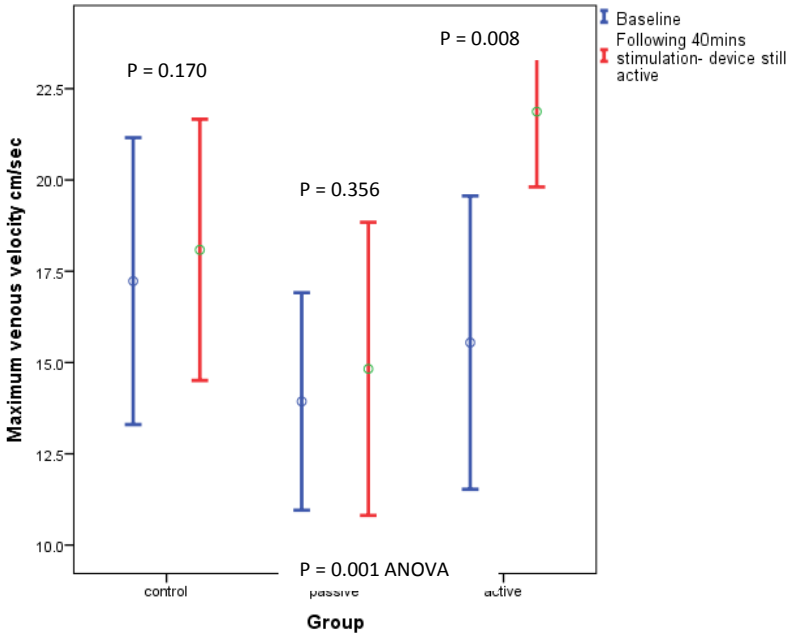


Figure 148: Active limbs: Change in maximum venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 5)

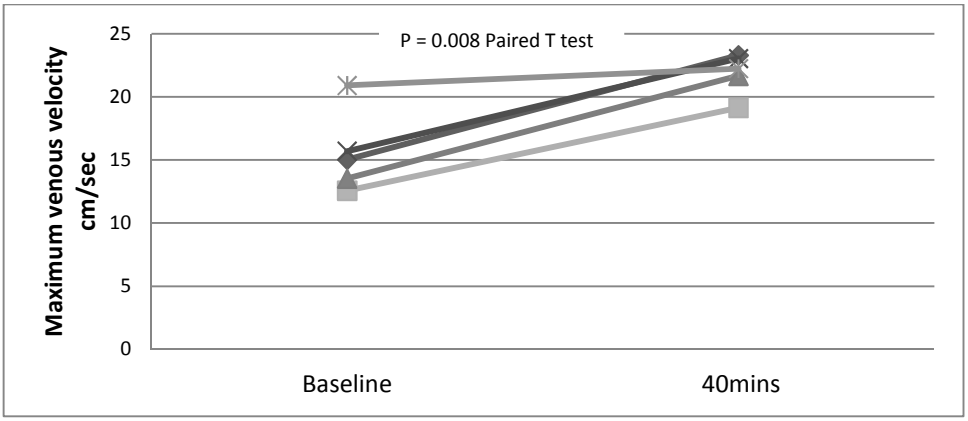


Figure 149: Passive limbs: Change in maximal venous velocity (cm/sec) from baseline with 40 minutes of stimulation of contralateral limb (n = 5)

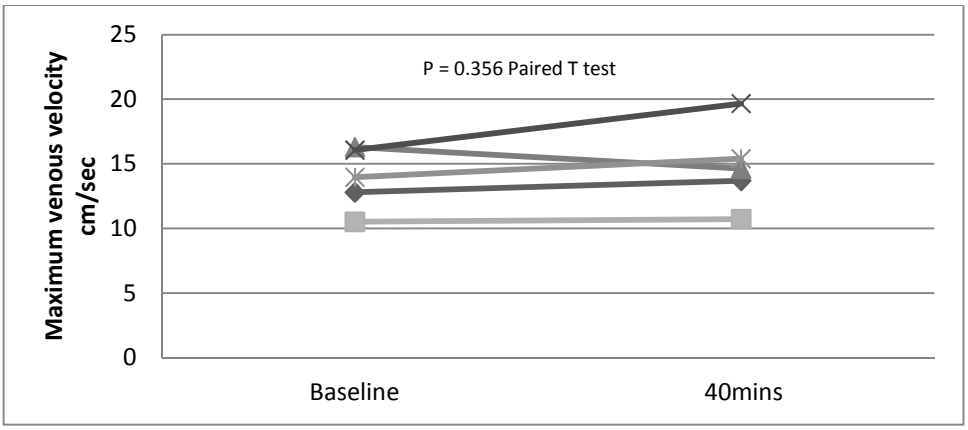
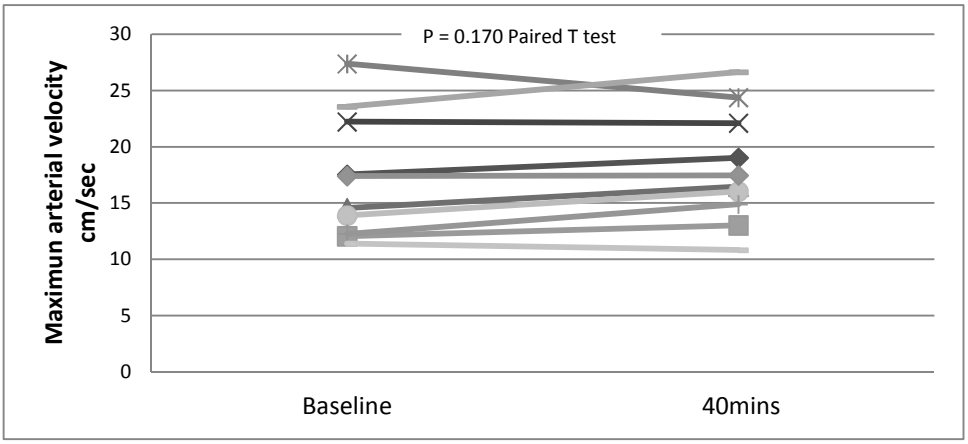


Figure 150: Control limbs: Change in maximal venous velocity (cm/sec) from baseline with 40 minutes of device applied to limbs but not activated (n = 10)



7.3.2.2.2 Mean venous velocity

The baseline values for mean venous velocity in the active limbs was 4.67 (SD 1.14), passive limbs 4.97 (SD 1.22) and control limbs 6.90 (SD 2.38) cm/sec. ANOVA analysis determined that the difference in baseline values was not statistically significant [P = 0.077].

The change in mean venous velocity in the active limbs was 1.27 (SD 0.18) [Paired T test P < 0.001], passive -0.45 (SD 0.23) [Paired T test P = 0.011] and control limbs 0.05 (SD 0.56) cm/sec [Paired T test P = 0.784] equating to percentage changes of 28.35% (SD 7.03), -9.35% (SD 4.25) and 0.85% (SD 9.01) respectively.

The difference in change from baseline between the active and passive groups was not statistically significant [Unpaired T Test: P < 0.001] whilst not for passive and

control [Unpaired T test P = 0.077]. The difference in change from baseline between the groups was statistically significant (ANOVA P < 0.001). Post hoc Bonferonni demonstrated that: active versus passive P < 0.001; active versus control P < 0.001 and control versus passive P = 0.140 *(Figure 151-154)*.

Figure 151: Mean venous velocity change from baseline

Error bar chart demonstrating the mean venous velocity at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

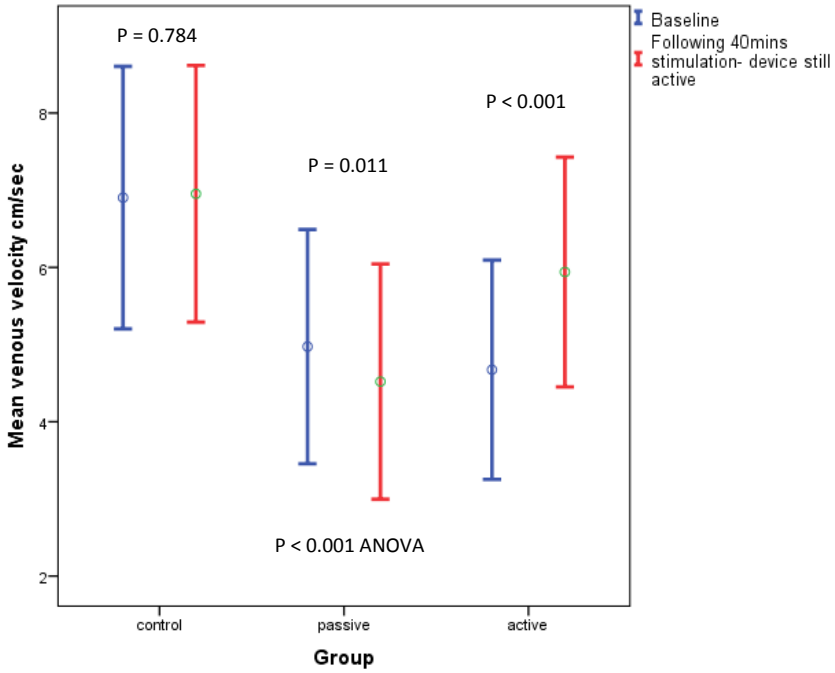


Figure 152: Active limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 5)

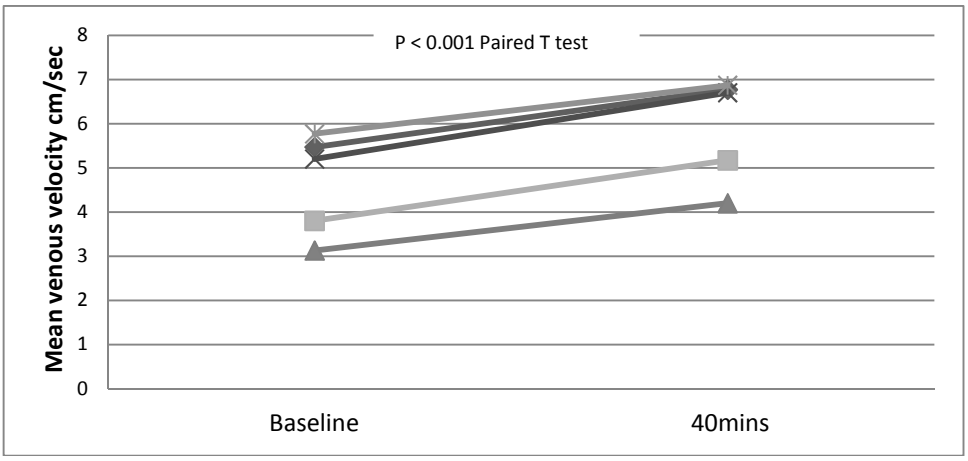


Figure 153: Passive limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 5)

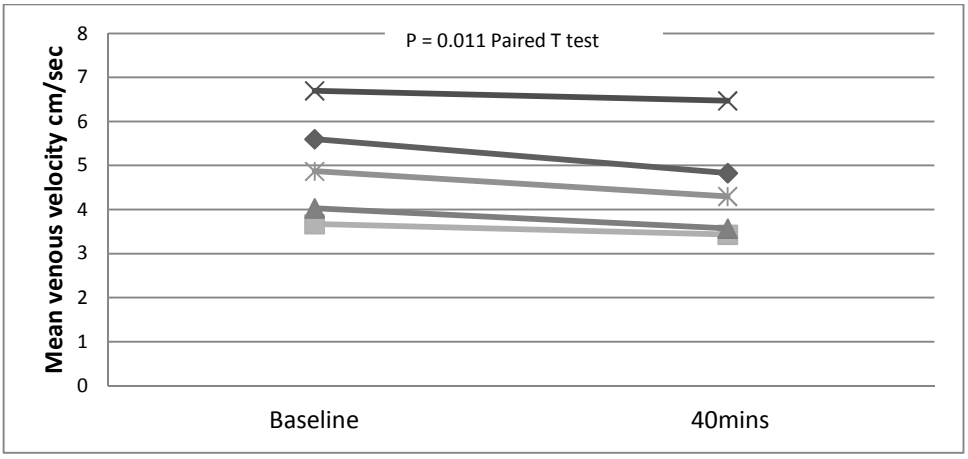
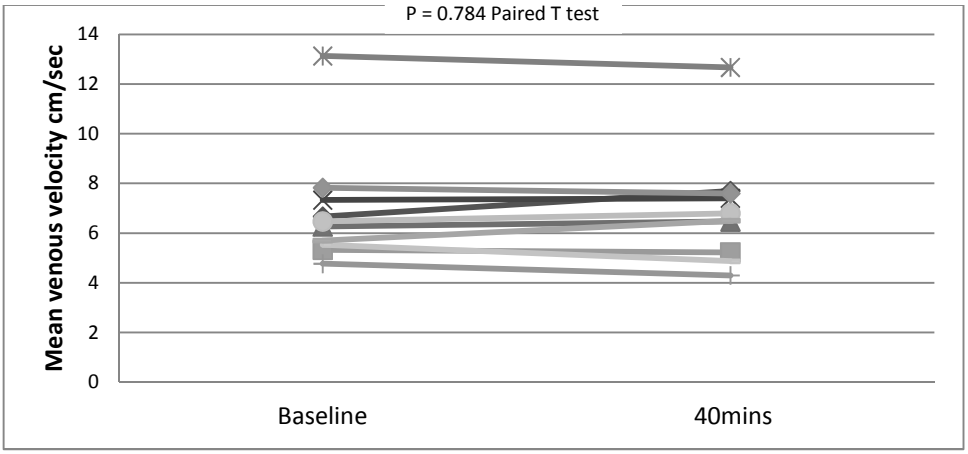


Figure 154: Control limbs: Change in mean venous velocity (cm/sec) from baseline with 40 minutes of stimulation (n = 10)



7.3.2.2.3 Venous diameter

The mean baseline venous diameter in the active group was 9.21 (SD 1.76), passive group 9.00 (SD 0.97) and control group 6.88 (SD 1.20) mm. The difference in baseline values was not statistically significant [P = 0.005 ANOVA, Active vs control P = 0.014, active vs passive P = 1.000, control vs passive P = 0.026]. The mean change in venous diameter in the active group was 0.03 (SD 0.10) [Paired T test P = 0.588], passive group 0.01 (SD 0.08)[Paired T test P = 0.862] and control group -0.03 (SD 0.05) mm [Paired T test P = 0.119], equating to percentage changes of 0.22% (SD 0.99), 0.13% (SD 0.88) and -0.42% (SD 0.72) respectively.

The difference in change from baseline between the active and passive groups was not statistically significant [Unpaired T Test: P =0.737], passive versus control P = 0.315 (Unpaired T Test). ANOVA analysis to investigate the change in diameter from baseline was statistically insignificant [P = 0.366].

7.3.2.2.4 Venous volume flow

The baseline values for venous volume flow for the active limbs was 0.29 (SD 0.11), passive group 0.31 (SD 0.16) and control group 0.25 (SD 0.13) L/min. The difference in baseline values was not statistically significant [P = 0.614 ANOVA]. The mean change in mean venous volume flow in the active limbs was 0.12 (SD 0.06) [Paired T test P = 0.008], passive limbs -0.02 (SD 0.02) [Paired T test P =

0.178] and control limbs 0.00 (SD 0.02) [Paired T Test P = 0.953] equating to percentage changes of 39.68% (SD 7.34), -5.60% (SD 8.26) and -1.31% (SD 7.53) respectively.

The difference in change from baseline between the active and passive groups was statistically significant [Unpaired T Test: P = 0.001] but not when comparing the passive and control groups [Unpaired T test P = 0.129]. This was confirmed on ANOVA analysis [P < 0.001] with post hoc Bonferonni: active versus passive P < 0.001; active versus control P < 0.001 and passive versus control P = 1.000 *(Figures 155-158)*.

Figure 155: Venous volume flow change from baseline

Error bar chart demonstrating the venous volume flow at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)

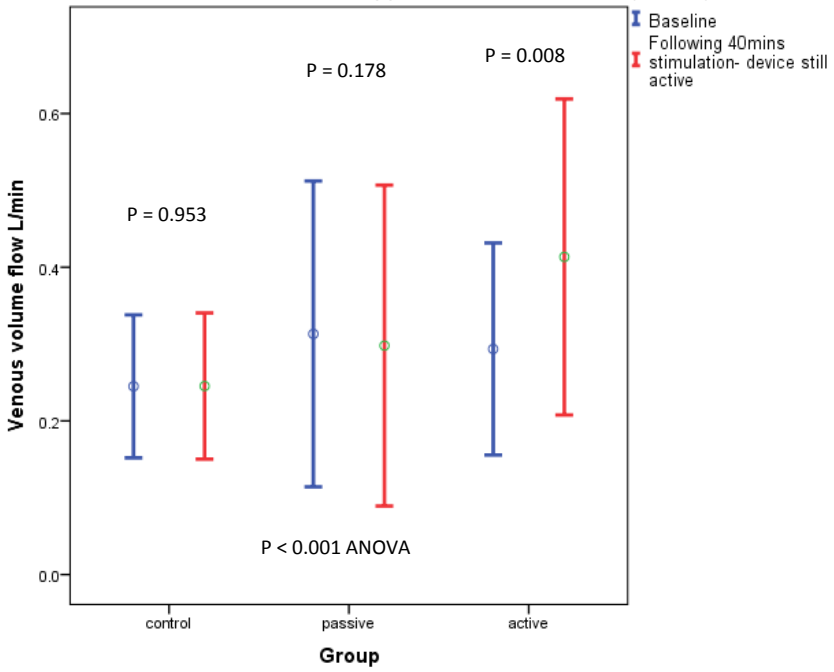


Figure 156: Active limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 5)

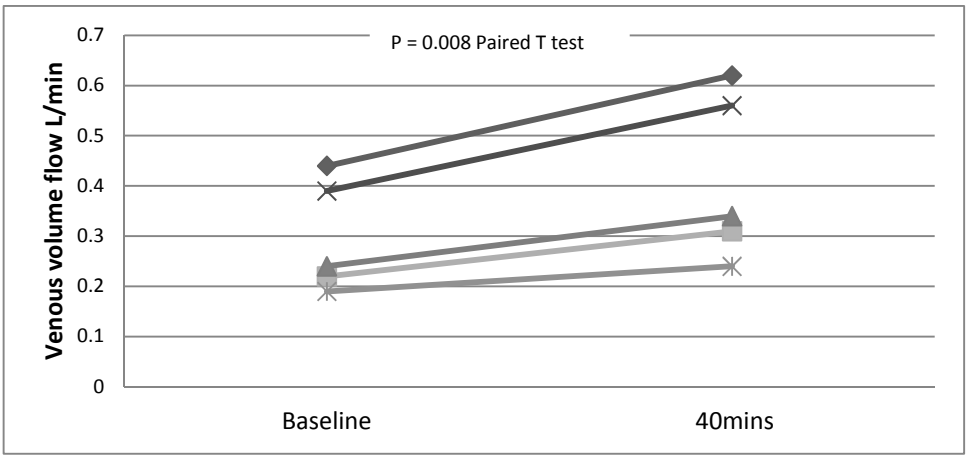


Figure 157: Passive limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 5)

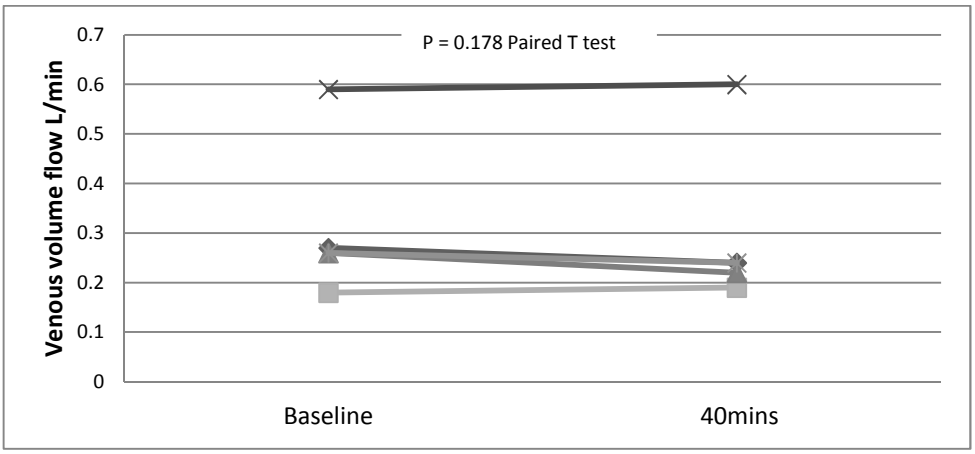
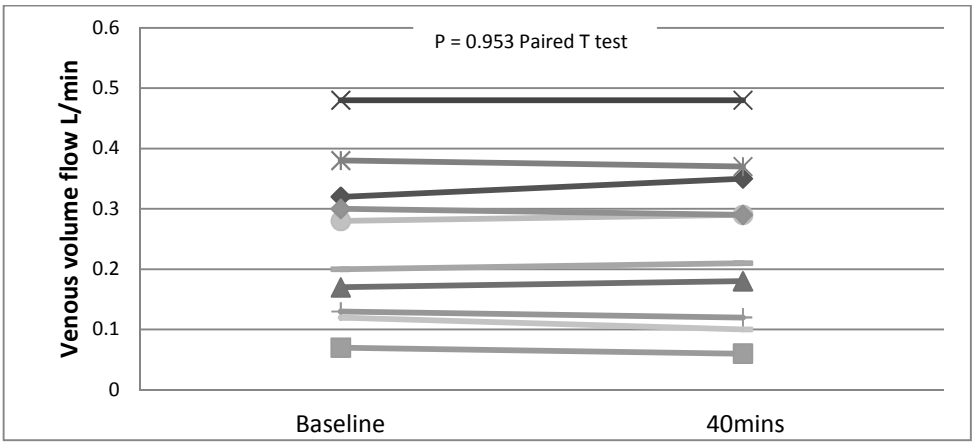


Figure 158: Control limbs: Change in venous volume flow (L/min) from baseline with 40 minutes of stimulation (n = 10)



7.3.2.3 Skin perfusion/ Laser Doppler flowmetry

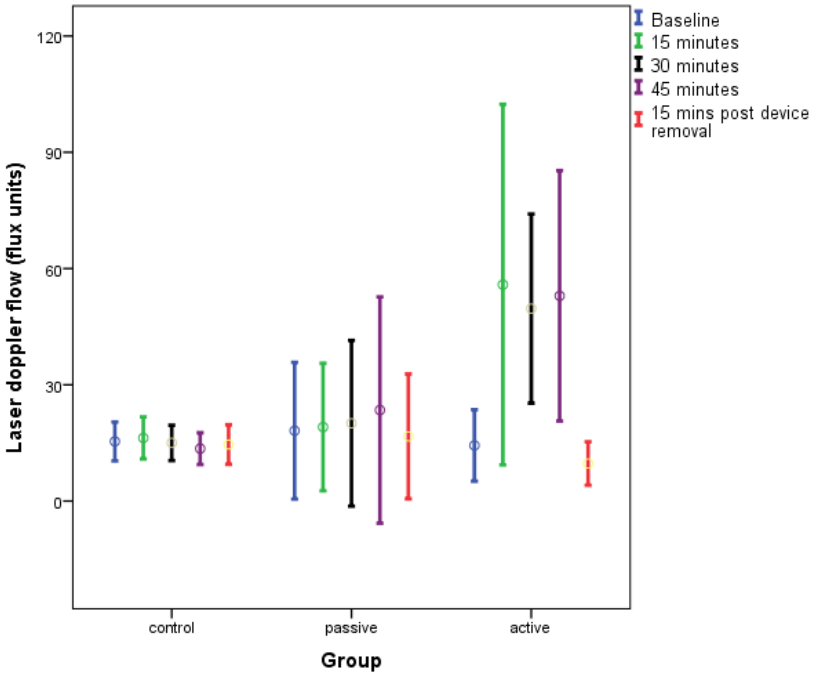
There was no appreciable change in skin temperature during the study period. Baseline temperature in the active group was 29.52 (SD 1.99), passive group 30.04 (SD 1.82) and control group 30.09 (SD 1.48)^{o}C. The difference in baseline values was not statistically significant [P = 0.819 ANOVA].
The change in temperature from baseline was active 0.50 (SD 0.78)[P = 0.224 Paired T test], passive group 0.36 (SD 0.92) and control group -0.10 (SD 0.30) ^{o}C [P = 0.322 Paired T test]. The differences in change from baseline between the two groups was not statistically significant [P = 0.121 ANOVA].

Baseline microcirculatory flow in the active groups was 14.36 (SD 7.44), passive group 18.16 (SD 14.20) and control group 15.40 (SD 7.04). The difference in baseline values seen in the groups was not statistically significant [P = 0.797 ANOVA]. The change at 15 minutes from baseline in the active group was 41.50 (SD 34.21) [P = 0.053 Paired T test], in the passive group was 0.94 (SD 3.99) [P = 0.626 Paired T test] and the control group 0.89 (SD 1.85) [P = 162 Paired T test]. Analysis of the differences seen between the active and passive groups demonstrated statistical significance [P = 0.001 ANOVA: active versus passive P = 0.004; active versus control P = 0.001; passive versus control P = 1.000].
The change at 30 minutes from baseline in the active groups was 35.30 (SD 20.67) [P = 0.019 Paired T test] and passive 1.92 (SD 5.48) [P = 0.477 Paired T test] and control -0.38 (SD 2.03) [P = 0.568 Paired T test]. Analysis of the differences in the changes seen was demonstrated to be statistically significant [P < 0.001 ANOVA. Bonferonni; active versus passive P < 0.001; active versus control P < 0.001 and passive versus control P = 1.000].
The change at 45 minutes from baseline in the active group was 38.60 (SD 25.36) [P = 0.027 Paired T test], passive 5.32 (SD 11.89) [P = 0.374 Paired T test] and control -1.86 (SD 1.84) [P = 0.011 Paired T test]. Analysis of the differences in the changes seen demonstrated statistical significance [P < 0.001 ANOVA, Bonferonni: active versus passive 0.004; active versus control P < 0.001; passive versus control P = 1.000].
The change at 15 minutes following device deactivation in the active groups was -4.66 (SD 3.51) [P = 0.041 Paired T test], passive -1.46 (SD 4.65) [P = 0.522 Paired T test] and control -0.81 (SD 2.00) [P = 0.231 Paired T test]. Analysis of the differences in the changes seen demonstrated non-significance [P = 0.110 ANOVA] *(Figure 159)*.

Figure 159: Laser Doppler flowmetry change from baseline

Error bar graph demonstrating mean laser Doppler flowmetry readings through the study period in each group. [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)

Active: limbs on which device is applied and active (n = 5)
Passive: contralateral limb to active limbs (n = 5)
Control: limbs on which device was applied but not activated (n = 10)



7.3.2.4 Cardiovascular effects

The mean heart rate at baseline in the active group was 65.20 (SD 7.92) and 80.00 (SD 9.80) beats per minute in the passive group. Comparison of the baseline values demonstrated statistical significance [P = 0.031 Unpaired T test].
After 30 minutes of stimulation the heart rate decreased by 1.20 (SD 6.06) in the active group [P = 0.681 Paired T test] and in the control group -5.80 (SD 3.96) [P = 0.031 Paired T test]. Comparison of the changes demonstrated non-significance [P = 0.193 Unpaired T test].
The heart rate increased by 0.80 (SD 4.60) [P = 0.717 Paired T test] in the active group and 0.60 (SD 3.51) in the control group [P = 0.722 Paired T test] following deactivation of the device. Unpaired T test analysis of the changes demonstrated non-significance [P = 0.940].

Systolic blood pressure at baseline in the active group was 142.60 (SD 28.59) and control 122.60 (SD 19.53) mmHg. Comparison of baseline values determined them

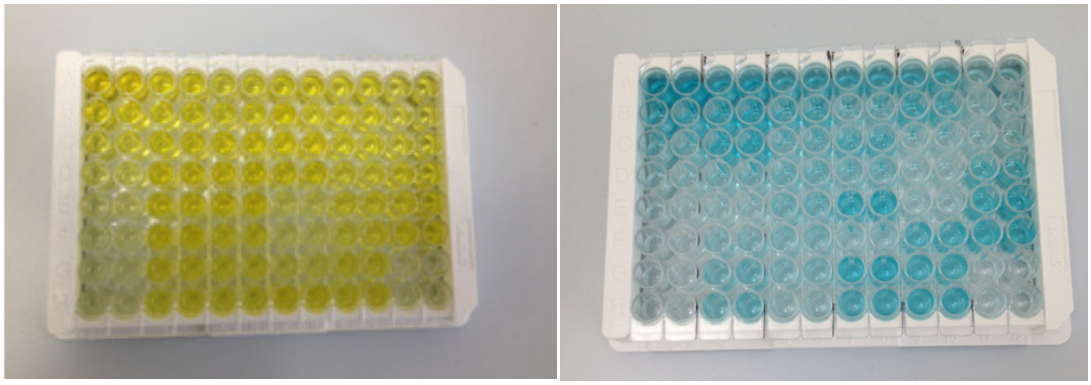
to be comparable [P = 0.233 Unpaired T test]. Systolic BP decreased at 30 minutes by 8.00 (SD 17.36) in the active group [P = 0.361 Paired T test] and 1.00 (SD 4.74) in the control group [P = 0.662 Paired T test]. Comparison of the differences seen demonstrated insignificance [P = 0.410 Unpaired T test]. Systolic BP increased from 30minutes to the end of the study period in the active group by 4.60 (SD 6.66) [P = 0.197 Paired T test] and 0.20 (SD 2.28) [P = 0.854 Paired T test] mmHg in the control group. Comparison of the differences seen demonstrated insignificance [P = 0.200 Unpaired T test].

Diastolic blood pressure at baseline in the active group was 77.4 (SD 9.94) and control 75.20 (SD 10.71) mmHg. Comparison of baseline values determined them to be comparable [P = 0.902 Unpaired T test]. Diastolic BP decreased at 30 minutes by 1.20 (SD 7.36) in the active group [P = 0.734 Paired T test] and increased by 0.60 (SD 4.98) in the control group [P = 0.801 Paired T test]. Comparison of the differences seen demonstrated insignificance [P = 0.582 Unpaired T test]. Diastolic BP increased from 30minutes to the end of the study period in the active group by 3.00 (SD 4.12) [P = 0.543 Paired T test] and 2.80 (SD 4.44) [P = 0.433 Paired T test] mmHg in the control group. Comparison of the differences seen demonstrated insignificance [P = 0.943 Unpaired T test].

Chapter Eight: Research question 6: The effects of peroneal nerve electrical neuromuscular stimulation on angiogenesis and fibrinolysis

A total of 77 subjects consented to participate in plasma sampling as part of the study: 30 claudicants (25 active and 5 controls); 25 infrainguinal bypass grafts (19 active and 6 control) and 21 varicose vein patients (17 active and 5 controls).

Figure 160: Examples of ELISA assay plates



8.1 Effect of geko™ on Tissue Plasminogen Activator levels

8.1.1 Claudicants

The mean baseline t-PA in the claudicants was 3714.3 pg/ml (SD 1368.1). The mean decrease in t-PA from baseline in the active group was 411.2pg/ml ($P < 0.001$), passive 259.5 pg/ml ($P = 0.001$) and control group 354.9 pg/ml ($P = 0.011$). The differences between groups were not statistically significant ($P = 0.295$).

8.1.2 Infra-inguinal bypass grafts

Mean baseline t-PA in the bypass patients was 5260.3 pg/ml (SD 2070.1). The mean reduction in t-PA levels from baseline the active limbs was 184.4 pg/ml (P = 0.140), passive limbs 271.1 pg/ml (P = 0.094) and control 111.2 pg/ml (P = 0.499). Intergroup analysis of the change from baseline demonstrated that the differences between groups was not statistically significant (P = 0.767).

8.1.3 Varicose veins

The mean baseline t-PA in the varicose vein patients was 3903.0 pg/ml (SD 1034.9). The mean decrease in t-PA levels from baseline in the active limbs was 227.4 pg/ml (P = 0.051), passive 289.1 pg/ml (P = 0.016) and control limbs 317.2 pg/ml (P = 0.002). The difference in the changes seen between groups is not statistically significant (P = 0.830).

8.1.4 All participants

The mean baseline t-PA level was significantly higher on ANOVA testing in the infra-inguinal bypass group than the claudicants (P < 0.001) and varicose vein group (P < 0.001) whilst there was no significant difference between the claudicants and varicose vein groups (P = 1.000).

The mean baseline t-PA level, for all participants, in the active and passive groups was 4260.0 (SD 1649.5) whilst the mean baseline t-PA level in the control group was 4291.4 pg/ml (SD 1723.0). The difference in mean t-PA at baseline was not statistically significant (P = 1.000).

Table 30: Comparison of baseline t-PA with and without the presence of risk factors or concomitant medications

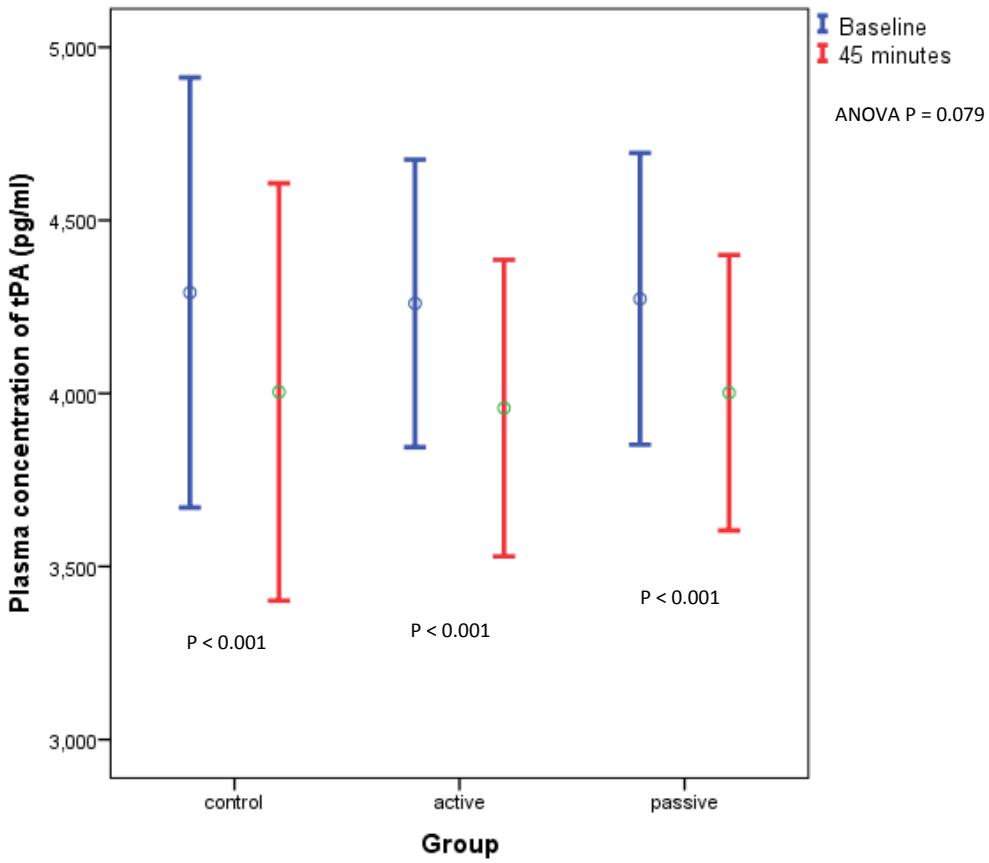
Variable	N=	Present Mean (pg/ml)	SD	N=	Absent Mean (pg/ml)	SD	Significance (P=)
Smoking	26	4077.7	1756.1	49	4359.6	1677.4	0.498
EtOH	57	4330.0	1620.4	18	4046.1	1960.7	0.540
Diabetes	11	4287.8	1805.7	64	4257.4	1694.5	0.957
Cerebrovascular	10	4956.2	1124.2	65	4155.1	1752.5	0.167
Hypertension	42	4698.7	1608.0	33	3706.0	1670.3	**0.011**
Respiratory	8	4308.9	1563.5	67	4256.3	1724.9	0.935
Cardiovascular	22	5127.7	1599.3	53	3902.5	1619.9	**0.004**
Antiplatelets	52	4359.3	1834.5	23	4041.7	1352.7	0.459
Aspirin	46	4097.7	1606.8	29	4522.3	1833.4	0.295
Clopidogrel	14	5321.3	1882.2	61	4018.7	1571.2	**0.009**
Warfarin	5	5983.6	2132.6	70	4138.9	1612.6	**0.018**
ACEI	27	4704.1	1645.3	48	4013.2	1694.0	0.091
Statin	52	4433.7	1781.2	23	3873.5	1456.9	0.190
ABPI <0.9	24	3872.2	1218.4	51	4445.3	1865.5	0.175

The baseline level of t-PA was statistically significantly lower in patients who were taking warfarin or clopidogrel and significantly higher in individuals with cardiovascular disease.

The mean change in t-PA in the active group was -302.4 pg/ml (SD 428.2) equating to a percentage change of -7.7% (Paired T-test $P < 0.001$). The mean change in t-PA in the passive group was -271.3 pg/ml (SD 467.8) equating to a percentage change of -5.5% ($P < 0.001$). The mean change in t-PA in the control group -287.2 pg/ml (SD 383.1) equating to a percentage change of -6.85% ($P < 0.001$).

Figure 161: tPA

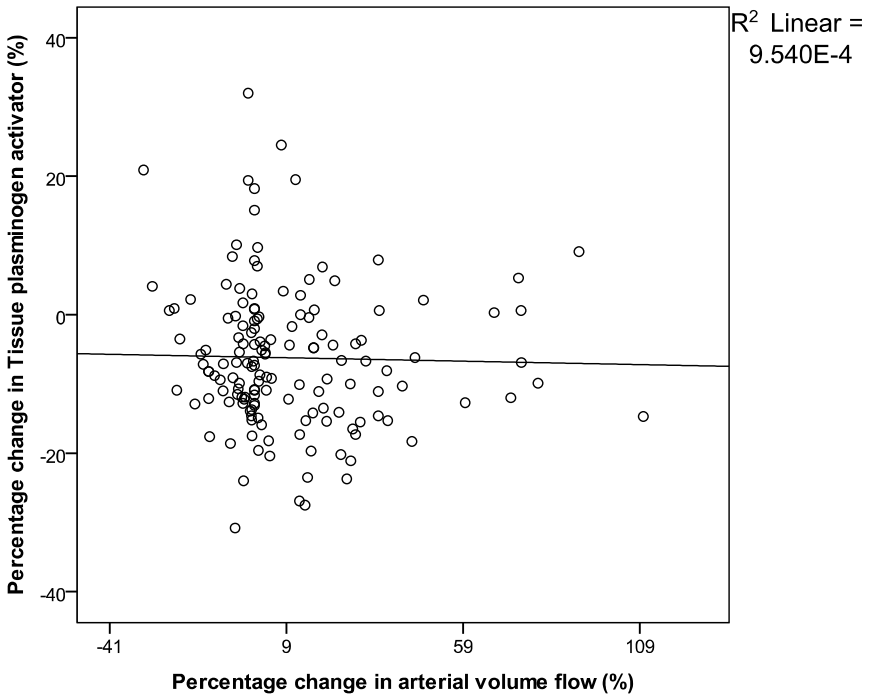
Error bar chart demonstrating the mean plasma tPA level at baseline and at 40 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)



Inter-group analysis [ANOVA] demonstrated that the differences in the changes seen in the three groups were not statistically significant (P = 0.079).

Linear regression did not demonstrate any relationship between the change in flow and the change in t-PA level.

Figure 162: Relationship between change in flow and change in t-PA level



8.2 Effect of geko™ on Plasminogen Activator Inhibitor-1 levels

8.2.1 Claudicants

The mean baseline PAI-1 in the claudicants was 185.5 ng/ml (SD 68.3). The reduction in PAI-1 from baseline in the active group was 36.5ng/ml (P = 0.001), passive 6.6ng/ml (P = 0.247) and control group 9.0ng/ml (P = 0.240). Intergroup analysis comparing the change from baseline demonstrated that the reduction in PAI-1 was statistically significant when comparing the active and passive groups (P = 0.021), whilst non-significant for active versus control (P = 0.162) and passive versus control (P = 1.000).

8.2.2 Infra-inguinal bypass grafts

Mean baseline PAI-1 in the bypass patients was 207.0 ng/ml (SD 71.9). The PAI-1 from baseline in the active group reduced by 44.1 ng/ml (P = 0.007), increased in the passive limbs by 1.1 ng/ml (P = 0.919) and decreased by 3.2 ng/ml in the control limbs (P = 0.514). ANOVA analysis demonstrated that the change in PAI-1 from baseline was statistically significant when comparing the active versus control (P = 0.038) and passive (P = 0.021) but non-significant when comparing passive and control (P = 1.000).

8.2.3 Varicose veins

The mean baseline PAI-1 in the varicose veins was 194.9 ng/ml (SD 106.2). The decrease in PAI-1 from baseline in the active limbs was 11.7 ng/ml (P = 0.316), passive 29.1 ng/ml (P = 0.098) and control 3.5 ng/ml (P = 0.668). The differences in mean change between the groups was not significant (P = 0.421).

8.2.4 All Patients

There was no statistically significant difference in baseline PAI-1 when comparing the bypass patients, claudicants or patients with varicose veins (P = 0.393).

Table 31: Comparison of baseline PAI-1 levels in presence and absence of risk factors and concomitant medications

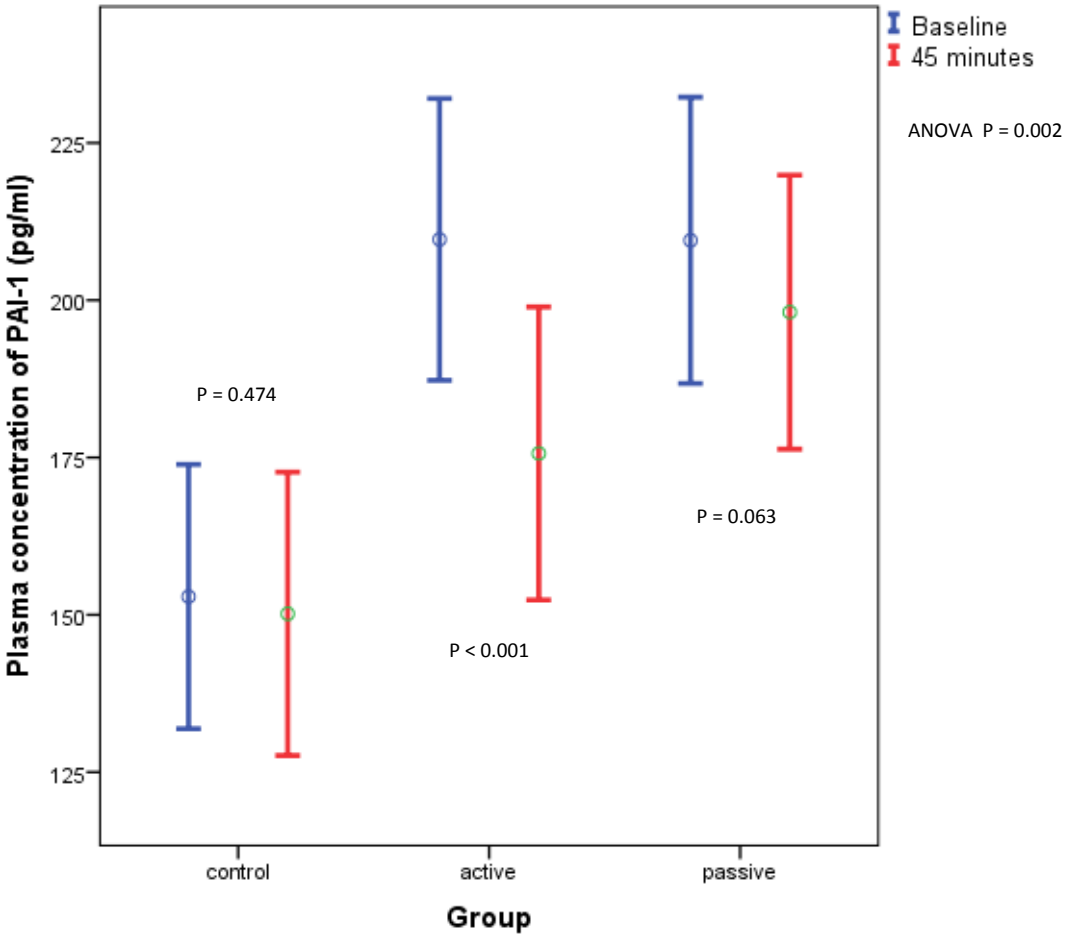
Variable	N=	Present Mean (ng/ml)	SD	N=	Absent Mean (ng/ml)	SD	Significance (P=)
Smoking	26	218.0	84.4	50	183.3	78.6	0.079
EtOH	57	204.6	85.3	19	167.0	64.1	0.083
Diabetes	11	213.5	105.5	65	192.1	77.7	0.424
Cerebrovascular	10	168.8	56.5	66	199.2	84.6	0.276
Hypertension	43	196.5	87.9	33	193.4	74.4	0.868
Respiratory	8	251.2	98.3	68	188.6	77.8	**0.040**
Cardiovascular	22	190.0	73.7	54	197.3	85.4	0.725
Antiplatelets	53	197.3	69.6	23	190.3	106.4	0.737
Aspirin	46	200.7	71.0	30	186.7	96.8	0.471
Clopidogrel	14	192.8	58.7	62	195.7	86.5	0.906
Warfarin	5	91.7	16.6	71	202.5	79.6	**0.003**
ACEI	28	202.1	95.8	48	191.1	73.2	0.574
Statin	53	203.8	81.4	23	175.3	81.0	0.165
ABPI <0.8	24	176.0	66.2	52	204.0	12.1	0.166

Mean baseline PAI-1 level for all participants in the active and passive groups was 209.7 ng/ml (SD 88.9) whilst the mean baseline PAI-1 level in the control group was 152.9 ng/ml (SD 58.3). The difference between the groups was statistically significant (P = 0.006).

The mean change in PAI-1 in the active group was -34.0 ng/ml (SD 52.2) equating to a percentage change of -16.2% (Paired T-test P < 0.001). The mean change in PAI-1 in the passive group was -11.4 (SD 47.4) equating to a percentage change of -3.6% (P = 0.063). The mean change in PAI-1 in the control group was -2.73ng/ml (SD 21.3), equating to a percentage change of -2.6% (P = 0.474).

Figure 163: PAI-1

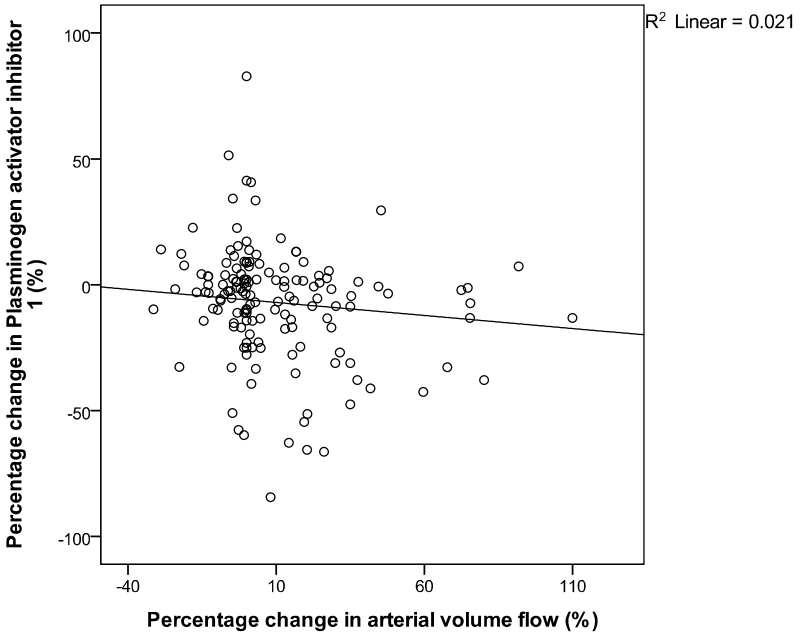
Error bar chart demonstrating the plasma concentration of PAI-1 at baseline and at 45 minutes in the active, passive and control groups [the mean is represented as a circle and the confidence interval is represented by a line protruding from the mean to a short horizontal line representing the limits of the confidence: 95% CI)



Inter-group analysis demonstrated that the difference in change in PAI-1 between the groups was statistically significant (P = 0.002). Post hoc Bonferonni testing demonstrated that the difference between the active and control limbs was statistically significant (P = 0.006) as was active and passive (P = 0.019) however the difference between the control and passive limbs was not statistically significant (P = 1.000).

Linear regression was performed to determine if the change in PAI-1 was related to the change in flow.

Figure 164: Relationship between change in flow and change in PAI-1 level



8.3 Effect of geko™ on VEGF levels

8.3.1 Claudicants

Mean baseline VEGF in the claudicants was 727.7 pg/ml (SD 376.8). The decrease in VEGF levels from baseline in the active limbs was 38.9 pg/ml (P = 0.037), the passive 3.4 pg/ml (P = 0.857) and control 23.6 pg/ml (P = 0.619). Statistical analysis demonstrated that the difference in change from baseline between groups was not statistically significant (P = 0.468).

8.3.2 Infra-inguinal bypass grafts

Mean baseline VEGF in the bypass patients was 742.0 pg/ml (SD 525.5). The reduction in VEGF levels from baseline in the active limbs was 76.1 pg/ml (P = 0.247), passive limbs 1.3 pg/ml (P = 0.966) and increased in the control limbs by 73.6 pg/ml (P = 0.119) groups. ANOVA analysis

demonstrated no significant differences in the changes between groups (P = 0.138).

8.3.3 Varicose veins

The mean baseline VEGF in the varicose vein patients was 542.3 pg/ml (SD 238.9). The change from baseline of VEGF in the active limbs was an increase of 1.1 pg/ml (P = 0.955) a decrease of 30.6 pg/ml in the passive limbs (P=0.376) and an increase of 31.5 pg/ml in the control group (P = 0.048) (ANOVA P = 0.292).

8.3.4 All patients

ANOVA analysis to compare the baseline VEGF levels between the infra-inguinal bypass patients, claudicants and patients with varicose veins suggested statistical significance (P = 0.034) however this was not demonstrated on Post hoc Bonferonni testing: bypass versus claudicants (P = 1.000); bypass versus varicose veins (P = 0.058); varicose veins versus claudicants (P = 0.071).

Table 32: Comparison of baseline VEGF level in the presence and absence of risk factors and concomitant medications

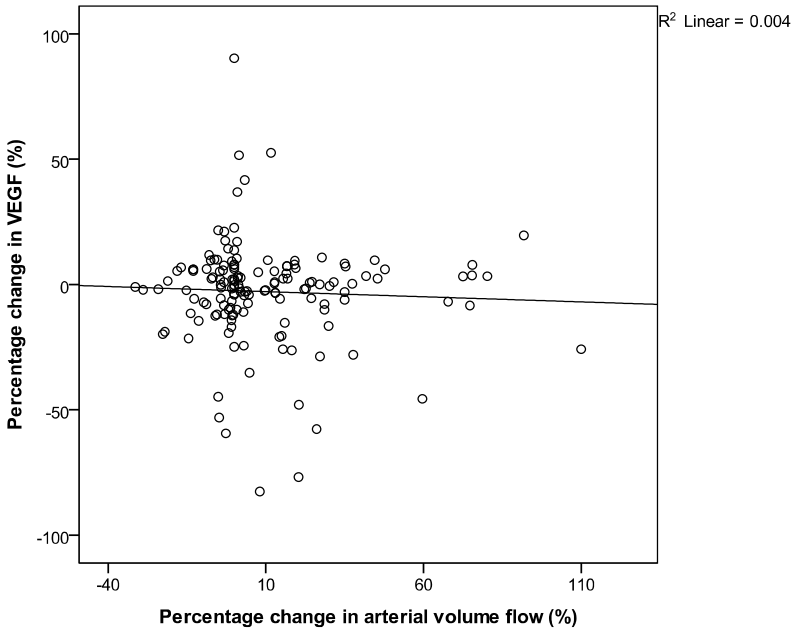
Variable	N=	Present Mean (pg/ml)	SD	N=	Absent Mean (pg/ml)	SD	Significance (P=)
Smoking	26	764.9	552.7	50	637.6	312.1	0.202
EtOH	57	680.2	412.0	19	684.0	420.3	0.972
Diabetes	11	721.4	517.8	65	674.3	395.0	0.728
Cerebrovascular	10	528.2	222.5	66	704.3	429.2	0.209
Hypertension	43	687.8	397.6	33	672.5	434.6	0.874
Respiratory	8	595.6	228.9	68	691.2	427.7	0.538
Cardiovascular	22	686.3	423.8	54	679.0	410.1	0.945
Antiplatelets	53	732.4	456.0	23	563.0	253.2	0.099
Aspirin	46	735.2	482.1	30	598.2	255.0	0.157
Clopidogrel	14	636.1	221.5	62	691.3	443.8	0.653
Warfarin	5	392.4	78.3	71	701.5	417.7	0.105
ACEI	28	677.3	410.9	48	683.4	415.8	0.951
Statin	53	740.6	452.4	23	544.1	254.4	0.055
ABPI <0.8	24	661.0	331.4	52	690.4	446.0	0.774

Mean baseline VEGF levels in the active and passive groups was 692.4 pg/ml (SD 394.1) whilst the mean baseline VEGF level in the control group was 730.5 pg/ml (SD 535.5). The difference in baseline values between the groups was not statistically significant (P = 0.903).

The mean change in VEGF in the active group was -46.5 pg/ml (SD176.3), equating to a percentage change of -8.6% (Paired T-test P = 0.40). The mean change in VEGF in the passive group was -3.5 pg/ml (SD 118.8), equating to a percentage change of -1.8% (P = 0.818). The mean change in VEGF in the control group was 30.1 pg/ml (SD 128.1), equating to a percentage change of 3.9% (P = 0.194) (ANOVA P = 0.014). Bonferonni testing revealed the difference between the active and control group to be statistically significant (P = 0.015) whilst the difference between the active and passive (P = 0.185) and passive and control (P = 0.591) were not.

Linear regression was performed to establish if the change in VEGF level was related to the change in flow.

Figure 165: Relationship between change in flow and change in VEGF level



Chapter Nine: Discussion

The studies provide a contribution to the evolving field of research focused upon the potential role of transcutaneous, peroneal nerve stimulation as a treatment adjunct in vascular disease.

This work evaluated five main questions; the first was patient centred and related to the ability of the device to produce effective muscle twitch in vascular patients and whether transcutaneous peroneal nerve stimulation was deemed tolerable. The second concerned the ability of the peroneal nerve stimulator to improve arterial, venous and microcirculatory flow in patients with claudication or varicose veins. The third considered the ability of the device to enhance flow through infra-inguinal bypass grafts. The fourth was to determine whether geko^{TM} usage affects plasma levels of vascular endothelial growth factor and finally the fifth to establish the impact of device use on levels of tissue plasminogen activator and plasminogen activator inhibitor 1.

9.1 geko^{TM} function and acceptability

9.1.1 Applicability

The geko^{TM} was unable to stimulate visible muscle twitch in 59% of the vascular patients tested, with the presence of moderate to severe oedema and a Toronto neuropathy score greater than 5 retaining prognostic significance on multivariable analysis. This led to the amendment of the protocol to include patients with claudication and exclude those with critical limb ischaemia in whom the device had proved ineffective.

This is clearly a marked limitation to its use as many vascular patients suffer from diabetes with reports of a 28% increase in the risk of developing PAOD with each 1% increase in HbA1c(81). The prevalence of neuropathy within

diabetic patients is related to the duration of the disease and it is estimated that approximately 50% of diabetics will develop a degree of neuropathy in their lifetime(429-431). The neuropathy seen in vascular patients can also be related to renal failure and ischaemia.

Substantial post operative oedema affects a high proportion of vascular patients, undergoing peripheral bypass surgery(432). Jacobs *et al* reported that 54.4% of patients post infrainguinal bypass surgery developed oedema(433). This will reduce the effectiveness of the device in patients who have recently undergone lower limb revascularisation. Peripheral oedema is also a known sequelae of chronic venous insufficiency(434). An adequate charge must be delivered to elicit nerve stimulation. Studies carried out by the anaesthetic community to establish the stimulation level required to obtain supra-maximal stimulation to determine the level of neuromuscular blockade in anaesthetised patients determined that, whilst a current of 50-50mA is sufficient to achieve this in the absence of oedema(435, 436), in grade 1 oedema at least 60mA was required and in grade 2 oedema 82.5mA was required. Harper *et al*(436) hypothesised that when oedema is present the current density available to stimulate the nerve is reduced. The presence of oedema is likely to reduce responsiveness to the stimulation delivered because it increases the distance from the skin to the nerve and the energy dissipates faster in fluids and gases than solid material.

It is important to note that a significant proportion of the patients trialled were inpatients awaiting treatment or post procedure. As such these individuals had advanced disease and therefore there was a greater incidence of oedema and higher neuropathy scores within this cohort.

The results of this study resulted in the development and production of the geko^{TM} mark 2 device with a pulse current twice that of the model utilised within this study. Whilst no studies to date have examined the efficacy of this device to produce muscle twitch such studies may demonstrate a larger group of participants in whom peroneal nerve stimulation may improve flow.

9.1.2 Tolerability and acceptance

The device has shown to be acceptable to patients, with all patients reporting that it is comfortable to wear, resulting in minimal distress. 24.6% of patients reported an immediate improvement in their symptoms.
Patient compliance is of paramount importance when investigating any new treatment such as selecting the most appropriate mechanical DVT prophylaxis(267). Kaplan *et al*(274) have stated that "The ideal device for out of hospital use should have the following characteristics: it should be safe, effective, portable, easy to use, inexpensive, and able to be worn while the patient is recumbent, sitting, standing or walking". In those patients in whom transcutaneous peroneal nerve stimulation produces visible muscle twitch the geko^{TM} device appears to fulfil these criteria, however as aforementioned the device was ineffective in a significant proportion of patients trialled.

In 75% of cases the device was reported to be easy or very easy to apply and the device rarely became detached. As such it should be possible to teach patients to self-apply the device. Considering the aging population interventions, such as these, which can be safely and easily applied by patients in the home environment should be considered. Studies are required to establish the feasibility of teaching patients, including the elderly and disabled, to self- apply the geko^{TM}.

9.2 Cardiovascular effects

9.2.1 Heart rate and blood pressure

In any study involving new technologies or treatments safety aspects must be considered. Vascular patients have an increased incidence of cardiovascular morbidity and as such heart rate and blood pressure were recorded at intervals throughout the study period. As can be seen from the results there

were no adverse cardiovascular effects associated with peroneal nerve stimulation with non-significant changes in heart rate and blood pressure when compared to the control group. Ishide *et al(437)* studied the effect of peroneal nerve stimulation in anaesthetised rats demonstrating a reduction in arterial pressure and heart rate. Indeed animal studies have shown that peripheral nerve stimulation may be beneficial for reducing the incidence of cardiac arrhythmias(438). Electrical muscle stimulation in patients during arthroplasty under general anaesthesia has also been shown to result in reduced heart rates(439) whilst evaluation of patients undergoing stimulation during bed rest demonstrated an increase in heart rate when compared to the control group(273).

The failure to demonstrate such a reduction within the patients in these studies may be due to either small patient numbers or patients' response to the unusual sensations associated with the nerve stimulation. Repeated applications of the device may result in reductions in heart rate and blood pressure.

9.2.2 Augmentation index and peripheral vascular resistance

The increase seen in microcirculatory flow in the active stimulation limbs, as evidenced by the laser Doppler flowmetry results, suggests up regulation of the skin microcirculation. This increase in the cross-sectional area of the vascular bed may suggest a decrease in peripheral vascular resistance. The Vicorder results in this study did not reflect this and may be as a result of insufficient numbers of patients and also the inherent inaccuracies with non-invasive measurements of systemic vascular resistance. A previous study to examine the effect of electrical muscle stimulation also failed to demonstrate any change in total peripheral resistance(439).

9.2.3 Cardiac Output

There was no significant change in cardiac output with use of the device. Whilst the gold standard method for assessing cardiac output would have been to utilise echocardiography this was not possible within the remit of this study and may explain the failure to detect a change. These results do not reflect the existing literature as it has previously been demonstrated that neuromuscular electrical stimulation increased cardiac output by up to 24%(439) . This was hypothesised to be due to increased venous return as the calf muscle pump empties the venous sinuses and as a result of reduced total peripheral resistance. Indeed it is known that exercise increases cardiac output by decreasing TPR with a resultant increase in flow to the tissues and hence increased venous return to the heart (440).

$$\text{Cardiac output} = \frac{\text{(Mean arterial pressure – Mean venous pressure)}}{\text{Total peripheral resistance (TPR)}}$$

Cardiac output is also known to be a function of heart rate and stroke volume. Previous work hypothesised that an increase in cardiac output may relate to an increase in heart rate secondary to a stress response to a previously unexperienced sensation, namely the electrical stimulation. However Faghri *et al* reported an increase in cardiac output with electrical muscle stimulation whilst heart rate decreased, and therefore was as a result of increased stroke volume(439). Within this study, there was no statistically significant change in heart rate at 30 minutes or following device removal. Future studies should include a greater sample size in order to detect smaller changes.

9.3 Haemodynamic efficacy

9.3.1 Venous volume flow

Peak venous flow velocity is often reported by device manufacturers as a surrogate marker i.e. an indication of device efficacy despite a lack of evidence that higher velocities reduce thrombosis risk, indeed a study by Proctor *et al* suggested the opposite(441). This study has demonstrated that peroneal nerve stimulation with the geko™ device significantly increased venous flow velocity and volume flow in all three patient groups, namely claudicants, patients with varicose veins and patients post infra-inguinal bypass grafts. This augmentation of flow is confined to the actively stimulated limbs with no significant differences found in comparison of passive (contralateral limb to stimulated limb) and control limbs (device applied but not active). These findings are in keeping with previously published literature. Kaplan *et al(274)* reported significant increases in femoral and popliteal vein blood flow associated with electrical stimulation of the foot muscles. Izumi *et al*(277) investigated the relative efficacy of peroneal nerve stimulation to increase flow within the popliteal vein when compared to IPC, electrical muscle stimulation, active ankle dorsiflexion and manual calf compression. They demonstrated that peroneal nerve stimulation produced greater increases in peak venous velocity than all other methods utilised. The geko™ peroneal nerve stimulator has been shown to be superior to intermittent compression devices at increasing venous flow(442) in healthy volunteers

Tucker *et al*(381) studied the impact of the geko™ device on venous flow in healthy volunteers and demonstrated a significant increase in venous volume flow and peak velocity. The increases observed in the patient cohorts in this study were less marked than demonstrated in healthy volunteers. This may reflect the differences in vessel compliance and cardiovascular physiology in these disease states.

The increase in venous velocity and volume flow demonstrated in this study would suggest that use of the device would therefore be a useful treatment adjunct within this cohort of patients. It is known that vascular patients, by the very nature of their disease are at increased risk of deep venous thrombosis, particularly if there are prolonged periods of immobility or after surgical procedures. Claudicants experience pain or discomfort in a group of muscles, most typically in the legs, which is brought on by exercise and alleviated with rest. Despite the initial management advice for such patients to increase their exercise levels, many are reluctant to do so due to concerns that they are doing themselves harm. The resultant relative immobility can increase the risk of thrombosis within this cohort. Reducing venous stasis, a known component of Virchow's triad, by increasing blood flow velocity, thus may reduce thrombosis risk. Indeed improving venous volume flow and velocity are known to facilitate clearance of the soleal sinuses and vein valve cusps, which are the principle sites for deep venous thrombosis formation(443, 444). Studies examining compression hosiery, intermittent pneumatic calf compression and calf muscle stimulation have demonstrated that all increase venous flow velocity and reduce the incidence of post-operative venous thrombosis.

Limitations exist for many of the physical devices currently utilised for DVT prophylaxis. Compression hosiery is contraindicated in patients with peripheral arterial occlusive disease due to the risk of reducing arterial flow. It is also known that difficulties exist in ensuring appropriate fit, thus avoiding complications, and compliance is poor. Intermittent pneumatic compression is expensive, cumbersome and requires the patient to be immobile whilst receiving the treatment and as such is not universally applicable in the outpatient setting. Calf muscle stimulation, whilst advantageous in the anaesthetised patient has been demonstrated to be uncomfortable in the conscious. Peroneal nerve stimulation would thus appear to be a useful and tolerable option in the prevention of deep venous thrombosis in patients unable or unwilling to wear compression hosiery. Studies to establish the relative cost effectiveness of the device compared to other more established regimes are indicated.

The improvements in venous flow demonstrated with the device are comparable to those seen with intermittent pneumatic compression (IPC), leading to the implication that the device could serve as an alternative to IPC. It has been suggested in several trials that IPC is a useful treatment adjunct in the management of chronic venous insufficiency, reducing the signs and symptoms(445, 446) and increases healing rates of chronic venous ulcers(447, 448). This may also be a future avenue of research for peroneal nerve stimulation.

The results from this study were utilised by Firstkind in the recent case for adoption of the technology as a method for prophylaxis for venous thromboembolism. This study represented the only study which had taken place in patients and was considered important for the determination by the National Institute of Health and Care Excellence that use of the geko^{TM} device is indicated in those at high risk of VTE in whom other methods, pharmacological or mechanical, are contraindicated(449).

9.3.2 Arterial flow

Arterial volume flow significantly increased in the active limbs in all three patient groups These findings correlate with the existing literature on the effect of peroneal nerve stimulation on arterial flow in healthy volunteers(381, 442). Whilst the use of transcutaneous electrical nerve stimulation is not an accepted component in the treatment of peripheral arterial disease the results of these studies suggest there may be a viable place for the device to be utilised as an adjunct in the management of patients with arterial disease, especially the immobile who are unable to exercise.

9.3.2.1 Claudicants

Exercise and walking have been shown to improve symptoms in claudicants. Despite this recommendation, patients are often non-compliant (450). The reasons for this include poor motivation(451), co-morbidities which preclude

exercise and weather or geographical obstacles. As a result therapies which can improve lower limb blood flow, improve patient capacity and slow disease progression are being sought and investigated. Recently several studies have investigated a possible role for intermittent calf pneumatic compression, however the devices are cumbersome and limit patient mobility(452, 453). Electrical muscle stimulation has been shown by Tsang *et al* to increase muscle blood flow and reduce muscle fatigue(454) however the failure of this technique to be adopted may be due to the discomfort experienced at higher stimulation levels with muscle stimulation. Peroneal nerve stimulation was found to be tolerable by patients with claudication and no reproduction of claudication symptoms was experienced which may encourage its use within this cohort of patients.

9.3.2.2 Infra-inguinal bypass grafts

Primary patency of infra-inguinal bypass grafts for claudication is approximately 85% whilst for critical limb ischaemia is approximately 15-25%. Early graft failure i.e. within 1 year of surgery, results most frequently from technical errors, thrombosis and intimal hyperplasia(455). Graft failures result in expensive salvage procedures and resource utilisation, and a not infrequent loss of limb with the incumbent disability and quality of life deterioration(456). It is known that flow is an important component of Virchow's triad for thrombus formation. Improving flow within bypass grafts as seen with peroneal nerve stimulation may reduce the incidence of graft thrombosis.

Venous bypass grafts undergo remodelling, early luminal enlargement, typically within the first month following surgery, followed by graft thickening and stiffening(457). These changes occur as a result of exposure to increased shear stress, wall tension and pulsatile flow. It has been shown that shear stress, which is a function of flow and the radius of the vessel, is the most important predictor of the luminal dilatation in the early post operative period(456). Improving flow within the bypass graft may lead to improved luminal dilatation and thus reduced failure rates. It is also possible that improvements in flow would reduce the incidence of graft thrombosis. It

is known that graft failure has significant implications, not only financial but also in terms of patient functionality and quality of life.

Use of peroneal nerve stimulation, may therefore be a useful treatment adjunct to improve patency of infra-inguinal bypass grafts. A prospective randomised controlled trial comparing standard care and the use of peroneal nerve stimulation in the immediate post-operative period to determine the impact on outcomes following infra-inguinal bypass procedures would be valuable. The improvements in flow may reduce the incidence of early graft failure, namely failure secondary to the formation of thrombus and the frequency of re-interventions. Such a trial would also enable determination of the cost effectiveness of the therapy.

9.3.3 Microcirculatory/ Laser doppler flow

Peroneal nerve stimulation improved microcirculatory flow, in all three patient groups, within 15 minutes of application and the flow augmentation persisted throughout the study period demonstrating that there is no 'tolerance effect'. This implies that peroneal nerve stimulation is beneficial for microcirculatory flow augmentation as long as there is active stimulation. The improvements seen within these patient cohorts have demonstrated that diseased vessel states do not negatively impact on the efficacy of peroneal nerve stimulation, which may be the case with other interventions.

The findings of these studies reflect the existing literature. A case study by Ogrin *et al* has previously demonstrated accelerated healing of venous ulcers with stimulation of the peroneal nerve(458). The positioning of the geko^{TM} device would enable continuation of compression therapy in such patients with concomitant electrical stimulation.

Electrical stimulation reduces inflammation and accelerated healing through the promotion of phagocytosis combined with the stimulation of cells such as fibroblasts and epithelial cells(170, 459, 460). Its use in wound care has been

increasing and its role as a treatment adjunct is gaining acceptance following the publication of several recent studies(461, 462) and the 2009 guidelines on pressure injury from the European Pressure Ulcer Advisory Panel and National Pressure Ulcer Advisory Panel(463). Standard techniques involve the use of electrodes, wires and a power source and patient mobility is adversely affected whilst the treatment is undertaken. The geko™ device is novel in that it is self contained promoting mobility and the potential for patients to self-apply may result in reduced nursing costs. These studies support further investigation of the potential role for peroneal nerve stimulation in the treatment of lower limb arterial, venous and in particular mixed ulceration, where patients would typically require revascularisation prior to commencement of compression therapy.

9.3.4 Proposed mechanisms of haemodynamic augmentation

Whilst the underlying mechanism for the improvement in venous flow is clear, namely activation of the muscle pumps of the foot and calf reducing venous stasis, the mechanism responsible for the improvements seen in arterial and microcirculatory flow are less comprehensible.

9.3.4.1 Starlings law

The geko™ device improves venous return which as such results in increased preload. This increase in load on the cardiac muscle fibres augments the strength of ventricular contraction and hence the stroke volume. This is likely in turn to increase the arterial volume flow. The Vicorder measurements from this study did not reflect this however this is likely to be due to non-optimal measuring techniques as aforementioned and a small sample size resulting in inadequate power and hence may be a false negative.

9.3.4.1 Sympathectomy

It is possible that the nerve stimulation acts in a manner similar to a temporary sympathectomy. Sympathetic blockade is known in healthy individuals to result in venous dilatation or increased blood flow, indicated by a reduction in capillary refill time, with abolition of the normal vasoconstrictor reflexes. Increased skin temperature, increased oxygen tension in the capillaries of the skin and increased venous oxygen tension have all been observed(464). This hypothesis is supported by the work of Kaada *et al* who demonstrated that the vasodilatory effects of transcutaneous nerve stimulation were blocked by cyproheptadine, implicating sympathetic blockade via a serotoninergic mechanism as the principle cause of the effect(465).
The improved skin perfusion has led to the belief that there will be improved ulcer healing with the use of chemical/ lumbar sympathectomy. These findings combined with the potential pain control effects has resulted in its use in the treatment of rest pain and/ or ulceration in patients with non-reconstructable peripheral arterial disease.

Histological studies have shown that sympathectomy results in relaxation of the smooth muscle of vessels at arteriovenous anastomoses in dogs(466, 467). Human studies have failed to conclusively demonstrate the same results(468, 469). The effect of diabetic neuropathy on the limb has however been demonstrated to increase skin blood flow.

The first study to investigate the effect of sympathectomy on macroscopic blood flow in humans was carried out by Barcroft and Swann(470) who investigated the effects on arm blood in patients who had the procedure for hyperhidrosis. They demonstrated an initial eightfold increase in blood flow however noted that this returned to normal within 8 days of the procedure. Blood flow in the feet did not increase until 2 days after lumbar sympathectomy and was significantly less marked returning to pre-procedural levels at a slower rate. The distribution of the effects seen in the limbs led to the conclusion that the effects were predominantly related to improved skin

blood flow and not muscle flow. Further studies to investigate the impact on muscle flow were disappointing with poor correlation between sympathectomy and muscle flow(471) and at best a temporary reduction in the peripheral vascular resistance of muscle(472). Cross *et al* were unable to demonstrate any changes in foot blood flow following sympathectomy(473).

Several studies have also investigated the role of concomitant sympathectomy at the time of arterial reconstruction. Whilst Terry *et al*(474) and Collins(475) demonstrated an immediate post procedural increase in flow of 30% two randomised controlled trials demonstrated no benefit in terms of graft patency rates(476, 477) despite reduction of peripheral vascular resistance(476). The effects of sympathectomy on rest pain secondary to critical limb ischaemia are more conclusive. In a RCT Cross *et al* reported symptom relief in 83.5% of patients at 1 month compared with 23.5% of controls (P<0.002) with 66.7% remaining pain free at 6 months(473). These findings are supported by several cohort studies(464, 478-481). Similarly Van Driel *et al* reported limb salvage, resolution of rest pain or ulcer healing in 48% of patients at 6 months. Amputation rates following sympathectomy range, in the literature, from early; 18.3(480) - 40%(478) and 24% (464) to 42.2% at 2 years(475).

To further elucidate the mechanism of action of transcutaneous peroneal nerve stimulation to improve arterial and microcirculatory flow, a study to investigate the effect of the device in patients following chemical/ surgical sympathectomy would be beneficial.

9.3.4.2 Nitric oxide

Nitric oxide has been shown to play an important role in the regulation of vascular tone and blood pressure, both at rest and following pharmacological or physiological stimulation(482, 483).
Vascular smooth muscle is partially innervated by neurons which demonstrate nitric oxide synthase immunoreactivity, termed nonadrenergic, noncholinergic (NANC[1]) nerves(484).

The local nature of the response seen with peripheral nerve stimulation suggests that it may be as a result of a locally released vasodilatory mediator(485). It has therefore been proposed that electrical stimulation results in the release of nitric oxide and vasodilatation via cyclic GMP(486-488).

Electroacupuncture has been shown to modulate sympathetic nerve activity and levels of nitric oxide, reducing blood pressure in animal studies(489). Studies in humans have also demonstrated that electroacupuncture increases the nitric oxide concentration in plasma with improved local circulation(490, 491).

ELISA analysis of plasma sampled prior to and following a period of stimulation could be performed to determine the effect of peroneal nerve stimulation on levels of nitric oxide. Ferroni *et al* demonstrated that levels of nitric oxide rose significantly the day following electrical nerve stimulation and hypothesised that this was due to VEGF stimulation of the endothelium to release NO(492).

9.4 Vascular endothelial growth factor

This study did not demonstrate any increase in VEGF following peroneal nerve stimulation in any of the patient groups. Ferroni *et al* have previously demonstrated increased levels of VEGF however determined that the maximum VEGF concentration occurred during the first 10 minutes of treatment, following which the levels declined to baseline(492). It is possible that the timing of samples within this study failed to catch this increase.

9.5 Fibrinolysis

9.5.1 Effect of disease states on fibrinolytic markers

In this study higher baseline levels of tissue plasminogen activator were present in patients with hypertension and cardiovascular disease including atrial fibrillation. This finding corresponds to the existing literature. t-PA levels have been demonstrated to be higher in patients with non-valvular atrial fibrillation than age-matched controls(493). The activity levels were found to decrease following treatment with warfarin or heparin. Higher t-PA and PAI-1 levels have also been shown to be associated with development of acute myocardial infarction and remain higher following the events than age-matched controls(494). Further studies have also demonstrated that elevated t-PA and PAI-1 correlated with serum levels of triglycerides and body mass index and that the t-PA/ PAI-1 complex is a risk factor for recurrent myocardial infarctions(495). Raised levels of t-PA has been shown to be an independently established risk factor for cardiovascular events(496). Elevated levels of t-PA antigen have also been reported in the literature to be independently associated with increased risk of ischaemic stroke in non-diabetic females(497) and to be associated with increased risk of developing diabetes mellitus(498).

This study failed to demonstrate an association between higher PAI-1 levels in patients with cardiovascular disease or its risk factors for vascular disease which contradicts the findings of some of the existing literature(494, 495, 499). However a systematic review by Gorog *et al* determined that fibrinolytic markers have a very limited role in predicting vascular disease or risk stratification due to the poor quality and low power of the existing studies(500).
Baseline plasma plasminogen activator inhibitor levels were determined in this study to be significantly higher in patients with respiratory disease. This finding reflects that reported in the existing literature, as fibrosis of the lungs results from the accumulation of extracellular matrix in both the interstitial tissues and the basement membranes as a result of imbalance in its

synthesis and degradation(501). Degradation of extracellular matrix is a function of the plasminogen activator system(502).

9.5.2 Effect of concomitant medications on fibrinolytic markers

This study has demonstrated that warfarin therapy is associated with statistically significantly lower levels of PAI-1 whilst clopidogrel and warfarin were associated with higher levels of t-PA. The impact of warfarin treatment on plasma levels of t-PA and PAI-1 have been investigated in several distinct patient populations with contradictory results. A study investigating the effects of warfarin and aspirin on thrombogenesis in patients with chronic atrial fibrillation demonstrated that following 8 weeks of treatment with warfarin alone PAI-1 levels were not altered, however combination therapy of aspirin and warfarin resulted in an increase in PAI-1(503). However treatment of patients post coronary artery bypass graft with 1mg of warfarin has not been shown to affect t-PA or PAI-1 levels(504). Similarly a study of chronic ambulatory peritoneal dialysis patients has shown that PAI-levels are not affected by warfarin therapy within this patient cohort (505).

Clopidogrel has been shown in studies to have a non-platelet related effect on levels of plasminogen activator inhibitor levels, resulting in lower levels(506, 507). This does not reflect the findings of this study as whilst lower, the difference in PAI-1 level did not reach statistical significance. This may be due to insufficient patient numbers as only 13 patients within the cohort were treated with clopidogrel.

In this study aspirin usage did not impact upon baseline levels of t-PA. Previous studies have shown that aspirin inhibits the normal physiological increase in t-PA activity following venous occlusion(508). However Levin *et al* demonstrated that aspirin affects t-PA activity by inhibiting the release of t-PA antigen(509)

9.5.3 Effect of nerve stimulation on fibrinolytic markers

This study has demonstrated that electrical stimulation of the peroneal nerve has no effect on tissue plasminogen activator levels when compared to the control participants. It is known that t-PA and u-PA are present in sensory neurons. Electrical cardiac stimulation has been shown to result in increased t-PA release into the circulation and activity with increases in heart rate, blood pressure and coronary blood flow(510, 511). No studies have investigated the effect of peripheral nerve electrical stimulation on plasma t-PA however Jawad *et al* reported a decrease in Tissue plasminogen activator antigen following electrical stimulation of the peroneal nerve(512).

This study has demonstrated a statistically significant reduction in plasma PAI-1 in vascular patients following a period of peroneal nerve stimulation. However the results are local and not systemic as seen by comparing the results of the active and passive samples. It is known that exercise reduces the levels of PAI-1 in those with metabolic syndrome but not those in normal men and women and is thought to be related to lipid profiles(513).
These findings reflect the existing literature. A study in 1979 demonstrated that electrical nerve stimulation resulted in release of tissue thromboplastin and stimulators of fibrinolysis(514). Another animal study examined vagal nerve electrical stimulation demonstrating an increase in plasminogen activator inhibitor 1(515). In these studies however it is believed that the increase in fibrinolytic factors is in response to increased blood coagulability and is a protective mechanism. More recently Katz *et al* examined the effect of electrical stimulation on patients with spinal cord injuries and determined that fibrinolytic activity was increased(516).

Increasing fibrinolytic activity by reducing levels of plasminogen activator inhibitor 1 could have many beneficial long term effects through the prevention of thrombosis. Increased PAI-1 levels have been shown to be implicated in the aging associated thrombosis and cardiovascular aging(517). As such the ability to reduce circulating levels may slow disease progression in the elderly. Reducing plasma levels of PAI-1 have also been shown in

experiments to slow progression of chronic kidney disease and may even result in a degree of disease resolution which has led to attempts to develop drugs and therapies which could target its activity(518). It has also been shown that high levels of PAI-1 may be responsible for the development of microvascular complications associated with Type II diabetes mellitus(519). Furthermore, studies have shown that a decreased fibrinolytic activity is a predictor of myocardial infarction(520, 521). It can therefore be seen that peroneal nerve stimulation would be a useful treatment adjunct or prophylaxis within the vascular disease population, many of whom have multiple risk factors for thrombotic disease.

Chapter Ten: Future avenues of research

10.1 Optimisation of patient use

The failure of the geko^{TM} device to produce visible muscle twitch in such a high proportion of vascular patients warrants attention. It is possible that by increasing the range of stimulation settings to beyond the abilities of the current geko^{TM} device that a higher proportion of this cohort could benefit from this device. Following on from this study a geko plus^{TM} has been developed which will deliver greater amplitude of current. Trials examining the efficacy of this device to produce muscle twitch are required.

Further work should also be carried out to establish whether increased stimulation intensities result in even greater improvements in haemodynamic flow. Prior work by Jawad(522) suggests that higher stimulation settings result in greater increases in flow within healthy volunteers, when 2 settings, high and low were utilised. Within this study patient tolerance was deemed of the utmost importance as such the stimulation level was increased only to the point of visible muscle twitch however many of the patients reported that whilst initially, upon device activation, there was some mild discomfort this rapidly eased and they developed a greater tolerance. It is possible that a gradual increase in the stimulation level over a period of time may facilitate higher stimulation levels whilst preserving patient comfort levels and this may further improve outcome measures. Griffin *et al* have previously demonstrated that higher rates of stimulation reduce peak systolic velocity but increase volume flow (523).This would allow determination of the optimal stimulation setting to maximally augment flow.

The optimal duration of stimulation also warrants study. Within this study, as participants were asked to remain reclined for the total study period, on a

couch, without moving, a longer study period was not deemed appropriate. However further measurements of arterial and venous flow would have been beneficial following deactivation of the device to establish whether any effects remained and for what time period. Previous studies examining use of spinal cord stimulation, TENS and peroneal nerve stimulation as therapeutic interventions have varied greatly in the duration of therapy applied to the subjects ranging from three stimulation periods lasting 20minutes per 24 hours(320) and continual stimulation(323). In healthy volunteers it has however been demonstrated that the effects of peroneal nerve stimulation on flow are sustained over a four hour period whilst the device remains active suggesting that there is no tolerance effect with the device(522).

10.2 Mechanism of action

Studies should be carried out to determine the actual mechanism of action of the device to augment flow. Cardiac output can be measured in a number of ways however the current gold standard is thermodilution which involves the insertion of a pulmonary artery catheter. Other techniques have been utilised however most involve the use of specialist equipment, highly trained operators and yet still there are issues with reproducibility. Indeed many also still rely upon the use of central venous catheters and/or arterial lines(524). The non-invasive use of photoplethysmogram sensors to estimate cardiac output and systemic vascular resistance has been studied previously however it has been determined to be imprecise and only of use to report change as opposed to absolute values(524). Thoracic bioreactance is gaining credence with the development of devices such as NICOM. The results from this show good correlation with those from thermodilution with high precision and responsiveness. Such a device may prove useful in future studies(525).

10.3 Long term clinical outcomes

Once the optimal duration and frequency of stimulation have been established further studies to determine the long term effects of device application should be undertaken.

10.2.1 Claudication

Current conservative management for individuals with claudication comprise best medical therapy, lifestyle modification and exercise. A randomised controlled trial to examine the impact of peroneal nerve stimulation in addition to current best management on claudication distance and quality of life scores should be undertaken.

10.2.2 Venous ulceration

The results of this study in relation to increased venous and microcirculatory flow raises the possibility that the healing of venous and mixed picture ulcers may be accelerated with use of the device. A randomised trial to examine the addition of peroneal nerve stimulation to current treatment regimes would be beneficial and protocols for such a study are being developed.

10.2.3 Bypass grafts

The augmentation of flow through venous bypass grafts may prove beneficial in long term graft patency. Studies to examine the effects of long term nerve stimulation following surgery should be carried out. Duplex ultrasound assessment is routinely carried out for surveillance of such grafts and as such patients would likely not require additional follow up appointments reducing the burden of the trial on participants.

10.2.4 Critical limb ischaemia

It was the initial intention that this study would examine the effect of the geko^{TM} on patients with critical limb ischaemia. Research study one highlighted the problem of poor device efficacy to produce a visible muscle twitch within this cohort of patients due to a combination of peripheral oedema and neuropathy. However it is within this cohort that flow optimisation would potentially yield the greatest results in terms of pain relief and limb salvage. Should studies examining the 'mark 2' device demonstrate greater efficacy it is within this cohort of vascular patients that studies examining the long term effects of use should be concentrated.

10.4 Angiogenesis

Further studies to investigate the effect of peroneal nerve stimulation on angiogenesis and VEGF should incorporate serial measurements of VEGF which would best be carried out through the insertion of lines at the start of the study period. This would enable repeated samplings without subjecting the patient to repeated needlings and would reduce vessel trauma.

A previous study examining the effect of a short period of exercise on angiogenesis also failed to demonstrate a change in the plasma levels of VEGF despite serial measurements however did demonstrate an increased expression of VEGF mRNA in calf muscle biopsies secondary to HIF1 alpha upregulation stimulating VEGF mRNA transcription(526). Core muscle biopsies would therefore provide additional information on the impact of nerve stimulation on angiogenesis and in studies of long term use would also enable histological examination of specimens to assess the impact of regular peroneal nerve stimulation on muscle capillary density(527). Research examining the impact of exercise on capillary muscle density in claudicants is currently underway at the University of Hull.

10.5 Fibrinolysis

Whilst traditionally assessments of fibrinolysis has involved the measurement of PAI-1 or Thrombin-activatable Fibrinolysis Inhibitor (TAFI) more recently viscoelastic hemostatic assays such as thromboelastography and thromboelastometry have been used.

Thromboelastography provides information about the viscoelastic properties of a blood clot during its formation and lysis. Data pertaining to coagulation factors i.e. the time taken to initiate the formation of a clot, fibrinogen and fibrin cross-linking, platelet function and fibrinolysis is obtained(528). The use of thromboelastography was reported by Jawad in the preliminary studies of peroneal nerve stimulation in healthy volunteers and contrary to expectations demonstrated a reduction in clotting time suggesting a reduction in clotting time whilst conversely they demonstrated a reduction in tPA antigen suggesting increased fibrinolysis. It is therefore clear that further research on this area would be beneficial. Another potential method of investigating fibrinolysis would be to utilise the thrombolytic assessment system (TAS). Point of care analysis of coagulation has become commonplace in recent years to assess patient's international normalised ratio whilst on treatment with warfarin(529). The TAS analyser has also been adapted to assess fibrinolysis and as such response to treatment or to screen for fibrinolytic defects. The measurement of lysis onset time may provide valuable information on effects of treatments on fibrinolysis(530, 531).

Chapter Eleven: Conclusion

Whilst studies have previously been undertaken in healthy volunteers and have demonstrated augmentation of blood flow this was the first ever research programme utilising the geko^{TM} peroneal nerve stimulation device in vascular patients. It has demonstrated that nerve stimulation increases arterial, venous and microcirculatory flow within this cohort.

The tolerability questionnaire results imply that device tolerance will be high. This combined with the small, portable and easily applied nature of the device raised the possibility that the device could be used in a number of settings, not just secondary care.

The findings reported within this thesis regarding device efficacy, i.e. the ability of the device to produce visible muscle twitch within the vascular population have already led to the redesign of the device to deliver a greater current to the nerve and reduce the number of non-responders. Efficacy studies for the 'mark 2' device are underway.

The data from these studies was also presented in the application for NICE medical technology approval and resulted in the recommendation that the device in indicated in the prevention of venous thromboembolism in those in whom alternation mechanical or medicinal prophylaxis is contraindicated.

The next priority must be to optimise the device use in terms of stimulation delivered and its duration prior to investigating the longer term clinical outcomes within these groups of patients.

- The increase in venous volume flow could lead to a role in the management of deep and superficial venous insufficiency in those who do not tolerate compression and for whom surgery is not an

option. The increase in arterial volume flow suggests a possible role for peroneal nerve stimulation as a treatment adjunct in patients with peripheral arterial occlusive disease both before and after surgical or endovascular intervention, to prevent disease progression and/ or prevent graft failure.

- The increase in microcirculatory flow seen with peroneal nerve stimulation indicates that it may play a beneficial role in the healing of chronic wounds and ulcers.
- The enhanced fibrinolytic activity, by decreasing levels of plasminogen activator inhibitor-1, is encouraging and may prove beneficial in prophylaxis against development and propagation of both arterial and venous thromboses.

Whilst the aims of the studies were met and answered the studies were not without their limitations. The sample sizes were small and the intervention period was only one hour, therefore further studies to fully investigate the optimal frequency and duration of intervention and the effects of long term usage on patient outcomes are required. This would also enable cost efficacy analysis to be calculated.

References

1. Shepherd JT, Vanhoutte, M.D. The Human Cardiovascular System: Facts and Concepts. illustrated, reprint ed. New York: Raven Press, 1979; 1999.
2. Katz AM. Ernest Henry Starling, his predecessors, and the "Law of the Heart". Circulation. 2002;106(23):2986-92. Epub 2002/12/04.
3. BiologyonlineUS. Available from: http://biologyonline.us/Online%20A&P/AP%201/Northland/AP1lab/Lab%20Online/Lab%2012/24.htm.
4. Wu KK, Thiagarajan P. Role of endothelium in thrombosis and hemostasis. Annual review of medicine. 1996;47:315-31. Epub 1996/01/01.
5. Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. Pflugers Archiv : European journal of physiology. 2000;440(5):653-66. Epub 2000/09/28.
6. Shireman PK, Pearce WH. Endothelial cell function: biologic and physiologic functions in health and disease. AJR American journal of roentgenology. 1996;166(1):7-13. Epub 1996/01/01.
7. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiological reviews. 2006;86(1):279-367. Epub 2005/12/24.
8. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;288(5789):373-6. Epub 1980/11/27.
9. Govers R, Rabelink TJ. Cellular regulation of endothelial nitric oxide synthase. American journal of physiology Renal physiology. 2001;280(2):F193-206. Epub 2001/02/24.
10. Rasche H. Haemostasis and Thrombosis: an overview. . Eur Heart J Suppl 2001;3(Supplement Q):Q3-7.
11. Cesarman- Maus G, Hajjar, K.A. . Molecular mechanisms of fibrinolysis. . British Journal of Haematology. 2005;129(3):307-21.
12. Ross R. Atherosclerosis--an inflammatory disease. The New England journal of medicine. 1999;340(2):115-26. Epub 1999/01/14.
13. Bennett PC, Silverman SH, Gill PS, Lip GY. Peripheral arterial disease and Virchow's triad. Thrombosis and haemostasis. 2009;101(6):1032-40. Epub 2009/06/06.
14. Coutinho T, Rooke TW, Kullo IJ. Arterial dysfunction and functional performance in patients with peripheral artery disease: a review. Vasc Med. 2011;16(3):203-11. Epub 2011/03/31.
15. Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation. 2003;107(22):2864-9. Epub 2003/06/11.
16. Arnett DK, Evans GW, Riley WA. Arterial stiffness: a new cardiovascular risk factor? American journal of epidemiology. 1994;140(8):669-82. Epub 1994/10/15.
17. de Bono D. Significance of raised plasma concentrations of tissue-type plasminogen activator and plasminogen activator inhibitor in patients at risk from ischaemic heart disease. British heart journal. 1994;71(6):504-7. Epub 1994/06/01.
18. Lowe GD, Danesh J, Lewington S, Walker M, Lennon L, Thomson A, et al. Tissue plasminogen activator antigen and coronary heart disease. Prospective study and meta-analysis. European heart journal. 2004;25(3):252-9. Epub 2004/02/20.
19. Juhan-Vague I, Alessi, M.C. Fibrinolysis and risk of coronary artery disease. . Fibrinolysis. 1996;10:127-37.

20. Smith FB, Fowkes FG, Rumley A, Lee AJ, Lowe GD, Hau CM. Tissue plasminogen activator and leucocyte elastase as predictors of cardiovascular events in subjects with angina pectoris: Edinburgh Artery Study. European heart journal. 2000;21(19):1607-13. Epub 2000/09/16.
21. Salomaa V, Stinson V, Kark JD, Folsom AR, Davis CE, Wu KK. Association of fibrinolytic parameters with early atherosclerosis. The ARIC Study. Atherosclerosis Risk in Communities Study. Circulation. 1995;91(2):284-90. Epub 1995/01/15.
22. Binder BR, Christ G, Gruber F, Grubic N, Hufnagl P, Krebs M, et al. Plasminogen activator inhibitor 1: physiological and pathophysiological roles. News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society. 2002;17:56-61. Epub 2002/03/23.
23. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. The New England journal of medicine. 2000;342(24):1792-801. Epub 2000/06/15.
24. Huber K. Plasminogen activator inhibitor type-1 (part two): role for failure of thrombolytic therapy. PAI-1 resistance as a potential benefit for new fibrinolytic agents. Journal of thrombosis and thrombolysis. 2001;11(3):195-202. Epub 2001/09/29.
25. Juhan-Vague I, Alessi MC. PAI-1, obesity, insulin resistance and risk of cardiovascular events. Thrombosis and haemostasis. 1997;78(1):656-60. Epub 1997/07/01.
26. Rey S, Semenza GL. Hypoxia-inducible factor-1-dependent mechanisms of vascularization and vascular remodelling. Cardiovascular research. 2010;86(2):236-42. Epub 2010/02/19.
27. Mariotti M, Maier, J. Angiogenesis: An Overview. New Frontiers in Angiogenesis 2006;1:1-29.
28. Klagsbrun M, Moses MA. Molecular angiogenesis. Chemistry & biology. 1999;6(8):R217-24. Epub 1999/07/28.
29. Ide AG, Baker, N.H., Warren, S.L. . Vascularisation of the Brown Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. . American Journal of Roentgenology. 1939;42:891-99.
30. Algire GH, Chalkley, H.W., Legallais, F.Y., Park, H.D. Vascular reactions of normal and malignant tissues in vivo. I. Vascular reactions of mice to wounds and to normal and neoplastic transplants. . Journal of National Cancer Institute. 1945;6:73-85.
31. Greenblatt M, Shubi P. Tumor angiogenesis: transfilter diffusion studies in the hamster by the transparent chamber technique. Journal of the National Cancer Institute. 1968;41(1):111-24. Epub 1968/07/01.
32. Ehrmann RL, Knoth M. Choriocarcinoma. Transfilter stimulation of vasoproliferation in the hamster cheek pouch. Studied by light and electron microscopy. Journal of the National Cancer Institute. 1968;41(6):1329-41. Epub 1968/12/01.
33. Folkman J. Tumor angiogenesis: therapeutic implications. The New England journal of medicine. 1971;285(21):1182-6. Epub 1971/11/18.
34. Ferrara N. Vascular endothelial growth factor. Arteriosclerosis, thrombosis, and vascular biology. 2009;29(6):789-91. Epub 2009/01/24.
35. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;219(4587):983-5. Epub 1983/02/25.
36. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochemical and biophysical research communications. 1989;161(2):851-8. Epub 1989/06/15.
37. Germani A, Di Campli C, Pompilio G, Biglioli P, Capogrossi MC. Regenerative therapy in peripheral artery disease. Cardiovascular therapeutics. 2009;27(4):289-304. Epub 2009/11/12.

38. Phillips HS, Hains J, Leung DW, Ferrara N. Vascular endothelial growth factor is expressed in rat corpus luteum. Endocrinology. 1990;127(2):965-7. Epub 1990/08/01.
39. Jakeman LB, Winer J, Bennett GL, Altar CA, Ferrara N. Binding sites for vascular endothelial growth factor are localized on endothelial cells in adult rat tissues. The Journal of clinical investigation. 1992;89(1):244-53. Epub 1992/01/01.
40. Ferrara N. VEGF and the quest for tumour angiogenesis factors. Nature reviews Cancer. 2002;2(10):795-803. Epub 2002/10/03.
41. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature. 1993;362(6423):841-4. Epub 1993/04/29.
42. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocrine reviews. 1997;18(1):4-25. Epub 1997/02/01.
43. Takeshita S, Zheng LP, Brogi E, Kearney M, Pu LQ, Bunting S, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. The Journal of clinical investigation. 1994;93(2):662-70. Epub 1994/02/01.
44. Bauters C, Asahara T, Zheng LP, Takeshita S, Bunting S, Ferrara N, et al. Recovery of disturbed endothelium-dependent flow in the collateral-perfused rabbit ischemic hindlimb after administration of vascular endothelial growth factor. Circulation. 1995;91(11):2802-9. Epub 1995/06/01.
45. Deiner C, Schwimmbeck PL, Koehler IS, Loddenkemper C, Noutsias M, Nikol S, et al. Adventitial VEGF165 gene transfer prevents lumen loss through induction of positive arterial remodeling after PTCA in porcine coronary arteries. Atherosclerosis. 2006;189(1):123-32. Epub 2006/01/26.
46. Rajagopalan S, Mohler ER, 3rd, Lederman RJ, Mendelsohn FO, Saucedo JF, Goldman CK, et al. Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: a phase II randomized, double-blind, controlled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. Circulation. 2003;108(16):1933-8. Epub 2003/09/25.
47. Isner JM, Pieczek A, Schainfeld R, Blair R, Haley L, Asahara T, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. Lancet. 1996;348(9024):370-4. Epub 1996/08/10.
48. Baumgartner I, Pieczek A, Manor O, Blair R, Kearney M, Walsh K, et al. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. Circulation. 1998;97(12):1114-23. Epub 1998/04/16.
49. Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation. 2003;107(10):1359-65. Epub 2003/03/19.
50. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocrine reviews. 2004;25(4):581-611. Epub 2004/08/06.
51. Dor Y, Djonov V, Abramovitch R, Itin A, Fishman GI, Carmeliet P, et al. Conditional switching of VEGF provides new insights into adult neovascularization and pro-angiogenic therapy. The EMBO journal. 2002;21(8):1939-47. Epub 2002/04/16.
52. Cao R, Brakenhielm E, Pawliuk R, Wariaro D, Post MJ, Wahlberg E, et al. Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. Nature medicine. 2003;9(5):604-13. Epub 2003/04/02.
53. Webb NJ, Bottomley MJ, Watson CJ, Brenchley PE. Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. Clin Sci (Lond). 1998;94(4):395-404. Epub 1998/06/26.

54. Ferrero S. Serum and plasma vascular endothelial growth factor levels in testicular cancer patients. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2004;15(6):989-90. Epub 2004/05/21.
55. George ML, Eccles SA, Tutton MG, Abulafi AM, Swift RI. Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: clinical evidence of platelet scavenging? Clinical cancer research : an official journal of the American Association for Cancer Research. 2000;6(8):3147-52. Epub 2000/08/24.
56. Lee JK, Hong YJ, Han CJ, Hwang DY, Hong SI. Clinical usefulness of serum and plasma vascular endothelial growth factor in cancer patients: which is the optimal specimen? International journal of oncology. 2000;17(1):149-52. Epub 2000/06/15.
57. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011;473(7347):317-25. Epub 2011/05/20.
58. Muir RL. Peripheral arterial disease: Pathophysiology, risk factors, diagnosis, treatment, and prevention. Journal of vascular nursing : official publication of the Society for Peripheral Vascular Nursing. 2009;27(2):26-30. Epub 2009/06/03.
59. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. Arteriosclerosis, thrombosis, and vascular biology. 2010;30(7):1282-92. Epub 2010/06/18.
60. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. 1995;26(3):485-90. Epub 1995/09/01.
61. Asmar RG, Topouchian JA, Benetos A, Sayegh FA, Mourad JJ, Safar ME. Non-invasive evaluation of arterial abnormalities in hypertensive patients. Journal of hypertension Supplement : official journal of the International Society of Hypertension. 1997;15(2):S99-107. Epub 1997/03/01.
62. Bramwell JC, Hill, A.V. The velocity of the pulse wave in man. Proc R Soc Lond B. 1922;93:298-306.
63. Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT, Tajik AJ. Noninvasive measurement of central vascular pressures with arterial tonometry: clinical revival of the pulse pressure waveform? Mayo Clinic proceedings Mayo Clinic. 2010;85(5):460-72. Epub 2010/05/04.
64. Yasmin, Brown MJ. Similarities and differences between augmentation index and pulse wave velocity in the assessment of arterial stiffness. QJM : monthly journal of the Association of Physicians. 1999;92(10):595-600. Epub 2000/01/11.
65. Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, et al. Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation. 1993;88(4 Pt 1):1456-62. Epub 1993/10/01.
66. Wilkinson IB, Mohammad NH, Tyrrell S, Hall IR, Webb DJ, Paul VE, et al. Heart rate dependency of pulse pressure amplification and arterial stiffness. American journal of hypertension. 2002;15(1 Pt 1):24-30. Epub 2002/02/05.
67. Kelly R, Daley J, Avolio A, O'Rourke M. Arterial dilation and reduced wave reflection. Benefit of dilevalol in hypertension. Hypertension. 1989;14(1):14-21. Epub 1989/07/01.
68. Saba PS, Roman MJ, Pini R, Spitzer M, Ganau A, Devereux RB. Relation of arterial pressure waveform to left ventricular and carotid anatomy in normotensive subjects. Journal of the American College of Cardiology. 1993;22(7):1873-80. Epub 1993/12/01.
69. McVeigh G, Brennan G, Hayes R, Cohn J, Finkelstein S, Johnston D. Vascular abnormalities in non-insulin-dependent diabetes mellitus identified by arterial waveform analysis. The American journal of medicine. 1993;95(4):424-30. Epub 1993/10/01.
70. Kahonen M, Ylitalo R, Koobi T, Turjanmaa V, Ylitalo P. Influences of nonselective, beta(1)-selective and vasodilatory beta(1)-selective beta-blockers on arterial pulse wave

velocity in normotensive subjects. General pharmacology. 2000;35(4):219-24. Epub 2002/02/06.
71. Chen CH, Ting CT, Lin SJ, Hsu TL, Yin FC, Siu CO, et al. Different effects of fosinopril and atenolol on wave reflections in hypertensive patients. Hypertension. 1995;25(5):1034-41. Epub 1995/05/01.
72. Hiatt WR, Hoag S, Hamman RF. Effect of diagnostic criteria on the prevalence of peripheral arterial disease. The San Luis Valley Diabetes Study. Circulation. 1995;91(5):1472-9. Epub 1995/03/01.
73. Fowkes FG, Housley E, Cawood EH, Macintyre CC, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. International journal of epidemiology. 1991;20(2):384-92. Epub 1991/06/01.
74. Shammas NW. Epidemiology, classification, and modifiable risk factors of peripheral arterial disease. Vascular health and risk management. 2007;3(2):229-34. Epub 2007/06/22.
75. Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, et al. Mortality over a period of 10 years in patients with peripheral arterial disease. The New England journal of medicine. 1992;326(6):381-6. Epub 1992/02/06.
76. Criqui MH, Ninomiya JK, Wingard DL, Ji M, Fronek A. Progression of peripheral arterial disease predicts cardiovascular disease morbidity and mortality. Journal of the American College of Cardiology. 2008;52(21):1736-42. Epub 2008/11/15.
77. Weitz JI, Byrne J, Clagett GP, Farkouh ME, Porter JM, Sackett DL, et al. Diagnosis and treatment of chronic arterial insufficiency of the lower extremities: a critical review. Circulation. 1996;94(11):3026-49. Epub 1996/12/01.
78. Second European Consensus Document on chronic critical leg ischemia. European journal of vascular surgery. 1992;6 Suppl A:1-32. Epub 1992/05/01.
79. Schroeder TV. The TASC supplement - international recommendations for management of peripheral arterial disease. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2000;19(6):563. Epub 2000/06/30.
80. Rutherford RB, Baker JD, Ernst C, Johnston KW, Porter JM, Ahn S, et al. Recommended standards for reports dealing with lower extremity ischemia: revised version. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 1997;26(3):517-38. Epub 1997/10/06.
81. Adler AI, Stevens RJ, Neil A, Stratton IM, Boulton AJ, Holman RR. UKPDS 59: hyperglycemia and other potentially modifiable risk factors for peripheral vascular disease in type 2 diabetes. Diabetes care. 2002;25(5):894-9. Epub 2002/04/30.
82. Dao HH, Essalihi R, Bouvet C, Moreau P. Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension. Cardiovascular research. 2005;66(2):307-17. Epub 2005/04/12.
83. Willigendael EM, Teijink JA, Bartelink ML, Peters RJ, Buller HR, Prins MH. Smoking and the patency of lower extremity bypass grafts: a meta-analysis. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2005;42(1):67-74. Epub 2005/07/14.
84. Shammas NW, Lemke JH, Dippel EJ, McKinney DE, Takes VS, Youngblut M, et al. In-hospital complications of peripheral vascular interventions using unfractionated heparin as the primary anticoagulant. The Journal of invasive cardiology. 2003;15(5):242-6. Epub 2003/05/06.

85. Guo X, Oldham MJ, Kleinman MT, Phalen RF, Kassab GS. Effect of cigarette smoking on nitric oxide, structural, and mechanical properties of mouse arteries. American journal of physiology Heart and circulatory physiology. 2006;291(5):H2354-61. Epub 2006/07/04.
86. Selvin E, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National Health and Nutrition Examination Survey, 1999-2000. Circulation. 2004;110(6):738-43. Epub 2004/07/21.
87. Meijer WT, Grobbee DE, Hunink MG, Hofman A, Hoes AW. Determinants of peripheral arterial disease in the elderly: the Rotterdam study. Archives of internal medicine. 2000;160(19):2934-8. Epub 2000/10/21.
88. NICE B. Hypertension: Management in adults in primary care: pharmacological update. Joint British Hypertension Society and National Institute for Healthcare Excellence Guidance. 2007.
89. Gorter PM, Olijhoek JK, van der Graaf Y, Algra A, Rabelink TJ, Visseren FL. Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm. Atherosclerosis. 2004;173(2):363-9. Epub 2004/04/06.
90. Planas A, Clara A, Pou JM, Vidal-Barraquer F, Gasol A, de Moner A, et al. Relationship of obesity distribution and peripheral arterial occlusive disease in elderly men. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2001;25(7):1068-70. Epub 2001/07/10.
91. Wyatt MG, Scott PM, Scott DJ, Poskitt K, Baird RN, Horrocks M. Effect of weight on claudication distance. The British journal of surgery. 1991;78(11):1386-8. Epub 1991/11/01.
92. Smith FC, Shearman CP, Simms MH, Gwynn BR. Falsely elevated ankle pressures in severe leg ischaemia: the pole test--an alternative approach. European journal of vascular surgery. 1994;8(4):408-12. Epub 1994/07/01.
93. Yao ST, Hobbs JT, Irvine WT. Pulse examination by an ultrasonic method. British medical journal. 1968;4(5630):555-7. Epub 1968/11/30.
94. Cao P, Eckstein HH, De Rango P, Setacci C, Ricco JB, de Donato G, et al. Chapter II: Diagnostic methods. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2011;42 Suppl 2:S13-32. Epub 2011/12/30.
95. Kaiser V, Kester AD, Stoffers HE, Kitslaar PJ, Knottnerus JA. The influence of experience on the reproducibility of the ankle-brachial systolic pressure ratio in peripheral arterial occlusive disease. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 1999;18(1):25-9. Epub 1999/07/02.
96. Fowkes FG. The measurement of atherosclerotic peripheral arterial disease in epidemiological surveys. International journal of epidemiology. 1988;17(2):248-54. Epub 1988/06/01.
97. McLafferty RB, Moneta GL, Taylor LM, Jr., Porter JM. Ability of ankle-brachial index to detect lower-extremity atherosclerotic disease progression. Arch Surg. 1997;132(8):836-40; discussion 40-1. Epub 1997/08/01.
98. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, et al. ACC/AHA 2005 guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): executive summary a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease) endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular

Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Journal of the American College of Cardiology. 2006;47(6):1239-312. Epub 2006/03/21.
99. Collins R, Burch J, Cranny G, Aguiar-Ibanez R, Craig D, Wright K, et al. Duplex ultrasonography, magnetic resonance angiography, and computed tomography angiography for diagnosis and assessment of symptomatic, lower limb peripheral arterial disease: systematic review. BMJ. 2007;334(7606):1257. Epub 2007/06/06.
100. Allard L, Cloutier G, Guo Z, Durand LG. Review of the assessment of single level and multilevel arterial occlusive disease in lower limbs by duplex ultrasound. Ultrasound in medicine & biology. 1999;25(4):495-502. Epub 1999/07/01.
101. Kock MC, Dijkshoorn ML, Pattynama PM, Myriam Hunink MG. Multi-detector row computed tomography angiography of peripheral arterial disease. European radiology. 2007;17(12):3208-22. Epub 2007/09/21.
102. Muluk SC, Muluk VS, Kelley ME, Whittle JC, Tierney JA, Webster MW, et al. Outcome events in patients with claudication: a 15-year study in 2777 patients. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2001;33(2):251-7; discussion 7-8. Epub 2001/02/15.
103. Solomon R. The role of osmolality in the incidence of contrast-induced nephropathy: a systematic review of angiographic contrast media in high risk patients. Kidney international. 2005;68(5):2256-63. Epub 2005/10/14.
104. Levy EM, Viscoli CM, Horwitz RI. The effect of acute renal failure on mortality. A cohort analysis. JAMA : the journal of the American Medical Association. 1996;275(19):1489-94. Epub 1996/05/15.
105. Menke J, Larsen J. Meta-analysis: Accuracy of contrast-enhanced magnetic resonance angiography for assessing steno-occlusions in peripheral arterial disease. Annals of internal medicine. 2010;153(5):325-34. Epub 2010/09/08.
106. Lee VS, Martin DJ, Krinsky GA, Rofsky NM. Gadolinium-enhanced MR angiography: artifacts and pitfalls. AJR American journal of roentgenology. 2000;175(1):197-205. Epub 2000/07/06.
107. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2007;45 Suppl S:S5-67. Epub 2007/01/16.
108. Izquierdo-Porrera AM, Gardner AW, Powell CC, Katzel LI. Effects of exercise rehabilitation on cardiovascular risk factors in older patients with peripheral arterial occlusive disease. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2000;31(4):670-7. Epub 2001/02/07.
109. Parmenter BJ, Raymond J, Dinnen P, Singh MA. A systematic review of randomized controlled trials: Walking versus alternative exercise prescription as treatment for intermittent claudication. Atherosclerosis. 2011;218(1):1-12. Epub 2011/05/24.
110. Watson L, Ellis B, Leng GC. Exercise for intermittent claudication. Cochrane Database Syst Rev. 2008(4):CD000990. Epub 2008/10/10.
111. Kruidenier LM, Nicolai SP, Hendriks EJ, Bollen EC, Prins MH, Teijink JA. Supervised exercise therapy for intermittent claudication in daily practice. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2009;49(2):363-70. Epub 2008/11/26.
112. Nicolai SP, Teijink JA, Prins MH. Multicenter randomized clinical trial of supervised exercise therapy with or without feedback versus walking advice for intermittent claudication. Journal of vascular surgery : official publication, the Society for Vascular

Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2010;52(2):348-55. Epub 2010/05/19.
113. Leng GC, Fowler B, Ernst E. Exercise for intermittent claudication. Cochrane Database Syst Rev. 2000(2):CD000990. Epub 2000/05/05.
114. Cook NR, Cutler JA, Obarzanek E, Buring JE, Rexrode KM, Kumanyika SK, et al. Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (TOHP). BMJ. 2007;334(7599):885-8. Epub 2007/04/24.
115. Law M, Tang JL. An analysis of the effectiveness of interventions intended to help people stop smoking. Archives of internal medicine. 1995;155(18):1933-41. Epub 1995/10/09.
116. Jorenby DE, Leischow SJ, Nides MA, Rennard SI, Johnston JA, Hughes AR, et al. A controlled trial of sustained-release bupropion, a nicotine patch, or both for smoking cessation. The New England journal of medicine. 1999;340(9):685-91. Epub 1999/03/04.
117. Rosenberg L, Palmer JR, Shapiro S. Decline in the risk of myocardial infarction among women who stop smoking. The New England journal of medicine. 1990;322(4):213-7. Epub 1990/01/25.
118. Secondary prevention of vascular disease by prolonged antiplatelet treatment. Antiplatelet Trialists' Collaboration. Br Med J (Clin Res Ed). 1988;296(6618):320-31. Epub 1988/01/30.
119. Dorffler-Melly J, Koopman MM, Adam DJ, Buller HR, Prins MH. Antiplatelet agents for preventing thrombosis after peripheral arterial bypass surgery. Cochrane Database Syst Rev. 2003(3):CD000535. Epub 2003/08/15.
120. Drouet L, Bal dit Sollier C, Henry P. [The basis of platelets: platelets and atherothrombosis: an understanding of the lack of efficacy of aspirin in peripheral arterial disease (PAD) and diabetic patients]. Drugs. 2010;70 Suppl 1:9-14. Epub 2010/10/28.
121. Dorffler-Melly J, Buller HR, Koopman MM, Prins MH. Antithrombotic agents for preventing thrombosis after infrainguinal arterial bypass surgery. Cochrane Database Syst Rev. 2003(4):CD000536. Epub 2003/10/30.
122. The HOPE (Heart Outcomes Prevention Evaluation) Study: the design of a large, simple randomized trial of an angiotensin-converting enzyme inhibitor (ramipril) and vitamin E in patients at high risk of cardiovascular events. The HOPE study investigators. The Canadian journal of cardiology. 1996;12(2):127-37. Epub 1996/02/01.
123. Estacio RO, Jeffers BW, Hiatt WR, Biggerstaff SL, Gifford N, Schrier RW. The effect of nisoldipine as compared with enalapril on cardiovascular outcomes in patients with non-insulin-dependent diabetes and hypertension. The New England journal of medicine. 1998;338(10):645-52. Epub 1998/03/05.
124. Overlack A, Adamczak M, Bachmann W, Bonner G, Bretzel RG, Derichs R, et al. ACE-inhibition with perindopril in essential hypertensive patients with concomitant diseases. The Perindopril Therapeutic Safety Collaborative Research Group. The American journal of medicine. 1994;97(2):126-34. Epub 1994/08/01.
125. Lane DA, Lip GY. Treatment of hypertension in peripheral arterial disease. Cochrane Database Syst Rev. 2009(4):CD003075. Epub 2009/10/13.
126. Hennekens CH, Albert CM, Godfried SL, Gaziano JM, Buring JE. Adjunctive drug therapy of acute myocardial infarction--evidence from clinical trials. The New England journal of medicine. 1996;335(22):1660-7. Epub 1996/11/28.
127. Radack K, Deck C. Beta-adrenergic blocker therapy does not worsen intermittent claudication in subjects with peripheral arterial disease. A meta-analysis of randomized controlled trials. Archives of internal medicine. 1991;151(9):1769-76. Epub 1991/09/01.

128. Aung PP, Maxwell HG, Jepson RG, Price JF, Leng GC. Lipid-lowering for peripheral arterial disease of the lower limb. Cochrane Database Syst Rev. 2007(4):CD000123. Epub 2007/10/19.
129. Aronow WS, Nayak D, Woodworth S, Ahn C. Effect of simvastatin versus placebo on treadmill exercise time until the onset of intermittent claudication in older patients with peripheral arterial disease at six months and at one year after treatment. The American journal of cardiology. 2003;92(6):711-2. Epub 2003/09/16.
130. Mondillo S, Ballo P, Barbati R, Guerrini F, Ammaturo T, Agricola E, et al. Effects of simvastatin on walking performance and symptoms of intermittent claudication in hypercholesterolemic patients with peripheral vascular disease. The American journal of medicine. 2003;114(5):359-64. Epub 2003/04/26.
131. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998;352(9131):837-53. Epub 1998/09/22.
132. Beebe HG, Dawson DL, Cutler BS, Herd JA, Strandness DE, Jr., Bortey EB, et al. A new pharmacological treatment for intermittent claudication: results of a randomized, multicenter trial. Archives of internal medicine. 1999;159(17):2041-50. Epub 1999/10/08.
133. Robless P, Mikhailidis DP, Stansby GP. Cilostazol for peripheral arterial disease. Cochrane Database Syst Rev. 2007(1):CD003748. Epub 2007/01/27.
134. Lehert P, Comte S, Gamand S, Brown TM. Naftidrofuryl in intermittent claudication: a retrospective analysis. Journal of cardiovascular pharmacology. 1994;23 Suppl 3:S48-52. Epub 1994/01/01.
135. NICE. NICE technology appraisals [TA223] :Cilostazol, naftidrofuryl oxalate, pentoxifylline and inositol nicotinate for the treatment of intermittent claudication in people with peripheral arterial disease. 2011.
136. Loosemore TM, Chalmers TC, Dormandy JA. A meta-analysis of randomized placebo control trials in Fontaine stages III and IV peripheral occlusive arterial disease. International angiology : a journal of the International Union of Angiology. 1994;13(2):133-42. Epub 1994/06/01.
137. Rofsky NM, Adelman MA. MR angiography in the evaluation of atherosclerotic peripheral vascular disease. Radiology. 2000;214(2):325-38. Epub 2000/02/15.
138. Pentecost MJ, Criqui MH, Dorros G, Goldstone J, Johnston KW, Martin EC, et al. Guidelines for peripheral percutaneous transluminal angioplasty of the abdominal aorta and lower extremity vessels. A statement for health professionals from a special writing group of the Councils on Cardiovascular Radiology, Arteriosclerosis, Cardio-Thoracic and Vascular Surgery, Clinical Cardiology, and Epidemiology and Prevention, the American Heart Association. Circulation. 1994;89(1):511-31. Epub 1994/01/01.
139. Ramaswami G, Tegos T, Nicolaides AN, Dhanjil S, Griffin M, Al-Kutoubi A, et al. Ultrasonic plaque character and outcome after lower limb angioplasty. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 1999;29(1):110-9; discussion 9-21. Epub 1999/01/12.
140. Brewster DC, Perler BA, Robison JG, Darling RC. Aortofemoral graft for multilevel occlusive disease. Predictors of success and need for distal bypass. Arch Surg. 1982;117(12):1593-600. Epub 1982/12/01.
141. Adam DJ, Beard JD, Cleveland T, Bell J, Bradbury AW, Forbes JF, et al. Bypass versus angioplasty in severe ischaemia of the leg (BASIL): multicentre, randomised controlled trial. Lancet. 2005;366(9501):1925-34. Epub 2005/12/06.
142. Nguyen BN, Conrad MF, Guest JM, Hackney L, Patel VI, Kwolek CJ, et al. Late outcomes of balloon angioplasty and angioplasty with selective stenting for superficial

femoral-popliteal disease are equivalent. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2011;54(4):1051-7 e1. Epub 2011/06/04.
143. Schillinger M, Sabeti S, Loewe C, Dick P, Amighi J, Mlekusch W, et al. Balloon angioplasty versus implantation of nitinol stents in the superficial femoral artery. The New England journal of medicine. 2006;354(18):1879-88. Epub 2006/05/05.
144. Laird JR, Katzen BT, Scheinert D, Lammer J, Carpenter J, Buchbinder M, et al. Nitinol stent implantation versus balloon angioplasty for lesions in the superficial femoral artery and proximal popliteal artery: twelve-month results from the RESILIENT randomized trial. Circulation Cardiovascular interventions. 2010;3(3):267-76. Epub 2010/05/21.
145. Simpson EL, Michaels JA, Thomas SM, Cantrell AJ. Systematic review and meta-analysis of additional technologies to enhance angioplasty for infrainguinal peripheral arterial occlusive disease. The British journal of surgery. 2013;100(9):1128-37. Epub 2013/07/12.
146. Klinkert P, Post PN, Breslau PJ, van Bockel JH. Saphenous vein versus PTFE for above-knee femoropopliteal bypass. A review of the literature. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2004;27(4):357-62. Epub 2004/03/12.
147. Veith FJ, Gupta SK, Ascer E, White-Flores S, Samson RH, Scher LA, et al. Six-year prospective multicenter randomized comparison of autologous saphenous vein and expanded polytetrafluoroethylene grafts in infrainguinal arterial reconstructions. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 1986;3(1):104-14. Epub 1986/01/01.
148. Wilson SE. New alternatives in management of the infected vascular prosthesis. Surgical infections. 2001;2(2):171-5; discussion 5-7. Epub 2003/02/22.
149. Schanzer A, Hevelone N, Owens CD, Belkin M, Bandyk DF, Clowes AW, et al. Technical factors affecting autogenous vein graft failure: observations from a large multicenter trial. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2007;46(6):1180-90; discussion 90. Epub 2007/12/25.
150. Faries PL, Arora S, Pomposelli FB, Jr., Pulling MC, Smakowski P, Rohan DI, et al. The use of arm vein in lower-extremity revascularization: results of 520 procedures performed in eight years. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2000;31(1 Pt 1):50-9. Epub 2000/01/22.
151. Roll S, Muller-Nordhorn J, Keil T, Scholz H, Eidt D, Greiner W, et al. Dacron vs. PTFE as bypass materials in peripheral vascular surgery--systematic review and meta-analysis. BMC surgery. 2008;8:22. Epub 2008/12/23.
152. Jensen LP, Lepantalo M, Fossdal JE, Roder OC, Jensen BS, Madsen MS, et al. Dacron or PTFE for above-knee femoropopliteal bypass. a multicenter randomised study. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2007;34(1):44-9. Epub 2007/04/03.
153. Coggia M, Javerliat I, Di Centa I, Colacchio G, Leschi JP, Kitzis M, et al. Total laparoscopic bypass for aortoiliac occlusive lesions: 93-case experience. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2004;40(5):899-906. Epub 2004/11/24.
154. Harrington ME, Harrington EB, Haimov M, Schanzer H, Jacobson JH, 2nd. Axillofemoral bypass: compromised bypass for compromised patients. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 1994;20(2):195-201. Epub 1994/08/01.

155. Vascular_Society. http://www.vascularsociety.org.uk/national-vascular-database.html2012 [cited 2013].
156. Griffiths GD, Nagy J, Black D, Stonebridge PA. Randomized clinical trial of distal anastomotic interposition vein cuff in infrainguinal polytetrafluoroethylene bypass grafting. The British journal of surgery. 2004;91(5):560-2. Epub 2004/05/04.
157. Pereira CE, Albers M, Romiti M, Brochado-Neto FC, Pereira CA. Meta-analysis of femoropopliteal bypass grafts for lower extremity arterial insufficiency. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2006;44(3):510-7. Epub 2006/09/05.
158. Dormandy JA, Rutherford RB. Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Consensus (TASC). Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2000;31(1 Pt 2):S1-S296. Epub 2000/02/09.
159. NICE. NICE Clinical Guideline 147: Lower limb peripheral arterial disease: diagnosis and management. 2012.
160. Melzack R, Wall PD. Pain mechanisms: a new theory. Science. 1965;150(3699):971-9. Epub 1965/11/19.
161. Melzack R. Gate Control Theory: on the evolution of pain concepts. . Pain Forum: Official Journal of the American Pain Society. 1996;5:128-38.
162. Cui JG, O'Connor WT, Ungerstedt U, Linderoth B, Meyerson BA. Spinal cord stimulation attenuates augmented dorsal horn release of excitatory amino acids in mononeuropathy via a GABAergic mechanism. Pain. 1997;73(1):87-95. Epub 1997/12/31.
163. Croom JE, Foreman RD, Chandler MJ, Barron KW. Cutaneous vasodilation during dorsal column stimulation is mediated by dorsal roots and CGRP. The American journal of physiology. 1997;272(2 Pt 2):H950-7. Epub 1997/02/01.
164. Petrakis IE, Sciacca V. Transcutaneous oxygen tension (TcPO2) in the testing period of spinal cord stimulation (SCS) in critical limb ischemia of the lower extremities. International surgery. 1999;84(2):122-8. Epub 1999/07/17.
165. Talebi G, Torkaman G, Firoozabadi M, Shariat S. Effect of anodal and cathodal microamperage direct current electrical stimulation on injury potential and wound size in guinea pigs. Journal of rehabilitation research and development. 2008;45(1):153-9. Epub 2008/06/21.
166. Orida N, Feldman JD. Directional protrusive pseudopodial activity and motility in macrophages induced by extracellular electric fields. Cell motility. 1982;2(3):243-55. Epub 1982/01/01.
167. Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, et al. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature. 2006;442(7101):457-60. Epub 2006/07/28.
168. Taskan I, Ozyazgan I, Tercan M, Kardas HY, Balkanli S, Saraymen R, et al. A comparative study of the effect of ultrasound and electrostimulation on wound healing in rats. Plastic and reconstructive surgery. 1997;100(4):966-72. Epub 1997/09/18.
169. Bach S, Bilgrav K, Gottrup F, Jorgensen TE. The effect of electrical current on healing skin incision. An experimental study. The European journal of surgery = Acta chirurgica. 1991;157(3):171-4. Epub 1991/03/01.
170. Zhao M, Bai H, Wang E, Forrester JV, McCaig CD. Electrical stimulation directly induces pre-angiogenic responses in vascular endothelial cells by signaling through VEGF receptors. Journal of cell science. 2004;117(Pt 3):397-405. Epub 2003/12/18.
171. Shiojima I, Walsh K. Role of Akt signaling in vascular homeostasis and angiogenesis. Circulation research. 2002;90(12):1243-50. Epub 2002/06/29.

172. Kanno S, Oda N, Abe M, Saito S, Hori K, Handa Y, et al. Establishment of a simple and practical procedure applicable to therapeutic angiogenesis. Circulation. 1999;99(20):2682-7. Epub 1999/05/25.
173. Ojingwa JC, Isseroff, R.R. Electrical stimulation and wound healing. . The Journal of Investigational Dermatology 2002;36:1-12.
174. Shealy CN, Taslitz N, Mortimer JT, Becker DP. Electrical inhibition of pain: experimental evaluation. Anesthesia and analgesia. 1967;46(3):299-305. Epub 1967/05/01.
175. Cook AW, Oygar A, Baggenstos P, Pacheco S, Kleriga E. Vascular disease of extremities. Electric stimulation of spinal cord and posterior roots. New York state journal of medicine. 1976;76(3):366-8. Epub 1976/03/01.
176. Ubbink DT, Vermeulen H. Spinal cord stimulation for non-reconstructable chronic critical leg ischaemia. Cochrane Database Syst Rev. 2005(3):CD004001. Epub 2005/07/22.
177. Burton C. Instrumentation for dorsal column stimulator implantation. Surgical neurology. 1974;2(1):39-40. Epub 1974/01/01.
178. Omura Y. Electrical parameters for safe and effective electro-acupuncture and transcutaneous electrical stimulation: threshold potentials for tingling, muscle contraction and pain; and how to prevent adverse effects of electro-therapy. Part 1. Acupuncture & electro-therapeutics research. 1985;10(4):335-7. Epub 1985/01/01.
179. Dubinsky RM, Miyasaki J. Assessment: efficacy of transcutaneous electric nerve stimulation in the treatment of pain in neurologic disorders (an evidence-based review): report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 2010;74(2):173-6. Epub 2010/01/01.
180. Omura Y. Non-invasive circulatory evaluation and electro-acupuncture & TES treatment of diseases difficult to treat in Western medicine. Acupuncture & electro-therapeutics research. 1983;8(3-4):177-256. Epub 1983/01/01.
181. Kaada B. Systemic sclerosis: successful treatment of ulcerations, pain, Raynaud's phenomenon, calcinosis, and dysphagia by transcutaneous nerve stimulation. A case report. Acupuncture & electro-therapeutics research. 1984;9(1):31-44. Epub 1984/01/01.
182. Debreceni L, Gyulai M, Debreceni A, Szabo K. Results of transcutaneous electrical stimulation (TES) in cure of lower extremity arterial disease. Angiology. 1995;46(7):613-8. Epub 1995/07/01.
183. Jacob AG, Driscoll DJ, Shaughnessy WJ, Stanson AW, Clay RP, Gloviczki P. Klippel-Trenaunay syndrome: spectrum and management. Mayo Clinic proceedings Mayo Clinic. 1998;73(1):28-36. Epub 1998/01/27.
184. Kihiczak GG, Meine JG, Schwartz RA, Janniger CK. Klippel-Trenaunay syndrome: a multisystem disorder possibly resulting from a pathogenic gene for vascular and tissue overgrowth. International journal of dermatology. 2006;45(8):883-90. Epub 2006/08/17.
185. Corcos L, Procacci T, Peruzzi G, Dini M, De Anna D. Sapheno-femoral valves. Histopathological observations and diagnostic approach before surgery. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 1996;22(10):873-80. Epub 1996/10/01.
186. Corcos L, De Anna D, Dini M, Macchi C, Ferrari PA, Dini S. Proximal long saphenous vein valves in primary venous insufficiency. Journal des maladies vasculaires. 2000;25(1):27-36. Epub 2000/03/08.
187. Psaila JV, Melhuish J. Viscoelastic properties and collagen content of the long saphenous vein in normal and varicose veins. The British journal of surgery. 1989;76(1):37-40. Epub 1989/01/01.
188. Evans CJ, Allan PL, Lee AJ, Bradbury AW, Ruckley CV, Fowkes FG. Prevalence of venous reflux in the general population on duplex scanning: the Edinburgh vein study. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and]

International Society for Cardiovascular Surgery, North American Chapter. 1998;28(5):767-76. Epub 1998/11/11.
189. Ruckley CV. Socioeconomic impact of chronic venous insufficiency and leg ulcers. Angiology. 1997;48(1):67-9. Epub 1997/01/01.
190. Kurz X, Kahn SR, Abenhaim L, Clement D, Norgren L, Baccaglini U, et al. Chronic venous disorders of the leg: epidemiology, outcomes, diagnosis and management. Summary of an evidence-based report of the VEINES task force. Venous Insufficiency Epidemiologic and Economic Studies. International angiology : a journal of the International Union of Angiology. 1999;18(2):83-102. Epub 1999/07/29.
191. Eklof B, Rutherford RB, Bergan JJ, Carpentier PH, Gloviczki P, Kistner RL, et al. Revision of the CEAP classification for chronic venous disorders: consensus statement. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2004;40(6):1248-52. Epub 2004/12/29.
192. Hallett J, W., Mills, J.L., Earnshaw, J.J., Reekers, J.A. Pathophysiology of varicose veins and chronic venous insufficiency. Edinburgh: Mosby; 2004.
193. Phillips T, Stanton B, Provan A, Lew R. A study of the impact of leg ulcers on quality of life: financial, social, and psychologic implications. Journal of the American Academy of Dermatology. 1994;31(1):49-53. Epub 1994/07/01.
194. Franks PJ, Moffatt CJ. Health related quality of life in patients with venous ulceration: use of the Nottingham health profile. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2001;10(8):693-700. Epub 2002/03/02.
195. van Korlaar I, Vossen C, Rosendaal F, Cameron L, Bovill E, Kaptein A. Quality of life in venous disease. Thrombosis and haemostasis. 2003;90(1):27-35. Epub 2003/07/24.
196. Walters SJ, Brazier JE. Comparison of the minimally important difference for two health state utility measures: EQ-5D and SF-6D. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2005;14(6):1523-32. Epub 2005/08/23.
197. Browse NL, Burnand, K.G., Irvine, A.T., Wilson, N.M. Diseases of the Veins. 2nd Edition ed. London: Arnold Publishers; 1998.
198. Criqui MH, Denenberg JO, Bergan J, Langer RD, Fronek A. Risk factors for chronic venous disease: the San Diego Population Study. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2007;46(2):331-7. Epub 2007/06/30.
199. Fowkes FG, Evans CJ, Lee AJ. Prevalence and risk factors of chronic venous insufficiency. Angiology. 2001;52 Suppl 1:S5-15. Epub 2001/08/21.
200. Labropoulos N, Kokkosis, A.A., Spentzouris, G., Gasparis, A.P., Tasiopoulos, A.K. The distribution and significance of varicosities in the saphenous trunks. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2010;51(1):96-103.
201. Kroeger K, Ose C, Rudofsky G, Roesener J, Hirche H. Risk factors for varicose veins. International angiology : a journal of the International Union of Angiology. 2004;23(1):29-34. Epub 2004/05/25.
202. Laurikka JO, Sisto T, Tarkka MR, Auvinen O, Hakama M. Risk indicators for varicose veins in forty- to sixty-year-olds in the Tampere varicose vein study. World journal of surgery. 2002;26(6):648-51. Epub 2002/06/08.
203. Dindelli M, Parazzini F, Basellini A, Rabaiotti E, Corsi G, Ferrari A. Risk factors for varicose disease before and during pregnancy. Angiology. 1993;44(5):361-7. Epub 1993/05/01.

204. Tuchsen F, Hannerz H, Burr H, Krause N. Prolonged standing at work and hospitalisation due to varicose veins: a 12 year prospective study of the Danish population. Occupational and environmental medicine. 2005;62(12):847-50. Epub 2005/11/22.
205. Tuchsen F, Krause N, Hannerz H, Burr H, Kristensen TS. Standing at work and varicose veins. Scandinavian journal of work, environment & health. 2000;26(5):414-20. Epub 2000/12/05.
206. Hoare MC, Royle JP. Doppler ultrasound detection of saphenofemoral and saphenopopliteal incompetence and operative venography to ensure precise saphenopopliteal ligation. The Australian and New Zealand journal of surgery. 1984;54(1):49-52. Epub 1984/02/01.
207. Kim J, Richards S, Kent PJ. Clinical examination of varicose veins--a validation study. Annals of the Royal College of Surgeons of England. 2000;82(3):171-5. Epub 2000/06/20.
208. Ernst CB, Stanley, J.C. Invasive methods of diagnosing venous disease. St Louis: Mosby; 2001.
209. Gloviczki P, Comerota AJ, Dalsing MC, Eklof BG, Gillespie DL, Gloviczki ML, et al. The care of patients with varicose veins and associated chronic venous diseases: clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2011;53(5 Suppl):2S-48S. Epub 2011/05/06.
210. Motykie GD, Caprini JA, Arcelus JI, Reyna JJ, Overom E, Mokhtee D. Evaluation of therapeutic compression stockings in the treatment of chronic venous insufficiency. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 1999;25(2):116-20. Epub 1999/02/26.
211. Hafner HM, Vollert B, Schlez A, Junger M. [Compression stocking in treatment of ulcus cruris. An efficient alternative to bandages]. Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete. 2000;51(12):925-30. Epub 2001/02/24. Kompressionsstrumpf zur Behandlung des venosen Ulcus cruris. Eine effiziente Alternative zum Verband.
212. Buchtemann AS, Steins A, Volkert B, Hahn M, Klyscz T, Junger M. The effect of compression therapy on venous haemodynamics in pregnant women. British journal of obstetrics and gynaecology. 1999;106(6):563-9. Epub 1999/07/30.
213. Ibegbuna V, Delis KT, Nicolaides AN, Aina O. Effect of elastic compression stockings on venous hemodynamics during walking. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2003;37(2):420-5. Epub 2003/02/04.
214. Partsch B, Partsch H. Calf compression pressure required to achieve venous closure from supine to standing positions. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2005;42(4):734-8. Epub 2005/10/26.
215. Amsler F, Blattler W. Compression therapy for occupational leg symptoms and chronic venous disorders - a meta-analysis of randomised controlled trials. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2008;35(3):366-72. Epub 2007/12/08.
216. O'Meara S, Cullum NA, Nelson EA. Compression for venous leg ulcers. Cochrane Database Syst Rev. 2009(1):CD000265. Epub 2009/01/23.
217. Partsch H, Flour M, Smith PC. Indications for compression therapy in venous and lymphatic disease consensus based on experimental data and scientific evidence. Under the auspices of the IUP. International angiology : a journal of the International Union of Angiology. 2008;27(3):193-219. Epub 2008/05/29.

218. Lurie F, Kistner RL. Trends in patient reported outcomes of conservative and surgical treatment of primary chronic venous disease contradict current practices. Annals of surgery. 2011;254(2):363-7. Epub 2011/05/03.
219. Raju S, Hollis K, Neglen P. Use of compression stockings in chronic venous disease: patient compliance and efficacy. Annals of vascular surgery. 2007;21(6):790-5. Epub 2007/11/06.
220. Pannier F, Hoffmann, B., Stang, A., Jockel, K-H., Rabe, E. Prevalence and acceptance of therapy with medical compression stockings: Results of the Bonn Vein Study. Phlebologie. 2007;5:245-9.
221. Gohel MS, Barwell JR, Taylor M, Chant T, Foy C, Earnshaw JJ, et al. Long term results of compression therapy alone versus compression plus surgery in chronic venous ulceration (ESCHAR): randomised controlled trial. BMJ. 2007;335(7610):83. Epub 2007/06/05.
222. Mendes RR, Marston WA, Farber MA, Keagy BA. Treatment of superficial and perforator venous incompetence without deep venous insufficiency: is routine perforator ligation necessary? Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2003;38(5):891-5. Epub 2003/11/07.
223. Puggioni A, Lurie F, Kistner RL, Eklof B. How often is deep venous reflux eliminated after saphenous vein ablation? Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2003;38(3):517-21. Epub 2003/08/30.
224. Michaels JA, Campbell WB, Brazier JE, Macintyre JB, Palfreyman SJ, Ratcliffe J, et al. Randomised clinical trial, observational study and assessment of cost-effectiveness of the treatment of varicose veins (REACTIV trial). Health Technol Assess. 2006;10(13):1-196, iii-iv. Epub 2006/05/19.
225. Luebke T, Brunkwall J. Systematic review and meta-analysis of endovenous radiofrequency obliteration, endovenous laser therapy, and foam sclerotherapy for primary varicosis. The Journal of cardiovascular surgery. 2008;49(2):213-33. Epub 2008/04/24.
226. Marsh P, Price BA, Holdstock J, Harrison C, Whiteley MS. Deep vein thrombosis (DVT) after venous thermoablation techniques: rates of endovenous heat-induced thrombosis (EHIT) and classical DVT after radiofrequency and endovenous laser ablation in a single centre. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2010;40(4):521-7. Epub 2010/07/27.
227. Pan Y, Zhao Z, Mei J, Shao M, Zhang J. Comparison of endovenous laser ablation and high ligation and stripping for varicose vein treatment: a meta-analysis. Phlebology / Venous Forum of the Royal Society of Medicine. 2013. Epub 2013/02/08.
228. Rasmussen L, Lawaetz M, Bjoern L, Blemings A, Eklof B. Randomized clinical trial comparing endovenous laser ablation and stripping of the great saphenous vein with clinical and duplex outcome after 5 years. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2013;58(2):421-6. Epub 2013/06/19.
229. Siribumrungwong B, Noorit P, Wilasrusmee C, Attia J, Thakkinstian A. A systematic review and meta-analysis of randomised controlled trials comparing endovenous ablation and surgical intervention in patients with varicose vein. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2012;44(2):214-23. Epub 2012/06/19.
230. NICE. Clinical guideline 168: Varicose veins in the legs: the diagnosis and management of varicose veins. In: NICE, editor. 2013.
231. Bradbury AW, Bate G, Pang K, Darvall KA, Adam DJ. Ultrasound-guided foam sclerotherapy is a safe and clinically effective treatment for superficial venous reflux. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and]

International Society for Cardiovascular Surgery, North American Chapter. 2010;52(4):939-45. Epub 2010/07/20.
232. van den Bos R, Arends L, Kockaert M, Neumann M, Nijsten T. Endovenous therapies of lower extremity varicosities: a meta-analysis. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2009;49(1):230-9. Epub 2008/08/12.
233. Jia X, Mowatt G, Burr JM, Cassar K, Cook J, Fraser C. Systematic review of foam sclerotherapy for varicose veins. The British journal of surgery. 2007;94(8):925-36. Epub 2007/07/20.
234. Sam RC, MacKenzie RK, Paisley AM, Ruckley CV, Bradbury AW. The effect of superficial venous surgery on generic health-related quality of life. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2004;28(3):253-6. Epub 2004/08/04.
235. Dwerryhouse S, Davies B, Harradine K, Earnshaw JJ. Stripping the long saphenous vein reduces the rate of reoperation for recurrent varicose veins: five-year results of a randomized trial. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 1999;29(4):589-92. Epub 1999/04/09.
236. Jones L, Braithwaite BD, Selwyn D, Cooke S, Earnshaw JJ. Neovascularisation is the principal cause of varicose vein recurrence: results of a randomised trial of stripping the long saphenous vein. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 1996;12(4):442-5. Epub 1996/11/01.
237. van Rij AM, Jiang P, Solomon C, Christie RA, Hill GB. Recurrence after varicose vein surgery: a prospective long-term clinical study with duplex ultrasound scanning and air plethysmography. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2003;38(5):935-43. Epub 2003/11/07.
238. Mekako AI, Chetter IC, Coughlin PA, Hatfield J, McCollum PT. Randomized clinical trial of co-amoxiclav versus no antibiotic prophylaxis in varicose vein surgery. The British journal of surgery. 2010;97(1):29-36. Epub 2009/12/17.
239. Lurie F, Creton D, Eklof B, Kabnick LS, Kistner RL, Pichot O, et al. Prospective randomized study of endovenous radiofrequency obliteration (closure procedure) versus ligation and stripping in a selected patient population (EVOLVeS Study). Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2003;38(2):207-14. Epub 2003/08/02.
240. Holme JB, Skajaa K, Holme K. Incidence of lesions of the saphenous nerve after partial or complete stripping of the long saphenous vein. Acta chirurgica Scandinavica. 1990;156(2):145-8. Epub 1990/02/01.
241. Atkin GK, Round T, Vattipally VR, Das SK. Common peroneal nerve injury as a complication of short saphenous vein surgery. Phlebology / Venous Forum of the Royal Society of Medicine. 2007;22(1):3-7. Epub 2008/02/13.
242. van Rij AM, Chai J, Hill GB, Christie RA. Incidence of deep vein thrombosis after varicose vein surgery. The British journal of surgery. 2004;91(12):1582-5. Epub 2004/09/24.
243. Pittler MH, Ernst E. Horse chestnut seed extract for chronic venous insufficiency. Cochrane Database Syst Rev. 2006(1):CD003230. Epub 2006/01/27.
244. Jull A, Arroll B, Parag V, Waters J. Pentoxifylline for treating venous leg ulcers. Cochrane Database Syst Rev. 2007(3):CD001733. Epub 2007/07/20.
245. Coleridge-Smith P, Lok C, Ramelet AA. Venous leg ulcer: a meta-analysis of adjunctive therapy with micronized purified flavonoid fraction. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2005;30(2):198-208. Epub 2005/06/07.

246. Heit JA, Melton LJ, 3rd, Lohse CM, Petterson TM, Silverstein MD, Mohr DN, et al. Incidence of venous thromboembolism in hospitalized patients vs community residents. Mayo Clinic proceedings Mayo Clinic. 2001;76(11):1102-10. Epub 2001/11/13.
247. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR. Cardiovascular risk factors and venous thromboembolism incidence: the longitudinal investigation of thromboembolism etiology. Archives of internal medicine. 2002;162(10):1182-9. Epub 2002/05/22.
248. Nordstrom M, Lindblad B, Bergqvist D, Kjellstrom T. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. Journal of internal medicine. 1992;232(2):155-60. Epub 1992/08/01.
249. Oger E. Incidence of venous thromboembolism: a community-based study in Western France. EPI-GETBP Study Group. Groupe d'Etude de la Thrombose de Bretagne Occidentale. Thrombosis and haemostasis. 2000;83(5):657-60. Epub 2000/05/24.
250. Cohen AT, Agnelli G, Anderson FA, Arcelus JI, Bergqvist D, Brecht JG, et al. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. Thrombosis and haemostasis. 2007;98(4):756-64. Epub 2007/10/17.
251. Heit JA, O'Fallon WM, Petterson TM, Lohse CM, Silverstein MD, Mohr DN, et al. Relative impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-based study. Archives of internal medicine. 2002;162(11):1245-8. Epub 2002/06/01.
252. Lindblad B, Eriksson A, Bergqvist D. Autopsy-verified pulmonary embolism in a surgical department: analysis of the period from 1951 to 1988. The British journal of surgery. 1991;78(7):849-52. Epub 1991/07/01.
253. Anderson FA, Jr., Spencer FA. Risk factors for venous thromboembolism. Circulation. 2003;107(23 Suppl 1):I9-16. Epub 2003/06/20.
254. Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. Journal of thrombosis and haemostasis : JTH. 2005;3(8):1611-7. Epub 2005/08/17.
255. Ashrani AA, Heit JA. Incidence and cost burden of post-thrombotic syndrome. Journal of thrombosis and thrombolysis. 2009;28(4):465-76. Epub 2009/02/19.
256. House_of_Commons_Health_Committee. The prevention of venous thromboembolism in hospitalised patients. In: Office LTS, editor. 2005.
257. Rashid ST, Thursz MR, Razvi NA, Voller R, Orchard T, Shlebak AA. Venous thromboprophylaxis in UK medical inpatients. Journal of the Royal Society of Medicine. 2005;98(11):507-12. Epub 2005/11/02.
258. Sigel B, Edelstein AL, Savitch L, Hasty JH, Felix WR, Jr. Type of compression for reducing venous stasis. A study of lower extremities during inactive recumbency. Arch Surg. 1975;110(2):171-5. Epub 1975/02/11.
259. Agu O, Hamilton G, Baker D. Graduated compression stockings in the prevention of venous thromboembolism. The British journal of surgery. 1999;86(8):992-1004. Epub 1999/08/25.
260. Amaragiri SV, Lees TA. Elastic compression stockings for prevention of deep vein thrombosis. Cochrane Database Syst Rev. 2000(3):CD001484. Epub 2000/07/25.
261. Sajid MS, Desai M, Morris RW, Hamilton G. Knee length versus thigh length graduated compression stockings for prevention of deep vein thrombosis in postoperative surgical patients. Cochrane Database Syst Rev. 2012;5:CD007162. Epub 2012/05/18.
262. Winslow EH, Brosz DL. Graduated compression stockings in hospitalized postoperative patients: correctness of usage and size. The American journal of nursing. 2008;108(9):40-50; quiz -1. Epub 2008/08/30.
263. Wilkins RW, Stanton JR. Elastic stockings in the prevention of pulmonary embolism. II. A progress report. The New England journal of medicine. 1953;248(26):1087-90. Epub 1953/06/25.

264. Callam MJ, Ruckley CV, Dale JJ, Harper DR. Hazards of compression treatment of the leg: an estimate from Scottish surgeons. Br Med J (Clin Res Ed). 1987;295(6610):1382. Epub 1987/11/28.
265. Merrett ND, Hanel KC. Ischaemic complications of graduated compression stockings in the treatment of deep venous thrombosis. Postgraduate medical journal. 1993;69(809):232-4. Epub 1993/03/01.
266. Dennis M, Sandercock PA, Reid J, Graham C, Murray G, Venables G, et al. Effectiveness of thigh-length graduated compression stockings to reduce the risk of deep vein thrombosis after stroke (CLOTS trial 1): a multicentre, randomised controlled trial. Lancet. 2009;373(9679):1958-65. Epub 2009/05/30.
267. Morris RJ, Woodcock JP. Evidence-based compression: prevention of stasis and deep vein thrombosis. Annals of surgery. 2004;239(2):162-71. Epub 2004/01/28.
268. Vanek VW. Meta-analysis of effectiveness of intermittent pneumatic compression devices with a comparison of thigh-high to knee-high sleeves. The American surgeon. 1998;64(11):1050-8. Epub 1998/11/03.
269. Cahan MA, Hanna DJ, Wiley LA, Cox DK, Killewich LA. External pneumatic compression and fibrinolysis in abdominal surgery. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2000;32(3):537-43. Epub 2000/08/25.
270. Comerota AJ, Chouhan V, Harada RN, Sun L, Hosking J, Veermansunemi R, et al. The fibrinolytic effects of intermittent pneumatic compression: mechanism of enhanced fibrinolysis. Annals of surgery. 1997;226(3):306-13; discussion 13-4. Epub 1997/10/27 20:30.
271. Allenby F, Boardman L, Pflug JJ, Calnan JS. Effects of external pneumatic intermittent compression on fibrinolysis in man. Lancet. 1973;2(7843):1412-4. Epub 1973/12/22.
272. Broderick BJ, O'Briain DE, Breen PP, Kearns SR, Olaighin G. A hemodynamic study of popliteal vein blood flow: the effect of bed rest and electrically elicited calf muscle contractions. Conference proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference. 2009;2009:2149-52. Epub 2009/12/08.
273. Broderick BJ, O'Briain DE, Breen PP, Kearns SR, Olaighin G. A pilot evaluation of a neuromuscular electrical stimulation (NMES) based methodology for the prevention of venous stasis during bed rest. Medical engineering & physics. 2010;32(4):349-55. Epub 2010/02/23.
274. Kaplan RE, Czyrny JJ, Fung TS, Unsworth JD, Hirsh J. Electrical foot stimulation and implications for the prevention of venous thromboembolic disease. Thrombosis and haemostasis. 2002;88(2):200-4. Epub 2002/08/28.
275. Warwick DJ, Shaikh A, Gadola S, Stokes M, Worsley P, Bain D, et al. Neuromuscular electrostimulation viathe common peroneal nerve promotes lower limb blood flow in a below-kneecast: A potential for thromboprophylaxis. Bone & joint research. 2013;2(9):179-85. Epub 2013/09/04.
276. Czyrny JJ, Kaplan RE, Wilding GE, Purdy CH, Hirsh J. Electrical foot stimulation: a potential new method of deep venous thrombosis prophylaxis. Vascular. 2010;18(1):20-7. Epub 2010/02/04.
277. Izumi M, Ikeuchi M, Mitani T, Taniguchi S, Tani T. Prevention of venous stasis in the lower limb by transcutaneous electrical nerve stimulation. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2010;39(5):642-5. Epub 2010/01/19.
278. Linhardt RJ, Gunay NS. Production and chemical processing of low molecular weight heparins. Seminars in thrombosis and hemostasis. 1999;25 Suppl 3:5-16. Epub 1999/11/05.

279. Eikelboom JW, Hankey GJ. Low molecular weight heparins and heparinoids. The Medical journal of Australia. 2002;177(7):379-83. Epub 2002/10/03.
280. Collins R, Scrimgeour A, Yusuf S, Peto R. Reduction in fatal pulmonary embolism and venous thrombosis by perioperative administration of subcutaneous heparin. Overview of results of randomized trials in general, orthopedic, and urologic surgery. The New England journal of medicine. 1988;318(18):1162-73. Epub 1988/05/05.
281. Clagett GP, Reisch JS. Prevention of venous thromboembolism in general surgical patients. Results of meta-analysis. Annals of surgery. 1988;208(2):227-40. Epub 1988/08/01.
282. Weitz JI. New anticoagulants for treatment of venous thromboembolism. Circulation. 2004;110(9 Suppl 1):I19-26. Epub 2004/09/02.
283. Geerts WH, Heit JA, Clagett GP, Pineo GF, Colwell CW, Anderson FA, Jr., et al. Prevention of venous thromboembolism. Chest. 2001;119(1 Suppl):132S-75S. Epub 2001/02/07.
284. Warkentin TE, Levine MN, Hirsh J, Horsewood P, Roberts RS, Gent M, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. The New England journal of medicine. 1995;332(20):1330-5. Epub 1995/05/18.
285. Hirsh J, Warkentin TE, Shaughnessy SG, Anand SS, Halperin JL, Raschke R, et al. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. Chest. 2001;119(1 Suppl):64S-94S. Epub 2001/02/07.
286. Yusuf S, Mehta SR, Chrolavicius S, Afzal R, Pogue J, Granger CB, et al. Comparison of fondaparinux and enoxaparin in acute coronary syndromes. The New England journal of medicine. 2006;354(14):1464-76. Epub 2006/03/16.
287. Long JB. Venous thromboembolism: pharmacological and nonpharmacological interventions. The Journal of cardiovascular nursing. 2009;24(6 Suppl):S8-13. Epub 2009/11/05.
288. Papadopoulos S, Flynn JD, Lewis DA. Fondaparinux as a treatment option for heparin-induced thrombocytopenia. Pharmacotherapy. 2007;27(6):921-6. Epub 2007/06/05.
289. Hyers TM. Management of venous thromboembolism: past, present, and future. Archives of internal medicine. 2003;163(7):759-68. Epub 2003/04/16.
290. Murabito JM, D'Agostino RB, Silbershatz H, Wilson WF. Intermittent claudication. A risk profile from The Framingham Heart Study. Circulation. 1997;96(1):44-9. Epub 1997/07/01.
291. Calle-Pascual AL, Romero, L., Durán A., Díaz J.A., Manrique, H., Charro A. . Silent peripheral vascular disease is prevalent in people with diabetic neuropathy. AV DIABETOL. 2004;20:123-6.
292. O'Hare AM, Glidden DV, Fox CS, Hsu CY. High prevalence of peripheral arterial disease in persons with renal insufficiency: results from the National Health and Nutrition Examination Survey 1999-2000. Circulation. 2004;109(3):320-3. Epub 2004/01/21.
293. Behse F, Buchthal F, Carlsen F. Nerve biopsy and conduction studies in diabetic neuropathy. Journal of neurology, neurosurgery, and psychiatry. 1977;40(11):1072-82. Epub 1977/11/01.
294. Perkins BA, Greene DA, Bril V. Glycemic control is related to the morphological severity of diabetic sensorimotor polyneuropathy. Diabetes care. 2001;24(4):748-52. Epub 2001/04/24.
295. Bril V, Tomioka S, Buchanan RA, Perkins BA. Reliability and validity of the modified Toronto Clinical Neuropathy Score in diabetic sensorimotor polyneuropathy. Diabetic medicine : a journal of the British Diabetic Association. 2009;26(3):240-6. Epub 2009/03/26.

296. Young MJ, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. Diabetes care. 1994;17(6):557-60. Epub 1994/06/01.
297. Over DS, Saxon, D., Shah, A. Quantitative assessment of diabetic peripheral neuropathy with use of the clanging tuning fork test. Endocr Pract. 2007;13(1):5-10.
298. Sundkvist G, Dahlin LB, Nilsson H, Eriksson KF, Lindgarde F, Rosen I, et al. Sorbitol and myo-inositol levels and morphology of sural nerve in relation to peripheral nerve function and clinical neuropathy in men with diabetic, impaired, and normal glucose tolerance. Diabetic medicine : a journal of the British Diabetic Association. 2000;17(4):259-68. Epub 2000/05/23.
299. Braddom RL, Hollis JB, Castell DO. Diabetic peripheral neuropathy: a correlation of nerve conduction studies and clinical findings. Archives of physical medicine and rehabilitation. 1977;58(7):308-13. Epub 1977/07/01.
300. Ewing DJ, Campbell IW, Clarke BF. The natural history of diabetic autonomic neuropathy. The Quarterly journal of medicine. 1980;49(193):95-108. Epub 1980/01/01.
301. Burn DJ, Bates D. Neurology and the kidney. Journal of neurology, neurosurgery, and psychiatry. 1998;65(6):810-21. Epub 1998/12/17.
302. Weinrauch LA, D'Elia JA, Gleason RE, Keough J, Mann D, Kennedy FP. Autonomic function in type I diabetes mellitus complicated by nephropathy. A cross-sectional analysis in the presymptomatic phase. American journal of hypertension. 1995;8(8):782-9. Epub 1995/08/01.
303. Sterner NG, Nilsson H, Rosen U, Lilja B, Sundkvist G. Relationships among glomerular filtration rate, albuminuria, and autonomic nerve function in insulin-dependent and non-insulin-dependent diabetes mellitus. Journal of diabetes and its complications. 1997;11(3):188-93. Epub 1997/05/01.
304. Di Paolo B, Cappelli P, Spisni C, Albertazzi A, Rossini PM, Marchionno L, et al. New electrophysiological assessments for the early diagnosis of encephalopathy and peripheral neuropathy in chronic uraemia. International journal of tissue reactions. 1982;4(4):301-7. Epub 1982/01/01.
305. Savazzi GM, Migone L, Cambi V. The influence of glomerular filtration rate on uremic polyneuropathy. Clinical nephrology. 1980;13(2):64-72. Epub 1980/02/01.
306. Bazzi C, Pagani C, Sorgato G, Albonico G, Fellin G, D'Amico G. Uremic polyneuropathy: a clinical and electrophysiological study in 135 short- and long-term hemodialyzed patients. Clinical nephrology. 1991;35(4):176-81. Epub 1991/04/01.
307. Weinberg DH, Simovic D, Isner J, Ropper AH. Chronic ischemic monomelic neuropathy from critical limb ischemia. Neurology. 2001;57(6):1008-12. Epub 2001/09/26.
308. Mulvey MR, Bagnall AM, Johnson MI, Marchant PR. Transcutaneous electrical nerve stimulation (TENS) for phantom pain and stump pain following amputation in adults. Cochrane Database Syst Rev. 2010(5):CD007264. Epub 2010/05/14.
309. Ephraim PL, Wegener ST, MacKenzie EJ, Dillingham TR, Pezzin LE. Phantom pain, residual limb pain, and back pain in amputees: results of a national survey. Archives of physical medicine and rehabilitation. 2005;86(10):1910-9. Epub 2005/10/11.
310. Nikolajsen L, Jensen TS. Phantom limb pain. British journal of anaesthesia. 2001;87(1):107-16. Epub 2001/07/20.
311. Carabelli RA, Kellerman WC. Phantom limb pain: relief by application of TENS to contralateral extremity. Archives of physical medicine and rehabilitation. 1985;66(7):466-7. Epub 1985/07/01.
312. Finsen V, Persen L, Lovlien M, Veslegaard EK, Simensen M, Gasvann AK, et al. Transcutaneous electrical nerve stimulation after major amputation. The Journal of bone and joint surgery British volume. 1988;70(1):109-12. Epub 1988/01/01.

313. Giuffrida O, Simpson L, Halligan PW. Contralateral stimulation, using TENS, of phantom limb pain: two confirmatory cases. Pain Med. 2010;11(1):133-41. Epub 2009/10/01.
314. Gyory AN, Caine DC. Electric pain control (EPC) of a painful forearm amputation stump. The Medical journal of Australia. 1977;2(5):156-8. Epub 1977/07/30.
315. Hirano K, Yamashiro H, Maeda N, Takeuchi T. [A case of long-standing phantom limb pain: complete relief of pain]. Masui The Japanese journal of anesthesiology. 1988;37(2):222-5. Epub 1988/02/01.
316. Katz J, France C, Melzack R. An association between phantom limb sensations and stump skin conductance during transcutaneous electrical nerve stimulation (TENS) applied to the contralateral leg: a case study. Pain. 1989;36(3):367-77. Epub 1989/03/01.
317. Miles J, Lipton S. Phantom limb pain treated by electrical stimulation. Pain. 1978;5(4):373-82. Epub 1978/12/01.
318. Sciacca V, Tamorri M, Rocco M, Mingoli A, Mattia C, Fiume D, et al. Modifications of transcutaneous oxygen tension in lower limb peripheral arterial occlusive disease patients treated with spinal cord stimulation. The Italian journal of surgical sciences / sponsored by Societa italiana di chirurgia. 1986;16(4):279-82. Epub 1986/01/01.
319. Broseta J, Barbera J, de Vera JA, Barcia-Salorio JL, Garcia-March G, Gonzalez-Darder J, et al. Spinal cord stimulation in peripheral arterial disease. A cooperative study. Journal of neurosurgery. 1986;64(1):71-80. Epub 1986/01/01.
320. Anderson SI, Whatling P, Hudlicka O, Gosling P, Simms M, Brown MD. Chronic transcutaneous electrical stimulation of calf muscles improves functional capacity without inducing systemic inflammation in claudicants. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2004;27(2):201-9. Epub 2004/01/14.
321. Tsang GMK, Green, M.A., Crow, A.J., Smith, F.C.T., Beck, S., Hudlicka, O., Shearman, C.P. Chronic muscle stimulation improves ischaemic muscle performance in patients with peripheral vascular disease. European journal of vascular surgery. 1994;8:419-22.
322. Tallis RC, Illis LS, Sedgwick EM, Hardwidge C, Garfield JS. Spinal cord stimulation in peripheral vascular disease. Journal of neurology, neurosurgery, and psychiatry. 1983;46(6):478-84. Epub 1983/06/01.
323. Amann W, Berg P, Gersbach P, Gamain J, Raphael JH, Ubbink DT. Spinal cord stimulation in the treatment of non-reconstructable stable critical leg ischaemia: results of the European Peripheral Vascular Disease Outcome Study (SCS-EPOS). European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2003;26(3):280-6. Epub 2003/09/27.
324. Claeys LG, Horsch S. Transcutaneous oxygen pressure as predictive parameter for ulcer healing in endstage vascular patients treated with spinal cord stimulation. International angiology : a journal of the International Union of Angiology. 1996;15(4):344-9. Epub 1996/12/01.
325. Klomp HM, Spincemaille GH, Steyerberg EW, Berger MY, Habbema JD, van Urk H. Design issues of a randomised controlled clinical trial on spinal cord stimulation in critical limb ischaemia. ESES Study Group. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 1995;10(4):478-85. Epub 1995/11/01.
326. Klomp HM, Spincemaille GH, Steyerberg EW, Habbema JD, van Urk H. Spinal-cord stimulation in critical limb ischaemia: a randomised trial. ESES Study Group. Lancet. 1999;353(9158):1040-4. Epub 1999/04/13.
327. Klomp HM, Steyerberg EW, van Urk H, Habbema JD. Spinal cord stimulation is not cost-effective for non-surgical management of critical limb ischaemia. European journal of

vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2006;31(5):500-8. Epub 2006/01/04.
328. Spincemaille GH, Klomp HM, Steyerberg EW, Habbema JD. Pain and quality of life in patients with critical limb ischaemia: results of a randomized controlled multicentre study on the effect of spinal cord stimulation. ESES study group. Eur J Pain. 2000;4(2):173-84. Epub 2000/08/25.
329. Spincemaille GH, Klomp HM, Steyerberg EW, van Urk H, Habbema JD. Technical data and complications of spinal cord stimulation: data from a randomized trial on critical limb ischemia. Stereotactic and functional neurosurgery. 2000;74(2):63-72. Epub 2001/03/17.
330. Ubbink DT, Spincemaille GH, Prins MH, Reneman RS, Jacobs MJ. Microcirculatory investigations to determine the effect of spinal cord stimulation for critical leg ischemia: the Dutch multicenter randomized controlled trial. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 1999;30(2):236-44. Epub 1999/08/07.
331. Jivegard LE, Augustinsson LE, Holm J, Risberg B, Ortenwall P. Effects of spinal cord stimulation (SCS) in patients with inoperable severe lower limb ischaemia: a prospective randomised controlled study. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 1995;9(4):421-5. Epub 1995/05/01.
332. Spincemaille GH, Klomp, H.M., Steyerberg, E.W., Habbema, J.D. Spinal cord stimulation in patients with critical limb ischaemia: A preliminary evaluation of a multicentre trial. Acta Chirurgica Austriaca. 2000;32:49-51.
333. Suy R, Gybels, J., Van Damme, H., Martin, D., van Maele, R., Delaporte, C. Spinal Cord Stimulation Stimulation for ischaemic rest pain. The Belgium randomised study. In: Horsch, S., Claeys, L editor(s). Spinal Cord Stimulation: An innovative method in the treatment of PVD Springer. 1994:197-202.
334. Ojingwa JC, Isseroff, R.R. Electrical stimulation and wound healing. The Journal of Investigational Dermatology. 2002;36:1-12.
335. Silva Ede F, Martins CC, Guirro EC, Guirro RR. High voltage electrical stimulation as an alternative treatment for chronic ulcers of the lower limbs. Anais brasileiros de dermatologia. 2010;85(4):567-9. Epub 2010/10/15.
336. Burdge JJ, Hartman JF, Wright ML. A study of HVPC as an adjunctive therapy in limb salvage for chronic diabetic wounds of the lower extremity. Ostomy/wound management. 2009;55(8):30-8. Epub 2009/09/01.
337. Lee BY, Wendell K, Al-Waili N, Butler G. Ultra-low microcurrent therapy: a novel approach for treatment of chronic resistant wounds. Advances in therapy. 2007;24(6):1202-9. Epub 2008/01/01.
338. Cosmo P, Svensson H, Bornmyr S, Wikstrom SO. Effects of transcutaneous nerve stimulation on the microcirculation in chronic leg ulcers. Scandinavian journal of plastic and reconstructive surgery and hand surgery / Nordisk plastikkirurgisk forening [and] Nordisk klubb for handkirurgi. 2000;34(1):61-4. Epub 2000/04/11.
339. Comorosan S, Vasilco R, Arghiropol M, Paslaru L, Jieanu V, Stelea S. The effect of diapulse therapy on the healing of decubitus ulcer. Romanian journal of physiology : physiological sciences / [Academia de Stiinte Medicale]. 1993;30(1-2):41-5. Epub 1993/01/01.
340. Feedar J, Kloth L, Gentzkow G. Chronic dermal ulcer healing enhanced with monophasic pulsed electrical stimulation. Physical therapy. 1992;72(7):539. Epub 1992/07/01.

341. Katelaris PM, Fletcher JP, Little JM, McEntyre RJ, Jeffcoate KW. Electrical stimulation in the treatment of chronic venous ulceration. The Australian and New Zealand journal of surgery. 1987;57(9):605-7. Epub 1987/09/01.
342. Kaada B. Promoted healing of chronic ulceration by transcutaneous nerve stimulation (TNS). VASA Zeitschrift fur Gefasskrankheiten Journal for vascular diseases. 1983;12(3):262-9. Epub 1983/01/01.
343. Mulder GD. Treatment of open-skin wounds with electric stimulation. Archives of physical medicine and rehabilitation. 1991;72(6):375-7. Epub 1991/05/01.
344. Lawson D, Petrofsky JS. A randomized control study on the effect of biphasic electrical stimulation in a warm room on skin blood flow and healing rates in chronic wounds of patients with and without diabetes. Medical science monitor : international medical journal of experimental and clinical research. 2007;13(6):CR258-63. Epub 2007/05/31.
345. Wolcott LE, Wheeler PC, Hardwicke HM, Rowley BA. Accelerated healing of skin ulcer by electrotherapy: preliminary clinical results. Southern medical journal. 1969;62(7):795-801. Epub 1969/07/01.
346. Gogia PP, Marquez RR, Minerbo GM. Effects of high voltage galvanic stimulation on wound healing. Ostomy/wound management. 1992;38(1):29-35. Epub 1992/01/01.
347. Gault WR, Gatens PF, Jr. Use of low intensity direct current in management of ischemic skin ulcers. Physical therapy. 1976;56(3):265-9. Epub 1976/03/11.
348. Barron JJ, Jacobson WE, Tidd G. Treatment of decubitus ulcers. A new approach. Minnesota medicine. 1985;68(2):103-6. Epub 1985/02/01.
349. Adegoke BOA, Badmos, K.A. Acceleration of pressure ulcer healing in spinal cord injured patients using interrupted direct current. . Afr J Med Med Sci 2001;30:195-7.
350. Ahmad ET. High-voltage pulsed galvanic stimulation: effect of treatment duration on healing of chronic pressure ulcers. Annals of burns and fire disasters. 2008;21(3):124-8. Epub 2008/09/30.
351. Gentzkow GD, Miller KH. Electrical stimulation for dermal wound healing. Clinics in podiatric medicine and surgery. 1991;8(4):827-41. Epub 1991/10/01.
352. Wood JM, Evans PE, 3rd, Schallreuter KU, Jacobson WE, Sufit R, Newman J, et al. A multicenter study on the use of pulsed low-intensity direct current for healing chronic stage II and stage III decubitus ulcers. Archives of dermatology. 1993;129(8):999-1009. Epub 1993/08/01.
353. Ogrin R, Darzins P, Khalil Z. The use of sensory nerve stimulation and compression bandaging to improve sensory nerve function and healing of chronic venous leg ulcers. Current aging science. 2009;2(1):72-80. Epub 2009/12/22.
354. Junger M, Arnold A, Zuder D, Stahl HW, Heising S. Local therapy and treatment costs of chronic, venous leg ulcers with electrical stimulation (Dermapulse): a prospective, placebo controlled, double blind trial. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2008;16(4):480-7. Epub 2008/07/22.
355. Carley PJ, Wainapel SF. Electrotherapy for acceleration of wound healing: low intensity direct current. Archives of physical medicine and rehabilitation. 1985;66(7):443-6. Epub 1985/07/01.
356. Griffin JW, Tooms RE, Mendius RA, Clifft JK, Vander Zwaag R, el-Zeky F. Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. Physical therapy. 1991;71(6):433-42; discussion 42-4. Epub 1991/06/01.
357. Goldman R, Rosen M, Brewley B, Golden M. Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. Advances in skin & wound care. 2004;17(6):284-94. Epub 2004/08/04.

358. Jercinovic A, Karba, R., Vodovnik, L., Stefanovska, A., Kroselj, P., Turk, R., Dzidic, I., Benko, H., Savrin, R. . Low Frequency Pulsed Current and Pressure Ulcer Healing. . IEEE Transactions on Rehabilitation Engineering 1994;2(4):225-33.
359. Jankovic A, Binic I. Frequency rhythmic electrical modulation system in the treatment of chronic painful leg ulcers. Archives of dermatological research. 2008;300(7):377-83. Epub 2008/07/17.
360. Asbjornsen G, Hernaes, B., Molvaer, G. The Effect of Transcutaneous Electrical Nerve Stimulation on Pressure Sores in Geriatric Patients. . J Clinical and Experimental Gerontology 1990;12(4):209-14.
361. Adunsky A, Ohry A. Decubitus direct current treatment (DDCT) of pressure ulcers: results of a randomized double-blinded placebo controlled study. Archives of gerontology and geriatrics. 2005;41(3):261-9. Epub 2005/07/07.
362. Houghton PE, Campbell KE, Fraser CH, Harris C, Keast DH, Potter PJ, et al. Electrical stimulation therapy increases rate of healing of pressure ulcers in community-dwelling people with spinal cord injury. Archives of physical medicine and rehabilitation. 2010;91(5):669-78. Epub 2010/05/04.
363. Houghton PE, Kincaid CB, Lovell M, Campbell KE, Keast DH, Woodbury MG, et al. Effect of electrical stimulation on chronic leg ulcer size and appearance. Physical therapy. 2003;83(1):17-28. Epub 2002/12/24.
364. Feedar JA, Kloth LC, Gentzkow GD. Chronic dermal ulcer healing enhanced with monophasic pulsed electrical stimulation. Physical therapy. 1991;71(9):639-49. Epub 1991/09/01.
365. Lundeberg TC, Eriksson SV, Malm M. Electrical nerve stimulation improves healing of diabetic ulcers. Annals of plastic surgery. 1992;29(4):328-31. Epub 1992/10/01.
366. Kloth LC, Feedar JA. Acceleration of wound healing with high voltage, monophasic, pulsed current. Physical therapy. 1988;68(4):503-8. Epub 1988/04/01.
367. Peters EJ, Lavery LA, Armstrong DG, Fleischli JG. Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. Archives of physical medicine and rehabilitation. 2001;82(6):721-5. Epub 2001/06/02.
368. Baker LL, Chambers R, DeMuth SK, Villar F. Effects of electrical stimulation on wound healing in patients with diabetic ulcers. Diabetes care. 1997;20(3):405-12. Epub 1997/03/01.
369. Baker LL, Rubayi S, Villar F, Demuth SK. Effect of electrical stimulation waveform on healing of ulcers in human beings with spinal cord injury. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 1996;4(1):21-8. Epub 1996/01/01.
370. Ojingwa JC, Isseroff, R.R. Stimulation of Wound Healing. JInvest Dermatol 2002;121:1-12.
371. Adegoke BO, Badmos KA. Acceleration of pressure ulcer healing in spinal cord injured patients using interrupted direct current. African journal of medicine and medical sciences. 2001;30(3):195-7. Epub 2003/09/27.
372. Gardner SE, Frantz RA, Schmidt FL. Effect of electrical stimulation on chronic wound healing: a meta-analysis. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 1999;7(6):495-503. Epub 2000/01/13.
373. Young S, Hampton S, Tadej M. Study to evaluate the effect of low-intensity pulsed electrical currents on levels of oedema in chronic non-healing wounds. Journal of wound care. 2011;20(8):368, 70-3. Epub 2011/08/16.
374. Taylor RR, Sladkevicius E, Guest JF. Modelling the cost-effectiveness of electric stimulation therapy in non-healing venous leg ulcers. Journal of wound care. 2011;20(10):464, 6, 8-72. Epub 2011/11/10.

375. Faghri PD, Votto JJ, Hovorka CF. Venous hemodynamics of the lower extremities in response to electrical stimulation. Archives of physical medicine and rehabilitation. 1998;79(7):842-8. Epub 1998/07/31.
376. Clarke Moloney M, Lyons GM, Breen P, Burke PE, Grace PA. Haemodynamic study examining the response of venous blood flow to electrical stimulation of the gastrocnemius muscle in patients with chronic venous disease. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2006;31(3):300-5. Epub 2005/10/26.
377. Corley GJ, Breen PP, Grace PA, G OL. The effect of surface neuromuscular electrical stimulation and compression hosiery applied to the lower limb, on the comfort and blood flow of healthy subjects. Conference proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference. 2008;2008:703-6. Epub 2009/01/24.
378. Bogachev VY, Golovanova OV, Kuznetsov AN, Shekoyan AO, Bogacheva NV. Electromuscular stimulation with VEINOPLUS(R) for the treatment of chronic venous edema. International angiology : a journal of the International Union of Angiology. 2011;30(6):567-90. Epub 2012/01/12.
379. Gray H. Anatomy of the Human Body. 20th ed. Philadelphia: Lea and Febiger; 1918.
380. Jawad H, Bain, D.S., Dawson, H., Adams, K., Johnston, A., Tucker, A.T. The Effect of OnpulseTM in Improving Lower Limb Blood Flow In Healthy Volunteers. UIP Congress 2011 Internation Congress of the Union Internationale de Phlebologie; Sept 15-17 2011; Czech Republic2011.
381. Tucker A, Maass A, Bain D, Chen LH, Azzam M, Dawson H, et al. Augmentation of venous, arterial and microvascular blood supply in the leg by isometric neuromuscular stimulation via the peroneal nerve. The International journal of angiology : official publication of the International College of Angiology, Inc. 2010;19(1):e31-7. Epub 2010/04/01.
382. Jawad H, Bain, D.S., Dawson, H., Adams, K., Johnston, A., Tucker, A.T. A comparative study investigating the effectiveness of the geko™ medical device versus intermittent pneumatic compression in enhancing lower limb blood flow in healthy subjects. . 2011.
383. WMA. World Medical Association Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects. http://www.wma.net2008.
384. Consensus statement on mandatory registration of clinical trials. The British journal of surgery. 2007;94(4):511-12.
385. DoH. Research Governance Framework for Health and Social Care. 2nd Edition. In: Health Do, editor. 2005.
386. Data Protection Act. In: HMSO, editor. Great Britain1998.
387. DoH. The Caldicott Committee: Report on the review of patient- identifiable information. 1997.
388. LaBan MM, Petty D, Hauser AM, Taylor RS. Peripheral nerve conduction stimulation: its effect on cardiac pacemakers. Archives of physical medicine and rehabilitation. 1988;69(5):358-62. Epub 1988/05/01.
389. Cramp FL, McCullough GR, Lowe AS, Walsh DM. Transcutaneous electric nerve stimulation: the effect of intensity on local and distal cutaneous blood flow and skin temperature in healthy subjects. Archives of physical medicine and rehabilitation. 2002;83(1):5-9. Epub 2002/01/10.
390. Bril V, Perkins BA. Validation of the Toronto Clinical Scoring System for diabetic polyneuropathy. Diabetes care. 2002;25(11):2048-52. Epub 2002/10/29.

391. Obeid AN, Barnett NJ, Dougherty G, Ward G. A critical review of laser Doppler flowmetry. Journal of medical engineering & technology. 1990;14(5):178-81. Epub 1990/09/01.
392. Oberg PA. Laser-Doppler flowmetry. Critical reviews in biomedical engineering. 1990;18(2):125-63. Epub 1990/01/01.
393. Kvernebo K, Slagsvold CE, Stranden E, Kroese A, Larsen S. Laser Doppler flowmetry in evaluation of lower limb resting skin circulation. A study in healthy controls and atherosclerotic patients. Scandinavian journal of clinical and laboratory investigation. 1988;48(7):621-6. Epub 1988/11/01.
394. Karanfilian RG, Lynch TG, Lee BC, Long JB, Hobson RW, 2nd. The assessment of skin blood flow in peripheral vascular disease by laser Doppler velocimetry. The American surgeon. 1984;50(12):641-4. Epub 1984/12/01.
395. Johnson JM, Taylor WF, Shepherd AP, Park MK. Laser-Doppler measurement of skin blood flow: comparison with plethysmography. Journal of applied physiology: respiratory, environmental and exercise physiology. 1984;56(3):798-803. Epub 1984/03/01.
396. Holloway GA, Jr., Watkins DW. Laser Doppler measurement of cutaneous blood flow. The Journal of investigative dermatology. 1977;69(3):306-9. Epub 1977/09/01.
397. Vongsavan N, Matthews B. Some aspects of the use of laser Doppler flow meters for recording tissue blood flow. Experimental physiology. 1993;78(1):1-14. Epub 1993/01/01.
398. Radiologists RCo. Standards for Ultrasound Equipment. 2005.
399. BMUS. Extending the provision of ultrasound services in the UK. 2004.
400. IPEM. Report 70: Testing of Doppler Ultrasound equipment. 1994.
401. Halliwell M. A tutorial on ultrasonic physics and imaging techniques. Proceedings of the Institution of Mechanical Engineers Part H, Journal of engineering in medicine. 2010;224(2):127-42. Epub 2010/03/31.
402. Kossoff G. Analysis of focusing action of spherically curved transducers. Ultrasound in medicine & biology. 1979;5(4):359-65. Epub 1979/01/01.
403. Coleridge-Smith P, Labropoulos N, Partsch H, Myers K, Nicolaides A, Cavezzi A. Duplex ultrasound investigation of the veins in chronic venous disease of the lower limbs--UIP consensus document. Part I. Basic principles. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2006;31(1):83-92. Epub 2005/10/18.
404. Dudley NJ, Griffith K. The importance of rigorous testing of circumference measuring callipers. Ultrasound in medicine & biology. 1996;22(8):1117-9. Epub 1996/01/01.
405. Ng A, Swanevelder, J. Resolution in ultrasound imaging. Contin Educ Anaesth Crit Care Pain. 2011;11(5):186-92.
406. Jago JR, Whittingham TA, Heslop R. The influence of ultrasound scanner beam width on femur length measurements. Ultrasound in medicine & biology. 1994;20(8):699-703. Epub 1994/01/01.
407. Machi J, Sigel B, Roberts AB, Kahn MB. Oversaturation of color may obscure small intraluminal partial occlusions in color Doppler imaging. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine. 1994;13(10):735-41; quiz 821-2. Epub 1994/10/01.
408. Wachsberg RH. B-flow imaging of the hepatic vasculature: correlation with color Doppler sonography. AJR American journal of roentgenology. 2007;188(6):W522-33. Epub 2007/05/23.
409. Pozniak MA, Zagzebski JA, Scanlan KA. Spectral and color Doppler artifacts. Radiographics : a review publication of the Radiological Society of North America, Inc. 1992;12(1):35-44. Epub 1992/01/01.

410. Kisslo JA, Adams, D.B., Belkin, R.N. Doppler Color Flow Imaging: Churchill Livingstone; 1988 25 Jan 1988. 184 p.
411. Brant WE, Helms, C.A. Fundamentals of Diagnostic Radiology. Third Edition ed: Lippincott Williams & Wilkins; 2007. 1559 p.
412. Kruskal JB, Newman PA, Sammons LG, Kane RA. Optimizing Doppler and color flow US: application to hepatic sonography. Radiographics : a review publication of the Radiological Society of North America, Inc. 2004;24(3):657-75. Epub 2004/05/15.
413. Shiota T. Automated cardiac flow measurement by digital colour Doppler echocardiography. Heart. 2002;88(3):211-2. Epub 2002/08/16.
414. Tahmasebpour HR, Buckley AR, Cooperberg PL, Fix CH. Sonographic examination of the carotid arteries. Radiographics : a review publication of the Radiological Society of North America, Inc. 2005;25(6):1561-75. Epub 2005/11/15.
415. Gooding GA, Effeney DJ, Goldstone J. The aortofemoral graft: detection and identification of healing complications by ultrasonography. Surgery. 1981;89(1):94-101. Epub 1981/01/01.
416. Lewis P, Psaila JV, Davies WT, McCarty K, Woodcock JP. Measurement of volume flow in the human common femoral artery using a duplex ultrasound system. Ultrasound in medicine & biology. 1986;12(10):777-84. Epub 1986/10/01.
417. Ogawa T, Lurie F, Kistner RL, Eklof B, Tabrah FL. Reproducibility of ultrasound scan in the assessment of volume flow in the veins of the lower extremities. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2002;35(3):527-31. Epub 2002/03/06.
418. Hoskins PR. Measurement of arterial blood flow by Doppler ultrasound. Clinical physics and physiological measurement : an official journal of the Hospital Physicists' Association, Deutsche Gesellschaft fur Medizinische Physik and the European Federation of Organisations for Medical Physics. 1990;11(1):1-26. Epub 1990/02/01.
419. Burns PN. Measuring volume flow with Doppler ultrasound-an old nut. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 1992;2(4):238-41. Epub 1992/07/01.
420. Zierler BK, Kirkman TR, Kraiss LW, Reiss WG, Horn JR, Bauer LA, et al. Accuracy of duplex scanning for measurement of arterial volume flow. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 1992;16(4):520-6. Epub 1992/10/01.
421. Pucci G, Cheriyan J, Hubsch A, Hickson SS, Gajendragadkar PR, Watson T, et al. Evaluation of the Vicorder, a novel cuff-based device for the noninvasive estimation of central blood pressure. Journal of hypertension. 2013;31(1):77-85. Epub 2012/10/20.
422. McGreevy C, Barry M, Bennett K, Williams D. Repeatability of the measurement of aortic pulse wave velocity (aPWV) in the clinical assessment of arterial stiffness in community-dwelling older patients using the Vicorder device. Scandinavian journal of clinical and laboratory investigation. 2013. Epub 2013/04/03.
423. Altman DG. Principles of statistical analysis. Practical statistics for Medical Research. Hall C, editor. London1991.
424. Student. The probable error of a mean. Biometrika. 1906;6(1):1-25.
425. Mann HB, Whitney, D.R. On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat. 1947;18:50-60.
426. Pearson K. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonable supposed to have arisen from random sampling. Philos Mag. 1900;50(5):157-75.
427. Fisher RA. On the interpretation of chi square from contingency tables, and the calculation of P. J Roy Stat Soc. 1922;85:87-94.

428. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychological bulletin. 1979;86(2):420-8. Epub 1979/03/01.
429. Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. Neurology. 1993;43(4):817-24. Epub 1993/04/01.
430. Dyck PJ, Litchy WJ, Lehman KA, Hokanson JL, Low PA, O'Brien PC. Variables influencing neuropathic endpoints: the Rochester Diabetic Neuropathy Study of Healthy Subjects. Neurology. 1995;45(6):1115-21. Epub 1995/06/01.
431. Edwards JL, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. Pharmacology & therapeutics. 2008;120(1):1-34. Epub 2008/07/12.
432. te Slaa A, Dolmans DE, Ho GH, Moll FL, van der Laan L. Pathophysiology and treatment of edema following femoropopliteal bypass surgery. Vascular. 2012;20(6):350-9. Epub 2012/09/18.
433. Jacobs MJ, Beckers RC, Jorning PJ, Slaaf DW, Reneman RS. Microcirculatory haemodynamics before and after vascular surgery in severe limb ischaemia--the relation to post-operative oedema formation. European journal of vascular surgery. 1990;4(5):525-9. Epub 1990/10/01.
434. Cho S, Atwood JE. Peripheral edema. The American journal of medicine. 2002;113(7):580-6. Epub 2002/12/03.
435. Kopman AF, Lawson D. Milliamperage requirements for supramaximal stimulation of the ulnar nerve with surface electrodes. Anesthesiology. 1984;61(1):83-5. Epub 1984/07/01.
436. Harper NJ, Greer R, Conway D. Neuromuscular monitoring in intensive care patients: milliamperage requirements for supramaximal stimulation. British journal of anaesthesia. 2001;87(4):625-7. Epub 2002/03/07.
437. Ishide T, Pearce WJ, Ally A. Cardiovascular responses during stimulation of hindlimb skeletal muscle nerves in anaesthetized rats. Clinical and experimental pharmacology & physiology. 2002;29(8):689-95. Epub 2002/07/09.
438. Zhou X, Wolf PD, Smith WM, Blanchard SM, Ideker RE. Effects of peroneal nerve stimulation on hypothalamic stimulation-induced ventricular arrhythmias in rabbits. The American journal of physiology. 1994;267(5 Pt 2):H2032-41. Epub 1994/11/01.
439. Faghri PD, Van Meerdervort HF, Glaser RM, Figoni SF. Electrical stimulation-induced contraction to reduce blood stasis during arthroplasty. IEEE transactions on rehabilitation engineering : a publication of the IEEE Engineering in Medicine and Biology Society. 1997;5(1):62-9. Epub 1997/03/01.
440. Hambrecht R, Gielen S, Linke A, Fiehn E, Yu J, Walther C, et al. Effects of exercise training on left ventricular function and peripheral resistance in patients with chronic heart failure: A randomized trial. JAMA : the journal of the American Medical Association. 2000;283(23):3095-101. Epub 2000/06/24.
441. Proctor MC, Greenfield LJ, Wakefield TW, Zajkowski PJ. A clinical comparison of pneumatic compression devices: the basis for selection. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2001;34(3):459-63; discussion 63-4. Epub 2001/09/05.
442. Jawad H BD, Dawson H, Adams K, Johnston A, Tucker AT. The effectiveness of a novel neuromuscular electrostimulation method versus intermittent pneumatic compression in enhancing lower limb blood flow. Journal of Vascular Surgery: Venous and Lymphatic Disorders. 2013;2(2):160-5.
443. Nicolaides AN, Kakkar VV, Renney JT. Soleal sinuses and stasis. The British journal of surgery. 1971;58(4):307. Epub 1971/04/01.

444. Nicolaides AN, Kakkar VV, Field ES, Renney JT. The origin of deep vein thrombosis: a venographic study. The British journal of radiology. 1971;44(525):653-63. Epub 1971/09/01.
445. Arcelus JI, Caprini, J.A. The home use of external pneumatic compression for the management of chronic venous insufficiency. International angiology : a journal of the International Union of Angiology. 1996;15(3 Suppl1):32-6.
446. Ginsberg JS, Brill-Edwards P, Kowalchuk G, Hirsh J. Intermittent compression units for the postphlebitic syndrome. A pilot study. Archives of internal medicine. 1989;149(7):1651-2. Epub 1989/07/01.
447. Smith PC, Sarin S, Hasty J, Scurr JH. Sequential gradient pneumatic compression enhances venous ulcer healing: a randomized trial. Surgery. 1990;108(5):871-5. Epub 1990/11/01.
448. Hazarika EZ, Wright DE. Chronic leg ulcers. The effect of pneumatic intermittent compression. The Practitioner. 1981;225(1352):189-92. Epub 1981/02/01.
449. NICE. The geko device for reducing the risk of venous thromboembolism. In: 19] NMTGM, editor. https://www.nice.org.uk/guidance/MTG19/chapter/1-recommendations2014.
450. Oka RK, Altman M, Giacomini JC, Szuba A, Cooke JP. Exercise patterns and cardiovascular fitness of patients with peripheral arterial disease. Journal of vascular nursing : official publication of the Society for Peripheral Vascular Nursing. 2004;22(4):109-14; quiz 15-6. Epub 2004/12/14.
451. Cheetham DR, Burgess L, Ellis M, Williams A, Greenhalgh RM, Davies AH. Does supervised exercise offer adjuvant benefit over exercise advice alone for the treatment of intermittent claudication? A randomised trial. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2004;27(1):17-23. Epub 2003/12/04.
452. de Haro J, Acin, F., Florez, A., Bleda, S., Fernandez, J.L. A prospective randomized controlled study with intermittent mechanical compression of the calf in patients with claudication. Journal of Vascular Surgery. 2012;51:857-62.
453. Morris RJ, Woodcock JP. Effects of supine intermittent compression on arterial inflow to the lower limb. Arch Surg. 2002;137(11):1269-73. Epub 2002/11/07.
454. Tsang GM, Green MA, Crow AJ, Smith FC, Beck S, Hudlicka O, et al. Chronic muscle stimulation improves ischaemic muscle performance in patients with peripheral vascular disease. European journal of vascular surgery. 1994;8(4):419-22. Epub 1994/07/01.
455. Sabik JF, 3rd. Understanding saphenous vein graft patency. Circulation. 2011;124(3):273-5. Epub 2011/07/20.
456. Owens CD, Ho KJ, Conte MS. Lower extremity vein graft failure: a translational approach. Vasc Med. 2008;13(1):63-74. Epub 2008/03/29.
457. Owens CD, Wake N, Jacot JG, Gerhard-Herman M, Gaccione P, Belkin M, et al. Early biomechanical changes in lower extremity vein grafts--distinct temporal phases of remodeling and wall stiffness. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter. 2006;44(4):740-6. Epub 2006/08/24.
458. Ogrin R, Darzins P, Khalil Z. Use of the sensory nerve stimulator to accelerate healing of a venous leg ulcer with sensory nerve dysfunction: a case study. International wound journal. 2005;2(3):242-51. Epub 2006/04/19.
459. Chakravarti B, Chakravarti DN. Phagocytosis: an overview. Pathology and immunopathology research. 1987;6(5-6):316-42. Epub 1987/01/01.
460. Kamrin BB. Induced collagenolytic activity by electrical stimulation of embryonic fibroblasts in tissue culture. Journal of dental research. 1974;53(6):1475-83. Epub 1974/11/01.

461. Barnes R, Shahin Y, Gohil R, Chetter I. Electrical stimulation vs. standard care for chronic ulcer healing: a systematic review and meta-analysis of randomised controlled trials. European journal of clinical investigation. 2014;44(4):429-40. Epub 2014/01/25.
462. Fernandez-Chimeno M HP, Holey L. Electrical stimulation for chronic wounds. Cochrane Database System Rev. 2004(1):CD004550.
463. European_Pressure_Ulcer_Advisory_Panel_and_National_Pressure_Ulcer_Advisory_Panel. Prevention and Treatment of Pressure Ulcers: Quick Reference Guide2009.
464. Alexander JP. Chemical lumbar sympathectomy in patients with severe lower limb ischaemia. The Ulster medical journal. 1994;63(2):137-43. Epub 1994/10/01.
465. Kaada B, Eielsen O. In search of mediators of skin vasodilation induced by transcutaneous nerve stimulation: II. Serotonin implicated. General pharmacology. 1983;14(6):635-41. Epub 1983/01/01.
466. Cronenwett JL, Lindenauer SM. Direct measurement of arteriovenous anastomotic blood flow after lumbar sympathectomy. Surgery. 1977;82(1):82-9. Epub 1977/07/01.
467. Scarpino JH, Delaney JP. Lumbar sympathectomy and arteriovenous shunting. Surgical forum. 1971;22:176-8. Epub 1971/01/01.
468. Moore WS, Hall AD. Effects of lumbar sympathectomy on skin capillary blood flow in arterial occlusive disease. The Journal of surgical research. 1973;14(2):151-7. Epub 1973/02/01.
469. Carr MJ, Crooks JA, Griffiths PA, Hopkinson BR. Capillary blood flow in ischemic limbs before and after surgery assessed by subcuticular injection of Xenon 133. American journal of surgery. 1977;133(5):584-6. Epub 1977/05/01.
470. Barcroft H, Swann, H.J.C. Sympathetic Control of Human Blood Vessels. London: Arnold; 1953.
471. Gillespie JA. Extent and permanence of denervation produced by lumbar sympathectomy. A quantitative investigation of its effects on sudomotor activity. British medical journal. 1961;1(5219):79-83. Epub 1961/01/14.
472. Dornhorst AC, Sharpey-Schafer EP. Collateral resistance in limbs with arterial obstruction: spontaneous changes and effects of sympathectomy. Clin Sci (Lond). 1951;10(3):371-81. Epub 1951/08/01.
473. Cross FW, Cotton LT. Chemical lumbar sympathectomy for ischemic rest pain. A randomized, prospective controlled clinical trial. American journal of surgery. 1985;150(3):341-5. Epub 1985/09/01.
474. Terry HJ, Allan JS, Taylor GW. The effect of adding lumbar sympathectomy to reconstructive arterial surgery in the lower limb. The British journal of surgery. 1970;57(1):51-5. Epub 1970/01/01.
475. Collins GJ, Jr., Rich NM, Clagett GP, Salander JM, Spebar MJ. Clinical results of lumbar sympathectomy. The American surgeon. 1981;47(1):31-5. Epub 1981/01/01.
476. Barnes RW, Baker WH, Shanik G, Maixner W, Hayes AC, Lin R, et al. Value of concomitant sympathectomy in aortoiliac reconstruction. Results of a prospective, randomized study. Arch Surg. 1977;112(11):1325-30. Epub 1977/11/01.
477. Satiani B, Liapis CD, Hayes JP, Kimmins S, Evans WE. Prospective randomized study of concomitant lumbar sympathectomy with aortoiliac reconstruction. American journal of surgery. 1982;143(6):755-60. Epub 1982/06/01.
478. Kim GE, Ibrahim IM, Imparato AM. Lumbar sympathectomy in end stage arterial occlusive disease. Annals of surgery. 1976;183(2):157-60. Epub 1976/02/01.
479. Keane FB. Phenol lumbar sympathectomy for severe arterial occlusive disease in the elderly. The British journal of surgery. 1977;64(7):519-21. Epub 1977/07/01.
480. Perez-Burkhardt JL, Gonzalez-Fajardo JA, Martin JF, Carpintero Mediavilla LA, Mateo Gutierrez AM. Lumbar sympathectomy as isolated technique for the treatment of

lower limbs chronic ischemia. The Journal of cardiovascular surgery. 1999;40(1):7-13. Epub 1999/04/30.
481. Mashiah A, Soroker D, Pasik S, Mashiah T. Phenol lumbar sympathetic block in diabetic lower limb ischemia. Journal of cardiovascular risk. 1995;2(5):467-9. Epub 1995/10/01.
482. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet. 1989;2(8670):997-1000. Epub 1989/10/28.
483. Scherrer U, Randin, D., Vollenweider, P., Vollenweider, L., Nicod, P. . Nitric oxide accounts for insulin's vascular effects in humans. J Clin Invest. 1995;94:2511-5.
484. Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(2):682-5. Epub 1990/01/01.
485. Northington FJ, Matherne GP, Berne RM. Competitive inhibition of nitric oxide synthase prevents the cortical hyperemia associated with peripheral nerve stimulation. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(14):6649-52. Epub 1992/07/15.
486. Schmidt HH, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biochimica et biophysica acta. 1993;1178(2):153-75. Epub 1993/08/18.
487. Denninger JW, Marletta MA. Guanylate cyclase and the .NO/cGMP signaling pathway. Biochimica et biophysica acta. 1999;1411(2-3):334-50. Epub 1999/05/13.
488. Ignarro LJ. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol 1999;30:535-60.
489. Paterno JC, Bergamaschi CT, Campos RR, Higa EM, Soares MF, Schor N, et al. Electroacupuncture and moxibustion decrease renal sympathetic nerve activity and retard progression of renal disease in rats. Kidney & blood pressure research. 2012;35(5):355-64. Epub 2012/04/05.
490. Li S, Chen K, Wu Y, Jiao J, Tao L. Effects of warm needling at zusanli (ST 36) on NO and IL-2 levels in the middle-aged and old people. Journal of traditional Chinese medicine = Chung i tsa chih ying wen pan / sponsored by All-China Association of Traditional Chinese Medicine, Academy of Traditional Chinese Medicine. 2003;23(2):127-8. Epub 2003/07/24.
491. Tsuchiya M, Sato EF, Inoue M, Asada A. Acupuncture enhances generation of nitric oxide and increases local circulation. Anesthesia and analgesia. 2007;104(2):301-7. Epub 2007/01/24.
492. Ferroni P, Roselli M, Guadagni F, Martini F, Mariotti S, Marchitelli E, et al. Biological effects of a software-controlled voltage pulse generator (PhyBack PBK-2C) on the release of vascular endothelial growth factor (VEGF). In Vivo. 2005;19(6):949-58. Epub 2005/11/10.
493. Mitusch R, Siemens HJ, Garbe M, Wagner T, Sheikhzadeh A, Diederich KW. Detection of a hypercoagulable state in nonvalvular atrial fibrillation and the effect of anticoagulant therapy. Thrombosis and haemostasis. 1996;75(2):219-23. Epub 1996/02/01.
494. Habib SS, Abdel Gader AG, Kurdi MI, Suriya MO, Al Aseri Z. Tissue plasminogen activator and plasminogen activator inhibitor-1 levels in patients with acute myocardial infarction and unstable angina. JPMA The Journal of the Pakistan Medical Association. 2012;62(7):681-5. Epub 2013/07/23.
495. Wiman B, Andersson T, Hallqvist J, Reuterwall C, Ahlbom A, deFaire U. Plasma levels of tissue plasminogen activator/plasminogen activator inhibitor-1 complex and von Willebrand factor are significant risk markers for recurrent myocardial infarction in the Stockholm Heart Epidemiology Program (SHEEP) study. Arteriosclerosis, thrombosis, and vascular biology. 2000;20(8):2019-23. Epub 2000/08/11.
496. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma

precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation. 1998;98(21):2241-7. Epub 1998/11/24.
497. Macko RF, Kittner SJ, Epstein A, Cox DK, Wozniak MA, Wityk RJ, et al. Elevated tissue plasminogen activator antigen and stroke risk: The Stroke Prevention In Young Women Study. Stroke; a journal of cerebral circulation. 1999;30(1):7-11. Epub 1999/01/08.
498. Wannamethee SG, Sattar N, Rumley A, Whincup PH, Lennon L, Lowe GD. Tissue plasminogen activator, von Willebrand factor, and risk of type 2 diabetes in older men. Diabetes care. 2008;31(5):995-1000. Epub 2008/02/01.
499. Segarra A, Chacon P, Martinez-Eyarre C, Argelaguer X, Vila J, Ruiz P, et al. Circulating levels of plasminogen activator inhibitor type-1, tissue plasminogen activator, and thrombomodulin in hemodialysis patients: biochemical correlations and role as independent predictors of coronary artery stenosis. Journal of the American Society of Nephrology : JASN. 2001;12(6):1255-63. Epub 2001/05/25.
500. Gorog DA. Prognostic value of plasma fibrinolysis activation markers in cardiovascular disease. Journal of the American College of Cardiology. 2010;55(24):2701-9. Epub 2010/06/12.
501. Senoo T, Hattori N, Tanimoto T, Furonaka M, Ishikawa N, Fujitaka K, et al. Suppression of plasminogen activator inhibitor-1 by RNA interference attenuates pulmonary fibrosis. Thorax. 2010;65(4):334-40. Epub 2010/04/15.
502. Liu RM. Oxidative stress, plasminogen activator inhibitor 1, and lung fibrosis. Antioxidants & redox signaling. 2008;10(2):303-19. Epub 2007/11/06.
503. Li-Saw-Hee FL, Blann AD, Lip GY. Effects of fixed low-dose warfarin, aspirin-warfarin combination therapy, and dose-adjusted warfarin on thrombogenesis in chronic atrial fibrillation. Stroke; a journal of cerebral circulation. 2000;31(4):828-33. Epub 2001/02/07.
504. Walenga JM, Hoppensteadt D, Pifarre R, Cressman MD, Hunninghake DB, Fox NL, et al. Hemostatic effects of 1 mg daily warfarin on post CABG patients. Post CABG Studies Investigators. Journal of thrombosis and thrombolysis. 1999;7(3):313-8. Epub 1999/06/22.
505. Kim SB, Lee SK, Park JS, Chi HS, Hong CD, Yang WS. Effects of fixed low-dose warfarin on hemostatic factors in continuous ambulatory peritoneal dialysis patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2001;37(2):343-7. Epub 2001/02/07.
506. Zhao L, Gray L, Leonardi-Bee J, Weaver CS, Heptinstall S, Bath PM. Effect of aspirin, clopidogrel and dipyridamole on soluble markers of vascular function in normal volunteers and patients with prior ischaemic stroke. Platelets. 2006;17(2):100-4. Epub 2006/01/20.
507. Peters H, Eisenberg R, Daig U, Liefeldt L, Westenfeld R, Gaedeke J, et al. Platelet inhibition limits TGF-beta overexpression and matrix expansion after induction of anti-thy1 glomerulonephritis. Kidney international. 2004;65(6):2238-48. Epub 2004/05/20.
508. Levin RI, Harpel PC, Weil D, Chang TS, Rifkin DB. Aspirin inhibits vascular plasminogen activator activity in vivo. Studies utilizing a new assay to quantify plasminogen activator activity. The Journal of clinical investigation. 1984;74(2):571-80. Epub 1984/08/01.
509. Levin RI, Harpel PC, Harpel JG, Recht PA. Inhibition of tissue plasminogen activator activity by aspirin in vivo and its relationship to levels of tissue plasminogen activator inhibitor antigen, plasminogen activator and their complexes. Blood. 1989;74(5):1635-43. Epub 1989/10/01.
510. Bjorkman JA, Jern S, Jern C. Cardiac sympathetic nerve stimulation triggers coronary t-PA release. Arteriosclerosis, thrombosis, and vascular biology. 2003;23(6):1091-7. Epub 2003/04/26.
511. Aspelin T, Eriksen M, Lindgaard AK, Lyberg T, Ilebekk A. Cardiac fibrinolytic capacity is markedly increased after brief periods of local myocardial ischemia, but declines

following successive periods in anesthetized pigs. Journal of thrombosis and haemostasis : JTH. 2005;3(9):1947-54. Epub 2005/08/17.
512. Jawad H BD, Dawson H, et al., editor. The effect of OnPulse in improving lower limb blood flow in healthy volunteers. Union Internationale de Phlebologie; 2011; Prague, Czech Republic.
513. Esmat S AASR, Rashed L. Effect of Exercise on Plasminogen Activator Inhibitor-1 (PAI-1) Level in patients with Metabolic Syndrome. Journal of American Science. 2010;6(12):1374-80.
514. Kuznik BI, Malezhik LP, Molchanova NL, Rusiaev VF. [Dynamics of peripheral nerve procoagulant and fibrinolytic activity upon electrical stimulation]. Fiziologicheskii zhurnal SSSR imeni I M Sechenova. 1979;65(3):414-20. Epub 1979/03/01. Dinamika prokoaguliantnoi i fibrinoliticheskoi aktivnosti perifericheskikh nervov pri ikh electricheskoi stimuliatsii.
515. van Westerloo DJ, Giebelen IA, Meijers JC, Daalhuisen J, de Vos AF, Levi M, et al. Vagus nerve stimulation inhibits activation of coagulation and fibrinolysis during endotoxemia in rats. Journal of thrombosis and haemostasis : JTH. 2006;4(9):1997-2002. Epub 2006/06/30.
516. Katz RT, Green D, Sullivan T, Yarkony G. Functional electric stimulation to enhance systemic fibrinolytic activity in spinal cord injury patients. Archives of physical medicine and rehabilitation. 1987;68(7):423-6. Epub 1987/07/01.
517. Yamamoto K, Takeshita K, Kojima T, Takamatsu J, Saito H. Aging and plasminogen activator inhibitor-1 (PAI-1) regulation: implication in the pathogenesis of thrombotic disorders in the elderly. Cardiovascular research. 2005;66(2):276-85. Epub 2005/04/12.
518. Eddy AA, Fogo AB. Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. Journal of the American Society of Nephrology : JASN. 2006;17(11):2999-3012. Epub 2006/10/13.
519. Erem C, Hacihasanoglu A, Celik S, Ovali E, Ersoz HO, Ukinc K, et al. Coagulation and fibrinolysis parameters in type 2 diabetic patients with and without diabetic vascular complications. Medical principles and practice : international journal of the Kuwait University, Health Science Centre. 2005;14(1):22-30. Epub 2004/12/21.
520. Held C, Hjemdahl P, Rehnqvist N, Wallen NH, Bjorkander I, Eriksson SV, et al. Fibrinolytic variables and cardiovascular prognosis in patients with stable angina pectoris treated with verapamil or metoprolol. Results from the Angina Prognosis study in Stockholm. Circulation. 1997;95(10):2380-6. Epub 1997/05/20.
521. Juhan-Vague I, Pyke SD, Alessi MC, Jespersen J, Haverkate F, Thompson SG. Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. ECAT Study Group. European Concerted Action on Thrombosis and Disabilities. Circulation. 1996;94(9):2057-63. Epub 1996/11/01.
522. Jawad H. The Effect of a Novel Electrical Stimulation Methods for Improving Lower Limb Blood Flow in Healthy Volunteers. https://qmro.qmul.ac.uk/xmlui/handle/123456789/31202012 [cited 2015 01/08/2015]; Available from: https://qmro.qmul.ac.uk/xmlui/handle/123456789/3120.
523. Griffin M, Nicolaides AN, Bond D, Geroulakos G, Kalodiki E. The efficacy of a new stimulation technology to increase venous flow and prevent venous stasis. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2010;40(6):766-71. Epub 2010/07/24.
524. Lee QY, Redmond SJ, Chan G, Middleton PM, Steel E, Malouf P, et al. Estimation of cardiac output and systemic vascular resistance using a multivariate regression model with features selected from the finger photoplethysmogram and routine cardiovascular measurements. Biomedical engineering online. 2013;12:19. Epub 2013/03/05.

525. Marik PE. Noninvasive cardiac output monitors: a state-of the-art review. Journal of cardiothoracic and vascular anesthesia. 2013;27(1):121-34. Epub 2012/05/23.
526. Palmer-Kazen U, Religa P, Wahlberg E. Exercise in patients with intermittent claudication elicits signs of inflammation and angiogenesis. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2009;38(6):689-96. Epub 2009/09/25.
527. Baum O, Gubeli J, Frese S, Torchetti E, Malik C, Odriozola A, et al. Angiogenesis-related ultrastructural changes to capillaries in human skeletal muscle in response to endurance exercise. J Appl Physiol (1985). 2015;119(10):1118-26. Epub 2015/09/19.
528. Panigada M, Zacchetti L, L'Acqua C, Cressoni M, Anzoletti MB, Bader R, et al. Assessment of Fibrinolysis in Sepsis Patients with Urokinase Modified Thromboelastography. PloS one. 2015;10(8):e0136463. Epub 2015/08/27.
529. Cachia PG, McGregor E, Adlakha S, Davey P, Goudie BM. Accuracy and precision of the TAS analyser for near-patient INR testing by non-pathology staff in the community. Journal of clinical pathology. 1998;51(1):68-72. Epub 1998/05/13.
530. Despotis GJ, Gravlee G, Filos K, Levy J. Anticoagulation monitoring during cardiac surgery: a review of current and emerging techniques. Anesthesiology. 1999;91(4):1122-51. Epub 1999/10/16.
531. Oberhardt BJ, Mize PD, Pritchard CG. Point-of-care fibrinolytic tests: the other side of blood coagulation. Clinical chemistry. 1997;43(9):1697-702. Epub 1997/09/23.

Appendices

Appendix Four: Patient information sheets *On headed paper

To be completed by the patient

Wearing the device

How long did you wear the geko™ device for?

Please enter the approx. number of minutes: _____minutes.

How comfortable was the device to wear once applied?

(please mark the appropriate box ☑)

1= very comfortable, 2= comfortable, 3= uncomfortable,
4=very uncomfortable, 5 = extremely uncomfortable)

1. ☐ 2. ☐ 3. ☐ 4. ☐ 5. ☐

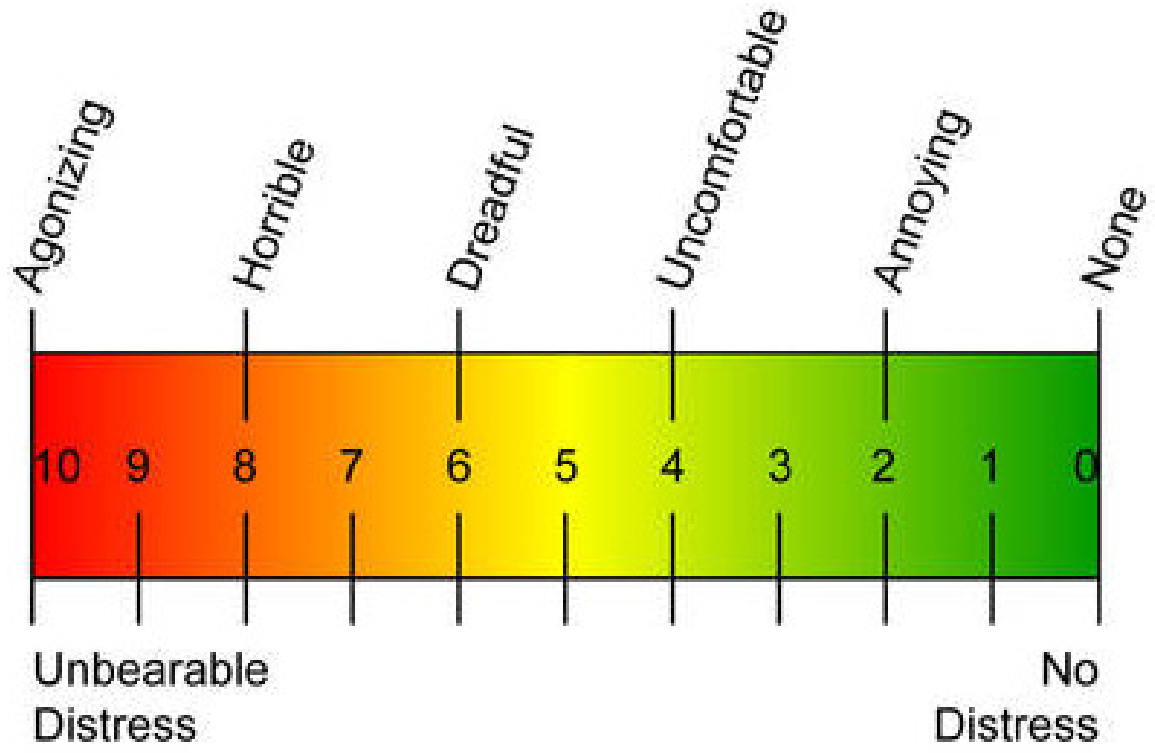
If ③④⑤ has been indicated please provide a comment below:

_____

_____

_____

Please mark on the diagram below how comfortable the device was to wear when it was working:



Contact

How well did the geko™ device stick to your leg?

1 = very well, 2 = well, 3 = with difficulty, 4 = extreme difficulty, 5 = unable to fit

1. ☐ 2. ☐ 3. ☐ 4. ☐ 5. ☐

If ③④⑤ has been indicated please provide a comment below:

Did the device become detached at any time during wear?

Yes ☐ No ☐

If yes, please indicate where on the device this occurred.

Head ☐ Tail ☐ Whole device ☐

If partial detachment occurred during wear, did this cause you to remove the device completely?

Yes ☐ No ☐

Were you able to re-apply the device easily?

Yes ☐ No ☐

If no, please comment below:

Effect of wearing the device

How does your leg feel after wearing the geko™ device?"

No difference ☐

Minimal improvement in comfort ☐

No heavy leg /swelling feeling ☐

A positive improvement ☐

Appendix Five: Patient information sheets *On headed paper

Prospective observational series to establish the haemodynamic efficacy and tolerability of the geko™ device in patients with lower limb vascular disease.

Claudicant Patient

Patient information sheet

Part 1

Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

What is the purpose of the study?

Your consultant believes you may be a suitable/ willing participant for a research study being carried out at Hull Royal Infirmary. The study is being carried out by a Research Registrar attached to the Department of Vascular Surgery, undertaking a research degree at Hull University.

You are being asked to take part in this study because you have been referred to the Vascular Department with impaired or reduced blood flow to one (or more) of your legs.

The reduced blood flow means that not enough blood and oxygen is reaching the tissues, and this often results in pain on walking, known as claudication.

The treatment of claudication and ischaemia aims to relieve your symptoms, improve your quality of life, heal areas of ulceration and ultimately prevent amputation. Unfortunately in some patients there is not a surgical treatment or surgery has failed to improve the symptoms.

In these situations treatment is difficult and as such new treatments are being investigated.

You have been invited to take part in a clinical trial to see if using a device called the geko™ can ***TEMPORARILY*** improve the circulation in your leg and establish how comfortable the device is to wear.

To help you decide if you would like to take part, please read this information sheet. It gives you details of what will be involved if you decide to take part and also who to contact if you would like to discuss the study or ask any questions.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to participate you will be given this information sheet to keep and be asked to sign a Consent form. You are still free to withdraw at any time and without giving a reason. Your non-participation or dropping out of the study will not affect your planned treatment and care in any way.

Before you can begin the study

You may read the full study protocol as well as this Patient Information Sheet, which gives you many details about the study. The recruiting Investigator will tell you about any potential adverse events that could occur in this study. You will be told exactly what the study entails and what will be required of you. You are encouraged to ask questions of the Investigators conducting the

recruitment interview until you are satisfied that you fully understand the nature of the study and the requirements.

What happens in the study?

If you think you might be interested in taking part in the study, you will have a short interview with one of the Researchers so we can collect some details from you and make sure you are eligible to join in the trial.

Once you are enrolled in the trial we will ask you to complete short questionnaires and we will perform a physical examination, which will include a non-invasive assessment of the nerve function in your legs.

The study and tests will take place in a clinical room at Hull Royal Infirmary. You will be asked to avoid fatty foods, tobacco and caffeine on your assessment day. It is advised that you wear light comfortable garments and your legs will need to be exposed.

At the start of the study you will be given 30 minutes to get used to your environment and relax. You will be given questionnaires to complete which will ask you about the pain you have been experiencing.

The geko™ device will then be applied to your leg, behind your knee, to stimulate a nerve for 60 minutes. Some patients will have the device applied but not turned on. You may experience the muscles in your leg and/or foot gently contracting/ twitching.

Two different types of scan will be performed to look at the blood flow in your leg before the device is turned on and at intervals following the application. One is an ultrasound scan which simply involves applying a probe to the skin and measuring blood flow. The other is called a laser Doppler scan and looks at skin blood flow using a small probe which is attached to your foot during the study. Both scans are safe and painless.

We will also do the same measurements on the other leg for comparison.

15-20mls of blood (one tablespoon) will be taken from a vein in the groin of each leg (at the top of the leg in the crease) before the device is activated and again after 45 minutes. This is to look for markers in the blood which may suggest a reduction in blood clots and also encourage the growth of new blood vessels.

After the device is removed you will be given a short questionnaire to fill in about how you found the experience and you will complete the pain questionnaires again.

Are there any risks to participating in the study?

Taking part in the trial will not alter the operation or treatment that you will receive for your circulation.

The blood collections should not cause you any significant discomfort.

What are the possible benefits of taking part?

This study may not benefit you directly; however it may lead to the development of a novel method for the future treatment of ischaemic legs. There may be some pain relief.

Could I come to any harm if I take part in the study?

You may be withdrawn from the study if the doctors feel it is best for you or if you do not comply with the requirements of the study.

If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel unacceptable levels of discomfort, or for any reason during the study you do not wish to continue, than we will stop the tests immediately.

The blood flow measurements and ultrasound are non-invasive, painless and known to be entirely safe.

All of the previous work using the system was found to be safe. When the device is applying an electrical stimulation, you will feel some muscle twitching and maybe tingling in your lower legs. That is how the device works and is intended in this study.

There are very few risks involved in using this type of equipment and the device is commonly used for therapeutic purposes to exercise muscles under the supervision of a Physiotherapist, as well as by members of the public for “toning” purposes in their own homes.

The blood tests may leave a small bruise, but are safe and will be performed by trained Medical Staff.

What happens when the research study stops?

When the study is complete, you will continue to be followed up by the Vascular team as planned.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

If you have a complaint, please contact the following in the first instance: Miss Rachel Barnes.

If you feel any significant discomfort or distress during the investigations, you must say so and we will stop the tests immediately at any time.

Any complaints about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

A contact number for complaints will be given.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

If you require any further information please contact:

Research team contact;

Miss Rachel Barnes,

Clinical Research Fellow,

Academic Vascular Surgery Unit,

Vascular Laboratory,

Hull Royal Infirmary,

Hull. HU3 2JZ

Tel: 01482 674178

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will need to use the data collected up to your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this trial, you should first ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain, you can do this via the NHS Complaints Procedure. Details can be obtained from;

Ms Janet Austin, Head of Complaints Department, Hull Royal Infirmary.

Tel: 01482 605284

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against

Hull and East Yorkshire Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you. In the highly unlikely event that you suffer from injury or illness as a result of participation in this study, indemnity will be provided by the Hull and East Yorkshire hospitals NHS Trust. Compensation will be by the usual NHS procedures.

Will my taking part in this study be kept confidential?

All the information obtained about you in the course of the study is confidential and will be kept in a secure locked room. The investigators performing the study and a study Monitor will have access to the data collected in this study. They may also be looked at by representatives of regulatory authorities and by authorised people from Hull Royal Infirmary to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen to the results of the research study?

The results of this study may be published or presented at meetings. You will not be identified in any report / publication or presentation. We would be happy to supply you with a copy of the results on request.

Who is organising and funding the study?

This study is organised and funded through the Academic Vascular Surgery Unit, Hull Royal Infirmary.

Who has reviewed this study?

The ethics behind this study have been reviewed and supported by the National Research Ethics Service Committee East of England- Cambridge East.

Further information/independent advice

Independent advice regarding this study or any other aspect of your care can be obtained from the Patients Advisory Liaison Service (PALS) using the details below;

PALS Office, Main Reception, Hull Royal Infirmary, Anlaby Road, HULL, HU3 2JZ

Tel. 01482 623065

Fax: 01482 622252

Email: pals@hey.nhs.uk

What happens next?

Please discuss this information with your family, friends or GP if you wish. Any questions can be answered then or please do not hesitate to contact the research team on the number below. Thank you very much for taking the time to read this information sheet and considering taking part in our research.

Prospective observational series to establish the haemodynamic efficacy and tolerability of the geko™ device in patients with lower limb vascular disease.

Volunteer Participant

Participant information sheet

Part 1

Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

What is the purpose of the study?

You are being asked to take part in this study because whilst you have varicose veins you have normal blood supply to your legs. The study is being carried out by a Research Registrar attached to the Department of Vascular Surgery, undertaking a research degree at Hull University.

Some patients have reduced blood flow which means that not enough blood and oxygen is reaching the tissues and this often results in pain, ulcers or tissue loss- known as ischaemia. The treatment of ischaemia aims to relieve symptoms, improve quality of life, heal areas of ulceration and ultimately prevent amputation.

For some of these patients there is not a surgical treatment or surgery has failed to improve the symptoms.

In these situations treatment is difficult and as such new treatments are being investigated.

You have been invited to take part in a clinical trial to see if using a device called the geko™ can ***TEMPORARILY*** improve the circulation and establish how comfortable the device is to wear. It is important to compare the effects seen in people without ischaemia, i.e. patients like you compared with ischaemic patients. This information will assist us to develop the device for patients with poor leg blood flow (ischaemia) and may be useful for other vascular conditions in the future such as varicose veins.

To help you decide if you would like to take part, please read this information sheet. It gives you details of what will be involved if you decide to take part and also who to contact if you would like to discuss the study or ask any questions.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to participate you will be given this information sheet to keep and be asked to sign a Consent form. You are still free to withdraw at any time and without giving a reason. Any future care you may need will not be affected in any way.

Before you can begin the study

You may read the full study protocol as well as this Patient Information Sheet, which gives you many details about the study. The recruiting Investigator will tell you about any potential adverse events that could occur in this study. You will be told exactly what the study entails and what will be required of you. You are

encouraged to ask questions of the Investigators conducting the recruitment interview until you are satisfied that you fully understand the nature of the study and the requirements.

What happens in the study?

If you think you might be interested in taking part in the study, you will have a short interview with one of the Researchers so we can collect some details from you and make sure you are eligible to join in the trial.

Once you are enrolled in the trial we will ask you to complete short questionnaires and we will perform a physical examination.

The study and tests will take place in a clinical room at Hull Royal Infirmary. You will be asked to avoid fatty foods, tobacco and caffeine on your assessment day. It is advised that you wear light comfortable garments and your legs will need to be exposed.

At the start of the study you will be given 30 minutes to get used to your environment and relax. You will be given questionnaires to complete which will ask you about the pain you have been experiencing.

The geko™ device will then be applied to your leg, behind the knee, to stimulate a nerve for 60 minutes. Some patients will have the device applied but not turned on. You may experience the muscles in your leg and/or foot gently contracting/ twitching.

Two different types of scan will be performed to look at the blood flow in your leg before the device is turned on and at intervals following the application. One is an ultrasound scan which simply involves moving a probe over the skin and measuring blood flow. The other is called a laser Doppler scan and looks at skin blood flow using a small probe which is attached to your foot during the study. Both scans are safe and painless.

We will also do the same measurements on the other leg for comparison.

15-20mls of blood (one tablespoon) will be taken from the vein in the groin of each leg (at the top of the leg in the crease) before the device is activated and again after 45 minutes. This is to look for markers in the blood which may suggest a reduction in the development of blood clots and also encourage the growth of new blood vessels.

After the device is removed you will be given a short questionnaire to fill in about how you found the experience and you will complete the pain questionnaires again.

Are there any risks to participating in the study?

There are no known risks associated with wearing the device.

The blood collections should not cause you any significant discomfort.

What are the possible benefits of taking part?

This study may not benefit you directly; however it may lead to the development of a novel method for the future treatment of ischaemic legs.

Could I come to any harm if I take part in the study?

You may be withdrawn from the study if the doctors feel it is best for you or if you do not comply with the requirements of the study.

If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel unacceptable levels of discomfort, or for any reason during the study you do not wish to continue, than we will stop the tests immediately.

The blood flow measurements and ultrasound are non-invasive, painless and known to be entirely safe.

All of the previous work using the system was found to be safe. When the device is applying an electrical stimulation, you will feel some muscle twitching and maybe tingling in your lower legs. That is how the device works and is intended in this study.

There are very few risks involved in using this type of equipment and the device is commonly used for therapeutic purposes to exercise muscles under the supervision of a Physiotherapist, as well as by members of the public for “toning” purposes in their own homes.

The blood tests may leave a small bruise, but are safe and will be performed by trained Medical Staff.

What happens when the research study stops?

When the study is complete there will be no change to any care you have been receiving or awaiting.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

If you have a complaint, please contact the following in the first instance: Miss Rachel Barnes.

If you feel any significant discomfort or distress during the investigations, you must say so and we will stop the tests immediately at any time.

Any complaints about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

A contact number for complaints will be given.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

If you require any further information please contact:

Research team contact;

Miss Rachel Barnes,

Clinical Research Fellow,

Academic Vascular Surgery Unit,

Vascular Laboratory,

Hull Royal Infirmary,

Hull. HU3 2JZ

Tel: 01482 674178

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will need to use the data collected up to your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this trial, you should first ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain, you can do this via the NHS Complaints Procedure. Details can be obtained from;

Ms Janet Austin, Head of Complaints Department, Hull Royal Infirmary.

Tel: 01482 605284

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Hull and East Yorkshire Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you. In the highly unlikely event that you suffer from injury or illness as a result of participation in this study, indemnity will be provided by the Hull and East Yorkshire hospitals NHS Trust. Compensation will be by the usual NHS procedures.

Will my taking part in this study be kept confidential?

All the information obtained about you in the course of the study is confidential and will be kept in a secure locked room. The investigators performing the study and a study Monitor will have access to the data collected in this study. They may also be looked at by representatives of regulatory authorities and by authorised people from Hull Royal Infirmary to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen to the results of the research study?

The results of this study may be published or presented at meetings. You will not be identified in any report / publication or presentation. We would be happy to supply you with a copy of the results on request.

Who is organising and funding the study?

This study is organised and funded through the Academic Vascular Surgery Unit, Hull Royal Infirmary.

Who has reviewed this study?

The ethics behind this study have been reviewed and supported by the National Research Ethics Service East of England Committee- Cambridge East.

Further information/independent advice

Independent advice regarding this study or any other aspect of your care can be obtained from the Patients Advisory Liaison Service (PALS) using the details below;

PALS Office, Main Reception, Hull Royal Infirmary, Anlaby Road, HULL, HU3 2JZ

Tel. 01482 623065

Fax: 01482 622252

Email: pals@hey.nhs.uk

What happens next?

Please discuss this information with your family, friends or GP if you wish. Any questions can be answered then or please do not hesitate to contact the research team on the number below. Thank you very much for taking the time to read this information sheet and considering taking part in our research.

A randomised control trial to establish if the use of geko™ post infra-inguinal surgical vein revascularisation increases flow through the graft

Lay Title: "Surgical Revascularisation and Nerve Stimulation Trial"

Patient information sheet

Part 1

Invitation

Your consultant believes you may be a suitable/ willing participant for a research study being carried out at Hull Royal Infirmary. The study is being carried out by a Research Registrar attached to the Department of Vascular Surgery, undertaking a research degree at Hull University.

Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part
- Part 2 gives you more detailed information about the conduct of the study

What is the purpose of the study?

You are suffering from arterial disease which is affecting the circulation in your leg(s). The lack of blood flow has meant that not enough blood and oxygen is reaching the tissues resulting in pain, ulcers or tissue loss. You have undergone a bypass graft to improve the blood flow and as such hopefully relieve your symptoms, improve your quality of life, heal areas of ulceration and ultimately prevent amputation.

As has been explained to you by your surgical team, unfortunately bypass grafts can block/ fail over time which can result in the return of your symptoms. We are investigating ways in which we can prevent this.

You have been invited to take part in a clinical trial to see if using a device called the geko™ may *TEMPORARILY* improve the blood flow through your bypass graft following your operation. In the active group it is hoped that this intervention may improve the patency (working) of the graft.

To help you decide if you would like to take part, please read this information sheet. It gives you details of what will be involved if you decide to take part and also who to contact if you would like to discuss the study or ask any questions.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. Your non-participation or dropping out of the study will not affect your planned treatment and care in any way.

Before you can begin the study

You may read the full study protocol as well as this Information sheet which gives you many details about the study. The

Recruiting Investigator will tell you about any potential adverse events that could occur in the study. You will be told exactly what the study entails and what will be required of you. You are encouraged to ask questions until you are satisfied that you fully understand the nature of the study and the requirements.

What happens in the study?

If you think you might be interested in taking part in the study, you will have a short interview with one of the researchers so we can collect some details from you and make sure there is no reason not to include you in the trial. Once you are enrolled in the trial we will ask you to complete 3 short questionnaires and we will perform an examination.

Study day

You will all attend the vascular lab in Hull Royal Infirmary (1st floor). You will be asked to avoid fatty foods, tobacco and caffeine on this assessment day. It is advised that you wear light comfortable garments and your legs will need to be exposed.

At the start of the study period you will be given 30 minutes to get used to your environment and relax. You will be given a questionnaire to complete which will ask you about the pain you have been experiencing.

If you are in the *active* part of the trial the geko™ device will then be applied to your leg to stimulate a nerve for 60 minutes. You will experience the muscles in your leg and/or foot gently contracting/ twitching. If you are in the *control* group the device will be applied to your leg but will not be activated.

Two different types of scan will be performed to look at the blood flow in your leg before the device is turned on and at intervals following the application. One is an ultrasound scan which simply involves applying a probe to the skin and measuring blood flow. The other is called a laser Doppler scan and looks at skin blood flow using a small probe which is continually attached to your foot during the study.

We will also do the same measurements on the other leg for comparison.

A measurement will also be taken using two small blood pressure cuffs, one of which will be attached to a strap around the neck and the other around the leg, The cuffs gently inflate to look at how stiff the walls of your blood vessels are and what affect the geko™ is having on the strength of your heart muscle contractions. The test is painless and will be performed before the device is activated and following its removal

15-20mls of blood (one tablespoon) will be taken from the groin of each leg (at the top of the leg in the crease) before the device is activated and again after 45 minutes. This will not be any more uncomfortable than a standard blood test and will be performed using the ultrasound to reduce the risks of discomfort, bleeding and bruising. The purpose of these tests is to look for markers in the blood which reduce blood clots and encourage the growth of new blood vessels. A systemic blood sample will also be taken from your arm at the beginning of the study period.

After the device is removed you will be given a short questionnaire to fill in about how you found the experience and you will complete the pain questionnaire again.

Are there any risks to participating in the study?

Taking part in the trial will not alter the operation or treatment that you will receive for your circulation. There is a small possibility of skin reactions to the gel on the device although this has been extensively tested. You may also experience some mild discomfort when the device is first turned on.

What are the potential benefits of taking part?

The research which has been carried out so far suggests that wearing the device may temporarily improve blood flow, but the effect on bypass grafts is unknown. If it is shown to improve flow in the graft it may be a useful treatment option in the future.

Could I come to any harm if I take part in the study?

You may be withdrawn from the study if the doctors feel it is best for you or if you do not comply with the requirements of the study.

If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel unacceptable discomfort, or for any reason during the study you do not wish to continue, than we will stop the tests immediately.

The blood flow measurements and ultrasound are non-invasive, painless and known to be entirely safe.

All of the previous work using the system was found to be safe. When the device is applying an electrical stimulation, you will feel some muscle twitching and maybe tingling in your lower legs. That is how the device works and is intended in this study.

There are very few risks involved in using this type of equipment and the device is commonly used for therapeutic purposes to exercise muscles under the supervision of a Physiotherapist, as well as by members of the public for “toning” purposes in their own homes.

What happens when the research study stops?

When the study is complete, you will continue to be followed up by the vascular team as usual.

What if there is a problem?

Any complaint or concerns about the way you have been dealt with during the study or potential harm you might suffer will be addressed. The detailed information on this is given in part 2.

If you have a complaint please contact the following in the first instance:

Miss Rachel Barnes

If you feel any discomfort or distress during the investigations, you must say so and we will stop the tests immediately at any time.

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

A contact number for complaints will be given.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

If you require any further information please contact:

Research team contact;

Miss Rachel Barnes, Clinical Research Fellow

Academic Vascular Surgery unit

Vascular Laboratory

Hull Royal Infirmary,

Hull. HU3 2JZ

Tel: 01482 674178

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will need to use the data collected up to your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this trial, you should first ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain, you can do this via the NHS Complaints Procedure. Details can be obtained from;

Ms Janet Austin, Head of Complaints Department, Hull Royal Infirmary. Tel: 01482 605284

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Hull and East Yorkshire Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you. In the highly unlikely event that you suffer from injury or illness as a result of participation in this study, indemnity will be provided by the Hull and East Yorkshire hospitals NHS Trust. Compensation will be by the usual NHS procedures.

Will my taking part in this study be kept confidential?

All the information obtained about you in the course of the study is confidential and will be kept in a secure locked room. The investigators performing the study and a study Monitor will have access to the data collected in this study. They may also be looked at by representatives of regulatory authorities and by authorised people from Hull Royal Infirmary to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen to the results of the research study?

The results of this study may be published or presented at meetings. You will not be identified in any report / publication or presentation. We would be happy to supply you with a copy of the results on request.

Who is organising and funding the study?

This study is organised and funded through the Academic Vascular Surgery unit, Hull Royal Infirmary.

Who has reviewed this study?

The ethics behind this study have been reviewed and supported by the National Research Ethics Service Committee- Sheffield .

Further information/independent advice

Independent advice regarding this study or any other aspect of your care can be obtained from the Patients Advisory Liaison Service (PALS) using the details below;

PALS Office, Main Reception, Hull Royal Infirmary, Anlaby Road, HULL, HU3 2JZ
Tel. 01482 623065
Fax: 01482 622252
Email: pals@hey.nhs.uk

What happens next?

Please discuss this information with your family, friends or GP if you wish. Any questions can be answered then or please do not hesitate to contact the research team on the number below. Thank you very much for taking the time to read this information sheet and considering taking part in our research.

Appendix Seven: Consent forms *On headed paper

Consent to participate in:

Prospective observational series to establish the haemodynamic efficacy and tolerability of the geko™ device in patients with lower limb vascular disease.

CLAUDICANT PATIENT

Please affix

Pt. Details sticker

	Participants Initials
I confirm that I have been given adequate time to read and understand all of the Patient Information Sheet version 1.2; Dated 21st February 2013 relating to the trial. I have had the opportunity to ask any questions and understood the responses.	
I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records	
I understand that participation in the trial is entirely voluntary and that I have the right to withdraw at any time without giving my reasons.	
I consent to my general practitioner and consultant vascular surgeons being informed of my participation in the trial.	
I agree to take part in the trial	
I consent to have details stored by the research team and understand that my details will not be available to anyone other than the research staff or database administrator.	
I understand that the results of the study may be presented at medical conferences and published in medical literature in an anonymous form. No identifiable details will be released to anyone outside of the research team without my permission.	

Participant Name: _____ date__/__/__

Signature: _____

Researcher Name: _____ date__/__/__

Signature _____

Consent to participate in:

Prospective observational series to establish the haemodynamic efficacy and tolerability of the geko™ device in patients with lower limb vascular disease.

VOLUNTEER PARTICIPANT

Please affix

Pt. Details sticker

	Participants Initials
I confirm that I have been given adequate time to read and understand all of the Patient Information Sheet version 1.2; Dated 21st February 2013 relating to the trial. I have had the opportunity to ask any questions and understood the responses.	
I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records	
I understand that participation in the trial is entirely voluntary and that I have the right to withdraw at any time without giving my reasons.	
I consent to my general practitioner and consultant vascular surgeons being informed of my participation in the trial.	
I agree to take part in the trial	
I consent to have details stored by the research team and understand that my details will not be available to anyone other than the research staff or database administrator.	
I understand that the results of the study may be presented at medical conferences and published in medical literature in an anonymous form. No identifiable details will be released to anyone outside of the research team without my permission.	

Participant Name: _____ date__/__/__

Signature: _____

Researcher Name: _____ date__/__/__

Signature _____

Consent to participate in:

A randomised control trial to establish if the use of geko™ post infra-inguinal surgical revascularisation increases flow through the graft.

The Surgical Revascularisation and Nerve Stimulation Trial (SRANS)

Please affix
Pt. Details sticker

	Participants Initials
I confirm that I have been given adequate time to read and understand all of the Patient Information Sheet version 1.1; Dated 16th November 2012, relating to the trial. I have had the opportunity to ask any questions and have understood the responses.	
I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records	
I understand that participation in the trial is entirely voluntary and that I have the right to withdraw at any time without giving my reasons.	
I consent to my general practitioner and consultant vascular surgeon being informed of my participation in the trial.	
I agree to take part in the trial	
I consent to have details stored by the research team and understand that my details will not be available to anyone other than the research staff or database administrator.	
I understand that the results of the study may be presented at medical conferences and published in medical literature in an anonymous form. No identifiable details will be released to anyone outside of the research team without my permission.	

Participant Name:_____ Date__/__/__

Signature: _____

Researcher Name:_____ Date__/__/__

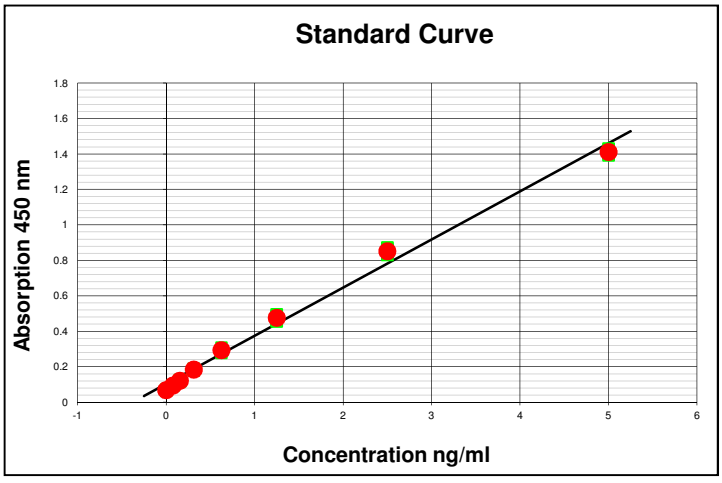
Signature: _____

Appendix Eight: ELISA plates

PAI plate 1

GK26 B	GK26 B	AH24 B	AH24 B	BC27 B	BC27 B	DD21 B	DD21 B	CM16 B	CM16 B
GK26 R	GK26 R	AH24 B	AH24 B	BC27 R	BC27 R	DD21 L	DD21 L	CM16 R	CM16 R
GK26 L	GK26 L	MP23 B	MP23 B	BC27 L	BC27 L	PA29 R	PA29 R	ST31 B	ST31 B
BC25 B	BC25 B	MP23 R	MP23 R	PH28 L	PH28 L	DD21 R	DD21 R	ST31 R	ST31 R
BC25 R	BC25 R	MP23 L	MP23 L	PH28 B	PH28 B	MH20 L	MH20 L	ST31 L	ST31 L
BC25 L	BC25 L	MG22 L	MG22 L	PH28 R	PH28 R	MH20 R	MH20 R	CE38 B	CE38 B
AH24 L	AH24 L	MG22 R	MG22 R	PA29 L	PA29 L	MH20 B	MH20 B	CE38 R	CE38 R
AH24 R	AH24 R	MG22 B	MG22 B	PA29 B	PA29 B	CM16 L	CM16 L	CE38 L	CE38 L

1	2	3	4	5	6	7	8	9	10
350.4	367.3	149.3	158.1	>525.0	>525.0	171.0	178.4	166.6	178.4
333.8	327.2	<0.0	184.6	440.6	441.4	216.7	225.5	<0.0	<0.0
297.7	334.9	119.8	125.7	404.2	395.3	274.5	297.7	351.1	339.7
218.9	232.9	94.7	106.2	162.5	164.7	424.1	432.2	365.5	337.9
75.2	80.0	<0.0	<0.0	184.6	179.5	197.5	209.3	362.9	378.4
297.3	305.8	<0.0	<0.0	127.2	123.5	98.0	98.8	219.6	209.7
136.0	128.6	28.1	26.2	280.4	286.7	136.7	126.8	118.3	114.6
209.7	224.8	157.0	144.8	37.3	481.5	83.3	73.4	256.5	>525.0

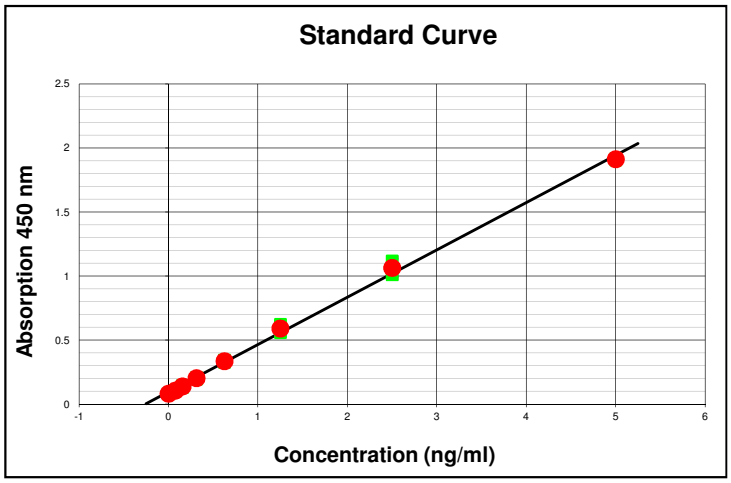


$R^2 = 0.9926$

PAI plate 2

GB14 B	GB14 B	RW12 L	RW12 L	PW39 B	PW39 B	PW39 L	PW39 L	MG15 L	MG15 L
GB14 L	GB14 L	RW12 R	RW12 R	JT36 B	JT36 B	PW39 R	PW39 R	MG15 R	MG15 R
GB14 R	GB14 R	NW37 B	NW37 B	JT36 R	JT36 R	CC30 B	CC30 B	MG15 B	MG15 B
RC11 B	RC11 B	NW37 R	NW37 R	JT36 L	JT36 L	CC30 R	CC30 R	SA13 R	SA13 R
RC11 R	RC11 R	NW37 L	NW37 L	KW33 B	KW33 B	CC30 L	CC30 L	SA13 L	SA13 L
RC11 L	RC11 L	CJ19 B	CJ19 B	KW33 R	KW33 R	JP17 B	JP17 B	SA13 B	SA13 B
NB18 B	NB18 B	CJ19 L	CJ19 L	KW33 L	KW33 L	JP17 R	JP17 R		
NB18 R	NB18 R	CJ19 R	CJ19 R	RW12 B	RW12 B	JP17 L	JP17 L		

1	2	3	4	5	6	7	8	9	10
256.5	256.8	265.7	280.9	173.9	170.9	154.4	192.0	171.5	172.6
245.4	241.6	287.6	305.5	113.0	117.3	157.4	165.8	192.0	187.2
232.9	236.5	247.8	261.6	211.5	209.6	237.8	247.3	170.1	180.1
99.2	105.9	248.9	254.3	112.7	112.7	226.7	227.5	208.0	201.0
42.6	44.2	236.7	254.6	266.0	259.5	19.8	225.6	219.1	216.7
98.4	99.2	123.3	126.0	41.0	41.0	255.9	199.4	255.9	279.2
219.1	218.3	111.9	116.0	270.8	276.5	147.9	158.2	<0.0	<0.0
229.7	237.3	42.9	43.1	271.7	279.8	168.8	173.1	<0.0	<0.0

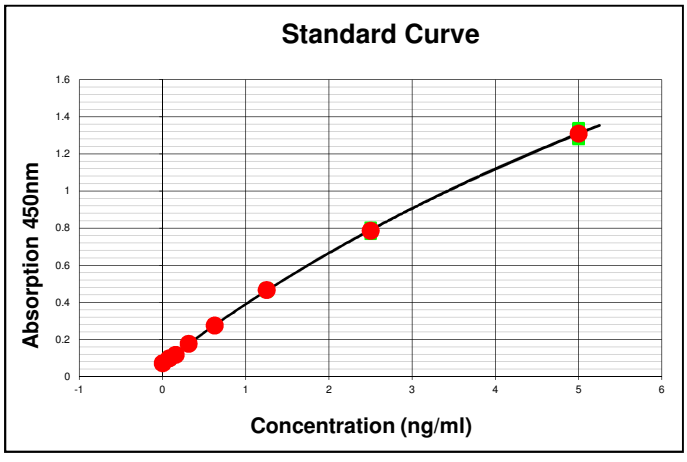


$R^2 = 0.9983$

PAI plate 3

PS41 B	PS41 B	PS41 R	PS41 R	PS41 L	PS41 L	JQ34 R	JQ34 R	JQ34 L	JQ34 L
KS42 B	KS42 B	KS42 R	KS42 R	KS42 L	KS42 L	JQ34 B	JQ34 B	CD35 L	CD35 L
MM44 B	MM44 B	MM44 L	MM44 L	MM44 R	MM44 R	CD35 R	CD35 R	CD35 B	CD35 B
SM45 B	SM45 B	SM45 L	SM45 L	SM45 R	SM45 R	CE50 L	CE50 L	CE50 R	CE50 R
BB46 B	BB46 B	BB46 L	BB46 L	BB46 R	BB46 R	CE50 B	CE50 B	CT51 B	CT51 B
EW47 B	EW47 B	EW47 R	EW47 R	EW47 L	EW47 L	CT51 L	CT51 L	CT51 R	CT51 R
BD48 B	BD48 B	BD48 L	BD48 L	BD48 R	BD48 R	MM40 B	MM40 B	MM40 R	MM40 R
DC49 B	DC49 B	DC49 L	DC49 L	DC49 R	DC49 R	MM40 L	MM40 L	MG22 L	MG22 L

1	2	3	4	5	6	7	8	9	10
76.4	74.5	101.4	110.7	49.6	49.6	80.5	84.5	178.2	184.7
98.0	103.8	176.3	193.5	127.7	136.5	117.0	122.6	209.5	225.1
250.8	277.1	231.9	271.1	191.3	214.8	121.3	129.3	245.9	232.7
233.2	230.3	164.2	170.6	188.7	193.1	64.9	76.5	109.0	123.2
193.1	291.8	138.6	146.3	146.0	147.9	55.3	66.7	269.4	296.2
210.7	238.1	158.4	165.6	145.2	145.5	167.5	198.9	291.4	317.2
151.8	154.4	102.9	101.0	94.7	95.4	66.9	63.6	87.3	94.0
195.1	220.2	244.3	147.1	146.8	143.1	42.3	47.0	87.0	115.9

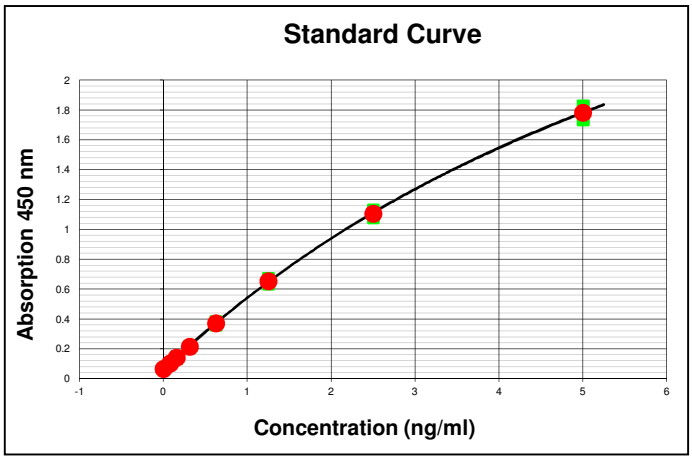


$R^2 = 0.9999$

PAI plate 4

ST60 B	ST60 B	ER80 B	ER80 B	MS67 R	MS67 R	SW32 B	SW32 B	RL64 R	RL64 R
ST60 R	ST60 R	JE82 L	JE82 L	MS67 L	MS67 L	AD69 B	AD69 B	SS66 L	SS66 L
ST60 L	ST60 L	MO63 B	MO63 B	JH81 B	JH81 B	AD69 L	AD69 L	SS66 R	SS66 R
ER80 L	ER80 L	MO63 R	MO63 R	JH81 R	JH81 R	AD69 R	AD69 R	DS65 B	DS65 B
ER80 R	ER80 R	MO63 L	MO63 L	JB68 B	JB68 B	SW32 R	SW32 R	DS65 R	DS65 R
MH62 B	MH62 B	JE82 R	JE82 R	JB68 R	JB68 R	SW32 L	SW32 L	DS65 L	DS65 L
MH62 R	MH62 R	JE82 B	JE82 B	JB68 L	JB68 L	RL64 B	RL64 B	SS66 B	SS66 B
MH62 L	MH62 L	MS67 B	MS67 B	JH81 L	JH81 L	RL64 L	RL64 L	CM16 R	CM16 R

1	2	3	4	5	6	7	8	9	10
164.3	168.2	130.9	145.2	100.2	100.0	97.0	93.8	212.9	240.2
190.1	187.9	60.1	66.1	99.0	95.3	223.4	213.5	210.6	220.6
173.2	188.4	192.0	207.2	56.3	65.0	230.6	220.9	217.4	242.3
71.0	64.3	180.4	164.9	104.4	110.8	239.7	254.4	132.4	144.0
178.2	179.6	143.8	155.2	132.2	126.0	84.7	84.5	134.8	147.2
94.5	100.5	96.1	95.8	118.8	113.5	70.5	68.8	109.7	124.4
90.0	88.4	81.5	87.3	100.0	99.2	94.8	95.0	251.4	266.2
82.0	85.1	105.6	100.7	73.9	69.3	137.4	178.5	145.7	165.1

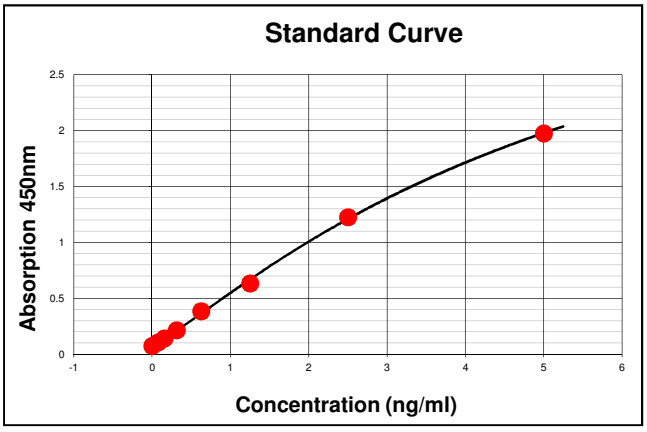


$R^2 = 1.000$

PAI plate 5

MP70 B	MP70 B	WS71 B	WS71 B	JP72 B	JP72 B	CM73 B	CM73 B	RM74 B	RM74 B
MP70 R	MP70 R	WS71 R	WS71 R	JP72 L	JP72 L	CM73 R	CM73 R	RM74 L	RM74 L
MP70 L	MP70 L	WS71 L	WS71 L	JP72 R	JP72 R	CM73 L	CM73 L	RM74 R	RM74 R
DM52 B	DM52 B	MA53 R	MA53 R	JA55 L	JA55 L	RM57 B	RM57 B	JE58 L	JE58 L
DM52 R	DM52 R	PG4 R	PG4 R	JA55 R	JA55 R	RM57 R	RM57 R	GB 59 B	GB 59 B
DM52 L	DM52 L	PG4 B	PG4 B	TW56 B	TW56 B	RM57 L	RM57 L	GB59 L	GB59 L
MA53 B	MA53 B	PG4 L	PG4 L	TW56 L	TW56 L	JE58 B	JE58 B	GB59 R	GB59 R
MA53 L	MA53 L	JA55 B	JA55 B	TW56 R	TW56 R	JE58 R	JE58 R		

1	2	3	4	5	6	7	8	9	10
166.9	160.0	83.8	91.9	178.8	176.5	214.2	196.1	303.1	300.5
186.6	190.5	143.7	147.3	215.4	208.7	36.4	36.0	201.5	203.7
202.0	198.2	156.3	164.0	207.5	214.2	188.2	187.0	293.5	320.8
144.3	142.1	151.2	172.2	212.3	210.8	268.6	206.7	374.0	155.2
161.5	156.5	322.9	338.9	271.2	163.5	230.4	252.2	150.6	156.0
158.2	165.5	218.56	226.9	311.2	342.9	245.3	260.9	237.9	255.6
159.3	160.2	233.1	256.7	409.9	392.0	166.9	168.2	154.1	164.2
168.7	180.2	183.4	204.8	336.4	347.8	176.1	162.2	<0.0	3.5

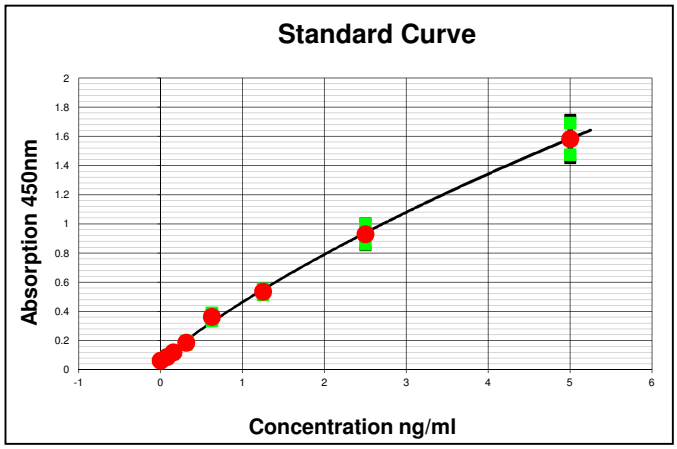


$R^2 = 0.9994$

PAI plate 6

CW3 L	CW3 L	PC2 R	PC2 R	MS5 L	MS5 L	PH79 B	PH79 B	SB75 L	SB75 L
CW3 R	CW3 R	PC2 B	PC2 B	MS5 B	MS5 B	PH79 R	PH79 R	SB75 R	SB75 R
CW3 B	CW3 B	PC2 L	PC2 L	MS5 R	MS5 R	PH79 L	PH79 L	SB75 B	SB75 B
JG7 B	JG7 B	MP10 B	MP10 B	CF6 B	CF6 B	KB78 B	KB78 B	MS76 B	MS76 B
JG7 L	JG7 L	MP10 L	MP10 L	CF6 R	CF6 R	KB78 R	KB78 R	MS76 R	MS76 R
JG7 R	JG7 R	MP10 R	MP10 R	CF6 L	CF6 L	KB78 L	KB78 L	MS76 L	MS76 L
GO0 B	GO0 B	GO9 L	GO9 L	RP8 B	RP8 B	DH77 B	DH77 B	DH77 L	DH77 L
GO9 R	GO9 R	RP8 L	RP8 L	RP8 R	RP8 R	DH77 R	DH77 R		

1	2	3	4	5	6	7	8	9	10
184.6	187.2	82.7	94.7	139.4	138.5	455.5	489.2	151.0	145.4
157.4	167.4	144.2	164.9	152.2	154.7	375.9	382.5	221.3	237.2
188.1	186.8	160.9	185.9	129.2	137.3	379.8	404.3	369.3	368.9
235.2	234.8	205.1	207.7	211.0	236.9	317.8	316.7	270.9	>525.0
149.4	166.8	230.1	253.7	204.1	200.5	284.4	316.4	321.5	342.1
263.1	272.6	216.6	232.1	204.7	183.9	284.4	334.2	338.0	396.3
349.0	337.2	309.7	363.9	192.6	193.9	371.6	399.5	403.5	437.0
166.5	167.1	180.4	190.1	62.8	67.0	388.4	414.7	2.8	2.8

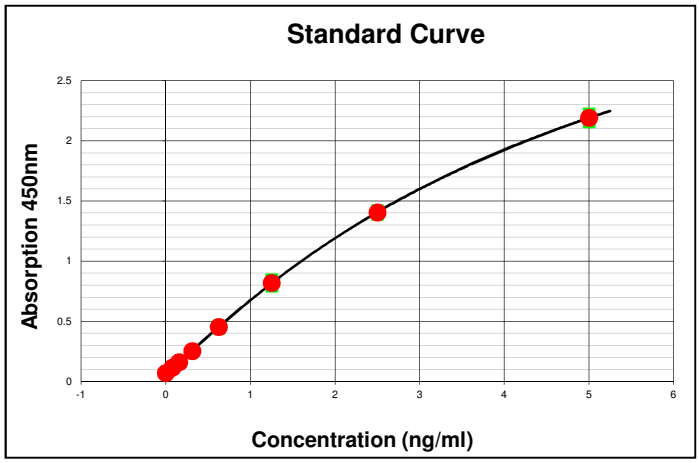


$R^2 = 0.9989$

PAI plate 7

MP23 B	MP23 B	MM40 L	MM40 L	MM20 L	MM20 L	DD21 R	DD21 R	KS42 L	KS42 L
MP23 R	MP23 R	MM40 B	MM40 B	MH20 R	MH20 R	DD21 L	DD21 L	KS42 B	KS42 B
MP23 L	MP23 L	MM40 R	MM40 R	MH20 B	MH20 B	DD21 B	DD21 B	KS42 R	KS42 R
JQ34 L	JQ34 L	JA55 B	JA55 B	BC25 B	BC25 B	PG4 B	PG4 B	MS76 L	MS76 L
JQ34 R	JQ34 R	JA55 L	JA55 L	BC25 R	BC25 R	PG4 L	PG4 L	MS76 R	MS76 R
JQ34 B	JQ34 B	JA55 R	JA55 R	BC25 L	BC25 L	PG4 R	PG4 R	MS76 B	MS76 B
GB59 R	GB59 R	GB59 B	GB59 B	RL64 L	RL64 L	CM73 R	CM73 R	CM73 L	CM73 L
GB59 L	GB59 L	RL64 R	RL64 R	RL64 B	RL64 B	CM73 B	CM73 B	KW33 B	KW33 B

1	2	3	4	5	6	7	8	9	10
76.3	79.8	58.1	62.0	131.1	153.3	128.2	137.5	175.0	195.2
67.7	66.2	68.2	76.5	123.9	126.1	140.7	148.0	178.9	183.7
72.3	79.7	71.8	78.0	161.0	171.1	139.4	151.3	177.1	182.9
133.8	147.1	140.4	150.4	229.6	249.0	176.9	190.5	215.8	210.6
88.8	90.1	163.8	183.3	56.5	59.4	211.3	215.6	215.8	233.9
138.1	136.2	163.2	173.8	206.2	218.3	167.8	185.2	206.0	220.1
105.8	111.3	110.4	113.9	162.6	178.9	112.5	118.5	141.5	142.3
113.9	112.9	164.8	167.8	156.2	172.7	135.4	142.7	217.6	228.5

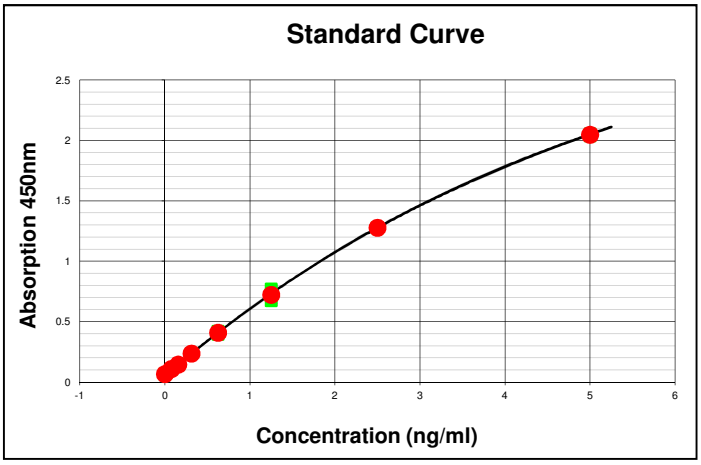


$R^2 = 1.0000$

PAI plate 8

CE38 R	CE38 R	AB1 B	AB1 B	CE38 L	CE38 L	JH81 B	JH81 B	CT51 L	CT51 L
CE38 B	CE38 B	AB1 R	AB1 R	CE38 R	CE38 R	JH81 R	JH81 R	CT51 B	CT51 B
CE38 L	CE38 L	AB1 L	AB1 L	CE38 B	CE38 B	JH81 L	JH81 L	CT51 R	CT51 R
BC27 R	BC27 R	PA29 L	PA29 L	DM52 B	DM52 B	CE50 B	CE50 B	JP72 L	JP72 L
BC27 B	BC27 B	PA29 R	PA29 R	DM52 R	DM52 R	CE50 L	CE50 L	JP72 B	JP72 B
BC27 L	BC27 L	PA29 B	PA29 B	DM52 L	DM52 L	CE50 R	CE50 R	JP72 R	JP72 R
DC49 R	DC49 R	MG22 B	MG22 B	MG22 L	MG22 L	WS71 B	WS71 B	KW33 R	KW33 R
DC49 L	DC49 L	MG22 R	MG22 R	WS71 R	WS71 R	WS71 L	WS71 L	KW33 L	KW33 L

1	2	3	4	5	6	7	8	9	10
154.1	161.9	153.9	170.5	107.5	113.7	76.4	85.2	264.0	269.1
154.6	158.9	161.9	166.8	155.2	167.9	91.5	99.8	273.6	265.9
114.1	120.5	169.0	179.3	165.2	174.5	65.8	80.0	266.7	272.8
>525.0	>525.0	203.6	215.3	94.8	100.6	95.9	106.3	140.7	140.7
>525.0	>525.0	180.4	170.3	97.5	96.3	93.8	100.8	141.4	148.8
>525.0	>525.0	194.9	181.1	99.0	100.6	100.2	106.7	146.8	150.9
200.7	204.5	95.3	94.0	82.5	85.7	119.9	127.6	37.8	38.0
167.0	199.3	34.2	36.2	118.7	117.7	119.1	124.8	219.7	238.6

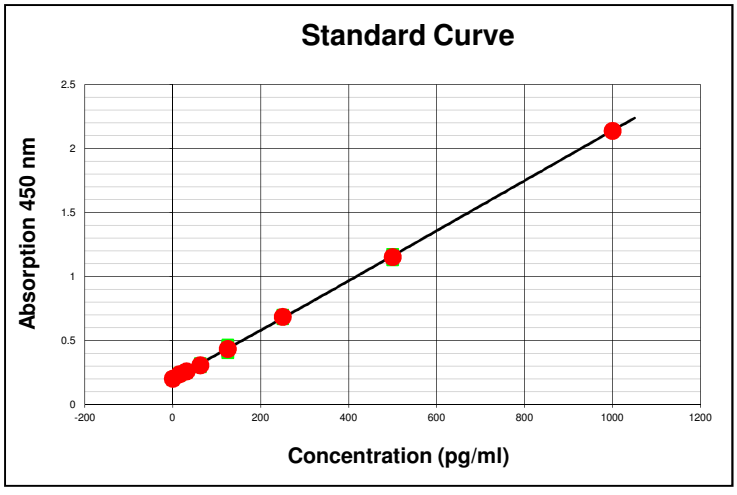


R^2 = 1.0000

TPA plate 1

GK26 B	GK26 B	AH24 B	AH24 B	BC27 B	BC27 B	DD21 B	DD21 B	CM16 B	CM16 B
GK26 R	GK26 R	AH24 B	AH24 B	BC27 R	BC27 R	DD21 L	DD21 L	CM16 R	CM16 R
GK26 L	GK26 L	MP23 B	MP23 B	BC27 L	BC27 L	PA29 R	PA29 R	ST31 B	ST31 B
BC25 B	BC25 B	MP23 R	MP23 R	PH28 L	PH28 L	DD21 R	DD21 R	ST31 R	ST31 R
BC25 R	BC25 R	MP23 L	MP23 L	PH28 B	PH28 B	MH20 L	MH20 L	ST31 L	ST31 L
BC25 L	BC25 L	MG22 L	MG22 L	PH28 R	PH28 R	MH20 R	MH20 R	CE38 B	CE38 B
AH24 L	AH24 L	MG22 R	MG22 R	PA29 L	PA29 L	MH20 B	MH20 B	CE38 R	CE38 R
AH24 R	AH24 R	MG22 B	MG22 B	PA29 B	PA29 B	CM16 L	CM16 L	CE38 L	CE38 L

1	2	3	4	5	6	7	8	9	10
7193.3	6871.5	4066.9	4159.3	3712.2	3604.1	3346.6	2711.3	5691.0	5466.0
6238.0	6478.2	4226.1	4851.7	2923.36	3181.6	2747.5	2426.1	5358.5	5578.5
6897.1	7029.9	2197.4	2301.4	3460.0	3573.3	6008.0	6432.2	5655.2	5496.7
5184.5	5266.4	1895.1	2082.9	2612.8	2519.5	2405.3	2545.4	5164.0	5496.7
4297.9	4539.0	1863.7	2009.9	2814.8	3093.8	3871.6	4087.4	5696.1	6238.0
4954.1	4897.8	3026.7	3228.0	2327.4	2923.4	4010.4	3609.3	3753.4	3779.1
3454.8	3490.9	2949.2	3016.4	6376.0	6759.2	4195.3	4380.0	3557.8	3480.6
4610.8	4544.1	4015.5	4215.8	6273.8	6386.2	4815.8	4974.6	3222.8	3485.7

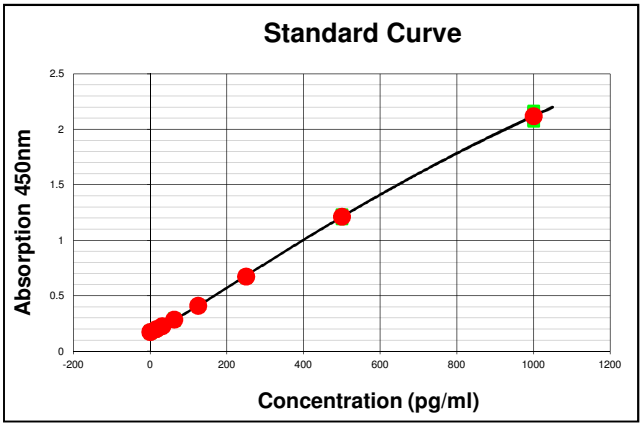


$R^2 = 0.9998$

TPA plate 2

GB14 B	GB14 B	RW12 L	RW12 L	PW39 B	PW39 B	PW39 L	PW39 L	MG15 L	MG15 L
GB14 L	GB14 L	RW12 R	RW12 R	JT36 B	JT36 B	PW39 R	PW39 R	MG15 R	MG15 R
GB14 R	GB14 R	NW37 B	NW37 B	JT36 R	JT36 R	CC30 B	CC30 B	MG15 B	MG15 B
RC11 B	RC11 B	NW37 R	NW37 R	JT36 L	JT36 L	CC30 R	CC30 R	SA13 R	SA13 R
RC11 R	RC11 R	NW37 L	NW37 L	KW33 B	KW33 B	CC30 L	CC30 L	SA13 L	SA13 L
RC11 L	RC11 L	CJ19 B	CJ19 B	KW33 R	KW33 R	JP17 B	JP17 B	SA13 B	SA13 B
NB18 B	NB18 B	CJ19 L	CJ19 L	KW33 L	KW33 L	JP17 R	JP17 R		
NB18 R	NB18 R	CJ19 R	CJ19 R	RW12 B	RW12 B	JP17 L	JP17 L		

1	2	3	4	5	6	7	8	9	10
4802.0	4987.4	2856.3	3142.5	4325.0	4603.5	3439.4	3926.4	2162.3	2373.7
3898.2	4507.2	3940.6	3978.4	7003.0	3509.2	4120.4	4177.3	2033.4	2332.3
4724.4	4632.4	5728.3	6208.4	5507.1	5951.8	3411.5	3555.9	2635.5	2851.
5542.1	5392.3	5784.0	6353.5	5179.1	5627.5	2755.1	2837.9	3804.1	4148.8
4768.0	4860.4	5819.5	6363.9	1130.7	1145.1	3304.6	3351.0	3818.2	4167.8
4865.3	5332.6	3416.1	3621.3	853.0	936.8	2304.8	3276.8	4339.4	4205.9
3397.5	3485.9	2907.0	2967.0	1518.8	1551.6	2953.1	3230.4	<0.0	<0.0
2732.1	2957.7	2741.3	2870.2	4320.3	3884.0	3309.2	3565.2	<0.0	<0.0

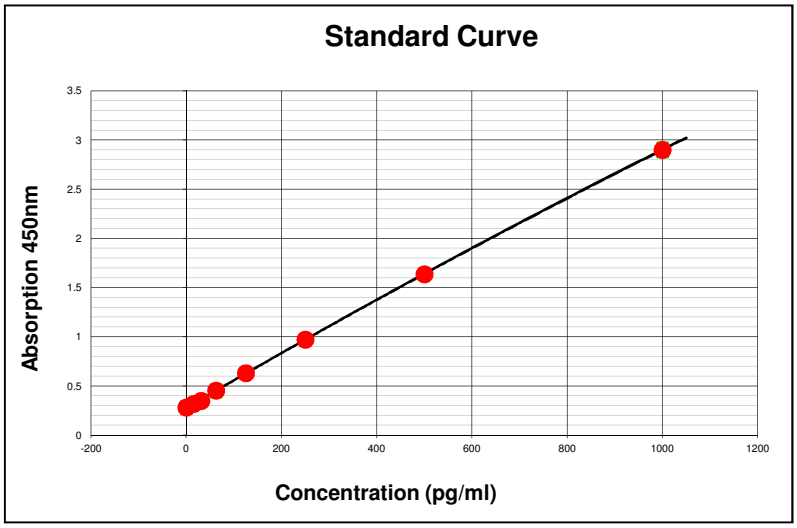


$R^2 = 0.9988$

TPA plate 3

PS41 B	PS41 B	PS41 R	PS41 R	PS41 L	PS41 L	JQ34 R	JQ34 R	JQ34 L	JQ34 L
KS42 B	KS42 B	KS42 R	KS42 R	KS42 L	KS42 L	JQ34 B	JQ34 B	CD35 L	CD35 L
MM44 B	MM44 B	MM44 L	MM44 L	MM44 R	MM44 R	CD35 R	CD35 R	CD35 B	CD35 B
SM45 B	SM45 B	SM45 L	SM45 L	SM45 R	SM45 R	CE50 L	CE50 L	CE50 R	CE50 R
BB46 B	BB46 B	BB46 L	BB46 L	BB46 R	BB46 R	CE50 B	CE50 B	CT51 B	CT51 B
EW47 B	EW47 B	EW47 R	EW47 R	EW47 L	EW47 L	CT51 L	CT51 L	CT51 R	CT51 R
BD48 B	BD48 B	BD48 L	BD48 L	BD48 R	BD48 R	MM40 B	MM40 B	MM40 R	MM40 R
DC49 B	DC49 B	DC49 L	DC49 L	DC49 R	DC49 R	MM40 L	MM40 L	MG22 L	MG22 L

1	2	3	4	5	6	7	8	9	10
7767.8	7951.2	6941.2	7410.5	7015.9	33.0	2500.6	2768.5	2753.8	2915.7
5927.8	6413.0	5584.6	6226.2	5615.4	10376.6	3030.1	3203.9	6354.5	6479.2
6475.3	6639.4	5488.5	5623.1	6428.5	6788.1	7979.2	8347.7	7291.9	7839.5
4418.8	4433.9	4524.6	4782.2	4309.4	4793.6	3616.2	3780.4	3552.9	3642.3
4554.8	4725.3	4061.2	118.4	6658.9	4181.5	3612.5	3825.2	8561.0	9331.7
5052.4	5048.5	75.7	4748.1	7485.8	4949.5	9299.0	9548.3	9233.9	9446.0
3750.5	3694.5	3452.4	3582.7	3341.0	3367.0	5189.8	3207.6	2676.6	2445.7
4725.3	2812.6	2941.5	3104.0	3590.1	3608.7	2518.9	2650.9	3044.9	3274.3

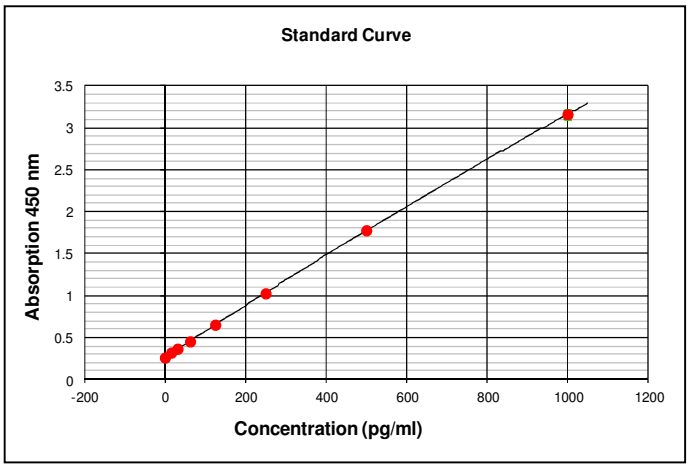


$R^2 = 0.9995$

TPA plate 4

ST60 B	ST60 B	ER80 B	ER80 B	MS67 R	MS67 R	SW32 B	SW32 B	RL64 R	RL64 R
ST60 R	ST60 R	JE82 L	JE82 L	MS67 L	MS67 L	AD69 B	AD69 B	SS66 L	SS66 L
ST60 L	ST60 L	MO63 B	MO63 B	JH81 B	JH81 B	AD69 L	AD69 L	SS66 R	SS66 R
ER80 L	ER80 L	MO63 R	MO63 R	JH81 R	JH81 R	AD69 R	AD69 R	DS65 B	DS65 B
ER80 R	ER80 R	MO63 L	MO63 L	JB68 B	JB68 B	SW32 R	SW32 R	DS65 R	DS65 R
MH62 B	MH62 B	JE82 R	JE82 R	JB68 R	JB68 R	SW32 L	SW32 L	DS65 L	DS65 L
MH62 R	MH62 R	JE82 B	JE82 B	JB68 L	JB68 L	RL64 B	RL64 B	SS66 B	SS66 B
MH62 L	MH62 L	MS67 B	MS67 B	JH81 L	JH81 L	RL64 L	RL64 L	CM16 R	CM16 R

1	2	3	4	5	6	7	8	9	10
3898.2	3824.2	2351.4	3214.9	1683.8	1745.7	2634.7	2909.2	3605.8	4192.1
4070.3	4399.1	3753.5	3807.3	1569.7	1644.6	3438.4	3425.1	3055.2	3498.7
3348.3	2935.7	3646.1	3804.0	3962.3	3773.7	2965.6	3334.9	2866.1	3408.4
2170.8	2124.9	3028.6	2760.2	4750.3	4491.0	3012.0	3525.4	3649.4	4077.1
2886.0	2082.3	1654.4	2661.1	4726.4	4822.2	3264.9	3115.0	3274.9	3874.7
1762.0	1540.4	3807.3	4511.4	4097.4	4552.3	2572.0	2595.1	3518.7	3787.1
1540.4	1641.4	3888.1	4443.3	3736.7	4073.7	3952.2	4897.6	3298.2	3972.4
1325.8	1452.6	1889.3	1722.9	4610.3	4743.5	3465.2	4266.7	4641.0	5010.9

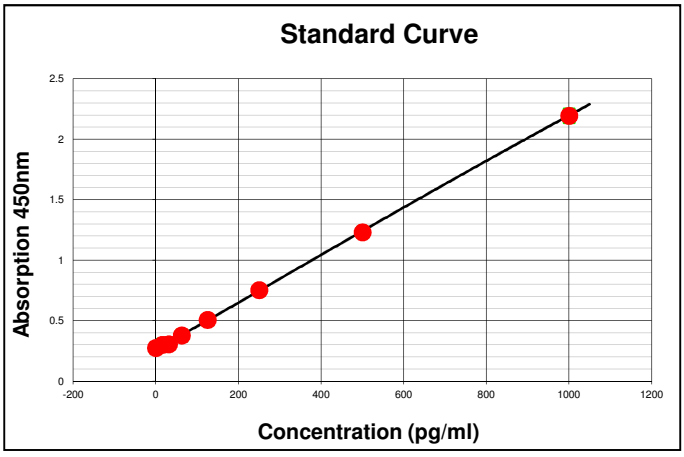


$R^2 = 0.9998$

TPA plate 5

MP70 B	MP70 B	WS71 B	WS71 B	JP72 B	JP72 B	CM73 B	CM73 B	RM74 B	RM74 B
MP70 R	MP70 R	WS71 R	WS71 R	JP72 L	JP72 L	CM73 R	CM73 R	RM74 L	RM74 L
MP70 L	MP70 L	WS71 L	WS71 L	JP72 R	JP72 R	CM73 L	CM73 L	RM74 R	RM74 R
DM52 B	DM52 B	MA53 R	MA53 R	JA55 L	JA55 L	RM57 B	RM57 B	JE58 L	JE58 L
DM52 R	DM52 R	PG4 R	PG4 R	JA55 R	JA55 R	RM57 R	RM57 R	GB 59 B	GB 59 B
DM52 L	DM52 L	PG4 B	PG4 B	TW56 B	TW56 B	RM57 L	RM57 L	GB59 L	GB59 L
MA53 B	MA53 B	PG4 L	PG4 L	TW56 L	TW56 L	JE58 B	JE58 B	GB59 R	GB59 R
MA53 L	MA53 L	JA55 B	JA55 B	TW56 R	TW56 R	JE58 R	JE58 R		

1	2	3	4	5	6	7	8	9	10
1591.6	1227.5	3187.9	3208.1	3572.0	3728.7	4427.78	3931.1	5400.3	4691.9
1119.0	1356.0	3006.0	3425.4	2965.6	3395.1	4062.7	4265.4	4783.4	4610.5
1422.7	1278.9	2586.6	3364.7	2975.7	3597.2	4245.2	4366.9	4992.1	5170.5
822.5	785.8	2530.9	2657.3	4448.1	4488.7	6344.9	6277.9	3647.8	3971.6
775.3	911.3	2899.9	2960.6	4727.5	4630.9	9677.5	6128.5	4722.4	5303.3
895.7	1005.0	3076.8	3122.2	<0.0	4752.9	5308.4	5702.3	4138.7	4351.6
2627.0	2647.2	2581.5	2990.9	2829.2	2697.8	3845.1	4336.4	4108.3	4148.9
2768.5	2915.1	5221.6	5282.8	2687.7	2505.6	4214.7	3915.9	<0.0	<0.0

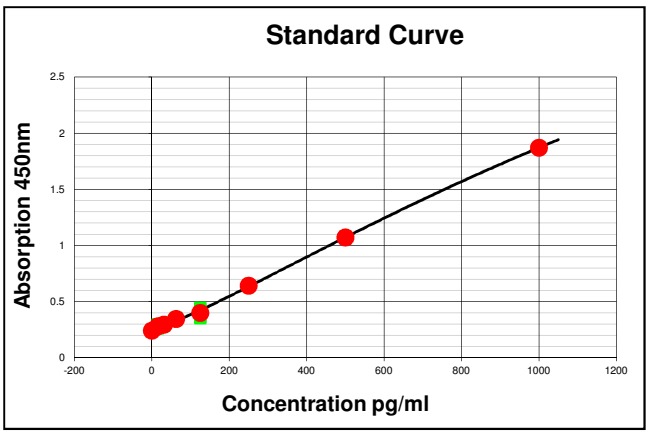


$R^2 = 0.9998$

TPA plate 6

CW3 L	CW3 L	PC2 R	PC2 R	MS5 L	MS5 L	PH79 B	PH79 B	SB75 L	SB75 L
CW3 R	CW3 R	PC2 B	PC2 B	MS5 B	MS5 B	PH79 R	PH79 R	SB75 R	SB75 R
CW3 B	CW3 B	PC2 L	PC2 L	MS5 R	MS5 R	PH79 L	PH79 L	SB75 B	SB75 B
JG7 B	JG7 B	MP10 B	MP10 B	CF6 B	CF6 B	KB78 B	KB78 B	MS76 B	MS76 B
JG7 L	JG7 L	MP10 L	MP10 L	CF6 R	CF6 R	KB78 R	KB78 R	MS76 R	MS76 R
JG7 R	JG7 R	MP10 R	MP10 R	CF6 L	CF6 L	KB78 L	KB78 L	MS76 L	MS76 L
GO0 B	GO0 B	GO9 L	GO9 L	RP8 B	RP8 B	DH77 B	DH77 B	DH77 L	DH77 L
GO9 R	GO9 R	RP8 L	RP8 L	RP8 R	RP8 R	DH77 R	DH77 R		

1	2	3	4	5	6	7	8	9	10
3415.8	3977.8	2446.0	2915.6	826.3	953.1	5303.7	6085.5	3841.4	5083.2
3092.1	2989.7	2938.4	3200.1	926.6	1025.0	5100.6	5807.2	4812.1	4800.6
3733.5	3977.8	2561.0	2909.9	744.3	920.0	4840.9	4313.7	5222.3	5234.0
4468.0	4445.1	1701.6	1856.6	3699.5	4325.2	4046.0	6414.1	2405.6	4405.1
4194.0	4800.6	972.8	1587.2	3512.2	3926.6	5760.1	7076.3	3336.3	4023.3
4485.1	4789.1	1635.5	1850.7	3801.7	4268.1	5943.1	6837.6	3580.3	4165.5
4405.1	4789.1	4194.0	5054.3	1968.8	2295.7	4760.3	4766.1	4691.4	5490.3
3847.1	4490.8	2015.9	2237.7	1526.5	1725.6	3972.1	5013.9	<0.0	<0.0

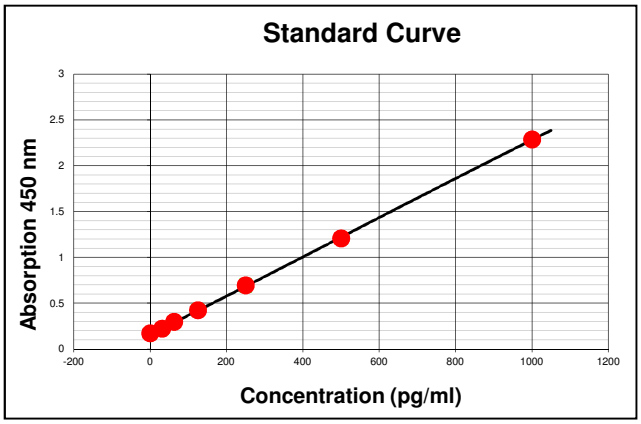


$R^2 = 0.9994$

TPA plate 7

KB78 B	KB78 B	PG4 B	PG4 B	WS71 R	WS71 R	EW47 B	EW47 B	ER80 L	ER80 L
KB78 R	KB78 R	PG4 R	PG4 R	WS71 B	WS71 B	EW47 R	EW47 R	MO63 B	MO63 B
KB78 L	KB78 L	PG4 L	PG4 L	WS71 L	WS71 L	EW47 L	EW47 L	MO63 R	MO63 R
TW56 B	TW56 B	JP17 R	JP17 R	BB46 B	BB46 B	DC49 B	DC49 B	ER80 B	ER80 B
TW56 L	TW56 L	JP17 B	JP17 B	BB46 R	BB46 R	DC49 L	DC49 L	MO63 L	MO63 L
TW56 R	TW56 R	JP17 L	JP17 L	BB46 L	BB46 L	DC49 R	DC49 R	ER80 R	ER80 R
ST60 L	ST60 L	AB1 B	AB1 B	AB1 L	AB1 L	JT36 B	JT36 B	KW33 R	KW33 R
ST60 R	ST60 R	AB1 R	AB1 R	AB1 R	AB1 R	JT36 R	JT36 R	KW33 L	KW33 L

1	2	3	4	5	6	7	8	9	10
6415.9	6668.4	4115.1	4143.0	4157.0	4324.4	<0.0	5948.9	2586.1	2684.4
7146.4	7263.7	3901.2	4003.5	4761.5	4659.2	5837.0	5953.6	4077.9	4245.3
7235.5	6954.1	4059.3	4017.5	4366.2	4515.0	4859.1	5347.7	3333.3	4687.1
3463.7	3626.7	4008.2	4170.9	5375.7	5487.4	6378.5	7710.3	3114.2	2838.7
3566.2	3682.5	4119.8	4031.4	8002.4	5222.1	3943.1	4384.8	3952.4	4152.3
4589.4	4240.7	3608.1	4129.1	4826.6	4915.0	4687.1	5063.8	2407.9	3174.8
3589.5	3780.3	2431.4	2558.0	2684.4	2759.3	8361.3	8754.2	1399.9	1347.2
4166.3	4315.1	2210.6	2276.4	7390.5	7531.5	7517.4	7540.9	1719.2	1785.6

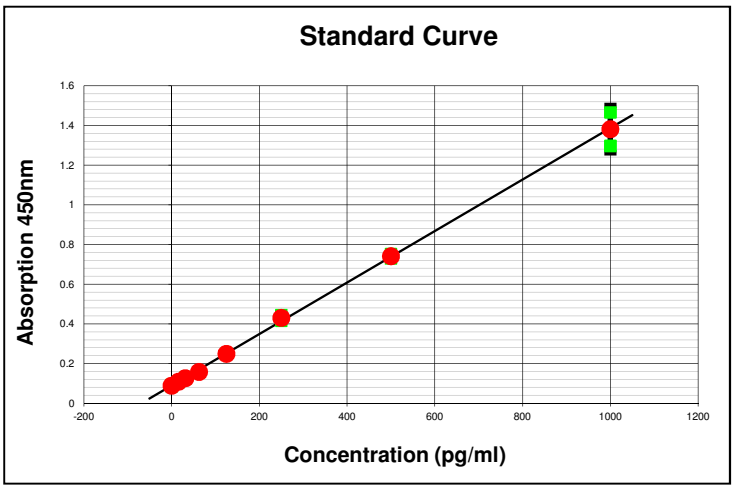


$R^2 = 0.9999$

VEGF plate 1

GK26 B	GK26 B	AH24 B	AH24 B	BC27 B	BC27 B	DD21 B	DD21 B	CM16 B	CM16 B
GK26 R	GK26 R	AH24 B	AH24 B	BC27 R	BC27 R	DD21 L	DD21 L	CM16 R	CM16 R
GK26 L	GK26 L	MP23 B	MP23 B	BC27 L	BC27 L	PA29 R	PA29 R	ST31 B	ST31 B
BC25 B	BC25 B	MP23 R	MP23 R	PH28 L	PH28 L	DD21 R	DD21 R	ST31 R	ST31 R
BC25 R	BC25 R	MP23 L	MP23 L	PH28 B	PH28 B	MH20 L	MH20 L	ST31 L	ST31 L
BC25 L	BC25 L	MG22 L	MG22 L	PH28 R	PH28 R	MH20 R	MH20 R	CE38 B	CE38 B
AH24 L	AH24 L	MG22 R	MG22 R	PA29 L	PA29 L	MH20 B	MH20 B	CE38 R	CE38 R
AH24 R	AH24 R	MG22 B	MG22 B	PA29 B	PA29 B	CM16 L	CM16 L	CE38 L	CE38 L

1	2	3	4	5	6	7	8	9	10
864.8	903.3	564.3	561.2	1379.5	1453.4	1095.9	1174.5	1102.1	985.0
901.8	926.4	482.6	539.6	1200.7	1248.5	1222.3	1268.5	1071.3	963.4
911.0	918.7	642.9	727.6	1447.3	1532.0	450.2	524.2	396.3	436.4
453.3	491.9	673.7	647.5	658.3	749.2	1225.4	1236.2	391.7	422.5
97.4	100.4	602.8	639.8	675.2	703.0	134.3	146.7	362.4	387.1
421.0	453.3	407.1	431.8	641.3	695.3	131.4	132.8	1203.8	1319.4
436.4	457.9	189.8	208.3	417.9	459.5	165.2	192.9	1274.7	1276.2
658.3	723.0	467.2	484.1	430.2	457.9	558.1	604.3	887.9	1008.1

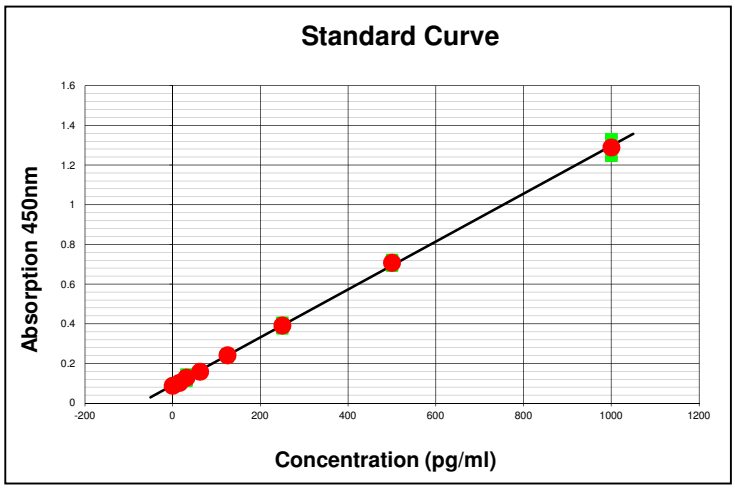


$R^2 = 0.9997$

VEGF plate 2

GB14 B	GB14 B	RW12 L	RW12 L	PW39 B	PW39 B	PW39 L	PW39 L	MG15 L	MG15 L
GB14 L	GB14 L	RW12 R	RW12 R	JT36 B	JT36 B	PW39 R	PW39 R	MG15 R	MG15 R
GB14 R	GB14 R	NW37 B	NW37 B	JT36 R	JT36 R	CC30 B	CC30 B	MG15 B	MG15 B
RC11 B	RC11 B	NW37 R	NW37 R	JT36 L	JT36 L	CC30 R	CC30 R	SA13 R	SA13 R
RC11 R	RC11 R	NW37 L	NW37 L	KW33 B	KW33 B	CC30 L	CC30 L	SA13 L	SA13 L
RC11 L	RC11 L	CJ19 B	CJ19 B	KW33 R	KW33 R	JP17 B	JP17 B	SA13 B	SA13 B
NB18 B	NB18 B	CJ19 L	CJ19 L	KW33 L	KW33 L	JP17 R	JP17 R		
NB18 R	NB18 R	CJ19 R	CJ19 R	RW12 B	RW12 B	JP17 L	JP17 L		

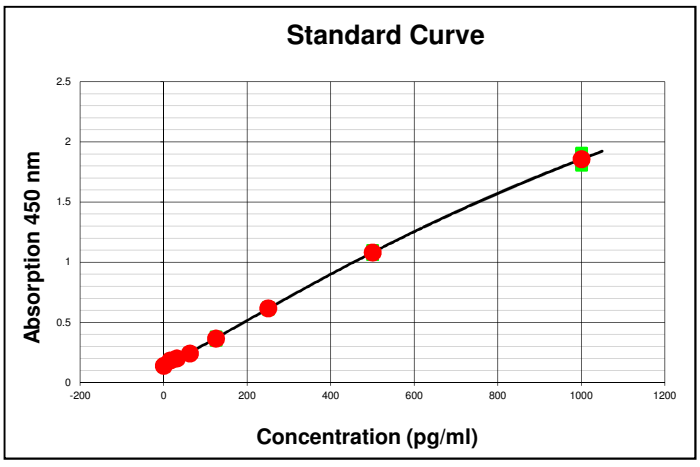
1	2	3	4	5	6	7	8	9	10
1247.8	1328.9	838.6	923.1	878.3	996.0	934.7	977.7	316.8	333.3
1208.0	1386.9	861.8	918.1	452.6	515.6	1042.3	947.9	330.0	321.7
1232.8	1294.1	517.2	573.5	896.6	946.3	243.9	265.4	326.7	378.1
437.7	452.6	532.1	568.6	475.8	523.8	210.7	220.7	1045.7	1009.2
176.0	185.9	561.9	595.1	1357.1	1449.9	229.0	224.0	1249.4	1415.1
369.8	412.8	396.3	427.8	232.3	255.5	1014.2	1088.7	1252.7	1342.2
605.0	656.4	399.6	437.7	1199.7	1325.6	972.3	987.7	2.0	15.3
600.0	674.6	131.2	142.8	817.0	938.0	1042.3	1118.5	15.3	10.3



VEGF plate 3

PS41 B	PS41 B	PS41 R	PS41 R	PS41 L	PS41 L	JQ34 R	JQ34 R	JQ34 L	JQ34 L
KS42 B	KS42 B	KS42 R	KS42 R	KS42 L	KS42 L	JQ34 B	JQ34 B	CD35 L	CD35 L
MM44 B	MM44 B	MM44 L	MM44 L	MM44 R	MM44 R	CD35 R	CD35 R	CD35 B	CD35 B
SM45 B	SM45 B	SM45 L	SM45 L	SM45 R	SM45 R	CE50 L	CE50 L	CE50 R	CE50 R
BB46 B	BB46 B	BB46 L	BB46 L	BB46 R	BB46 R	CE50 B	CE50 B	CT51 B	CT51 B
EW47 B	EW47 B	EW47 R	EW47 R	EW47 L	EW47 L	CT51 L	CT51 L	CT51 R	CT51 R
BD48 B	BD48 B	BD48 L	BD48 L	BD48 R	BD48 R	MM40 B	MM40 B	MM40 R	MM40 R
DC49 B	DC49 B	DC49 L	DC49 L	DC49 R	DC49 R	MM40 L	MM40 L	MG22 L	MG22 L

1	2	3	4	5	6	7	8	9	10
308.2	293.9	430.5	482.6	325.6	308.2	362.3	347.0	370.4	373.5
282.6	281.6	291.8	307.2	283.6	264.1	422.4	427.5	1868.8	2021.8
977.2	998.6	913.8	949.2	973.8	1159.7	1913.7	1956.2	2042.9	>2100.0
927.0	908.2	914.9	963.8	844.7	923.7	475.4	518.4	454.0	472.4
853.4	1000.8	958.2	959.3	912.7	913.8	434.6	472.4	712.9	693.9
1039.3	1124.6	1149.1	1166.7	1135.1	1123.4	618.6	669.8	608.2	633.2
460.1	485.6	442.8	462.1	534.9	441.7	382.6	446.8	368.4	414.2
760.7	818.7	766.1	761.8	604.0	785.4	347.0	321.5	366.4	386.7

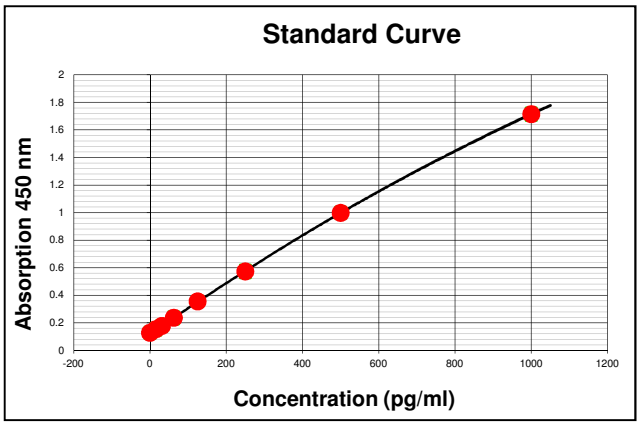


$R^2 = 0.9997$

VEGF plate 4

ST60 B	ST60 B	ER80 B	ER80 B	MS67 R	MS67 R	SW32 B	SW32 B	RL64 R	RL64 R
ST60 R	ST60 R	JE82 L	JE82 L	MS67 L	MS67 L	AD69 B	AD69 B	SS66 L	SS66 L
ST60 L	ST60 L	MO63 B	MO63 B	JH81 B	JH81 B	AD69 L	AD69 L	SS66 R	SS66 R
ER80 L	ER80 L	MO63 R	MO63 R	JH81 R	JH81 R	AD69 R	AD69 R	DS65 B	DS65 B
ER80 R	ER80 R	MO63 L	MO63 L	JB68 B	JB68 B	SW32 R	SW32 R	DS65 R	DS65 R
MH62 B	MH62 B	JE82 R	JE82 R	JB68 R	JB68 R	SW32 L	SW32 L	DS65 L	DS65 L
MH62 R	MH62 R	JE82 B	JE82 B	JB68 L	JB68 L	RL64 B	RL64 B	SS66 B	SS66 B
MH62 L	MH62 L	MS67 B	MS67 B	JH81 L	JH81 L	RL64 L	RL64 L	CM16 R	CM16 R

1	2	3	4	5	6	7	8	9	10
1966.4	>2100.0	405.4	433.4	757.2	702.8	1106.2	1137.0	531.6	518.0
>2100.0	>2100.0	261.8	267.3	762.0	758.4	984.9	1023.7	454.7	488.5
>2100.0	>2100.0	262.9	269.5	323.4	323.4	1006.1	1124.2	475.0	508.9
184.2	209.3	249.8	260.7	498.7	488.5	1031.3	1124.2	823.0	775.1
409.9	449.1	226.8	230.1	423.3	426.7	1249.3	1301.1	867.9	877.6
344.4	388.7	436.7	460.3	409.9	379.8	1129.3	1137.0	782.2	814.6
334.0	388.7	396.5	420.0	411.0	416.6	481.7	511.1	486.2	513.4
351.0	361.0	865.4	784.6	306.9	333.3	484.0	531.6	1066.7	1155.1

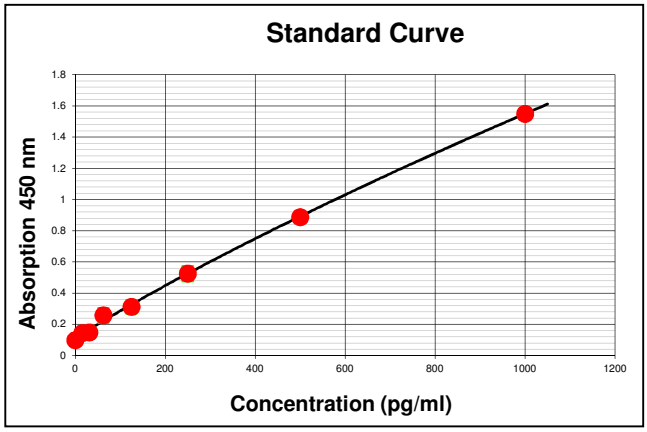


$R^2 = 1.0000$

VEGF plate 5

MP70 B	MP70 B	WS71 B	WS71 B	JP72 B	JP72 B	CM73 B	CM73 B	RM74 B	RM74 B
MP70 R	MP70 R	WS71 R	WS71 R	JP72 L	JP72 L	CM73 R	CM73 R	RM74 L	RM74 L
MP70 L	MP70 L	WS71 L	WS71 L	JP72 R	JP72 R	CM73 L	CM73 L	RM74 R	RM74 R
DM52 B	DM52 B	MA53 R	MA53 R	JA55 L	JA55 L	RM57 B	RM57 B	JE58 L	JE58 L
DM52 R	DM52 R	PG4 R	PG4 R	JA55 R	JA55 R	RM57 R	RM57 R	GB 59 B	GB 59 B
DM52 L	DM52 L	PG4 B	PG4 B	TW56 B	TW56 B	RM57 L	RM57 L	GB59 L	GB59 L
MA53 B	MA53 B	PG4 L	PG4 L	TW56 L	TW56 L	JE58 B	JE58 B	GB59 R	GB59 R
MA53 L	MA53 L	JA55 B	JA55 B	TW56 R	TW56 R	JE58 R	JE58 R		

1	2	3	4	5	6	7	8	9	10
432.3	465.8	871.7	958.6	702.7	787.3	317.4	314.9	913.6	971.3
493.0	562.4	934.7	1028.2	790.0	989.8	220.6	248.4	578.2	648.8
521.7	566.3	1061.1	1031.0	735.3	859.1	255.7	298.8	922.0	1008.2
349.9	380.1	>2100.0	>2100.0	1217.7	1222.1	1415.2	1604.1	512.5	529.5
349.9	368.8	>2100.0	?????	1217.7	1307.6	1655.1	1607.2	324.8	415.7
331.1	368.8	>2100.0	?????	392.8	420.8	1525.9	1723.6	339.8	525.6
>2100.0	>2100.0	?????	?????	450.3	406.8	474.9	491.7	343.6	354.9
>2100.0	>2100.0	895.4	887.0	395.3	458.1	1163.6	533.5	<0.0	<0.0

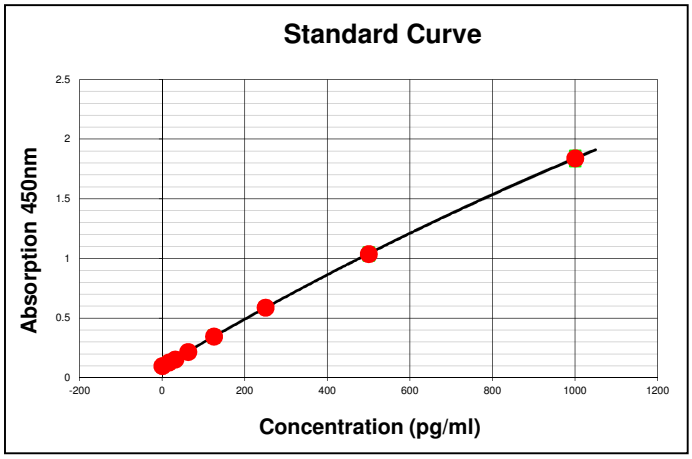


$R^2 = 0.9989$

VEGF plate 6

CW3 L	CW3 L	PC2 R	PC2 R	MS5 L	MS5 L	PH79 B	PH79 B	SB75 L	SB75 L
CW3 R	CW3 R	PC2 B	PC2 B	MS5 B	MS5 B	PH79 R	PH79 R	SB75 R	SB75 R
CW3 B	CW3 B	PC2 L	PC2 L	MS5 R	MS5 R	PH79 L	PH79 L	SB75 B	SB75 B
JG7 B	JG7 B	MP10 B	MP10 B	CF6 B	CF6 B	KB78 B	KB78 B	MS76 B	MS76 B
JG7 L	JG7 L	MP10 L	MP10 L	CF6 R	CF6 R	KB78 R	KB78 R	MS76 R	MS76 R
JG7 R	JG7 R	MP10 R	MP10 R	CF6 L	CF6 L	KB78 L	KB78 L	MS76 L	MS76 L
GO0 B	GO0 B	GO9 L	GO9 L	RP8 B	RP8 B	DH77 B	DH77 B	DH77 L	DH77 L
GO9 R	GO9 R	RP8 L	RP8 L	RP8 R	RP8 R	DH77 R	DH77 R		

1	2	3	4	5	6	7	8	9	10
133.4	142.5	299.7	319.1	377.9	387.3	308.9	364.5	404.9	449.8
115.3	130.4	550.1	586.4	412.2	482.4	321.2	369.6	486.6	538.4
163.6	180.7	436.2	486.6	319.1	344.9	296.6	308.9	467.6	560.8
503.5	544.8	562.9	617.6	444.6	487.6	399.7	451.9	212.1	201.0
409.1	431.0	559.7	605.7	485.5	519.3	277.2	507.7	201.0	256.8
510.9	515.1	581.1	617.6	470.8	495.0	435.2	501.3	219.2	216.1
497.1	481.3	458.2	501.3	262.9	273.1	621.9	667.3	696.7	715.3
243.5	264.9	260.8	275.1	97.2	129.4	603.6	656.4	<0.0	4.3

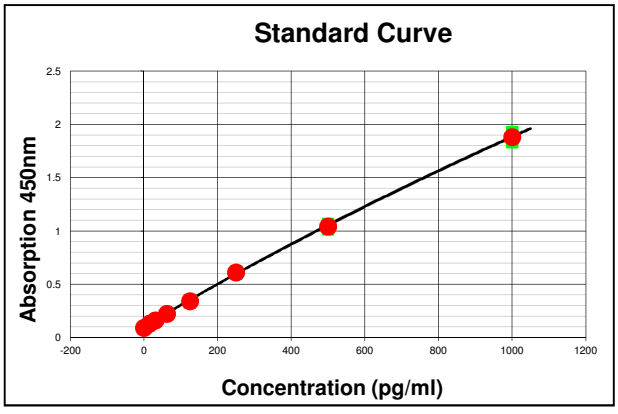


$R^2 = 1.0000$

VEGF plate 7

PG4 B	PG4 B	ST60 R	ST60 R	CE38 L	CE38 L	ER80 L	ER80 L	RP8 R	RP8 R
PG4 R	PG4 R	MA53 R	MA53 R	JT36 R	JT36 R	CJ19 R	CJ19 R		
PG4 L	PG4 L	MA53 B	MA53 B	KW33 R	KW33 R	RC11 R	RC11 R		
CD35 L	CD35 L	MA53 L	MA53 L	RM74 L	RM74 L	BC25 B	BC25 B		
CD35 R	CD35 R	AB1 L	AB1 L	KB78 R	KB78 R	BC25 R	BC25 R		
CD35 B	CD35 B	AB1 R	AB1 R	JP72 B	JP72 B	BC25 L	BC25 L		
ST60 L	ST60 L	AB1 B	AB1 B	JP72 L	JP72 L	GO9 R	GO9 R		
ST60 B	ST60 B	JE82 L	JE82 L	JP72 R	JP72 R	GB59 L	GB59 L		

1	2	3	4	5	6	7	8	9	10
1278.7	1562.4	1987.2	2356.2	817.8	861.0	245.6	289.0	119.1	111.3
1464.3	1937.6	1907.8	2036.9	781.4	854.3	96.8	101.7	<0.0	<0.0
1690.4	1798.9	2136.4	2166.3	159.2	175.9	115.2	139.6	<0.0	<0.0
1858.3	1997.1	2146.4	2146.4	493.4	547.2	379.9	414.1	<0.0	<0.0
1967.3	2096.6	784.7	801.2	477.6	557.8	86.3	97.8	<0.0	<0.0
1591.9	2076.7	659.5	736.5	543.0	587.6	292.0	308.3	<0.0	<0.0
1858.3	1957.4	634.7	690.9	577.0	630.5	244.6	272.8	<0.0	<0.0
1631.3	2076.7	139.6	167.1	548.3	583.4	253.7	279.9	<0.0	<0.0



$R^2 = 1.0000$