

REPERFUSION AS A MARKER OF SUCCESS OF DISTAL REVASCULARISATION

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ABSTRACT

Objectives: To determine whether skin microvascular perfusion and reactivity in critical limb ischaemia (CLI) is more enhanced following surgical, compared to endovascular treatment and if this is supplemented with concomitant improvement in clinical and haemodynamic parameters.

Design: Prospective observational study of forty CLI patients with multi-level atherosclerotic arterial disease requiring infra-inguinal revascularisation.

Methods: Clinical assignment to surgical (n=30) or endovascular (n=10) treatment with six-week follow-up. Laser Doppler fluxmetry (LDF) was used to assess the time to peak (Tp), basal flux and vasoconstrictor mechanisms. Outcomes were compared with clinical improvement and pressure changes at the ankle and toe. Anthropometric measures and tissue tension were assessed for evidence of reactive lower limb oedema.

Results: The surgical group showed a significant decrease in Tp (100 ± 4 to 59 ± 7 , $p < 0.001$) and some restoration of autoregulation, notably vasoconstriction in the foot on sitting (72.4 ± 6.8 to 52.2 ± 2.3 , $p 0.05$). Improvement in the anatomical severity of disease correlated with concomitant rise in toe pressures ($p < 0.001$) but not with pressures at the ankle. Reactive oedema was more evident post-surgery, with associated increases in lower-limb tissue tension. Endovascular intervention ameliorates symptoms in the short-term but did not provide the same microvascular restoration.

Conclusions: Microvascular perfusion and reactivity is greatly improved at six-weeks after surgical revascularisation but not after endovascular intervention.

“Wonder is the beginning of wisdom”
Socrates

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ABBREVIATIONS

ABS	Arterial Bypass Surgery	L-NMMA	NG monomethyl-L-arginine
ABP	Ankle Blood Pressure	MAP	Mean Arterial Pressure
ABPI	Ankle-Brachial Pressure Index	MRA	Magnetic Resonance Angiography
Ach	Acetylcholine	MTH	Metatarsal Head
APU	Arbitrary Perfusion Units	NO	Nitric Oxide
AT	Anterior Tibial	NSBI	Nicolson-Simms-Brown Index
AU	Arbitrary Units	PA	Popliteal Artery
AV	Arterio-venous	PAOD	Peripheral Arterial Occlusive Disease
CAD	Coronary Artery Disease	PG	Prostaglandin
CBV	Capillary Blood Velocity	PN	Peroneal
CFA	Common Femoral Artery	PORH	Post Occlusive Reactive Hyperaemia
CFC	Capillary Filtration Coefficient	PT	Posterior Tibial
CGRP	Calcitonin Gene Related Peptide	PTA	Percutaneous Transluminal Angioplasty
CIA	Common Iliac Artery	PTFE	Polytetrafluoroethylene
CLI	Critical Limb Ischaemia	PU	Perfusion Units
CT/A	Computed Tomography/Angiography	PVD	Peripheral Vascular Disease
CVD	Cerebrovascular Disease	RBC	Red Blood Cell
DAP	Digital Artery Pressure	RH	Reactive Hyperaemia
DM	Diabetes Mellitus	SFA	Superficial Femoral Artery
DP	Dorsalis Pedis	SNP	Sodium Nitroprusside
DPN	Diabetic Peripheral Neuropathy	SPP	Skin Perfusion Pressure
DSA	Digital Subtraction Angiography	SWM	Semmes-Weinstein Monofilament
DVT	Deep Vein Thrombosis	TASC	Trans-Atlantic Inter-Society Consensus
EDRF	Endothelial Derived Relaxation Factor	TBP	Toe Blood Pressure
EIA	External Iliac Artery	TBPI	Toe-Brachial Pressure Index
ESVS	European Society Vascular Surgery	TcP02	Transcutaneous Oxygen Pressure
FFT	Fast Fourier Transformation	Tp	Time to peak
HHD	Hand-Held Doppler	VAR	Veno-Arteriolar Reflex
IC	Intermittent Claudication	VDT	Vibration Detection Threshold
IIA	Internal Iliac Artery	VPT	Vibration Perception Threshold
LDF	Laser Doppler Fluxmetry	Xe¹³³	Xenon isotope

INTRODUCTION

1.1 Peripheral Vascular Disease

All mammalian species depend on the circulation of blood, propelled by the heart through a vascular network, in order to supply tissues with oxygen and nutrients and remove metabolites. Cardiac output, balanced by variable arteriolar tone in the periphery, creates pulsatile pressure in the arteries and enables tissue perfusion to be varied to meet local metabolic demands. The relatively higher pulse pressures and flow velocities applied to arteries, as distinct from veins, create shear stress, which is an important factor in the development of the degenerative process of atherosclerosis. Atherosclerosis is characterized by the progressive accumulation of lipoprotein plaques in the sub-endothelial layers of large arteries, particularly at points of branching where shear forces tend to be concentrated. The particular susceptibility of man to atherosclerosis is probably related to longevity as well as exposure to toxins and nutritional imbalance. As atherosclerotic plaques extend they tend to produce loss of arterial wall compliance and reduction of luminal diameter resulting in stenosis and later occlusion with restriction in nutritional blood flow to the tissues served, known clinically as Peripheral Arterial Occlusive disease (PAOD).

In its early stages PAOD is silent but as arterial stenosis develops the ability to up-regulate blood flow distal to the stenosis in order to fuel increased tissue activity is lost. In the case of skeletal muscle in limbs this functional ischaemia manifests as “intermittent claudication” (IC), where exercising muscles receiving insufficient blood flow develop oxygen debt, leading to acidosis perceived as local pain and fatigue. IC is commonly associated with

arterial stenosis/occlusion at a single anatomical level in the limb. Beyond this point of occlusion there is usually a measurable fall in systolic arterial pressure compared to an uninvolved limb (usually an arm), allowing calculation of the “ankle/brachial pressure index” (ABPI), usually less than 0.9 and showing a further fall when the affected limb is exercised sufficiently to produce IC. Compensatory increase of flow in branch networks having the effect of redirecting blood around the axial occlusion is known as collateral development.

In its later stages PAOD impairs nutritive blood flow to the extent that the basal metabolic requirements of tissues are not met, producing sensorimotor dysfunction and failure of growth and repair. In the limbs this combination of pain and trophic changes is known as “critical limb ischaemia” (CLI) and its development usually implies arterial occlusion involving two or more anatomical levels. ABPI may be 0.5 or considerably lower and sustained CLI, if not corrected or not compensated by collateral development can progress to gangrene and amputation (Figure 1). Whilst only 5% of patients with IC require any vascular intervention, the risk of amputation in uncorrected CLI is 40% at six months (Falluji and Mukherjee, 2012, Norgren et al., 2007).

Therapeutic interventions in PAOD, either by surgical reconstruction or endovascular recanalization, are designed to relieve claudication or prevent amputation and their efficacy may be judged by their clinical success in achieving these objectives. However the impacts of these forms of intervention on circulatory pathophysiology are subtly different and change over time. This work was undertaken to see whether scientific measurement of the peripheral circulation in a spectrum of limbs with CLI before and after different forms of treatment could analyse their impact and possibly inform their selection.



Figure 1: Photographic illustration of distal limb ischaemia, with wet gangrene of the forefoot and toes, with soft tissue swelling (Grey et al., 2006).

CLI is most commonly caused by obstructive atherosclerotic arterial disease but rarely may follow vasculitis, thromboangiitis obliterans, cystic adventitial disease, popliteal entrapment or trauma. These mechanisms will not be considered further in this study due to their infrequency (Gresele et al., 2011).

Aetiology

The aetiology of atherosclerotic CLI corresponds to the risk factors for the development of atherosclerosis in other vascular beds. Figure 2 demonstrates the independent risk factors for CLI, which tend to act independently and are additive.

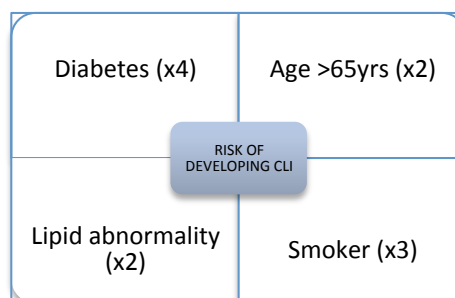


Figure 2: Independent risk factors for the development of CLI, with approximate magnitude of their effect on the development of CLI in parentheses (Norgren et al., 2007).

Other than increasing age, many of these risk factors are modifiable. Approximately 55% of patients with PAOD also suffer with hypertension (Clair et al., 2012). Diabetes confers around a four-fold increased risk for developing CLI, giving rise to predominantly diffuse, poorly collateralised, infra-geniculate disease. Smoking contributes significantly and the risk rises proportionately with cigarette consumption (Becker et al., 2011, Lepantalo et al., 2011, Norgren et al., 2007). Furthermore, the risk of developing CLI is significantly greater with a lower ABPI particularly <0.5 (Norgren et al., 2007). The increasing prevalence of these risk factors continues to fuel the increasing public health burden of PAOD.

Some other conditions, not primarily related to atherosclerosis, can lead to or cause progression to CLI without being actual risk factors. These include congenital or acquired hypercoagulable states, embolic disease (cardioembolic/popliteal aneurysms) and vasculitic conditions.

Disease Assessment

CLI is defined on the basis of specific assessment criteria made by clinical, haemodynamic and anatomical evaluation as follows.

Clinical

Assessment of vascular compromise can be made by clinical history and examination. Simple objective bedside tests can be employed to assess the macro- and/or microcirculation and together they should provide sufficient information to determine the presence, location/level and severity of the disease and whether revascularisation is possible or appropriate. Clinical signs pertinent to CLI are related to evidence of haemodynamic changes. Signs of severe rest

pain can be assessed by the patient's position and their willingness to elevate the limb to a horizontal position (Table 1). Ulcerations and gangrene typically occur at the extremity of the limb, the lateral aspect of the gaiter region and in areas of high pressure such as the heel or the metatarsal heads and in this context, should be due to objectively proven arterial occlusive disease.

Degree of rest pain	Limb position and relief of pain
First	Pain starts when horizontal but declines quickly
Second	Pain is only relieved when the limb is placed in the dependent position
Third	Patient has to remain in the seated position to relieve the pain

Table 1: The three degrees of rest pain and associated positioning of the limb. (Becker et al., 2011)

Trophic changes of skin, nail beds and toe pulp atrophy will be evident, together with a prolonged capillary refill time and poor venous refilling. Pallor of the foot on elevation and rubor in dependency is typical of low perfusion pressure and vasomotor paralysis (*see section on 'postural vasoconstriction' for more detail*).

Haemodynamic

Objective haemodynamic tests are performed to quantify disease severity and aid treatment planning:

Ankle-brachial Pressure Index (ABPI)

Yao introduced measurement of ABPI in 1969, demonstrating an objective pressure drop proportional to the severity of occlusive lesions and this remains the first line of evaluation

today (Becker et al., 2011). Ankle pressures non-invasively measure a drop in blood pressure across an area of increased arterial resistance, even in areas where flow levels can remain normal (Rutherford, 2000). It tends to decrease alongside the functional and anatomical severity of the arterial disease.

ABPI	Disease
0.91-1.3	Normal
0.90-0.71	Mild
0.70-0.40	Moderate
<0.40	Severe

Table 2: Pressure readings and their association with disease severity

Reliably, daily variation of ankle pressure indices do not tend to exceed 0.15mmHg, making it a reproducible test (Wahlberg et al., 1995, Line et al., 1996). Their ratio with upper limb pressure is used as a clinical outcome measure for disease severity and subsequent success of intervention or for follow-up. They are typically assessed with Doppler, as auscultation is extremely challenging in diseased vessels with low pressures. The value of ABPI varies slightly with the anatomical location of the lesion/s, but multi-level disease tends to produce the greatest reduction in indices (Rutherford, 2000).

Ankle pressures can be represented in a number of formats, either as an independent ankle systolic pressure, as an index with the brachial systolic pressure, or as a gradient across the vessel (brachial systolic pressure – ankle systolic pressure). Flow-limiting disease tends to be better detected using the latter two, whereas detection of critical limb ischaemia can be done with absolute pressures <50-70mmHg (Ouriel and Zarins, 1982). Nevertheless, clinical

discretion is fundamental to interpretation of results. With resting ankle pressures alone being of limited sensitivity in mild disease, comparison with post-exercise pressures can identify more subtle pathology, through augmentation of blood flow to calf muscle to demonstrate a larger pressure drop (Ota et al., 2005). In a similar way, ankle pressures have also been used in combination with reactive hyperaemia by measuring the systolic pressure in the ankle immediately after cuff occlusion and comparing it with that prior to arrest of inflow. They have been shown to be as reliable as exercise tests but avert the problems associated with poor mobility in elderly patients or patients with co-morbidities thus reducing compliance (Wyatt et al., 1990, Fowkes et al., 1988). They are, however, subject to variability, which can be reduced with serial measurements over time (Fowkes et al., 1988).

Measurements in patients with diabetic disease, uraemia or end stage renal failure and in the very elderly can also be inherently unreliable as a consequence of medial calcinosis, reflecting arterial wall rigidity from calcification of smooth muscle cells. Vessel incompressibility gives a high number of false negative readings in up to 25% of diabetics and less severe calcification may result in a normal ABI despite clinically significant PAOD (Lepantalo et al., 2011, Saucy et al., 2006).

Despite this, overall ankle pressures are an extremely useful and reliable indicator of disease in the macrocirculation in the vast majority of patients but their limitations can be exposed, particularly in some patient sub-groups, hence they should be used in conjunction with other clinical and anatomical findings and physiological parameters. They do not, however, provide information on nutritive blood supply in the microcirculation, particularly in the foot, nor about disease distal to the cuff (Jelnes, 1986, Stalc and Poredos, 2002). Moreover their

ability to predict limb survival is not good and in a study of 111 critically ischaemic limbs the mean ankle pressures and their associated indices did not differ significantly between patients who underwent amputation and those who did not (34mmHg and 0.22 vs. 43mmHg and 0.27) (Ubbink et al., 1999).

Toe-brachial Pressure Index (TBPI)

Toe pressure also correlates with disease severity and is a predictor of foot viability. It should be used in all patients suspected of having CLI as it reflects the overall obstruction in the arterial tree proximal to the measured digits (Tsai et al., 2000). Normally the toe pressure is about 30mmHg less than the ankle pressure (Falluji and Mukherjee, 2012). The great advantage of toe pressures is that they can provide accurate information on arterial pressures even if larger vessels are incompressible, as arterial calcification at this level is not a common occurrence. Therefore, they are a great adjunct in the assessment of diabetic patients. Toe pressures can also be represented as an index of brachial systolic pressure, which is typically 10-20mmHg below that of the arm or a gradient from the ankle of less than 60mmHg in healthy individuals (Rutherford, 2000). Like ankle pressures the degree of vascular impairment correlates strongly with the absolute toe pressure, with critically ischaemic patients having an absolute toe pressure less than 30-50mmHg or a TBPI <0.25 (Rutherford, 2000).

Cuff and strain gauge or photoplethysmography techniques can be employed to indirectly measure systolic arterial pressure at the level of the toe (Eickhoff, 1985). However, due to the requirement for specialist equipment it is not routinely used in all units. Limitations also exist, predominantly due to the ability of the equipment to detect the return of pressure as a

corollary of anatomical abnormalities like thickened nails and callus, absent toes usually from previous amputation, or even the presence of ulcers and gangrene can interfere with the readings. In one study of diabetics with CLI, inability to perform readings was as high as 16% (Lepantalo et al., 2011).

Skin Perfusion Pressures (SPP) and Digital Artery Pressure (DAP)

As alternatives to toe pressure measurements when these are practically unfeasible due to digital necrosis, skin perfusion or digital artery pressure can be determined. ‘The minimal external counter pressure on the underlying skin elicited by the pressure cuff, above which skin blood flow or digital artery flow ceases’, defines SPP and DAP respectively and both can be measured non-invasively (Yamada et al., 2008). They reflect perfusion in the smaller vessels and can provide a quantitative assessment of disease severity and predict the ability of a wound to heal.

The counter pressure is applied using a standard pneumatic cuff and increased above systolic pressure to achieve a near zero perfusion pressure. As cuff pressure is gradually reduced, often 5-10mmHg at a time, arterial inflow will increase with a rise in systolic pressure in microvessels in the skin. This can be detected either by laser Doppler, photoplethysmography or radioisotope washout techniques (Yamada et al., 2008). If the site of measurement is distal to the cuff, rather than beneath the cuff, distal arterial pressure (DAP) is measured as opposed to SPP. This reflects the pressure acting on the digital arteries serving the tip of the toe. Whilst it has been shown to correlate with SPP ($r=0.82$) DAP does tend to provide a more consistent reading amongst individuals (Fischer et al., 1995).

In normal individuals there is a physiological pressure drop between the ankle and the DAP of around 70%. This gradient is greater in patients with PAOD, indicating low driving pressure and flow since arteriolar resistance is already low, as vessels are dilated. A similar mean pressure drop between ankle and SPP exists in these two groups (Fischer et al., 1995). Thus, restoration of microcirculation/capillary flow in a healthy individual is around 50-70mmHg. The value is determined by the reappearance of pulsatile flow; however in patients with increasing severity of disease pulsatile flux is lost and distinguishing a rise in flux can be quite challenging (Fischer et al., 1995).

The sensitivity of SPP is increased with concurrent use of TBP but is still valuable when used in isolation, to assess disease severity and predict wound healing (Castronuovo et al., 1997, Yamada et al., 2008). Using a laser Doppler, Castronuovo et al characterised CLI by an SPP reading of <30mmHg (Castronuovo et al., 1997). Its advantage over TBP is that it can be used in the presence of toe ulceration, gangrene or toe amputation by assessing viable tissue. Furthermore, it overcomes the limitations of assessment of patients with medial calcinosis that ankle pressure is prone to (*see section 'ABPI' above*). Where accurate toe and ankle pressures are both available they have been shown to correlate well ($r=0.87$). This is true in both diabetic and non-diabetic patients ($r=0.85$ and 0.93 respectively) (Tsai et al., 2000, Yamada et al., 2008).

Whilst there are many factors to consider to achieve wound healing, patients with ulceration and a skin perfusion pressure of <30-40mmHg are significantly less likely to heal than those with perfusion pressures greater than this. In Castronuovo's series, 4 patients with SPP <30mHg did ultimately heal but it took much longer than the rest of the cohort who had all

healed within 6 weeks (Castronuovo et al., 1997). Therefore SPP can accurately predict wound healing with a greater reliability than other measures of distal perfusion (Castronuovo et al., 1997, Yamada et al., 2008).

There are a number of other measures of tissue perfusion, including methods such as transcutaneous oxygen pressure (TcPO₂), which will be discussed in more detail in '*How to assess skin circulation*'. By and large, these measures are not routine in clinical practice and tend to be reserved for research and trials.

Anatomical

Imaging techniques are used to assess the morphology of the disease and the inflow and outflow to occlusive lesions. They do not offer knowledge on the physiology and functional significance of the disease state but they do assist with detailed planning for revascularisation and the anatomical level at which re-perfusion is required. Primary imaging is done with Duplex ultrasonography, which is non-invasive and offers haemodynamic evaluation as well as an imaging modality (Cao et al., 2011). In experienced hands it is highly sensitive and specific and in infrainguinal disease there is excellent reproducibility with digital subtraction angiography, the gold standard (Eiberg et al., 2002). Its use can be limited in the visualisation of iliac disease due to the presence of overlying bowel gas or in patients who have extensive ulceration and therefore cannot tolerate examination over this area. With extremely poor inflow, significant oedema, obesity and/or highly calcified vessels images may be sub-standard (Clair et al., 2012). It is also the method of choice for surveillance following infrainguinal revascularisation.

Further multi-planar imaging with computed tomography (CT) or magnetic resonance imaging (MRI) and/or contrast angiography with digital subtraction (DSA) is performed depending on the individual, the unit and the requirement for accurate planning. Three-dimensional magnetic resonance angiography (MRA) is non-invasive, does not require the use of radiation and negates the need for intravenous contrast, which is advantageous in this population who are more susceptible to renal impairment. It has a tendency to over-estimate the degree and length of stenotic lesions but accuracy is further improved with the use of gadolinium and it is not affected by arterial calcification unlike CTA, which is useful particularly in the diabetic population. These non-invasive methods of imaging are now much more frequently used than traditional angiography, although this may be invoked if supplementary images or haemodynamic pressure assessments are required for treatment planning.

Disease Classification

The natural history of CLI is different from peripheral arterial disease as a whole and it is important to objectively distinguish this sub-group using the assessment criteria – clinical, haemodynamic and anatomical – as described above. PAOD does not necessarily progress through a stepwise trajectory through the clinical stages e.g. with intermittent claudication prior to the onset of rest pain, with many sufferers progressing from asymptomatic to CLI. This is particularly common in patients who are limited by other comorbidities and their functional status. Their clinical manifestation will depend on the extent of ischaemia and the time course of its development (Gresele et al., 2011).

Disease can be categorised clinically using the Fontaine and/or Rutherford classifications:

FONTAINE		RUTHERFORD	
I)	Asymptomatic	0)	Asymptomatic
II)	Claudication	1)	Mild claudication
	a) mild	2)	Moderate claudication
	b) moderate to severe	3)	Severe claudication
III)	Ischaemic rest pain	4)	Ischaemic rest pain
IV)	Ulceration and/or gangrene	5)	Minor tissue loss
		6)	Ulceration or gangrene

Table 3: The Fontaine and Rutherford classification of peripheral vascular disease (Dormandy and Rutherford, 2000).

The shortfall of these classification systems is the lack of delineation between symptoms and vascular status, as they do not take haemodynamic criteria into account. For example, ulceration may be due to arterial insufficiency or be caused by other pathologies such as chronic venous hypertension, peripheral sensory neuropathy and low flow states like congestive cardiac failure. Furthermore, these co-morbidities can also exacerbate less severe arterial occlusive disease and can complicate the clinical course (Dawson and Mills, 2007).

The changing definitions and inconsistent use of the term ‘CLI’ since it was first described in 1982, has made the incidence and prevalence of CLI difficult to accurately quantify. Definitions from working groups like the TransAtlantic Inter-Society Consensus (TASC) group have evolved over time:

‘Ischaemic rest pain with an ankle systolic pressure less than 50mmHg or toe systolic pressure <30mmHg; or if there is ulceration or gangrene and the ankle systolic pressure is <70mmHg or toe pressure <50mmHg.’

Figure 3: TASC II definition of critical limb ischaemia (Norgren et al., 2007).

Comparable definitions by the Modified/International vascular symposium working parties and the First/Second European Working groups have also been produced. They are not all

uniform with respect to exact haemodynamic parameters but have similarly depleted ankle and toe pressures. Some of the definitions of CLI from notable working groups are shown in the table below (Figure 4). There should be a causal link between symptoms and the severity of PAOD. In the case of CLI, one would expect failure to improve the arterial blood supply to result in a major amputation in the next six months to one year (Norgren et al., 2007).

Definitions of Critical Limb Ischaemia				
Fontaine Classification	International Vascular Symposium Working Party Definition	Modified International Vascular Symposium Working Party Definition	First European Working Group Definition	Second European Consensus Document
1. Stage 3 Rest Pain caused by arterial disease	1. Several weeks rest pain requiring opiate analgesia for at least 4 weeks <i>and either</i>	1. Severe rest pain requiring opiate analgesia for at least 4 weeks <i>and either</i>	1. Severe rest pain requiring opiate analgesia for at least 2 weeks <i>or</i>	1. Persistently recurring ischaemic rest pain requiring analgesia >2 weeks <i>and</i> ankle systolic pressure <50mmHg <i>and/or</i> toe systolic <30mmHg <i>or</i>
2. Stage 4 Ulceration and/or gangrene caused by arterial disease	2. Ankle Pressure <40mmHg <i>or</i>	2. Ankle Pressure <40mmHg <i>or</i>	2. Ulceration or gangrene <i>and</i>	2. Ulceration or gangrene of the foot or toes <i>and</i> ankle systolic pressure <50mmHg <i>and/or</i> toe systolic <30mmHg
	3. Ankle pressure <60mmHg in the presence of tissue necrosis or digital gangrene	3. Tissue necrosis or digital gangrene	3. Ankle Pressure <50mmHg	

Figure 4: Definitions of critical limb ischaemia (Wolfe and Wyatt, 1997).

All the definitions in use reflect the chronicity of the condition with symptoms lasting greater than two weeks, distinguishing it from acute limb ischaemia. Some groups also recommend the use of techniques to quantify the local microcirculation such as capillary microscopy, transcutaneous oxygen pressure (TcPO₂) or laser Doppler (1991).

Epidemiology

Difficulty stratifying the severity of ischaemia not only complicates estimation of the incidence and prevalence of CLI, but also our understanding of the natural history of the disease and the interpretation and prediction of the outcomes of treatments. PAOD in general has a reported prevalence of around 3-10% in the general population, increasing to 15-20% in those aged over seventy years. Estimates of PAOD incidence are currently around 50-100 per 100 000 per annum, with approximately 5-10% progressing from asymptomatic or claudicant to CLI at 5yrs (Adam et al., 2005, Falluji and Mukherjee, 2012, Gresele et al., 2011, Norgren et al., 2007). The prevalence of CLI is estimated at approximately 1% in the 60-90 year old population (Becker et al., 2011, Sigvant et al., 2007). In PAOD, there remains a significant male preponderance of approximately 3:1. In a Swedish study, this difference is reported to equalise when data is age-matched for CLI alone. However, whilst equivalent definitions to TASC were used, only patients with rest pain and an ABPI <0.5 were included (Sigvant et al., 2007). Some other studies however report gender differences in CLI prevalence with a 3:1 male preponderance similar to PAOD (Norgren et al., 2007).

Tyrell and Wolfe prospectively collected follow up data on critically ischaemic patients based on different working definitions of CLI. They aimed to identify patients who were likely to require amputation in the absence of successful reconstruction, given that a proportion of CLI patients will not progress to amputation without treatment (Tyrell and Wolfe, 1993). None of the definitions proved sensitive or specific in predicting outcomes and indeed results were worse still in the diabetic population. Four years later, Wolfe attempted to stratify severe ischaemia into high risk and low risk groups to be more representative of outcome (Wolfe and Wyatt, 1997). He analysed 6118 patients from recent publications and grouped them as high

risk/critical if patients presented with tissue loss and/or ankle pressure <40mmHg and low risk/subcritical if they had rest pain and/or ankle pressure >40mmHg. In the high-risk group only 5% of patients survived with an intact limb unless they underwent successful surgical intervention, compared to 27% in the lower risk group (Wolfe and Wyatt, 1997). Whilst both groups carry a high risk of limb loss the difference between the two groups was statistically significant and it suggests that revascularisation procedures are essential in the high risk group and other modalities of treatment such as sympathectomy or pharmacotherapy will not be effective in the high-risk group, but may prove to be of benefit in the subcritical group.

Confounding and Associated Conditions

CLI is the end-stage of arterial occlusive disease in the peripheries and therefore there is a high incidence of concurrent disease in other vascular territories, given that they are vulnerable to the same risk factors. The incidence of associated cerebrovascular disease (CVD) and severe cardiovascular disease (CAD) in this population is approximately 50-75% and 20% respectively (Bhatt et al., 2006). Coronary vessel assessment with angiography however, suggested up to 90% of patients would have some coronary disease present. For each pressure drop of 0.1 at the ankle, there is a 10% increase in the risk of a major cardiovascular event (Fowkes et al., 1991, Norgren et al., 2007). Intervention for CLI therefore carries a significant cardiovascular and cerebrovascular morbidity and mortality (~3%).

CLI is 10-20 times more common in the diabetic population than non-diabetics and the severity of their symptoms correlates with the severity and duration of their diabetes (Clair et al., 2012). Indeed, every 1% increase in glycosylated haemoglobin corresponds to a 25-28%

increase in relative risk of PAD (Lepantalo et al., 2011). In relation to this, Fosse found that diabetics have a 12x higher incidence of lower limb amputation than non-diabetics in a study of over 17000 amputations (Fosse et al., 2009). This risk climbs to 23-fold in those aged 65-74yrs (Lepantalo et al., 2011). In part this is due to the effects of diabetic vasculopathy on the microcirculation. In addition, it can confound the diagnosis of CLI as sensory neuropathy may mask symptoms of ischaemic rest pain and cause an underestimation of disease severity until further complications occur and significant vessel calcification can cause an overestimate of pressures. This is also true of the very elderly population who can have similarly rigid arteries. Infectious complications are more common in the diabetic population, which can precipitate wound breakdown and poor healing (*see section 'Diabetes'*). Furthermore, a sub-group of patients can be found to have critically low perfusion pressures but remain asymptomatic. This cohort of patients tends to be sedentary and may be diabetic with peripheral sensory neuropathy (Norgren et al., 2007).

Patient Prognosis

This patient population has a very poor prognosis. They are at exceptionally high risk of limb loss without some form of revascularisation procedure and approximately 25% will undergo amputation at first presentation. A significant number of patients will have other medical conditions and will be immobile or unable to live independently. Indeed, primary amputation may be preferable in these patients rather than aggressive revascularisation. At one-year the mortality rate is approximately 25% and the 5-year survival is 56% for class III and 33% for class IV patients, reflecting the underlying systemic atherosclerotic disease burden (Dorros et al., 2001).

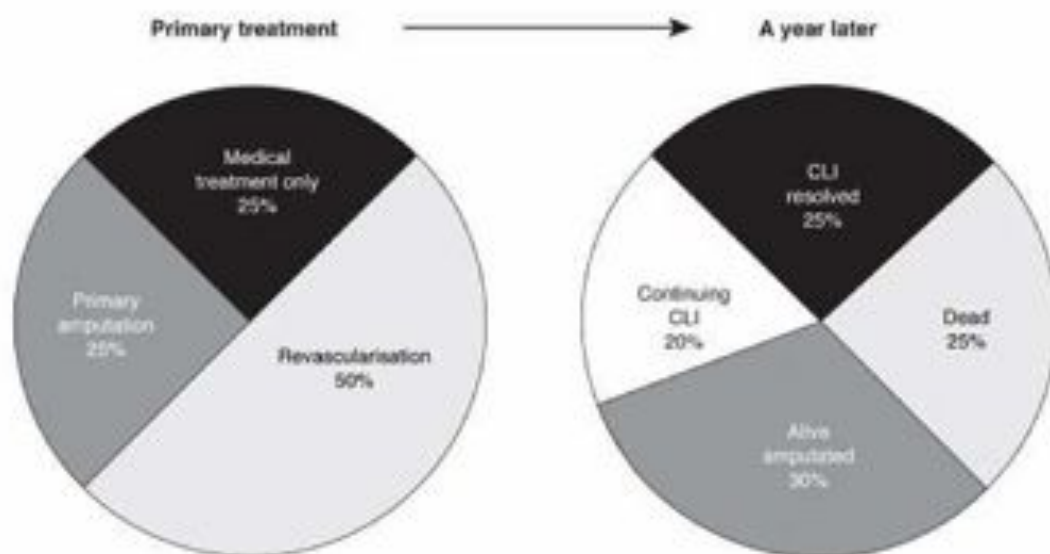


Figure 5: Percentage of the type of treatment patients with CLI undergo and their outcomes one year later (Norgren et al., 2007).

In an American prospective study, a cohort of 142 patients with uncomplicated stable ischaemic ulceration who were deemed unsuitable for revascularisation were followed up with non-interventional methods, predominantly dedicated wound care. Diabetes was present in over 70% of cases and chronic renal insufficiency featured in over a quarter of the patients. Patients who had complex or necrotising infections were not included and they usually required a revascularisation procedure. By 12 months 23% had required limb amputation and those whose wounds healed often took a year (52%) or more to achieve, which involved significant expense and limited activity by the patients. The study included all patients with ABI <0.7 however, those with an ABI <0.5 were significantly more likely to require amputation in keeping with CLI (15.7% vs. 30.8%, p0.02). Other measures of microcirculatory perfusion were not routinely assessed (Marston et al., 2006). Figure 6 below details the natural history of PAOD.

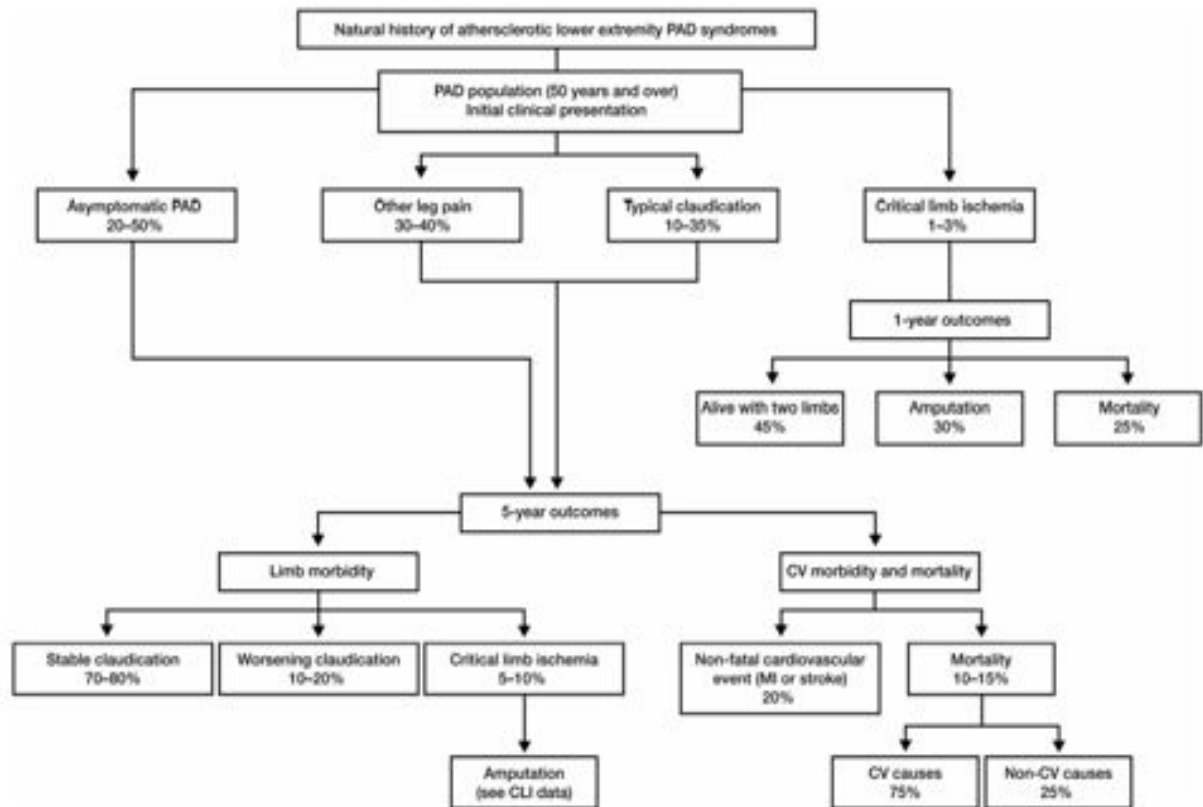


Figure 6: The natural history of atherosclerotic lower extremity peripheral arterial disease syndromes. PAD – peripheral arterial disease, CLI – critical limb ischaemia, CV – cardiovascular, MI – myocardial ischaemia (Norgren et al., 2007).

In summary, CLI is a complex disease process with attendant atherosclerosis in multiple vessel beds. It requires careful clinical, haemodynamic and anatomical assessment to ensure appropriate and timely intervention. In particular, diabetic patients can prove difficult to diagnose due to associated neuropathic and microcirculatory changes. Concomitant disturbance in flow in the microcirculation makes the skin most vulnerable to gangrene and ulceration.

1.2 Pathophysiology of Skin Circulation in CLI

Ulceration of the lower extremities is the typical manifestation of chronic ischaemic conditions, which is a state of low pressure and hypoperfusion. The ensuing complex processes of both macrovascular and microvascular dysfunction result in rest pain, trophic changes and impaired wound healing, pathognomonic of CLI. These features, representing a combination of anatomical deterioration and regulatory dysfunction of the skin resistance vessels and microcirculation, are predominantly due to inflammation, loss of sympathetic auto-regulatory mechanisms with oedema, altered blood composition and thrombosis, which culminate in further tissue damage (Abularrage et al., 2005).

Macrocirculation

The primary cause of CLI is the development of atherosclerotic plaques, which affect large and medium sized vessels. They are commonly found at arterial bifurcations where flow is more turbulent but they can occur anywhere in the arterial tree and can be very extensive. In early disease macrophages enter the sub-endothelial layer, encouraging accumulation and deposition of lipids. The adherent macrophages and the overlying endothelial cells promote inflammation and intimal hypertrophy through cell-signalling pathways. This results in an increase in the intima-media thickness of the vessel wall. Small areas of damage and erosion in the endothelium expose underlying collagen and stimulate formation of thrombus. Fissuring or rupture of the collagenous cap promotes further thrombus and haemorrhage into the plaque (Underwood, 2000). The progressive enlargement of fibro-lipid plaques, complicated by haemorrhage and rupture, and smooth muscle hypertrophy causes stenosis and obstruction within the vessel, which presents a high resistance to flow.

As a consequence, circulation downstream of multi-level multi-segment stenoses or occlusions has a critically low perfusion pressure and a reduction in blood supply to the tissues. The lack of pulsatile flow/pressure-dependent mechanical forces and shear stress on the vessel wall causes a respective deficiency in arterial wall remodelling and dilatation through endothelial mediators such as nitric oxide (NO) (Coats and Wadsworth, 2005, Kvernmo et al., 1999, Moncada and Higgs, 1991, Vallance et al., 1989). This causes further deterioration and limitation of exercise i.e. inadequate functional hyperaemia.

Compensatory angiogenesis and collateral formation recompense for a period of time but it is not sufficient in the longer term. Dependent positioning of the foot also increases local hydrostatic pressure and therefore increases nutritional blood supply to the ischaemic limb, but this too is of limited durability before tissue loss ensues.

Microcirculation

The skin microcirculation is a complex and dynamic system and an intimate relationship exists between pressure, flow and vascular structure and function (Berardesca et al., 2002). It is essential for ensuring adequate nutrition to the skin, preserving its barrier functions, and maintaining thermoregulatory regulation. Its control is mediated by local, systemic and reflex mechanisms induced by factors such as heat, posture and stress, in addition to its own inherent circadian rhythm to support tissue homeostasis.

The morphology of skin microcirculation has been studied in animals and humans alike and many similarities exist functionally and anatomically. The resistance arterial vessels – 10 - 150µm in diameter – control flow through two horizontal plexuses, one situated 1-1.5 mm

below the skin surface and the other at the dermal-subcutaneous junction. Ascending arterioles and descending venules are paired as they connect the two plexuses. From the upper layer, arterial capillaries rise to form the dermal papillary loops that represent the nutritive component of the skin circulation. The composition of skin microcirculation varies significantly depending on the location and therefore skin blood flow is not homogeneous (Fullerton et al., 2002). Glabrous or non-hairy skin such as that found at the tips of fingers and pulps of the toes contains predominantly (75%) arterio-venous (AV) shunts, giving rise to low resistance and high flow approximately 3-4x higher than skin without shunts (Kvernebo, 1988). These large-diameter dynamic microvessels are ideal for their primary function of thermoregulation, achieved via sympathetic modulation of vascular tone and perfusion; hence glabrous areas undergo wide fluctuations in flow (Abularrage et al., 2005, Fullerton et al., 2002, Jorreskog, 2012). On the other hand, in non-glabrous or hairy skin that does not possess AV shunts, nutritive flow is through capillaries supplied by resistance vessels that maintain a low basal flow but are responsive to autonomic sympathetic neural input, both vasoconstrictor and vasodilator, as well as local chemical and axon reflex influences. The metabolic demands of the skin only require a small fraction of the blood entering the skin (Rendell et al., 1998, Shepherd and Oberg, 1989). Optimal flow is also preserved by vascular myogenic activity, dependent on intra-luminal pressure dynamics to maintain basal vascular tone and total peripheral resistance (Coats and Hillier, 2000, Coats and Wadsworth, 2005).

Resistance vessels in CLI

The skin resistance arteriolar vessels that govern microcirculatory flow demonstrate structural and functional abnormalities in the pathogenesis of CLI long before the onset of clinical

symptoms (Celermajer et al., 1992). In early ischaemia electron microscopy demonstrates derangement in vessel architecture, secondary to hypoxic damage. There is a thicker basal lamina and endothelial swelling with associated 'gaps' between the elongated cells proximally, with collapsed and degenerated cells distally (Kelsall et al., 2001, Anvar et al., 2000c). This is further compounded by a reduction in the adventitia:lumen and media:lumen ratios and cross sectional areas, giving an overall reduction in wall thickness of around 30% (Coats, 2003, Coats and Hillier, 2000). This may be from atrophy, hypoplasia or remodelling of the cells, but regardless, this has a direct affect on the diameter/pressure relationship (Hillier et al., 1999). Interestingly, the media cross-sectional area is not significantly reduced in CLI, suggesting no loss of smooth muscle i.e. not hypoplastic (Anvar et al., 2000c, Hillier et al., 1999). The structure of a vessel wall is largely contingent on pressure-dependent vascular wall stress and shear stress (Coats, 2003, Coats and Wadsworth, 2005). Equally, the ability of a vessel to resist pressure-dependent change via myogenic response depends on its structural properties. Chronic ischaemia also leads to derangement of resistance vessel reactivity that has deleterious consequences for nutritive skin perfusion. Arterioles remain in a state of maximal vasodilatation in CLI such that their responses to the autonomic vasoconstrictor stimuli, adrenaline and noradrenaline, that maintain normal vascular tone are attenuated (Coats, 2003). Both active (myogenic) and passive (mechanical) tone may also be reduced. The constant low pressure and flow within the ischaemic microcirculation interfere with shear-dependent endothelial synthesis and activity of substances such as nitric oxide, causing additional loss of vasorelaxation and contributing to a situation of vasomotor paralysis. Over time with progression of disease, even the compensatory processes of vasodilation, angiogenesis and opening of collateral vessels are not sufficient to relieve symptoms (Figure 7).



Figure 7: Postulated overview of physiological adaptations of the vasculature in critical limb ischaemia (Coats and Wadsworth, 2005).

The consequences of these changes are evident in the disturbed distribution of blood flow to the nutritive microcirculation. Before examining this, it is pertinent to consider the methodology that is available for investigation of human skin microcirculation.

Techniques for assessment of skin microcirculation in CLI

There is no single standardised method for assessing and quantifying microvascular reactivity and the associated reflex and control mechanisms, rather an array of available modalities. This reflects the complexities of the microvasculature, in part due to the wide variation in skin blood flow on a day-to-day basis and between different sites (Sundberg, 1984). Furthermore,

arterioles are not readily accessible for observation and hence most techniques use indirect measures. The diversity of methodology and investigative models in the literature is based on studies of human or animal subjects assessed in health or with pathology, which may have been produced artificially as in the case of many animal studies. In early studies, indirect methods such as thermography, radioisotope washout or limb plethysmography were used to measure skin perfusion in areas such as the forearm or lower limb/hind leg that provide readily accessible vascular beds, following a series of interventions which elicit different substances suspected to have a key role in ischaemia-induced vasodilatation. In addition, blood flow could be assessed after periods of exercise or occlusion at rest to assess active and reactive hyperaemia respectively. These methods are, however, not necessarily specific to cutaneous tissue and therefore confounded by ischaemic alterations in such as adipose or muscular tissues.

More recent techniques permit direct observations of basal skin perfusion and responses to the different interventions that provoke specific regulatory mechanisms. These have been reviewed by (Roustit and Cracowski, 2012) and (Abularrage et al., 2005), the latter in relation to vascular disease. The following table summarises common methods employed for structural and functional assessment of the skin microcirculation in CLI:

Method	Technique	Assess	Measure
Laser Doppler Fluxmetry (LDF)	Non-invasive, continuous measure of allowing detection of short-term variations. Can be used with a variety of provocations / interventions including iontophoresis (see below)	Flow	Direct
Laser Doppler Imager	Utilises Doppler principle & directs laser with a large mirror to raster across skin, capturing a more sizeable area. Can be used with iontophoresis.	Flow	Direct
Iontophoresis	Non-invasive technique delivering vasoactive substances (NO agonists Ach & SNP, and antagonists) transdermally, using an electrical potential difference. Can be used simultaneously with LDF.	Flow	Direct
Video / Dynamic capillary nailfold microscopy	Basic light microscopy looking at density and morphology Quantitative measure of capillary flow velocity in a small number of capillaries. Can also be combined with fluorescein to show flow patterns	Flow	Direct
Transcutaneous tissue oxygenation (TcpO₂)	Non-invasive quantification of oxygen transfer to the skin after heating. Reacts very slowly to changes in blood flow.	Oxygenation	Direct

Table 4: Methods of assessing functional measures of skin microcirculation. NO – nitric oxide, Ach – Acetylcholine, SNP – sodium nitroprusside, AV – arterio-venous (Abularrage et al., 2005, Schabauer and Rooke, 1994, Kubli et al., 2000, Johnson et al., 1984, Winsor et al., 1987).

Laser Doppler Fluxmetry (LDF)

LDF is non-invasive and based on the changes in frequency of low energy laser light (Doppler effect) when it is scattered by moving red blood cells in the microcirculation. It is a continuous dynamic measure easily performed at any skin location allowing detection of short-term variations in blood flow (Bircher et al., 1994). It correlates well with other modalities, which all measure the microcirculation at slightly different levels of penetration (Kvernebo, 1988, Saumet et al., 1988). LDF detects sub-capillary flow as well as flow in the nutritive capillary bed and AV anastomoses. This is of importance in conditions where blood supply is redistributed pathologically i.e. in diabetes (Tooke et al., 1983) (*See section ‘Diabetes’*). It does not interfere with venous haemodynamics or cause injection trauma that

may alter local circulation (Orlandi et al., 1988). LDF can be used in isolation or coupled with microdialysis or iontophoresis in order to study perfusion and flow responses to vasoactive substances (Fullerton et al., 2002, Vongsavan and Matthews, 1993).

‘Normal’ basal or resting flux values are subject to significant spatial and temporal variations, particularly in areas of AV shunts and are therefore referred to as arbitrary perfusion units (APUs) (Sundberg, 1984). The coefficient of variation in healthy human volunteers has been shown to be as high as 47.6%, even within a short time frame (Orlandi et al., 1988). Significant differences in basal flux have been reported between healthy subjects and those with structural vascular abnormalities such as PAOD, although in some instances it has not always shown sufficient differentiation between normal limbs and those with disease due to a degree of overlap (Shepherd and Oberg, 1989, Morales et al., 2005, Wahlberg et al., 1990, Abu-Own et al., 1993, Van den Brande and Welch, 1988, Seifert et al., 1988). On the other hand, it does permit real-time examination of skin perfusion during specific interventions that invoke vasoreactivity and as such enables characterisation of deficits in CLI. Furthermore, the recent development of laser Doppler imaging gives regional integrative data over larger areas than the conventional single or multi-probe LDF, thus compensating for heterogeneity of perfusion.

LDF KEY POINTS

- Acceptance of the LDF technique depicted through its widespread use
- Generally a linear relationship between LDF and blood flow
- It is specific to superficial cutaneous vessels
- Allows simple, continuous, non-invasive measurement
- Heterogeneous nature of microvascular architecture does not allow absolute readings of blood flow to be measured

Dynamic capillaroscopy

Capillaroscopy uses optical microscopy to visualise the 5-7 μ m diameter surface microvessels and their associated networks in vivo. Capillary morphology such as number, tortuosity and diameter can be recorded and/or viewed on a video monitor (Wright et al., 2006). Functional measurements of erythrocyte transit times within capillaries allow assessments of flow (capillary blood cell velocity, CBV), which may be more sensitive to early changes in disease processes (Chang et al., 1997). More sophisticated software is used in dynamic capillaroscopy to enable measurement of flow dynamics and permeability (Abularrage et al., 2005). The use of fluorescein gives much clearer images and better functionally assesses the capillary system by progressive diffusion of dye into the interstitial medium, confirming perfusing capillaries (Berardesca et al., 2002). The main limitation is the relatively small sample area that can be studied (Tooke et al., 1983).

In healthy individuals, Lamah and colleagues confirmed that the total numbers of capillaries were not influenced by foot position, with a maximum 4% difference in observed capillary numbers between different foot positions over the period of one month (Lamah et al., 2001).

They also demonstrated the percentage ratio of perfused to total capillaries was 54.2% suggesting that under physiological conditions half of the nutritive capillaries are not perfused, allowing recruitment of capillaries to take place on physiological demand (Lamah et al., 2001). Capillaroscopy can also be used synchronously with other techniques, including LDF, which measures plexus and anastomotic vessels rather than just capillary flow, yielding complementary results particularly in conditions where blood flow may be pathologically redistributed such as diabetes (Tooke et al., 1983). This group also looked at the ratio of perfused to total skin capillaries in the PAOD population using dynamic capillaroscopy and found a higher proportion of perfused capillaries in this group compared with controls, although this was shown to be a consequence of a decreased total number of capillaries in the PVD group. The microcirculation was not simultaneously measured to assess flow (Abularrage et al., 2005). Furthermore, in critical limb ischaemia CBV in the supine position was found to be significantly lower than in claudicants or healthy subjects (0.04 vs. 0.20 vs. 0.19mm/s respectively, $p=0.005$), whilst capillary density and LDF did not differ. This is in keeping with lower ankle and toe pressures in the CLI group (de Graaff et al., 2003).

TcpO₂

This technique is used to assess the metabolic state of the limb. Electrodes on the skin surface are heated to 44°C and measure the oxygen diffusing through the skin from superficial vessels. This is reflective of the tissue perfusion and vascularity of the limb (Falluji and Mukherjee, 2012). A normal TcpO₂ value is around 60mmHg. The diagnosis of CLI is confirmed with a value <30mmHg (Norgren et al., 2007). Certainly a TcPO₂ >40mmHg is likely to see greater than 90% of neuroischaemic ulcers heal and accordingly patients who fail to improve following surgical revascularisation have an associated lower TcpO₂ than those

who improve (18.3 ± 25.3 vs. 33.5 ± 18.7) (Ray et al., 1997a). Pressure measurements alone have a poor predictive value and do not accurately predict the outcome of intervention and are not reflective of $TcpO_2$ readings. $TcpO_2$ correlates well with other microcirculatory parameters such as LDF time to peak with both measures having better association with clinical improvement ($p0.04$ and $p0.02$ respectively, ABPI and toe pressures were not statistically significant) (Ray et al., 1997a). Interestingly in percutaneous procedures $TcpO_2$ undergoes a transient decrease immediately following the intervention, before improving if it has been successful. This is likely to be a reflection of endothelial dysfunction caused by contrast material and does not correlate with macrocirculatory measures such as ABPI (Stalc and Poredos, 2002, Wagner et al., 2003, Wildgruber et al., 2007).

Whilst $TcpO_2$ is shown to be a sensitive indicator of CLI, the disadvantage is that it is time consuming to perform due to the time required for the process of heating. It is also less reliable in the presence of oedema (Abularrage et al., 2005). Furthermore, assessment of perfusion using LDF and reactive hyperaemia has been shown to be marginally more accurate than $TcpO_2$ in predicting clinical response to revascularisation in CLI ($p0.02$ vs. $p0.04$ respectively) (Ray et al., 1997a). For this reason, it was not used in this study and will not be considered further.

Assessing microcirculatory reactivity in CLI

The predominant mechanisms for regulation of skin microcirculation are neural, myogenic and endothelial. Cutaneous sympathetic neural activity induces vasoconstriction in response to cold or autonomic reflexes in all types of skin. In glabrous (non-hairy) skin, which is rich in AV shunts this is the only neurogenic mechanism but in non-glabrous (hairy) skin, there is

dual innervation of skin vessels with a non-adrenergic vasodilator pathway for vasodilation for thermoregulatory purposes (Charkoudian, 2010, Hodges and Johnson, 2009). The vascular myogenic response is an inherent property of smooth muscle in the walls of small arteries and arterioles, allowing these principal resistance segments of the microcirculation to respond to changes in transmural pressure. Elevated intraluminal pressure leads to myogenic constriction, whereas reduced pressure leads to myogenic dilation. This mechanism plays an important role in the fluctuations of flow within cutaneous microvascular networks (Lossius & Eriksen, 1995). The microvascular endothelium seeks to control vascular tone to maintain pressure, flow and distribution at an appropriate level. Several endothelial factors play a role in its regulation, of which the principal regulator is nitric oxide (NO), a powerful endothelial-derived vasodilator. NO regulates skin and other vascular beds both at rest and during periods of increased flow (Wong et al., 2003). It can be inhibited by NG monomethyl-L-arginine (L-NMMA) in vascular endothelial cells and intra-arterial administration causes a dose-dependent decrease in skin blood flow secondary to vasoconstriction. L-NMMA has no intrinsic vasoconstrictive properties itself and hence the mechanism of its inhibitory action on vasodilation is via NO (Meredith et al., 1996). This can be reversed by L-arginine, the amino acid it is synthesised from (Figure 8) (Moncada and Higgs, 1991, Vallance et al., 1989).

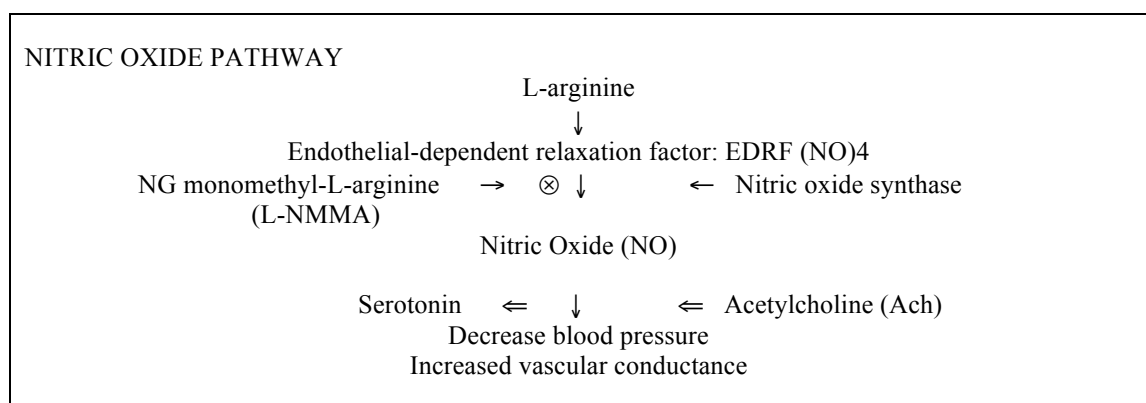


Figure 8: The nitric oxide pathway and its effect on blood pressure and vascular conductance.

Chemical stimulation with substances such as acetylcholine or substance P cause NO to constantly emanate from the arterial endothelium during periods of basal flow, to maintain a dilator tone and lower vascular resistance (Moncada and Higgs, 1991). In synergy with prostacyclin, platelets also produce NO as a method of regulating platelet aggregation. Therefore, impairment of NO synthesis results in increased platelet aggregation and adherence to the vessel wall, as well as vasoconstriction. In contrast, sodium nitroprusside (SNP) acts directly on cGMP in vascular smooth muscle cells and can be used to test endothelial-independent dilator function (Kvernmo et al., 1999, Jagren et al., 2002). The inherent characteristics and action of these substances make them ideal for investigating endothelial dysfunction and for use in iontophoresis.

The impact of CLI on these mechanisms regulating skin perfusion can be investigated in a number of different ways, one of which is the examination of patterns of microcirculatory flowmotion, or oscillations, in resting tissue. In addition, vascular tone is dependent on control by a number of variables including the autonomic nervous system, emotional and environmental stimuli, medication and orthostatic position. It is normally maintained in a relatively constricted state through inherent efferent nerve activity and external stimuli can also trigger sympathetic reflex responses to evoke changes in vessel resistance (Fullerton et al., 2002, Hagbarth et al., 1972). Provocation tests can therefore be employed which permit quantification of a change from baseline resting flux level, as a result of the given stimulus. This allows evaluation of microcirculatory control mechanisms depending on the nature of the stimulus.

The most common tests used are:

- Reactive hyperaemia – to test myogenic / metabolic mechanisms
- Local heating or cooling – to test dilator / constrictor capacity
- Maximal heating – to test axon reflex dilation and nitric oxide function
- Iontophoresis – to test mainly endothelial reactivity
- Postural vasoconstriction
- FFT analysis of LDF signals during reactive hyperaemia, heating or iontophoresis

Flowmotion

Vasomotion or flowmotion is the rhythmical variation in blood flow within tissues and is distinct from flux. It is composed of waves or ‘oscillations’ of different amplitudes and frequencies from vascular smooth muscle and reflects differing vascular regulatory processes (Cracowski et al., 2006, Tikhonova et al., 2010, Kvernmo et al., 2003). Measurement of resting skin blood flow by LDF will continuously change with the microcirculatory milieu however; characteristic frequency bands have emerged over time originating from different mechanisms of vascular regulation. These waves were originally separated into ‘large’ and ‘small’ reflecting a difference in amplitude and a concomitant variation in frequency. The former are more often seen in healthy individuals, with smaller waves of higher frequency seen in patients with increasing severity of vascular disease (Seifert et al., 1988). Later, three bands emerged to include the addition of a ‘pulsatile frequency’, shown to be consistent with heart rate (Hoffmann et al., 1993). Spectral analysis of LDF in the time and frequency domains has subsequently allowed discrimination between oscillations in skin perfusion.

Specific frequency ranges present in the signal can be sifted out and the total and individual power ascribed to them and their physiological origin can be identified. The five spectra and their associated frequency intervals are shown below:

Interval	Frequency (Hz)	Physiological Origin
I	0.009-0.02	Endothelial/Metabolic
II	0.02-0.06	Neurogenic
III	0.06-0.15	Myogenic
IV	0.15-0.6	Respiratory
V	0.6-2.0	Heart rate

Table 5: Frequency bands of flow motion waves (Avery et al., 2009, Kvernmo et al., 1999, Stewart et al., 2004, Tikhonova et al., 2010).

Minor variations exist in the exact intervals of frequency bands in the reported literature. Their origins are determined through systematic stimulation and blockade of substances known to have an effect on the specific pathways controlling vascular tone. The lowest frequency band reflects the intrinsic activity of the endothelium and contributes around 20% of blood perfusion in a healthy individual, despite oscillations only occurring at a frequency of approximately one minute (Kvernmo et al., 1999, Saumet et al., 1988, Kvernmo et al., 2003). Around 0.04Hz spontaneous fluctuations depend on axon reflex-mediated responses from autonomic sympathetic modulation. Administration of local anaesthetics or vagal stimulation can induce sympathetic denervation (Berardesca et al., 2002, Saumet et al., 1988). Myogenic vasomotion is due to inherent spontaneous variations in myogenic activity of the vascular smooth muscle cells of arteries and arterioles causing a change in microvascular calibre through contraction and dilatation. The respiratory element corresponds to the changes in venous return, causing peripheral vasoconstriction and dilatation. The highest

frequency corresponds to the vascular wall transmission of the stroke volume. By assessing these parameters in normal and pathological conditions, the regulation of the microcirculation can be studied and deviation from the normal microvascular oscillations can be used as a marker of disease.

Reactive Hyperaemia (RH)

Post-occlusive reactive hyperaemia is a simple and non-invasive provocation test combined with LDF to measure skin perfusion. It is defined as ‘the temporary increase in skin blood flow above baseline levels following the release of a brief arterial occlusion’ (Cracowski et al., 2006). It is a quantitative global assessment of microvascular function, representing both the function of the cutaneous microvasculature and the haemodynamics of larger vessels, through its ability to compensate after a period of ischaemia (Arora et al., 1998, Wong et al., 2003). In a normal healthy individual with a functionally intact vascular bed, there would be a rapid yet ephemeral peak in flux within a few seconds of removal of the occlusion and then a sustained hyperaemia, before a return to baseline within minutes (Figure 9) (Van den Brande and Welch, 1988, Zhao et al., 2004). The increase in blood flow allows repayment of the nutritional debt and clearance of metabolites, which accumulate during periods of circulatory compromise (Larkin and Williams, 1993, Wahlberg et al., 1995). This is supported by the finding that the total hyperaemic response time and the peak flow values are increased with increasing arterial occlusion times in healthy individuals (Wong et al., 2003). It is suggested that the area under the curve is the equivalent of the debt accrued during the period of circulatory arrest, representing the excess blood flow ‘repayment’ during the hyperaemic period (Patterson and Whelan, 1955).

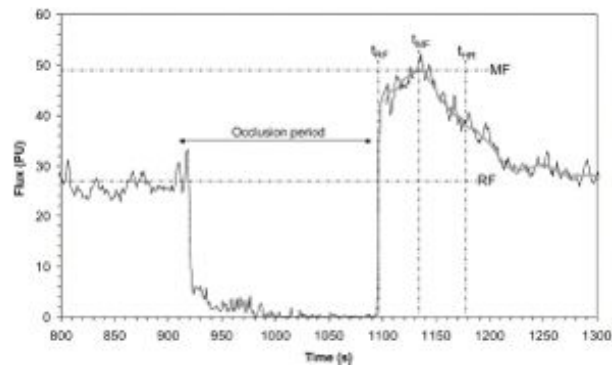


Figure 9: A PORH response in a control subject. The black line is the signal averaged over one second. The blood flux value drops almost immediately after cuff inflation, with a small residual output still present due to continuous red cell flux or vasomotion in the microcirculation. PU – perfusion units, t_{RF} – time to resting flux, t_{MF} – time to maximum flux, t_{HR} – time to half recovery of PORH, MF- maximum flux, RF – resting flux (Morales et al., 2005).

The exact mechanisms behind the response are complex and not fully defined. Interactions and facilitation of other pathways and/or mediators, such as NO, are likely to be occurring simultaneously (Cracowski et al., 2006, Zhao et al., 2004). Proposed mechanisms include:

1. Vasodilator metabolite release from ischaemic tissues i.e. prostaglandins, adenosine, histamine which is released into circulation with removal of the cuff (Patterson and Whelan, 1955)
2. Smooth muscle response to reduced oxygen tension
3. Myogenic response to a reduction in the transmural pressure of a static circulation
4. Stimulation of perivascular sensory nerve fibres causing release of potent vasodilators such as calcitonin gene related peptide (CGRP) and substance P neuropeptides (Larkin and Williams, 1993, Wong et al., 2003, Zhao et al., 2004)
5. Shear stress on the vessel wall following restoration of flow, acting as a stimulus for prostaglandin and nitric oxide production (Binggeli et al., 2003)

The complexity of the response is further highlighted by the inability to completely abolish the vasodilatory response when one or more of these mechanisms are blocked, either by inhibitors, antagonists or local anaesthetics (Larkin and Williams, 1993, Nowak and Wennmalm, 1979).

Many parameters can be quantified from a single PORH response. The commonest and most discriminative method is the time to peak (Tp). This is defined as the time of the highest value after release of the pressure cuff and is determined by flow velocity (Wahlberg et al., 1995). Tp is affected by vessel compliance, capacitance of run-off vessels and blood viscosity. Some descriptive measures have also been used to describe the shapes of the responses, such as whether it has a 'single or double' humped peak (Line et al., 1996). Additional quantitative parameters to Tp are used but many are exposed to more inter- and intra-subject variability than data expressed as maximum vasodilatation:

- ❖ Raw value of the peak
- ❖ Peak percentage from baseline value
- ❖ Time to second peak
- ❖ T/2 overshoot - time to reach 50% of the peak value on the down slope
- ❖ T/2 recovery - time to reach 50% of basal blood flow
- ❖ T recovery - time to reach basal blood flow value
- ❖ Area under the curve (AUC) - total blood flow in excess of the resting level or total hyperaemic response (Wong et al., 2003)

Successive measures of reactive hyperaemia at the toe pulp and the ankle of the lower limb, in healthy subjects and/or PAOD subjects, have been shown to be highly reproducible using laser Doppler fluxmetry (Kvernebo et al., 1989, Van den Brande and Welch, 1988). Reproducibility has also been demonstrated between left and right upper limbs under the same conditions, suggesting that one limb can be used as a control for the other (Patterson and Whelan, 1955). The average coefficient of variation for skin blood flow at the peak of the response was 8% in a group of 20 healthy volunteers, with 2 readings taken on the same day 1 hour apart (Orlandi et al., 1988).

Local heating or cooling

Temperature changes are provoked either directly from within the probe or by immersing the test area in cool water of around 15°C or water with a temperature of around 42°C to allow testing of dilator capacity of vessels. This technique is not widely used in the context of PAOD as they already have maximally dilated vessels as a consequence of the disease process.

Maximum heating

Local thermal hyperaemia causes a temperature-dependent sustained increase in cutaneous flow, with maximal dilatation between 42-44°C, corresponding to the maximum vasodilator capacity of the vessels. This is mediated initially by a local axon reflex causing a rapid ephemeral rise in flow and subsequently by nitric oxide with a characteristic 20-30 minute plateau (Cracowski et al., 2006). Whilst it provides some characteristic traces in certain

disease processes, it is difficult to extrapolate data due to differing responses to heating in different pathologies and not being able to distinguish between functional and structural deficiencies (Cracowski et al., 2006).

Iontophoresis

Whereas RH invokes a complex mechanism of vasodilation detectable by LDF, an alternative for assessment of specific vascular control mechanisms are the more invasive technique of iontophoresis. This utilises the principle that a charged vasoactive drug in solution will migrate across the skin under influence of a direct low-intensity electric current and chemically stimulate the endothelial release of nitric oxide, resulting in an effect on perfusion measured as a change in red cell flux (Cracowski et al., 2006). The most commonly used test agent is acetylcholine (Ach), which stimulates nitric oxide production, hence mimicking NO-dependent endothelial vasodilation and allowing assessment of endothelial vascular function. Other drugs are sodium nitroprusside, a NO donor used to test smooth muscle function, and the autonomic constrictors adrenaline and noradrenaline (Kvernmo et al., 1999).

Postural vasoconstriction (PVC)

PVC is a response of resistance vessels triggered by an increase in hydrostatic pressure in the microcirculation either on assuming the upright from the supine position (orthostasis), or on limb dependency. It is thought to be a protective mechanism that exists to minimise the effects of the increased pressure being transmitted to capillaries in order to prevent formation of oedema (Shepherd and Oberg, 1989, Wahlberg et al., 1990). During orthostasis control of systemic arterial pressure is mediated by the central baroreflex as blood pools in the lower

body and central arterial and cardiopulmonary receptors are stimulated by the reduction in mean arterial pressure to generate sympathetic neural activation. In conjunction with this, there are two contributory local constrictor mechanisms, the myogenic response and the veno-arteriolar response (VAR). The former occurs with the rise in arterial hydrostatic pressure, increasing pre-capillary arteriolar wall stretch and triggering intrinsic vascular smooth muscle contraction in the dependent limb /tissues. The latter is evoked by hydrostatic elevation of venous transmural pressure above a threshold level of 25mmHg, which distends venous stretch receptors and induces pre-capillary vasoconstriction through a local sympathetic neural pathway (Okazaki et al., 2005). The effect of both of these mechanisms can be eliminated with the use of local anaesthetic blockade or alpha antagonists to block adrenergic stimulation, suggesting the response is co-ordinated locally through nervous influence (Henriksen, 1976, Okazaki et al., 2005). The VAR is independent of central control as it is observed during spinal block and is thought to be neural as it can be inhibited by local anaesthetic application, and to be an autonomic sympathetic neural mechanism as adrenergic antagonists attenuate it. The VAR can reduce skin flux by 50-70% in healthy individuals (Jelnes, 1986, Jepsen and Gaehtgens, 1995, Henriksen, 1976).

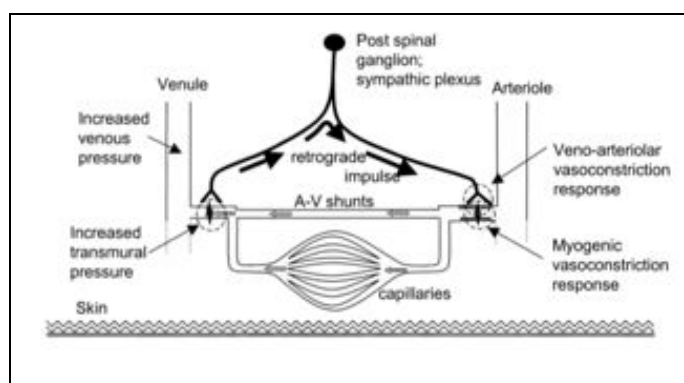


Figure 10: Schema of microcirculatory regulation mechanisms of skin perfusion with change to the sitting position. Increase in orthostatic pressure with dependency activates two local vasoconstriction responses, the myogenic response and the venoarteriolar response (de Graaff et al., 2003).

Height dependent vasoconstriction enables smaller central changes in resistance to maintain a constant mean arterial blood pressure (MAP), thereby limiting increasing afterload and redistributing flow to non-dependent tissues (Jepsen and Gaehtgens, 1995). It is coupled to respiratory movement, with deep inspiration causing vasoconstriction even in the presence of dependency (Hagbarth et al., 1972, Jepsen and Gaehtgens, 1995). The latter is likely to represent a more uniform response at rest and in orthostasis, to maintain steady flow despite alterations in systemic pressure. Whereas the veno-arteriolar reflex (VAR), is generated when transmural pressures are increased locally (Jepsen and Gaehtgens, 1995, Rordam et al., 1988). The effect of the VAR is highlighted by the use of a foot pump. In the dependent position, emptying the veins and reducing hydrostatic pressure attenuates the VAR. This is due to the alteration in the AV pressure gradient, mediating a significant increase in flow, as detected on LDF. This effect is not demonstrated in the supine position because hydrostatic pressures are not present in the lower limb (Abu-Own et al., 1993).

The changes associated with an upright posture vary according to skin site along the axis of the body because of height-dependent hydrostatic loading. From a series of LDF readings from the forehead to the lower limb, the postural vasoconstrictor response and associated reduction in flow become sequentially more pronounced with more distal readings; LDF decreased by $18.0 \pm 7.1\%$ above heart level, by $30.3 \pm 9.1\%$ at heart level and by $69.6 \pm 9.6\%$ at upper thigh level and in the more dependent skin regions, $p < 0.01$ (Jepsen and Gaehtgens, 1995). In the lower limb, constrictions were found to be comparable to one another, not demonstrating a height-dependent difference in response. Changes in LDF values on the plantar surface of the toe and palmar surface of the finger are related to the presence of

arteriovenous anastomoses and therefore a difference in flux would be expected between toe and shin locations but not between other skin regions of the lower limb such as shin and foot.

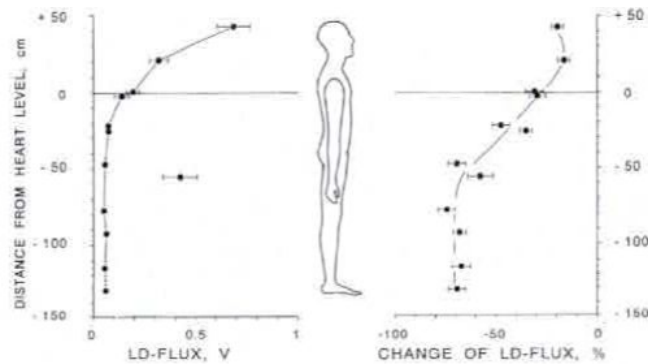


Figure 11: Left: skin LD-flux in the standing subject. Height dependent LD flux is only seen above heart level. Single stray points represent measurements at finger. Right: percent changes on standing up from recumbency of all measuring sites in relation to distance from heart level. Values are means \pm SE of experiments in 6 subjects (Jepsen and Gaehtgens, 1995).

When analysis was made between readings taken at the finger and toe in supine and standing positions, but with the finger or toe held at heart level, the relative reductions seen in LDF were not significantly different ($29.3 \pm 13.0\%$) (Jepsen and Gaehtgens, 1995). This technique eliminates the effect of hydrostatic pressure. Similarly if only the position of the hand or foot relative to the heart level is altered without a change in body posture, alterations in LDF are also seen (Jepsen and Gaehtgens, 1995).

Measuring PVC by LDF

LDF is an ideal tool for directly measuring reflex cutaneous vascular responses to simulated orthostasis, which can be used in conjunction with sympathetic nerve blockade. Xenon washout techniques have also been used to measure blood flow in subcutaneous tissues in response to stimuli of vasoconstriction (Eickhoff and Henriksen, 1985, Henriksen, 1976, Rordam et al., 1988).

Independent assessment of the VAR has been measured using proximal cuff inflation at approximately 40-50mmHg, to induce venous congestion. Whilst its advantage lies in not activating the myogenic arm of the response, it does however reduce the local perfusion pressure between arteries and veins and may subsequently overestimate the blood flow, independently to the VAR (Okazaki et al., 2005). Assessing LDF in recumbence with only one limb in a dependent position without the use of proximal cuff inflation can alleviate this perfusion problem. There will be no postural change and therefore no stimulation of the central baroreceptor reflex, so heart rate and MAP do not change. It will however provoke the myogenic response due to the increase in arterial hydrostatic pressure gradient between the heart and limb (Okazaki et al., 2005).

1.3 Control Mechanisms in CLI

Basal perfusion of skin in CLI

In early studies the development of direct observational techniques enabled visualisation of skin perfusion in patients with peripheral arterial disease of varying degrees including CLI. Overall skin perfusion measured by LDF was reduced at rest in severe ischaemia in the plantar foot and heel (Saucy et al., 2006) and at the toes (Kvernebo, 1988), although there was some overlap in patients with less severe ischaemia. Video microscopy established in healthy individuals that the capillary density in the toe was considerably greater than in the foot dorsum, the latter showing marked heterogeneity in distribution of capillary vessels (Lamah et al., 1996). In patients with CLI and ulceration, capillary numbers in both regions were 2/3 but of these ‘anatomically present’ vessels in the dorsum, a much higher proportion (86%) were

perfused after Na fluorescein injection than in controls (55%) (Lamah et al., 1999). Bongard and Fagrell found that total blood flow evaluated by LDF was significantly increased in the toes of PAOD patients, which may be explained by this phenomenon of proportional capillary perfusion (Bongard and Fagrell, 1990). In addition, perfusion of foot capillaries was inhomogeneous and the vessels were dilated, displaying fluorescein leakage typical of ischaemic damage that promotes oedema formation (Junger et al., 1989). This maldistribution of nutritive microcirculatory flow is typical when the supplying arterioles are maximally dilated in their state of vasomotor paralysis, as a mechanism for increasing oxygen and nutrient delivery to ischaemic skin. As a consequence of disrupted nutritive microcirculation, tissue oxygenation in the distal extremities is reduced in CLI. Scheffler & Rieger (1992) reviewed the utility of $TcpO_2$ data in peripheral arterial disease, underlining that values in the symptomatic foot $< 10\text{mmHg}$ when supine and 45mmHg when dependent were characteristic of chronic CLI and predictive of future amputation (Scheffler and Rieger, 1992).

Flowmotion in CLI

Flowmotion is the rhythmical, cyclical variation of blood perfusion in a tissue due to changes in smooth muscle tone in the wall of small arteries and arterioles (Anvar et al., 2000b). It is difficult to distinguish the corresponding origins of some of the early descriptions of ‘large’ and ‘small’ waves in reports of flowmotion in ischaemic patients, although a distinction based on different flowmotion patterns was certainly seen between healthy subjects and CLI patients. ‘Large’ waves ($0.051 \pm 0.02\text{Hz}$) were less evident with more severe ischaemia and ‘small’ waves ($0.362 \pm 0.07\text{Hz}$) were detected almost exclusively in patients with PAOD and were thought to be independent of respiratory frequency (Seifert et al., 1988). Subsequently,

descriptive terminology was used to evaluate oscillation patterns such as ‘aperiodical’, ‘sinoidal’, and ‘missing and small’ waves (Scheffler and Rieger, 1992). Although many seemed to have overlapping frequency bands ascribed to them an attempt was made to associate each with a systolic or ankle pressure index range. With worsening arterial disease a transition from aperiodical to sinusoidal occurred, with missing or small waves in CLI signifying a flatter trace, although this did not appear to be highly predictive (Scheffler and Rieger, 1992).

In recent years, Rossi and colleagues looked at cutaneous flowmotion in 20 patients with intermittent claudication, both at baseline and during reactive hyperaemia using wavelet analysis and the 5 characteristic frequency bands. A severely blunted post-ischemic increase in skin endothelial, sympathetic and myogenic-dependent vasomotion was found in the diseased leg of stage II PAOD patients, when compared to the amplified response of these bands in healthy subjects. A preserved leg skin vasomotion was observed under basal conditions in the same PAOD patients, although amplification of endothelial, sympathetic and myogenic activity was observed and may represent compensatory change at rest (Rossi et al., 2005, Rossi et al., 2008). The reduction in frequency bands associated with heart rate at rest may simply reflect poor transmission of output along the length of disease vessels. Respiratory frequency band was found to be unchanged at baseline and during reactive hyperaemia. Overall, in non-critical limbs flowmotion is not impaired during baseline but reacts with reduced vasomotion during periods of physiological demand (Rossi et al., 2005). In addition, Anvar and Khiabani observed a basally reduced skin endothelial, sympathetic and myogenic-dependent vasomotion power in the diseased leg of patients with CLI, suggesting

increasing degrees of impairment in vasomotion in more severe PAOD (Anvar et al., 2000b, Rossi et al., 2008).

Reactive Hyperaemia

Reactive hyperaemia causes augmentation of blood flow through stenotic areas or high resistance collaterals. Through provoking a hyperaemic response, the functional reserve of the limb is assessed and better differentiates PAOD patients from healthy subjects. In PAOD the response is delayed, reduced, or even abolished, particularly in the most distal tissues, providing good discrimination and reproducibility of microvascular function (Figure 12) (Leonardo et al., 1987, Morales et al., 2005, Van den Brande and Welch, 1988). Reactive hyperaemia has been shown to correlate with the severity of disease i.e. functional vs. critical ischaemia and also can identify the level in the arterial tree at which the occlusion or stenosis occurs (*see page 49*). Even distinction of sub-clinical disease in contralateral limbs can be made (Leonardo et al., 1987).

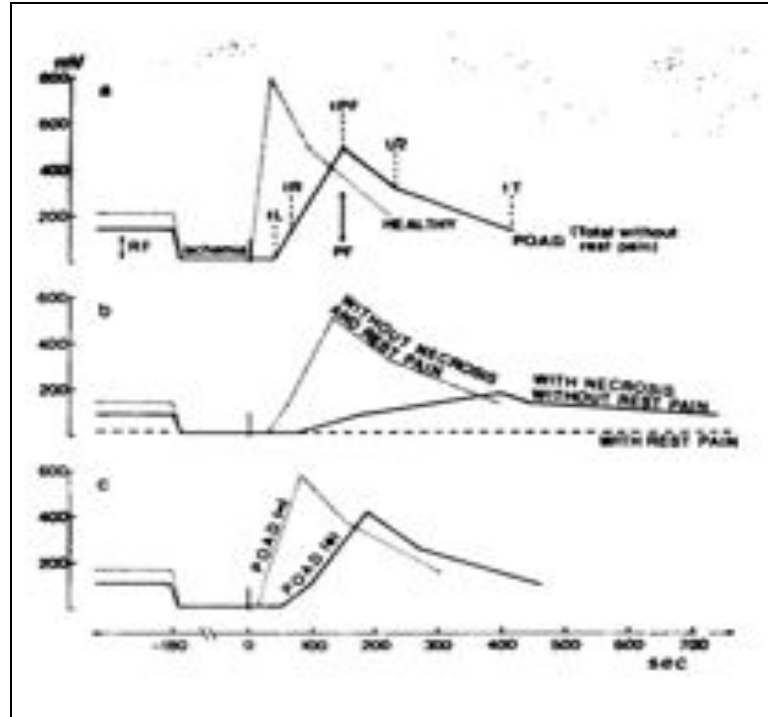


Figure 12: Typical reactive hyperaemia response curve in (a) totally healthy and arteriopathic limb, (b) arteriopathic limbs subdivided according to clinical picture, (c) limbs with more severe and less severe symptomatology of each patient. The reaction is delayed and flatter, with a prolonged return to baseline (Leonardo et al., 1987).

There have been a number of studies looking at reactive hyperaemia in healthy and diseased limbs in animals and humans. They all use a similar technique but vary the location of the probe and the cuff. Most studies use an occlusion time of 3 minutes and a static occlusion pressure of 250-350mmHg. Alternatively the pressure used can vary in relation to the individual's systolic pressure. The table below summarises reactive hyperaemia studies in PAOD.

Author & Year	Number	Probe location	Cuff location	Occlusion time	Pressure
Del Guercio et al. 1987	138 PAOD 71 healthy limbs	Pulp hallux	Thigh	3 min	250 mmHg
Van den Brande et al 1988	58 PAOD 21 normal limbs	Pulp hallux	Upper calf	3 min	300mmHg
Kvernebo et al 1989	85 healthy, elderly, IC, CLI	Hallux pulp & leg	Thigh	3 min	300 mmHg
Kvernebo K et al 1992	6 pigs with stenosis	Hind limb	'Proximal limb'	3 min	350 mmHg
Wahlberg E et al 1994	40 pigs with stenosis	Hind limb	'Proximal limb'	3 min	350 mmHg
Kvernebo et al 1996	9 pigs with varying stenoses	Hind limb	'Proximal limb'	3 min	350 mmHg
Orlandi C et al. 1998	20 HTN 20 healthy	Dorsum foot	Ankle	3 min	'Supra- systolic'
Ray S et al 1999	69 PAOD 15 controls	Dorsum foot	Ankle	2 min	100mmHg above ankle pressure
Morales F et al 2005	54 PAOD 30 controls	Dorsum foot	Thigh	3 min	30mmHg above systolic arm pressure

Table 6: Summary of studies recording reactive hyperaemia in animal and human subjects with and without stenoses, the location of the probe and the location of the cuff and its associated pressure and occlusion times.

Many parameters can be quantified from a single PORH response and each has their own inherent advantages and disadvantages. Prolongation of T_p is the one single outcome measure used consistently in studies, as it is a good discriminator of disease and it makes good sense to have a delay in response due to pressure losses across a stenosis (Wahlberg et al., 1994). Interestingly, hypertensive patients have been observed to have a slower rise to peak flux when compared with normotensive patients, suggesting early impairment of endothelial cell function. Neither the value at the peak of flux nor the recovery phase, assessed by $t/2$ recovery, have been shown to be consistently discriminative (Orlandi et al.,

1988). Table 7 below summarises some of the parameters used in the literature and whether they altered significantly with disease severity.

Author & Year	FRT/ fL	t/2 recovery	t recovery	Tp/t max	Peak flow	t/2 overshoot	tT
Del Guercio et al. 1987	Higher	Longer	Longer *	Longer	Lower	-	Longer
Van den Brande 1988	Higher	-	-	Longer	Lower	-	Longer *
Kvernebo et al 1989	NS	-	-	Longer	Lower	-	-
Kvernebo et al 1992	NS	-	-	Longer	-	-	-
Wahlberg E et al 1994	-	-	-	Longer	-	-	-
Kvernebo et al 1996	NS	-	-	Longer	NS	-	-
Orlandi C et al. 1998	-	Longer	Longer	Longer *	NS	NS	-
Ray S et al 1999	-	-	-	Longer	-	-	-
Morales F et al 2005	-	Longer	-	Longer	NS	-	Longer *

Table 7: The parameters measured in analysis of the hyperaemic curve and how they changed with disease severity. FRT – flux reappearance time or fL – flux latency, t/2 recovery – time to reach 50% of basal blood flow, t recovery – time lapse required to recover resting flux value, recorded prior to peak flux, Tp – time to peak or tmax – time to maximum peak, t/2 overshoot – time to reach 50% of the peak value on the down slope, tT – total hyperaemia time, *most discriminative measure in the study. NS not significant, - not tested.

Severity and location of stenosis

Wahlberg induced varying degrees of stenosis, from 5-100mmHg-pressure difference, in the peripheral vasculature of pigs (Wahlberg et al., 1994). He showed a relationship between the severity of stenosis and the time to peak hyperaemic response with more severe occlusive

disease giving a greater the prolongation of Tp ($r=0.86$ $p<0.0001$) and clearly distinguishing occlusive disease from healthy controls. This difference was most evident at larger pressures presumably because autoregulation is still present at lower pressure gradients (Wahlberg et al., 1994).

Similarly in humans, Kvernebo found that time to peak flux was intimately related to increasing disease burden and Tp was the parameter which most clearly separated the groups (Kvernebo et al., 1989). Others have also supported this quantitative finding (Larkin and Williams, 1993, Line et al., 1992, Line et al., 1996, Wahlberg et al., 1995). Tp was shorter in healthy volunteers of all ages compared to claudicants ($p<0.01$) and claudicants compared to CLI ($p<0.01$). Indeed many of the patients with critical ischaemia did not even have sufficient systolic pressure or remaining vasomotor function to the foot to show a pulp reactive hyperaemia response at all (Kvernebo et al., 1989). Diabetics were excluded. In contrast to Wahlberg, Kvernebo found that peak hyperaemic flux values also displayed a significant difference between patients and controls (Kvernebo et al., 1989, Wahlberg et al., 1994).

It has been suggested that the shape and pattern of the curve/s detected following release of the cuff, may provide insight into the anatomical distribution and physiological importance of arterial pathology. When Kvernebo experimented on 9 pigs with stenoses at different levels from aorta to superficial femoral artery, the time to peak was not significantly different between sites (Line et al., 1996). It is therefore likely that Tp reflects the vascular resistance of the limb as a whole.

Shape of the curve

Kvernebo also investigated whether the shape of the curve reflected peripheral vascular resistance, from lesions proximal to the tourniquet. He found that pig subjects without significant stenosis had an immediate single peak response following cuff release (Line et al., 1992, Line et al., 1996). 'Double-humped' curves where two or more peaks of hyperaemia were shown in quick succession were present when a significant arterial pressure gradient was present, irrespective of the site of stenosis ($p < 0.0001$) (Line et al., 1992, Line et al., 1996). The greater the pressure gradient, the more pronounced the double humped appearance became, as measured by f_{diff} (the difference between the flux value of the first peak and the minimum flux value between the two curves). The critical stenosis required to produce a difference had to exceed 70mmHg more proximally in the aorta but was less as the stenosis moved more distally to the CFA (40mmHg, $p < 0.05$) (Line et al., 1996). It seems likely that this initial first peak, of a 'double-humped' response is unrelated to flow, rather the transmission of pressure from the arterial wall and surrounding tissues detected by the sensitivity of the laser Doppler at the time of cuff insufflation. The finding that the onset of the first hump was timed as central blood pressure exceeds distal tourniquet pressure reinforces this (Line et al., 1992). The distal vasculature is subsequently in a vasodilated state, whilst transiently without volume flow. The delayed flow from the hyperaemic reperfusion is then seen as the second hump in diseased vessels (Line et al., 1996). These two phases are indistinguishable in subjects with healthy vessels. The number and size of the collateral circulation filling the microvasculature may explain why more proximal disease has a less pronounced first peak, until the severity of the stenosis reaches a critical point. The minimum flux between the two humps is thought to reflect the lowest distal pressure at the same time as the maximal arterial flow, as a consequence of pressure drop across the stenosis

from turbulent flow. Despite this initial shape change of the curve, the site of the lesion did not affect the overall time to peak significantly; only the increasing severity of stenosis would alter this. This is in favour of time to peak hyperaemia reflecting total vascular resistance of the limb (Line et al., 1996). This study however does not give insight into how more complex multi-level disease and influences the shape of the curve, or the effect that collateral circulation may have in lowering sensitivity to proximal disease.

Similarly Ray et al looked at the shapes of the hyperaemic curves in 69 PAOD patients and 15 controls and compared them to the anatomical distribution of disease and the ABPI of the subjects. They described the output as type I, II and III, rather than single or double humped curves. In this population they found type I curves with an immediate large amplitude peak was related to either normal vessels or proximal arteries (supra-geniculate vessels) with a single lesion (PPV 77%), consistent with low resistance transmission of the pressure/flow. Furthermore, the Tp in type I curves was always less than 10secs (Ray et al., 1999). Type II curves had multiple large amplitude peaks with delay in the highest peak and were associated with multiple proximal lesions (PPV 61%). It appeared that the stenoses and/or the presence of collaterals gave a disparate transmission of pressure and volume, represented by the numerous peaks. Finally, type III curves where there was a gradual increase in LDF over 1 minute or more towards pre-occlusion levels but did not show any large peaks. This pattern correlated with discontinuous run-off below the knee suggested that a patent tibial vessel is necessary to produce an LDF peak. (PPV 86%) Types I and II were strongly associated with one or more patent vessels from knee to ankle. Of note, the mean ABPI did not change significantly across the three groups, but there was a tendency for it to be lower with progression from type I to type III (Ray et al., 1999).

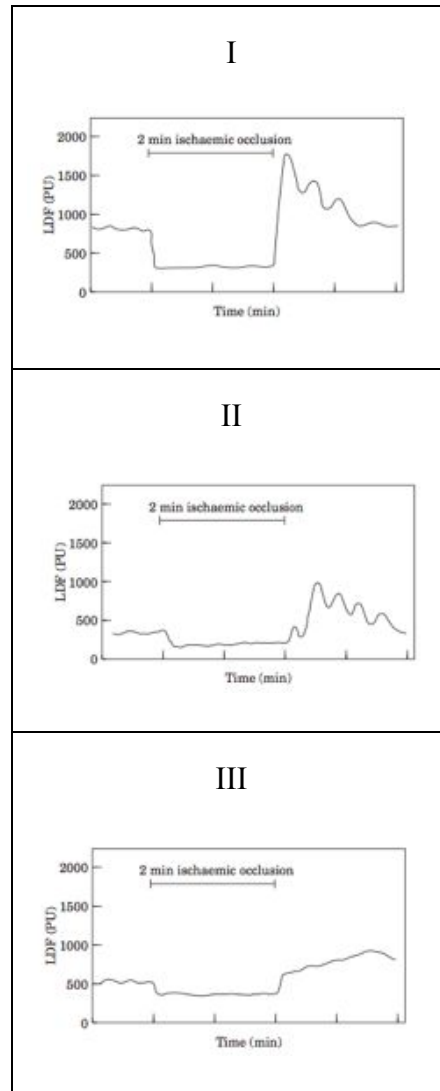


Figure 13: The three graph types I, II, III laser Doppler curve following 2 min arterial ischemia (Ray et al., 1999).

Van De Brande and Welch however, suggested that the time that the hyperaemic response starts after release of occlusion (T_0 , or FRT) is the most discriminating parameter between PAOD and normal subjects (Van den Brande and Welch, 1988). In a small subset of PAOD patients however, they described a T_0 of zero and an ‘s-shape’ response which infers analogous findings to the ‘double-humped’ response described above.

Location of cuff and probe

If Tp is a reflection of total limb resistance rather than outflow peripheral resistance, the position of cuff should not affect the time. Wahlberg et al showed that the Tp unaffected by location of tourniquet above or below the stenosis (Wahlberg et al., 1994). Probe placement and distance from the tourniquet should be standardised in any hyperaemia study as this has been shown to affect the time to peak (Line et al., 1996). Kvernebo found that whilst the Tp was not significantly prolonged by the location of the stenosis per se, it was prolonged by the location of the probe in relation to cuff. The further away the probe was sited from the tourniquet, the longer the time to peak in femoral artery stenosis, with a median difference of 15.6secs 22cm vs. 15cm ($p<0.05$). There was an insignificant trend towards a longer peak time was identified in more proximal iliac disease (Line et al., 1996).

The curve pattern was also different with a lower first-peak flux value with a longer distance from the tourniquet in the femoral stenoses. This may simply represent swifter detection of a change in pressure and flow in the arterial wall over a shorter distance, which is amplified when in a more distal stenosis, as the relative increase from the cuff is greater than a more proximal stenosis. This would explain the trend found in iliac disease rather than a significant difference.

Kvernebo also looked at RH at sites with and without AV anastomosis (hallux pulp vs. ankle) in 85 patients, at different stages of PAOD. Subjects were divided into four groups; young healthy, elderly healthy, claudicants and CLI. Resting flux and peak flux values were consistently higher in the toe pulp (3-6 times) compared to values from the leg ($p<0.01$) consistent with greater AV anastomoses. The time to peak had a tendency to be longer at the

toe pulp across all the groups, although this was not statistically significant (Kvernebo et al., 1989).

Reactive Hyperaemia:

- Severity of stenosis
- Magnitude of collaterals
- Distal disease
- Distance between tourniquet & probe
- Time to peak most consistent variable related to severity of disease

Postural Vasoconstriction in CLI

Perhaps rather counter intuitively after the PVC mechanisms described above, patients with critical limb ischaemia hang their leg in a dependent position to get relief from their symptoms, suggesting this provides an increase in peripheral flow and a reduction in local ischaemia. In CLI, the reduction in blood pressure at the ankle is compensated with regional passive dilatation and lowered peripheral resistance, thus increased capillary flow from raised hydrostatic pressure. Furthermore, increased venous transmural pressure and venous distension does not evoke the VAR in individuals with CLI and consequently there is a rise in capillary pressure. Therefore, patients with severe disease react with an increase in flow in dependency. Subsequently, the foot is suffused with blood and becomes hyperaemic in dependency. Equally elevation causes pallor, as a result of microcirculatory dysfunction.

Those with non-critical or functional ischaemia do not present this finding, although they do display a comparative reduction in peripheral vascular resistance compared to healthy

individuals. This is reflected by a similar horizontal resting flux on LDF, but a significantly higher flux on sitting, compared to healthy individuals, see figure 14 below (Abu-Own et al., 1993, Delis et al., 2001, Otah et al., 2005). This has also been demonstrated using Xenon washout techniques, with claudicants having a significantly higher local blood flow during dependency than normal subjects ($p=0.01$), suggesting a reduction in local peripheral resistance (Eickhoff, 1980).

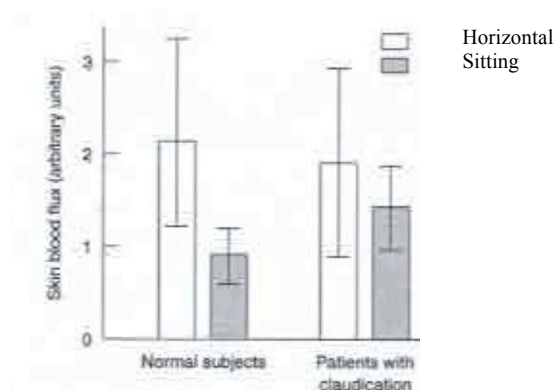


Figure 14: Median skin blood flux in horizontal and sitting positions in normal subjects and patients with stable intermittent claudication. There was no significant difference in flux in the horizontal position between the groups. In the sitting position, skin flux in claudicants was higher than in the normal subjects ($p<0.001$) (Delis et al., 2001).

Eickhoff and Henriksen also demonstrated that during lowering, blood flow in the limbs with intermittent claudication decreased less than in the normal limbs ($p=0.04$), however the calculated local vascular resistance was not found to be significantly different to that of normal individuals suggesting that autoregulation is preserved in non-critical limbs (Eickhoff and Henriksen, 1985). That said claudicants do exhibit more of a decrease in blood flow on elevation than normal subjects, indicating some impairment early in the disease process. Nevertheless, despite attenuation at this stage they are able to adapt and compensate through an intact reflex mechanism and increase their local vascular resistance.

In limbs experiencing rest pain, blood flow increased by 28% during lowering from the supine position (Eickhoff and Henriksen, 1985). It was however suggested that this was not due to passive dilatation of resistance vessels, but a consequence of an associated additional increase in arterial pressure on lowering of the limb, as there had still been a calculated increase in local vascular resistance of 21% suggesting that some vasoconstriction was still occurring (Eickhoff and Henriksen, 1985). A greater orthostatic response was also seen in a group of critically ischaemic patients compared to normal subjects across three different anatomical areas as a result of increased flow in dependency on LDF; pulp of the great toe, level of the second metatarsal body and anterolateral part of the ankle ($p<0.001$, $p<0.008$ and $p<0.001$ respectively). This response was twice as great at the toe compared to the foot and ankle locations, predominantly because the perfusion during dependency was much greater than in the horizontal position at the toe ($p<0.008$), with no significant differences at the foot and ankle positions between supine and dependency. It may be that the presence of AV shunts in the toe gives the greatest potential for skin perfusion to increase (Khiabani et al., 2000a). In addition transmural pressure will be greater at the toe because its location is more distal. It is not clear whether this is primarily due to a deranged nervous reflex mechanism or diminished contraction of smooth muscle due to structural or functional adaptation. In healthy individuals postural change in flow is not affected by location (Khiabani et al., 2000a).

In a small cohort of normal subjects ($n=12$), claudicants ($n=8$) and critically ischaemic patients ($n=7$), ankle and toe pressures were found to increase significantly from supine to sitting in all groups, as expected (de Graaff et al., 2003). There was however a significant increase in capillary pressure on sitting in all three groups, which was related to orthostatic

blood pressure increase i.e. the postural increase in toe pressure in all groups was not significantly different from the postural increase in capillary pressure ($p=0.88$, $p=0.26$, $p=0.46$ respectively), suggesting that capillary pressure is maintained at the expense of capillary perfusion in response to lower arterial pressure (de Graaff et al., 2003).

Oedema

The postural vasoconstriction mechanism is thought of by some as the “oedema protection reflex”, (Abu-Own et al., 1993) as persistent vasomotor dilatation/paralysis is suggested to favour oedema, secondary to increases in arterial hydrostatic pressure. More than 50% of CLI patients have oedema at presentation and there can be as much as $13 \pm 9\%$ mean volume difference between contralateral limbs without CLI (Anvar et al., 2000a, Anvar et al., 2001, Khiabani et al., 1999b). In addition to the reduction in peripheral vascular resistance in this cohort there is a relative increase in the time spent in the dependent position due to second or third degree rest pain, which necessitates limb dependency or even a constant seated position to relieve the pain. The associated increase in tissue pressure exceeds capillary pressure favouring transudation of fluid into the interstitial space. The oedema tends to be localised to the distal leg and foot and is pitting in nature and hence represents extracellular interstitial fluid (Khiabani et al., 1999a). As a consequence the tissues are saturated, nutritive capillaries may be compressed and the diffusion distance for nutrient exchange is greater and may in itself lead to further deterioration in tissue oxygenation. (Coats, 2003, Falluji and Mukherjee, 2012, Khiabani et al., 1999b). The normal integrity of tissue cannot be maintained and certainly once a wound or an ulcer has been formed, nutritional blood flow and extraction of oxygen cannot be increased by normal means through increasing total blood flow or by redistributing flow from non-nutritional to nutritional circulation via AV shunts. Infection

also raises the metabolic demands of the tissues and must be treated immediately (Khiabani et al., 1999a, Gresele et al., 2011). Presumably, persistently low distal pressures explain the absence of oedema observed in some patients suffering CLI.

Starling forces regulate fluid homeostasis, which represents the balance between hydrostatic and colloid oncotic pressures intra-luminally and in the interstitium (Anvar et al., 2001, Khiabani et al., 2000b, Khiabani et al., 1999b). A disparity in this balance is evident in CLI patients such that hydrostatic pressure in the capillaries is increased due to a reduction in pre-capillary resistance. Techniques to collect interstitial fluid have shown the colloid osmotic pressure in limbs with CLI to be significantly lower than in the limbs without CLI (2.3 SD 0.5mmHg vs. 3.1 SD 0.7mmHg, $p<0.0001$), indicating a reduction in proteins in this fluid. (Khiabani et al., 2000b) In the plasma, osmotic pressure is reduced predominantly due to a reduction in albumin with total protein levels being within normal range (Khiabani et al., 2000b, Khiabani et al., 1999b). In fifteen CLI patients Khiabani and colleagues found the mean plasma albumin concentration, which usually accounts for more than two-thirds of plasma oncotic pressure, to be $28.5\pm6.6\text{g/l}$, significantly lower than reference intervals (Khiabani et al., 2000b, Khiabani et al., 1999b). The net result is an increase in interstitial fluid formation, which accumulates in the compliant subcutaneous tissue. Whilst the reduction in albumin would account for bilateral oedema, it does not explain unilateral swelling and hence it is not felt to be the only source of oedema formation.

Furthermore, the capillary filtration coefficient (CFC), which is a functionality of the 'leakiness' of the capillaries combined with their available surface area, has been shown to be twice as great in patients with oedema ($p<0.01$), compared to either the contralateral limb or

control subjects (Anvar et al., 2000a). This increase in 'hydrodynamic conductivity' may be as a direct result of the disruption to the endothelium. One of the functions of the vascular endothelium is to regulate permeability, which may be damaged as consequence of metabolites produced by ischaemia (Khiabani et al., 2000b). Studies utilising transmission electron microscopy in CLI reveal swollen and deranged endothelial cells with large intercellular gap junctions, which are predominantly manifested at more distal locations (Anvar et al., 2000a, Anvar et al., 2000c). There was, however, no significant correlation found between total leg-foot volume and CFC ($p>0.05$) (Anvar et al., 2000a). Of note, there were no significant differences found in CLI patients with and without limb oedema, on analysis of flowmotion FFT in horizontal and dependent positions. Both low (endothelial, myogenic and neurogenic) and high frequency (respiratory) oscillations did not differ between these two groups (Anvar et al., 2000b).

The impairment of VAR is likely to play a contributing role with elevated venous pressure transmitting to the capillaries. Khiabani and colleagues however have demonstrated that whilst there is an impaired orthostatic response in the toe pulp of patients with CLI, this does not differ significantly between those with and without oedema pre-operatively, nor were they able to demonstrate a correlation between the degree of oedema and the orthostatic response (Khiabani et al., 2000a). Functionally significant DVT as a cause of limb swelling due to relative stasis has been excluded using plethysmography and colour flow duplex techniques (Khiabani et al., 2000a, Khiabani et al., 1999b). All of these potential contributory factors suggest that the development of oedema is a multifactorial process, not solely as a result of attrition of the VAR, CFC or a change in haemodynamics, particularly as there does not

appear to be a linear relationship between attenuation of these individual factors and limb volume.

Measurement of oedema through measuring limb volume can be achieved with a number of different methods including water displacement volumetry (WDV), which assumes that displacement of 1ml of water is equivalent to 1g volumes (Khiabani et al., 1999b). Alternative methods include infrared optoelectronic volumetry, which is expensive, and the disc model methods that assume a truncated cone for shape of lower limb for estimation. This is easier and cheaper to perform although may be less reliable if the oedema is localised to foot and ankle. In our experience the oedema is often involving the calf in addition to the ankle.

1.4 Diabetes

Diabetes adds extra complexities to the recognition, diagnosis and management of CLI. Approximately 2million people in the UK are affected by diabetes and its prevalence is expected to double in the next two decades (Price et al., 2008). Not only is diabetes a risk factor for the development of ischaemic foot ulceration, it also has its own inherent complexities as a consequence of the pathophysiological changes that occur in the foot. These are not exclusively related to ischaemia, but also the associated sensory motor impairment and autonomic dysfunction (Schramm et al., 2006). They also have a significantly reduced resistance to infection, helping to account for the 12-15x higher amputation rates in this group (Abbott et al., 1998, Fosse et al., 2009). Furthermore, the typical symptoms of CLI are not always present in this population.

The skin circulation in diabetes

Atherosclerotic disease tends to be more severe compared to non-diabetics, with early large vessel involvement and poor collateralisation. This is accelerated by poor glucose control, with every 1% increase in HbA1c giving rise to a 25-28% relative risk of peripheral arterial disease (Jorneskog, 2012, Lepantalo et al., 2011). In contrast to non-diabetics, men and women affected equally (Jorneskog, 2012). Fosse et al found that 95% of lower limb amputations in diabetics were associated with PAD (Fosse et al., 2009).

Poor glycaemic control also contributes to metabolic dysfunction and an impoverished microvasculature, with increased vascular permeability and impaired autoregulation of blood flow and tone (Schramm et al., 2006). Subsequently, wounds fail to heal from relatively minor trauma. Peripheral neuropathy synergistically acts to reduce capillary blood flow in the foot and it has been suggested that this is the main cause for reduced microvascular reactivity (Brooks et al., 2008, Schramm et al., 2006) (See below). Equally, microvascular abnormalities may contribute to endoneural perfusion and hypoxia, exacerbating peripheral neuropathy (Brooks et al., 2008).

As previously mentioned, ABPI measurements are inherently unreliable in the diabetic population and toe pressures can be difficult to measure in the presence of digital amputations or gangrene. Even when these measures are feasible, the value and accuracy at which limbs are considered 'critical' is at a different threshold to non-diabetics, particularly in view of the effects of neuropathy.

Neuropathy

Diabetic peripheral neuropathy (DPN), most commonly a distal symmetrical sensory polyneuropathy, is a feature of diabetic disease in 28% of type one diabetics and up to 50% of patients with type two diabetes and its development and progression is related to defective glycaemic control (Apelqvist et al., 2008, Aring et al., 2005, Boulton et al., 2006, Parry, 1999). It is therefore, the commonest complication of diabetes and can present clinically or be in apparent and often symptoms do not correspond with nerve function (Arezzo, 1999, Garrow and Boulton, 2006). It has been estimated that 15% of all diabetics will develop a foot ulcer in their lifetime (Miranda-Palma et al., 2005) and DPN is the causal factor in most foot ulcerations, (Garrow and Boulton, 2006) although the pathway to ulceration often requires two or more of the following risk factors to be present:

1. Metabolic and microcirculatory compromise
2. Atherosclerosis
3. Neuropathy
4. Mechanical deformity
5. Infection (Boulton et al., 2006)

Neuropathy in combination with one of these risk factors raises the incidence of ulceration to 25-30%, a twelve-fold higher risk (Frykberg et al., 1998, Lepantalo et al., 2011). The EURODIAB prospective study showed that this risk factor profile for the development of DPN is similar for the development of PAD including age, duration of diabetes, hypertension, smoking, hyperlipidaemia in addition to poor glycaemic control (Tesfaye et al., 2005). Consequently, a relatively lesser degree of macrovascular compromise may exist in the

diabetic patients, to give rise to comparatively more extensive tissue loss than in a non-diabetic patient. Of course, once ulceration ensues healing is far more difficult to achieve as a consequence of the ischaemia and microvascular compromise that already exists.

Concurrent autonomic neuropathy causes similar disturbances in microcirculatory flow by causing AV shunts to open, diverting blood away from nutritive beds into sub-papillary vessels and depriving capillaries of blood flow, hence reducing skin perfusion. Overall there is a normal or increased total skin microcirculation but a reduction in capillary circulation. This shunting occurs because autonomic neuropathy effectively ‘denervates’ the A-V shunts and loss of sympathetic vasoconstrictor tone allows these vessels to stay open with blood flow by-passing the capillaries. Paradoxically patients are seen to have a warm, pink and dry foot, as total skin circulation may be normal or over perfused (Schramm et al., 2006). This accounts for similar TcpO₂ values recorded in diabetics as non-diabetics with PAOD (Junger et al. 1989; Creuzig et al. 1991) while capillary circulation is markedly reduced. Jorreskog et al named this ‘chronic capillary ischaemia’, (Figure 15) (Jorreskog, 2012). These neuroischaemic features of the diabetic foot make typical symptoms such as rest pain and pallor less common. Certainly the diabetic circulation cannot initiate a hyperaemic response to further increase flow during periods of increased requirements such as ulceration or tissue necrosis. Patients should be screened regularly to identify insidious loss of this sensation and is crucial for preventing further deterioration in high-risk feet.

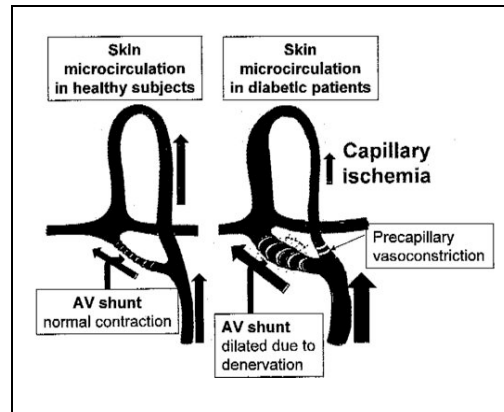


Figure 15: Chronic capillary ischaemia in the diabetic foot. 1) Thermoregulating arteriovenous (AV) shunts are innervated by the sympathetic nerve system. In diabetes, autonomic neuropathy may lead to denervation of the AV shunts, which lose their normal contraction leading to blood passing through these shunts instead of the capillaries. 2) Endothelial dysfunction with a disturbed balance between endogenous vasodilators and vasoconstrictors leading to precapillary vasoconstriction 3) Hemorheological alterations such as elevated levels of plasma fibrinogen (Jorneskog, 2012).

Furthermore, injury and damage to the foot is much more likely as sensation is deficient in peripheral neuropathy and anatomy and weight bearing dynamics of the foot are altered, further increasing the risk of ulceration (Lepantalo et al., 2011). Structural diabetic microangiopathy also serves to increase capillary leakage and venous pooling, thereby further impairing oxygen delivery to the tissues. Reduced inflammatory response to tissue injury and decreased resistance to infection increases the risk of foot ulcer and infections for diabetic CLI patients. Impaired sensory feedback means that the ischaemic process progresses silently (Lumsden et al., 2009). These complex processes and haemodynamic changes are difficult to detect with conventional assessment alone, such as ankle and toe pressures (Jorneskog, 2012).

Methods of assessing DPN

There are several well-defined screening methods to assess the functional integrity of sensory fibres through light touch, pressure or vibration sense (Goldberg and Lindblom, 1979). Assessment of the presence or absence of neuropathy and/or the degree of neuropathy present

can be used reliably to predict the likelihood of foot ulceration (Young et al., 1994). Clinical examination is supplemented with non-invasive screening tests such as the 10g Semmes-Weinstein Monofilament (SWM) to assess touch/pressure sensation, the Rydel-Seiffer tuning fork for vibration sense and a Neurothesiometer to quantitatively assess vibration perception threshold. In addition, electrophysiological studies of peripheral nerve function can be employed to look for attenuation of evoked nerve action potentials.

Neurothesiometer

The Neurothesiometer is an electromechanical instrument used to objectively evaluate sensory function in large and myelinated nerve fibres, by delivering vibrations of increasing strength (Krishnan et al., 2004, O'Neill et al., 2006). An increased VPT value (the lowest voltage at which vibration can be detected at least 50% of the time) (Garrow and Boulton, 2006) is one of the first clinical signs of peripheral neuropathy (van Deursen et al., 2001). It is quick, portable and relatively inexpensive (Thomson et al., 1992). Quantitative sensory testing has been shown to have a higher positive predictive value in detecting abnormalities than by clinical examination alone, thus it can be used to detect and monitor neuropathic progression (Abbott et al., 1998, Miranda-Palma et al., 2005). However, patients do need to be motivated and focused to comply with the testing, in order to achieve accuracy.

It is thought to relate better to clinical function than the non-parametric tests such as the standard non-graduated 128Hz tuning fork, which is only able to grossly identify the presence or absence of vibration perception, rather than the amplitude at which vibration is perceived (Garrow and Boulton, 2006). Kastenbauer et al and O'Neil et al showed that there was high agreement in vibration perception threshold (VPT) on the great toe pulp of diabetics between

these two devices, but there was greater inter-investigator agreement for the neurothesiometer than for the graduated tuning fork (O'Neill et al., 2006, Kastenbauer et al., 2004). Furthermore, VPTs procure a higher positive predictive value for ulceration than SWM. (Garrow and Boulton, 2006) Incidentally, VPTs were also higher in patients with higher HbA1c's and a longer duration of diabetes (Kastenbauer et al., 2004). This correlation was also found by Bril and Perkins in a larger cohort n=478 (Bril and Perkins, 2002).

Young et al conducted a prospective study and illustrated that a VPT value of >25V in at least one foot was associated with a high cumulative incidence of neuropathic ulceration compared to patients with a VPT of <15V (19.8% vs. 2.9%, $p<0.01$) (Young et al., 1994). This was further substantiated by a large prospective multi-centre trial (n=1035), confirming that VPT was an independent prognostic factor for foot ulceration in patients with established DPN, with a seven-fold risk for foot ulceration >25V and a 5.6% increase in the hazard of a first foot ulcer for every 1V increase thereafter (Abbott et al., 1998, Frykberg et al., 1998). The monofilament was not found to have the same predictive properties. High sensitivity is reported for VPTs at 92%, while the specificity appears much lower at around 39%, although appreciably over caution with good foot care and education as a result is not harmful (Miranda-Palma et al., 2005). Accordingly, Rayman et al found significant differences in VPT (12.0, IQR7.9-18.6V vs. 51.0, IQR40.0-51.0V) and VDT (21.0, IQR19.3- 22.3 vs. 26.0, IQR 22.6-26.0) $p<0.0001$, in diabetic patients with and without existing ulceration using the neurothesiometer (Krishnan et al., 2004).

Bril and Perkins also found that VPT values correlated strongly with the electrophysiological parameters, specifically sural nerve action potentials, which in turn corresponds with structural nerve abnormalities observed in diabetics (Bril and Perkins, 2002).

The VPT increases with age, implying an additional age-related neuropathy (Frykberg et al., 1998, Goldberg and Lindblom, 1979, Williams et al., 1988, Bergin et al., 1995, van Deursen et al., 2001, Kastenbauer et al., 2004). Bloom calculated estimates of centiles in relation to age in 519 non-diabetic subjects (see Figure 16 below). The data was arranged as centiles according to age. There was a linear relationship between the log transformation of the threshold readings and age. Interpretation of VPT in the elderly is much more difficult due to the broader ‘normal range’ of VPT in this group.

Age (years)	Thumbs			Great toes			Medial malleoli		
	2.5	50	97.5	2.5	50	97.5	2.5	50	97.5
10	2.3	4.7	9.6	2.1	4.9	11.3	2.9	6.6	15.0
20	2.6	5.3	10.7	2.7	6.1	14.2	3.6	8.1	18.4
30	2.9	5.9	11.9	3.3	7.7	17.8	4.4	9.9	22.5
40	3.2	6.5	13.2	4.2	9.6	22.3	5.4	12.2	27.7
50	3.6	7.2	14.7	5.2	12.1	28.0	6.6	14.9	33.9
60	4.0	8.1	16.3	6.6	15.2	35.1	8.1	18.3	41.7
70	4.4	9.0	18.1	8.3	19.1	44.0	9.9	22.5	>50
80	4.9	10.0	20.1	10.4	23.9	>50	12.2	27.6	>50
90	5.5	11.1	22.4	13.0	30.0	>50	14.9	33.9	>50

Figure 16: Mean and range of vibration threshold with age (2.5 and 97.5 centiles enclose 95% range) (Bloom et al., 1984).

It is important to recognise that vibration sensitivity is age dependent so that normal and pathological thresholds can be distinguished. Interestingly, Karvestedt et al conducted a population-based study on type two diabetics and as well as substantiating the link between DPN and age, they also found that it was independently associated with retinopathy. Patients with retinopathy have a longer disease history and also poor glucose control, which may explain the preponderance for more associated complications. Furthermore, the alliance

between evidence of DPN and peripheral vascular disease +/- ulceration was particularly prominent in patients who did not have associated retinopathy. This suggests a causal link with macrovascular, as well as microvascular disease in the pathophysiology of DPN. (Frykberg et al., 1998) Equally, the presence of peripheral pulses in diabetics has been shown to be protective for ulceration ($p=0.000$) (Frykberg et al., 1998).

Using the biothesiometer, an earlier device, Williams et al demonstrated wide variation of VPTs between different sites in the same subject, both ipsilateral and contralateral. They used a comparatively younger, but wider age range of 15-65years than Bloom. The variability was however less among non-diabetics, as expected if there is an element of neuropathy present (Williams et al., 1988). Although there was variation between the same sites on different sides of the body in all individuals ($\sim 30\%$), there was still a highly significant positive correlation between the two sites overall ($r=0.85$). Other investigators have also found correlation between different limbs, at the same site (Cassella et al., 2000, (Bloom et al., 1984, Thomson et al., 1992). Williams does not specifically make reference to consecutive readings at each site, although the methodology of the two studies differs, with the Williams disregarding the first set of readings, which may potentially alter overall accuracy. In contrast, Bril et al only found the variability to be between 6-8% using the neurothesiometer on the toes, concluding that it was in fact reflective of peripheral nerve function (Bril et al., 1997). It should be noted that much of the data using a biothesiometer, not a neurothesiometer, was attained greater than twenty years ago and this had inherent discrepancies in calibration, electrical safety and sensitivity at both higher and lower voltages. The more recent neurothesiometer should have alleviated many of these concerns (Thomson et al., 1992, Williams et al., 1988, van Deursen et al., 2001).

As far as different anatomical locations are concerned, the evidence is quite unanimous that distinct sites such as finger, ankle, toe, will provide different readings (Armstrong et al., 1998, Bloom et al., 1984, Dimitrakoudis and Bril, 2002, Goldberg and Lindblom, 1979, Thomson et al., 1992, Williams et al., 1988). Variation in stiffness of different tissues will affect the mechanical impedance from the neurothesiometer and is therefore likely to affect the vibration experienced, potentially explaining some of the variability. Testing should therefore take place in identical locations for comparison and multi-site testing is unlikely to yield a large benefit.

Comparison of VPT at least 6 weeks following revascularisation, in 56 patients with insulin dependent diabetes, did not show any improvement from pre- to post-intervention, despite a significant increase in transcutaneous oxygen tension (Veves et al., 1996).

Which control mechanisms are affected?

Basal flow and frequency analysis

Commonly, resting basal flow has not been shown to be different between diabetics and controls using LDF, nor in patients with diabetes and PVD (Forst et al., 1998, Rayman et al., 1986, Jorreskog et al., 1995, Parkhouse and Le Quesne, 1988).

Analysis of basal flow for its oscillatory components using wavelet analysis has not shown any significant differences between diabetic patients and controls, with or without PAD (Urbancic-Rovan et al., 2006). In general lower values were observed in diabetics compared with controls, which may be due to the large compensatory reserve of the skin in its basal state. However, a positive correlation was identified between ankle and brachial systolic

pressure and endothelial activity in the diabetic group. This was not identified in the control group suggesting that systemic pressure may have a greater influence on endothelial activity in diabetic disease (Urbancic-Rovan et al., 2006). In contrast, Jaffer et al used fast Fourier transformation in spectral analysis of resting vasomotion and found that the frequency was significantly raised in diabetes compared to controls (Jaffer et al., 2008).

Veno-arteriolar Reflex

Peripheral neuropathy affects both the somatic and autonomic nerves. Consequently, the sympathetically mediated VAR is attenuated in diabetics, approximately one third of the response expected in healthy individuals (Abularrage et al., 2005, Wahlberg et al., 1990). The subsequent increase in transmural pressure causes an increase in arteriovenous shunting with increased capillary pressure and long-term effects such as thickening of the basement membrane and dysregulation of microvascular function prevails (Cacciatori et al., 1997).

In a cohort of 47 insulin diabetics without PAD, significant correlation was found between vibration perception thresholds suggesting impairment of large somatic fibres, and VAR, impairment of peripheral sympathetic fibres (Cacciatori et al., 1997). This was progressive through from controls, to patients with out clinical signs of neuropathy to those with overt foot ulceration ($p < 0.001$). Even those patients with diabetes but without clinical evidence of neuropathy demonstrated an impaired VAR, despite similar resting values (Cacciatori et al., 1997). The investigators were careful to measure VAR with the patient in the supine position and the leg lowered below the level of the heart to ensure central mechanisms were not taken into account. Evidence of this impaired mechanism is also apparent in post-pubertal children with diabetes compared to their healthy peers (Shore et al., 1994). In this instance no

association was found with diabetic control or duration of diabetes and it had manifested before any clinically detectable microvascular complications.

Belcaro et al studied 300 subjects, equally divided between control and diabetic with/out neuropathy. They found the VAR to be 13% in diabetics with neuropathy, 28% in those without neuropathy and 46% in control subjects ($p < 0.02$) (Belcaro et al., 1992). On review of these patients 3 years later, the VAR was further attenuated, with an associated increase in capillary filtration in both groups of diabetics. They did not assess peripheral oedema in this group to see if this was allied to greater capillary filtration.

Most papers have excluded PAD as a potentially conflicting variable in assessing the microcirculation in diabetes. Wahlberg et al specifically looked at VAR in PAD (claudicants and CLI) patients with and without diabetes. On dependency, the reduction in flow of the diabetic group was one third of the reduction seen in the controls and in the PAOD group the average flux actually increased by a small amount (Wahlberg et al., 1990). This may be explained by the higher resting flux that was initially observed in the diabetic group when supine, thereby being unable to further increase flow on dependency.

Microcirculatory Control Mechanisms

Functional changes in the endothelium occur early in the pathogenesis of diabetes and even those with a family history and at increased risk of developing diabetes show reduction in vessel reactivity compared to controls, at the foot and the forearm, using iontophoretic techniques and heating of the skin (Arora et al., 1998, Caballero et al., 1999, Hamdy et al., 2001, Tooke et al., 1983). Impairment in vasodilatory responses including post-occlusive

reactive hyperaemia is representative of the normal response to injury and is therefore important when considering wound healing even in the absence of large vessel disease. Neuropathy renders the foot functionally ischaemic as blood flow fails to increase during periods of stress (Schramm et al., 2006).

Nerve-Axon Reflex

In addition to the endothelium-dependent pathways, impairment of the nerve-axon reflex mechanism contributes significantly to impaired vasodilatation in this population (Arora et al., 1998, Schramm et al., 2006, Veves et al., 1996). By monitoring capillary blood flow following neurovascular stimulation with acetylcholine, Forst and colleagues found that flow increased significantly in the control group and in diabetics without clinical neuropathy and did not increase significantly in those with neuropathy. These findings were attributed to loss of nociceptive C fibre function in the neuropathic group (Forst et al., 1998). Disturbances in capillary flow in the foot using video capillaroscopy were evident in diabetic patients following arterial occlusion (Jorneskog et al., 1995). However, the total skin blood flow assessed by LDF was normal, suggesting redistribution via the AV anastomotic network in these patients due to impaired nerve-axon pathway. Stansberry et al likened this neurovascular deterioration in diabetics to advancing age in non-diabetics, when they tested vasoconstriction and dilatation at the finger with LDF and found that blood flow in younger diabetics was similar to that of older controls (Stansberry et al., 1997). Rayman and colleagues found that an impaired hyperaemic response to minor thermal and needle trauma was related to duration of type I diabetes (Rayman et al., 1986). This occurred at sites on the feet and on the abdominal wall, suggesting a global impairment of the microcirculation. It is unclear whether the severity of impairment is a result of a reduction on vasoactive mediators,

structural microvascular abnormality limiting vasodilatory capacity or indeed a combination of these.

Local Heating

Analysing hyperaemia in diabetic patients with and without foot ulceration by their response to heating, did not declare a difference between the two groups. There was only a notable and statistical difference between control and diabetics overall, rather than the subgroups. The neurological parameters however did discriminate between patients with and without ulceration (Krishnan et al., 2004).

Reactive Hyperaemia

The hyperaemic response of the microcirculation to stimuli is attenuated in the diabetic population compared to healthy controls. Jaffer et al used a three-minute cuff occlusion time in a prospective case controlled study and found that both the percentage increase in flux from baseline (234 ± 62 vs. $453 \pm 155\%$, $p < 0.001$) and the time to peak (21.4 ± 0.4 vs. 12.8 ± 5.4 sec. $p < 0.05$) using LDF, were significantly different compared to controls (Jaffer et al., 2008). However, the coexistence of peripheral neuropathy and peripheral vascular disease has not been shown to further reduce endothelium-dependent vasodilatation any further than neuropathy alone (Schramm et al., 2006). In a single study specifically looking at the interaction between PAOD patients with and without diabetes, it was found that patients with diabetes had a shorter time to peak when compared to patients without diabetes (Wahlberg et al., 1990). This may be explained by the rigid nature of the diabetic vessels and the propensity of the pressure wave down it giving rise to a primary peak prior to the onset of the formal red cell flux. When the hyperaemia was repeated in the sitting position both PAOD

groups with and without diabetes had a significant increase in flux, in contrast to the reduction seen in the controls indicating the passive nature of filling in the vascular bed in patients who do not exhibit pre-capillary constriction (Wahlberg et al., 1990).

It is the case with all of these studies that many patients are excluded due to coexisting morbidities such as peripheral vascular disease and hypertension and the use of vasoactive medications. Many reactive hyperaemia papers exclude diabetics due to their stiffer arterial walls exhibiting a shorter Tp (Wahlberg et al., 1994).

DIABETES:

- Neuropathy is the result of a functional disturbance or pathological change, due to impaired endothelial function and impaired C-fibre axon reflex dilation.
- Impaired sympathetic vasoconstrictive responses to a variety of stimuli including postural change
- Impairment in vasodilatory responses including post-occlusive reactive hyperaemia

The microcirculation is a complex and dynamic environment, intimately associated with the macrocirculation. Normal homeostatic mechanisms to maintain local pressure and perfusion are affected early by ischaemia and are initiated before the onset of clinical signs and symptoms and are not amenable to detection through routine pressure measurements. Parameters of microvascular dysfunction are numerous and will often correlate with severity of disease, such as reactive hyperaemia and time to peak. Attenuation of mechanisms such as

PVC can potentially result in significant limb oedema and exacerbate nutrient exchange and integrity of the tissues. Diabetic patients represent an increasingly challenging cohort as neuropathy renders the foot functionally ischaemic and can complicate interpretation of microcirculatory investigations. Specialist attention is required in disease detection and instigating the appropriate treatment pathway if prognosis is to be improved.

Summary

The table below broadly summarises the changes observed in the microcirculation in patients with CLI, with diabetes and those who have both CLI and diabetes. Of note, lower limb oedema in the diabetic population is not differentiated from patients with or without CLI in the literature.

Variable	CLI	Diabetes	CLI & Diabetes
Basal Flux	↓	→	→
Reactive Hyperaemia	↓	↓	Shorter Tp
VAR	↓	↓	→
Oedema	>50%	NC	NC

Table 8: Summary of changes in microcirculatory control mechanisms in CLI and in diabetes compared to controls and in patients with CLI AND diabetes compared to CLI alone. ↓attenuated, → the same response as comparative group. NC, not specifically commented on in literature.

1.5 Treatment

Recommendations

CLI is treated with revascularisation of the limb, either with endovascular or open surgical techniques. Appropriate treatment is dependent upon the outcomes of the haemodynamic, physiological and anatomical assessments and the availability of autogenous conduits.

The TASC II classification categorises patients based on:

- ❖ Anatomical distribution of disease
- ❖ Extent of disease - multiple vs. single level
- ❖ Degree of stenosis/occlusion (Table 9)

The recommendation for treatment of choice is endovascular with TASC A classification and the preference progresses to surgery as the level and severity of disease progresses. The long-term prognosis and patient survival are directly related to the extent of the disease (Conte, 2010a).

Classification	Femoro-popliteal lesions	Aorto-Iliac lesions
TASC A	<ul style="list-style-type: none"> -Single stenosis <10cm in length -Single occlusion <5cm in length 	<ul style="list-style-type: none"> -Unilateral or bilateral stenoses of CIA Unilateral or bilateral single short stenosis ≤3cm of EIA
TASC B	<ul style="list-style-type: none"> -Multiple lesions (S or O) ≤5cm in length -Single S or O ≤15cm not involving infragenicular PA -Single or multiple lesions in the absence of continuous tibial vessels to improve flow for a distal bypass -Heavily calcified occlusion ≤5cm - Single popliteal stenosis 	<ul style="list-style-type: none"> Short ≤3cm stenosis of infrarenal aorta -Unilateral CIA occlusion -Single or multiple stenosis totalling 3-10cm involving EIA & not extending into CFA -Unilateral EIA occlusion not involving the origins of IIA or CFA
TASC C	<ul style="list-style-type: none"> -Multiple S or O totalling >15cm with/out heavy calcification -Recurrent stenoses or occlusions that need treatment after 2 endovascular interventions 	<ul style="list-style-type: none"> -Bilateral CIA occlusions -Bilateral EIA stenoses 3-10cm long not extending into CFA -Unilateral EIA stenosis extending into CFA -Unilateral EIA occlusion that involves the origins of IIA and/or CFA -Heavy calcified unilateral EIA occlusion with/out involvement of origins of IIA and/or CFA
TASC D	<ul style="list-style-type: none"> -Chronic total occlusions of the CFA or SFA (≥20cm involving PA) -Chronic total occlusions of PA and proximal trifurcation vessels 	<ul style="list-style-type: none"> -Infrarenal aortic occlusion -Diffuse disease involving aorta & both iliac arteries requiring treatment -Diffuse multiple stenoses involving the unilateral CIA, EIA, CFA -Unilateral occlusions of both CIA & EIA -Bilateral occlusions of EIA -Iliac stenoses in patients with AAA requiring treatment & not amenable to endograft placement or other lesions requiring aortic or iliac surgery

Table 9: TASC II classification of iliac and femoro-popliteal arterial lesions. S – stenosis, O – occlusion, AAA – abdominal aortic aneurysm, CIA – common iliac artery, EIA – external iliac artery, IIA – internal iliac artery, CFA – common femoral artery, SFA – superficial femoral artery, PA – popliteal artery (Norgren et al., 2007).

In addition, the co-morbid status of the patient is of crucial importance. They are a high-risk group of patients that often have significant disease in other vascular beds, limiting their lifespan and their ability to withstand high-risk procedures. There should be a clear aim to the treatment, which may include wound healing, limb salvage, ambulation, and/or long-term patency. The aim should be consistent with the expected longevity of the patient (Bradbury et al., 2010). Primary amputation should be reserved for cases that are non-salvageable due to extensive tissue loss or those with limited functional capacity. Timely decisions regarding primary amputation however, can reduce associated morbidity and mortality if appropriate rehabilitation is put in place (Dawson and Mills, 2007).

Treatment Comparison and Outcome Measures

The goals of treatment for CLI include the restoration of pulsatile, inline flow to the foot to assist wound healing, the relief of rest pain, the avoidance of major amputation, preservation of mobility and improvement of patient function and quality of life (Lumsden et al., 2009, Subherwal et al., 2012). The composite outcome of limb salvage and survival (amputation free survival AFS) is a commonly used outcome measure (Conte, 2010a). These goals are predominantly achieved with endovascular or open surgical techniques; although attempts at medical therapies have been tried and biological treatments are under investigation. Bypass with autogenous vein is still considered to be the gold standard (Conte et al., 2009, Conte et al., 2006). The morphology of the arterial lesion (e.g. site, length, type) and the patient characteristics influence the treatment and prognosis (Muradin et al., 2001).

Outcomes of recanalisation procedures can be measured using clinical endpoints or quantified from a haemodynamic standpoint. The table below summarises commonly used outcome measures:

Outcome	Endpoint	Measure
CLINICAL	Limb salvage	Amputation Amputation-free survival
	Anatomical	Patency rates
	Reintervention	Reintervention rates
	Patient perspective	Symptom relief Quality of life
HAEMODYNAMIC	Haemodynamics	Pressures
	Physiology	Tissue perfusion

Table 10: Outcome measures following revascularisation

Clearly, clinical outcome measures are easily accessible to record and are of paramount importance to the patient. Haemodynamic markers assess the magnitude and durability of any improved perfusion to correlate with treatment efficacy (Conte, 2010a). What is sometimes difficult to address is whether there is a direct relationship between outcomes and treatment failure, particularly with respect to limb salvage & mortality.

Clinical

Limb Salvage

Limb salvage or amputation free survival has traditionally been the benchmark of successful revascularisation in CLI. Whilst they are crude markers of success they are easy to measure and encompass the primary aim of treatment of CLI; limb salvage. It assumes that amputation rates will reduce as successful revascularisation procedures increase.

Patency Rates

Patency rates measure the success of the intervention itself and can be considered as primary or secondary patency depending on the requirement for reintervention. A radiological residual stenosis of $<30\%$ would be considered 'patent'. However, closure of surgical grafts or restenosis of an angioplastied vessel does not always represent failure if wounds have healed or rest pain has been resolved. Limb salvage rates have been shown to exceed graft patency rates and not all limbs become re-threatened (Dawson and Mills, 2007, Dorros et al., 2001). Patency rates vary depending on the anatomical site and severity of disease, and the type of graft material used (Conte, 2010a). Reintervention free survival takes into account the need for secondary procedures and is an important outcome which few studies make reference to.

Patient Perspective

Symptom relief is an important outcome measure. Amelioration of pain and wound healing are key and are not always synonymous with patency rates. Quality of life outcomes show a marked improvement in those patients who undergo successful revascularisation and consequently require less hospital care (Adam et al., 2005). Increases in walking distance or improvements in mobility are seldom recorded.

Haemodynamics

Pressures

Following a successful intervention, it seems intuitive that a post-intervention increase in ABPI's of at least 0.1 would signify an increased perfusion pressure and therefore an efficacious outcome. The increase often occurs in the first 24 hours but maximal

improvement can take up to one month. Jelnes and Wagner both showed a clear improvement from baseline ABPI following open ($p<0.001$) and endovascular ($p<0.05$) treatment respectively, in a mixed cohort of claudicants and CLI patients (Jelnes, 1986, Wagner et al., 2003). Saucy et al demonstrated a mean ABPI rise of 0.4 to 0.8, $p=0.002$ following successful surgical revascularisation in CLI and an associated rise in toe pressures from 21 to 40mmHg, $p=0.001$, in the immediate post-operative phase (Saucy et al., 2006). In a cohort of claudicants undergoing PTA, Husmann and colleagues however found that ABI was not significantly different up to six months post-intervention compared to pre-intervention (0.89, $r=0.72-1.10$ vs. 0.71, $r=0.52-0.85$ respectively) (Husmann et al., 2006).

ABPI is, of course, still subject to the same problems that it suffers when diagnosing CLI, particularly in regard to medial calcinosis and its inability to identify disease distal to the cuff and the overall circulation of the foot. It may therefore, not be a sensitive indicator of predicting success and patency in the longer term. In a study by Ray et al looking at outcomes of 41 patients with CLI (Fontaine stage III and IV), toe pressure measurements before the intervention were similar in patients who showed a clinical response to treatment and those that did not benefit at six months (19.4 ± 20.4 vs. 20.0 ± 20.8 , NS). The positive predictive value of a toe systolic pressure <30 mmHg was 21% (Ray et al., 1997a). Equally, pre-intervention ABPI's did not correlate with clinical outcome at six months either (0.35 ± 0.24 vs. 0.27 ± 0.27) (Ray et al., 1997a).

ABPIs have been adopted by some as a clinical screening tool for surveillance of grafts or angioplastied vessels. The intention is to increase detection of disease progression and those requiring further screening or observation. However, they are not very specific in detecting

those grafts, which may be at risk of failure (Wyatt et al., 1990). A large retrospective study of 270 infrainguinal bypasses, demonstrated a poor predictive value for ABPI <0.2 and ankle pulse volume recording (APVR) $<5\text{mm}$ on detecting either early or late graft closures (Samson et al., 1985). Furthermore, it is not sensitive in predicting wound healing as there was shown to be no significant difference in the ABPI between those whose ulcer did and did not heal (Castronuovo et al., 1997).

Normalising perfusion of the large vessels may only be one part of a multifaceted process and may not reflect transformations that occur in the microcirculation in CLI.

Improvements in Outcome Measures

Conventional treatment outcome measures have focused on clinical and haemodynamic markers of success, which have not shown a longer-term predictive value or correlation with patency or symptom relief. The adaptive microvascular changes and endothelial dysfunction that occur in critical ischaemia are well documented and contribute significantly to the clinical picture, but are not delineated by these methods. Whether these microcirculatory changes are reversible or permanent affects the clinical outcome and directly measuring them is a useful complement to the established techniques to detect success of treatments in terms of skin perfusion (Norgren et al., 2007).

Physiology: skin perfusion pressure

Restoration of microcirculatory flow after a period of occlusion is measured using a laser Doppler to detect the blood pressure at which this occurs. CLI is distinguished by a SPP of $<30\text{mmHg}$. Whilst it has been shown to be a reliable, non-invasive method of detecting

severity of disease and predicting ulcer healing, its continued monitoring following intervention is under utilised.

A novel angiographic technique suggested as an early surrogate marker of limb salvage is 'wound blush', comprising contrast opacification of the vessels around the wound immediately after endovascular treatment (Utsunomiya et al., 2012). In 93 limbs with Rutherford 5 or 6 disease, this correlated with limb salvage at one year and also with a higher pre-intervention SPP $26.1 \pm 12.4 \text{ mmHg}$ vs. $20.9 \pm 8.5 \text{ mmHg}$, $p=0.027$. This phenomenon may characterise patients with preservation of their microcirculatory function (Utsunomiya et al., 2012).

Surgery

Patients requiring surgical management are a particularly challenging group, as peri-operative morbidity and mortality is high and long-term patency rates can be poor. Whilst procedures can be performed under local anaesthesia, regional or general anaesthetics are commonplace. A period of stay in hospital is required, in the region of five to ten days, and may be longer in patients who need more extensive rehabilitation or suffer complications. In the BASIL trial the 30-day mortality rates were equal between the two arms but the surgical group suffered higher morbidity with longer hospital stay, a two-fold increase in myocardial infarctions and a three-fold increase in the risk of stroke (Falluji and Mukherjee, 2012).

Whilst there is no uniform or successful way to predict whether grafts will remain patent, good general principles can be applied to increase the chances of success. If the graft fails in the short term it is usually due to a technical failure. Embolectomy or graft revision can be

performed but subsequent risk of failure remains high. In the longer term 30-50% of lower extremity bypasses are reported to stenose or occlude within three to five years (Rutherford, 2000). Inflammatory, haemodynamic and humoral injury have been cited as causes (Conte et al., 2006).

Grafts should preferentially be performed using an autologous venous conduit such as the long saphenous vein, which has superior patency to cephalic or short saphenous vein at one year (64.4% vs. 51.5% respectively) (Conte et al., 2006). Thromboembolic complications occur more frequently with prosthetic grafts, especially when they are used more distally and are significantly inferior to vein grafts at any level (66% vein vs. 33-47% below and above knee PTFE respectively at 5 years) (Beard, 2008). Furthermore, they should not be used in the presence of active infection in the bone or soft tissue. The length and diameter of the graft also have an effect on long-term patency; with smaller (<3mm) and shorter (<40cm) vein grafts achieving lower patency rates at one year (diameter <3mm 42.4% and >3mm 68.4%, length <40cm 69.0% and >60cm 53.7%) (Conte et al., 2006).

The inflow and outflow should ideally be continuous with the pedal circulation to further augment success rates (Dawson and Mills, 2007). High vascular outflow resistance makes it more likely that the graft will fail. Inflow procedures can be performed in conjunction with bypass grafts, as can vessel endarterectomy. Complex, multi-level disease may necessitate sequential ± composite grafts to more than one outflow vessel.

Complications of surgery are early and late and include wound or chest infection, bleeding, myocardial infarction, stroke, graft failure and limb loss.

Endovascular

Until the latter two decades of the twentieth century it was conventional for all patients with CLI to be offered treatment with open arterial bypass surgery, which had been an established and effective technique since the 1950's, by surgeons including Kunlin and Hall (Goodney et al., 2011, Friedman, 2005). This policy reflected the absence of any alternative therapeutic options but it has been increasingly challenged over 20-30 years by the development of endovascular-based approaches. As a consequence, the number of infrainguinal bypass graft performed over the last decade has reduced significantly and the versatility of endovascular devices has developed (Beard, 2008). Advances in endovascular approaches in terms of imaging, equipment and technical ability have allowed greater numbers of patients to be deemed 'suitable' for this treatment and for more complex disease to be treated by minimally invasive methods. The term 'endovascular' encompasses percutaneous balloon angioplasty and more complex techniques, including an armamentarium of stents (un/covered, medicated) or atherectomy devices to improve success rates. The superiority of covered vs. uncovered stents has not been demonstrated nor a consistent and proven benefit of other accessory devices such as atherectomy balloons and laser (Beard, 2008, Lumsden et al., 2009). Angioplasty can be performed using conventional anatomic luminal techniques or newer sub-intimal techniques to treat long segment lower extremity disease, involving femoro-popliteal, crural or pedal levels. By directing a more flexible wire intentionally out of the lumen in a subluminal passage, very diffuse and/or calcified disease can be crossed before directing the wire back into the true lumen. The changes in clinical practice and new data available have rendered some of the TASC criteria out of date (Adam and Bradbury, 2007). That said, approximately one quarter of CLI patients may not be amenable to endovascular revascularisation because of unsuitable anatomy (Lumsden et al., 2009).

The advantages of angioplasty may be substantial in cohorts of patients with considerable co-morbidities. Firstly, the procedure is performed under local anaesthetic and therefore does not necessitate a general or regional anaesthesia or impose the stresses of surgery. Most cases can be performed as a day case. It can treat both inflow and outflow disease. The technical success rates for endovascular procedures are high; however, longer-term success is more intangible, particularly in the CLI population. Certainly the techniques do not tend to be as durable as bypass surgery if the disease is very distal, involves a long segment, or is very extensive (Dawson and Mills, 2007). As one might expect stenotic compared to occlusive disease is more amenable to successful intervention, which often delineates claudicants from critical limb ischaemia.

Kandarpa et al reported a technical success rate of 90% and 75% in CLI patients with occlusions and stenoses respectively. However at one-year patency rates were 62% at one year and 54% at 3yrs in those treated for stenotic disease and 26% and 18% at 1 and 3 years in those treated with occlusion (Kandarpa et al., 2001). Focal disease, in non-calcified vessels, with restorable run-off vessels is preferable for this treatment option, rather than diffuse disease with compromised distal vessels (Dawson and Mills, 2007). That said, interventions in below knee vessels area being increasingly used for treatment of CLI (*see infrapopliteal section*) (Clair et al., 2012).

Most reports of ‘success’ following angioplasty are made on clinical grounds from resolution of clinical symptoms or limb salvage, and morphologically from immediate technical success and long-term patency with serial ultrasound scans. A residual stenosis <30% is generally the accepted level of success. If the procedure is deemed to have ‘failed’ in the longer term it

may still provide enough distal perfusion to aid healing or resolve rest pain, particularly if it is single-level disease and has been described as a 'temporary bypass' to provide wound healing and limb salvage by Met et al (Met et al., 2008). This phenomenon is brought about by the discrepancy between vessel patency and limb salvage rates in the endovascular cohort. In a systematic review of subintimal angioplasty Met et al found a technical success rate of 80-90% in infrainguinal vessels, with primary patency rates of 50% at one year and clinical success varied from 50-70%. Limb salvage rates however, were substantially higher than this at one year (80-90%) (Met et al., 2008). Of note, this review included a mixture of patients with claudication and CLI and the associated technical success was lower in the CLI group compared to the claudicants. Failure is more likely if there is extensive disease or there are infected tissues (Dawson and Mills, 2007). Furthermore, all the articles Met reviewed were observational studies and the materials and experience with sub-intimal angioplasty were only just evolving in some of the earlier papers reviewed from the 1990s.

Whilst there is a significant restenosis rate in the longer term, this treatment is thought to preserve surgical options, if required at a later date or for additional endovascular procedures. However, on later analysis the BASIL trial found that a failed angioplasty and subsequent bypass surgery resulted in poorer outcomes than those who went straight to surgery and as such, should not be regarded as a risk-free procedure (Figure 17) (Conte, 2010a, Bradbury et al., 2010).

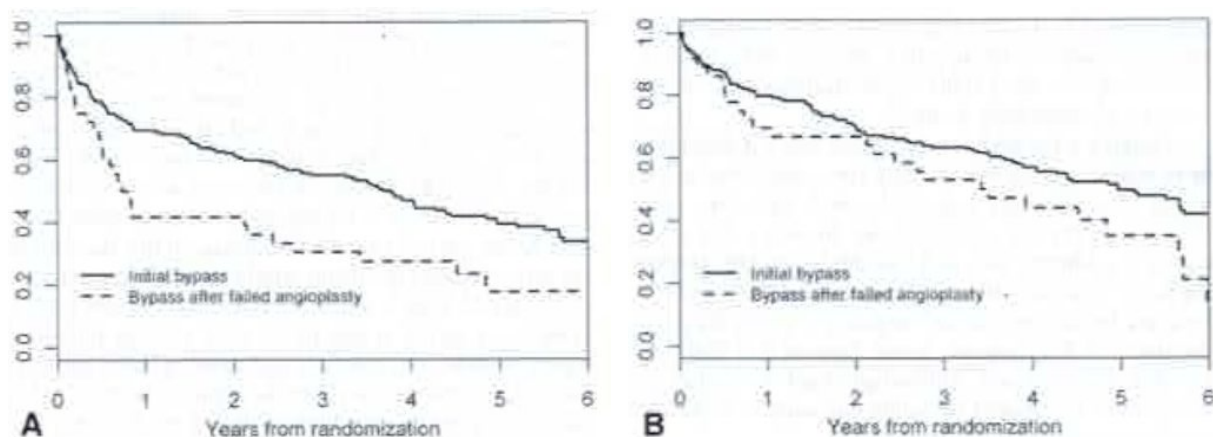


Figure 17: A) Amputation free survival and B) overall survival in patients randomised to and undergoing bypass surgery and in patients undergoing bypass surgery after failed balloon angioplasty (Bradbury et al., 2010).

CLI multi-level disease does often necessitate multiple-site angioplasty to achieve cure (Lumsden et al., 2009). Ryer et al found that 18% of patients with chronic limb ischaemia had recurrent stenoses (82% within 18 months) following PTA with or without stenting and of those 82% underwent repeat endovascular procedures, 11% had surgical revascularisation and 4% went straight to amputation. The limb salvage rate at 12months was 86% in a mixed cohort (Ryer et al., 2006).

Complications of endovascular management range from 8-17% and include bleeding, haematoma, vessels perforation, contrast-induced renal failure and emergency surgery from a dissection or embolus. On a microvascular level, trauma to the arterial wall causing distal emboli can cause microvascular impairment (Bongard et al., 1994, Met et al., 2008).

Femoropopliteal Disease

A number of adjuncts, predominantly stents, which provide a constant radial force on the arterial wall, are available to assist in primary patency of PTA (Lumsden et al., 2009). A

meta-analysis of stent implantation and balloon angioplasty of the femoro-popliteal segment in PAOD, found that stents might give a more favourable outcome than balloon angioplasty in more severe cases of CLI (3yr patency 63-66% vs. 30-43% respectively). The numbers of studies using stents in the analysis however were low as most of the stent data relates to claudicants (Muradin et al., 2001). Clearly stents carry their own risk particularly in reference to thromboembolism and there is usually a requirement for subsequent antiplatelet agents. In addition, most stents are used based on the anatomy of the lesion rather than the patient characteristics and symptomatology, making comparison of outcomes difficult.

Infrapopliteal Disease

Angioplasty below the knee was traditionally reserved for life and limb-threatening disease due to the likelihood of occluding the tibial vessels and rendering the limb non-viable. Whilst this still remains a significant risk, the procedure has been shown to be effective with technical success rates approaching 100% in some studies (Lumsden et al., 2009). Primary patency rates however, vary greatly with reports from 13%-81% at one year and stenoses tend to do better than occlusive lesions, as one may expect (Lumsden et al., 2009). As with femoropopliteal disease, longer lesions are most often approached with sub intimal recanalization whereas stenoses and short occlusions are generally managed with intraluminal angioplasty.

Primary stent treatment of infrapopliteal lesions has been used with array of success with the aim of securing inline flow to the foot. Proximal crural lesions have better one-year patency and limb salvage rates than more distal lesions (85.1% and 48.5% patency and 100% vs. 81.8% limb salvage respectively) usually as vessels tend to be more tortuous and calcified

more distally (Lumsden et al., 2009). Accordingly, technical success is usually lower in crural interventions compared with femoral procedures (Met et al., 2008). There are no trials of stent vs. surgical bypass grafts to the infrapopliteal region, which is most often required in critically ischaemic patients (Beard, 2008).

In a series of 284 limbs that underwent tibio-peroneal angioplasty 168 (59%) of ischaemic limbs also required inflow angioplasty (Dorros et al., 2001). Patients with class III disease were more likely to have a successful outcome than those with class IV disease (99% vs. 90%), reflected by fewer bypasses required as secondary treatment for class III disease (3% vs. 16%) and a greater number of subsequent amputations in class IV patients at 5 years (Dorros et al., 2001).

Iliac, Femoral and Infrapopliteal Disease

In a retrospective cohort study by Kudo et al of exclusively 138 CLI limbs undergoing PTA, the 5-year primary patency was 31.4%, with assisted primary and secondary patency rates of 75.5% and 79.6% respectively, with a limb salvage rate of 89.1%. The low primary patency rate is consistent with severe occlusive disease (Kudo et al., 2005). Nine limbs required major limb amputation; five (3.6%) were within 30 days and 2/3 were diabetic with unreconstructable distal vessels and/or advanced gangrene in all but one. Twenty-four percent of patients were over the age of 80 years. Average ABI was 0.4 with a range of 0.19-0.89, excluding 10% who had significant medial calcinosis. Primary patency rates reduced with more distal disease (iliac $51.6 \pm 11.0\%$, fem-pop $49.4 \pm 11.9\%$, below-knee $23.5 \pm 10.7\%$ at 36 months). Patients whose main lesions were distal however, were not always treated with

concomitant proximal angioplasty and they carried more risk factors (60% diabetic, 1/3 CRF, 1/3 >80yrs) (Kudo et al., 2005).

Most patients were TASC C (54%), 37% were TASC D and 9% were TASC B. However, this study encompassed selected CLI patients who had not fulfilled the criteria for open surgery (1, bilateral common iliac disease 2, unilateral external iliac occlusion 3, long >10cm infrainguinal occlusion starting from the orifice of the SFA). In one third of cases iliac angioplasty was the most distal artery treated and 55% of patients only had one segment treated. On univariate analysis, TASC classification was the most striking independent variable which significantly reduced primary± assisted patency (p0.02 & 0.0015), secondary patency (p0.098), clinical improvement (p0.053) and ultimately limb salvage (p0.0034). The number of segments treated and the most distal vessel treated significantly affected primary and secondary patency but not clinical improvement or limb salvage. Of note, 9.4% underwent subsequent surgical revascularisation within 30days following PTA, although the individual details of these patients are not mentioned (Kudo et al., 2005).

Certainly the long-term limb salvage rates are high in this study and PTA may simply be increasing the perfusion required to heal the wounds and allow collateral channels to form. This would be consistent with the almost 60% difference in primary patency rates and limb salvage at 5 years. It is however, difficult to gauge the severity of disease in this population given the numbers of patients who had ABI >0.4 and those who only required single segment revascularisation and hence whether they strictly fall into the classification of CLI. Consistent with mortality reports in CLI populations, only 44% of patients were alive at 5 years and those with the most severe TASC D disease did the least well (Kudo et al., 2005).

Surgery and Endovascular

Al-Omran et al retrospectively analysed over 27,000 endovascular and open procedures over a seven year period and showed that overall the 5-year survival rate for patients undergoing open surgery was $61.5\% \pm 0.38\%$ compared with $69\% \pm 0.6\%$ for those who underwent endovascular treatment. Furthermore, amputation free-survival at 5-year was also better in the endovascular group than the surgical group ($92.2\% \pm 0.34\%$ vs. $83.4\% \pm 0.37\%$ respectively) (Al-Omran et al., 2003). However, patients were not stratified according to their disease classification or whether they were non/critical or acute/chronic ischaemia and these two intervention groups have very different characteristics and anatomy of disease. The pattern of disease plays a huge role in the outcome of the procedure, as demonstrated by the vast difference in death rates between supra-inguinal and infra-inguinal procedure survival outcomes of 74.7% and 56.8% respectively. They did comment that the endovascular group was significantly younger than the open group. Male sex and increasing age was a common risk factor for risk of death to both groups and coronary artery disease and diabetes were significant risk factors in the surgical group. As such, the two groups are likely to represent very different patient populations with different disease profiles.

The Bypass versus Angioplasty in Severe Ischaemia of the Leg (BASIL) trial is the only randomised trial to date comparing open and endovascular interventions for severe and CLI. It ran over a 5-year period with 27 centres, randomising 452 patients (Adam et al., 2005). Patients were considered eligible if they met Rutherford classification 4-6, with no strict haemodynamic cut-off per se, hence encompassing patients considered as severe limb ischaemia (SLI) as well as critical limb ischaemia (Conte, 2010a). Of all patients presenting with severe/CLI, 10% considered to be in clinical equipoise with regards to the optimum

treatment were enrolled in the trial. There were however, no anatomic or clinical guidelines considered as 'equipoise', and therefore the individual centres dictated this (Conte, 2010a). The outcomes after 2-year follow up showed that overall survival and amputation free survival were not statistically different between the two groups, with an increased cost associated with surgery. In keeping with previous trial results on conduit material, BASIL found that prosthetic material was associated with significantly worse outcomes compared to autologous vein. Prosthetic graft constituted 25% of the surgical group. Post hoc analysis ten years on has demonstrated notably enhanced clinical outcomes in the group randomised to surgery. Seventy percent of patients survived beyond 2 years and in this group overall survival and amputation free survival increased. (Bradbury et al., 2010)

Skin Control Mechanisms after Treatment

The assessment of skin microcirculation has a predictive capability in patients with non-reconstructable disease and whether or not they will require a limb amputation over the next 18 months (Ubbink et al., 1999). Macrocirculatory measures such as ABPI does not offer any predictive capacity as all subjects share a similar reading in CLI. Combined assessment with capillary microscopy, transcutaneous oximetry and laser Doppler perfusion has shown additional capacity to distinguish those with particularly poor circulation. With cut off values of capillary density $<20\text{mm}^2$, $\text{TcpO}_2 <30\text{mmHg}$ and absent reactive hyperaemia there is a specificity of 87% and predictive value of 73% for amputation (Ubbink et al., 1999).

The success of revascularisation in CLI is dependent on reconstituting a normal and effective skin microcirculation. To this end, measures of skin haemodynamics should be a useful

adjunct in predicting outcomes and establishing what happens to control mechanisms in the skin after treatment.

Surgical Revascularisation

Laser Doppler Fluxmetry (LDF) and Laser Doppler Imaging (LDI)

In a small prospective study of ten patients undergoing infrainguinal reconstruction, with a pre-operative mean toe pressure of 21mmHg, a global increase was found in foot perfusion ten days following surgery using laser Doppler imaging (LDI) (Saucy et al., 2006). This was irrespective of which crural and foot vessels were patent pre-operatively and included three diabetic patients. In addition, it was associated with significant rises in ankle and toe pressures ($p=0.002$ and $p=0.001$) and subsequent ulcer healing in the affected limb. Interestingly, they found that skin blood flow reduced after surgery on the untreated side. Perhaps this was a consequence of neural stimulation from a relative increase in flow and pressure (*see 'VAR' below*). This study demonstrates the importance of skin microcirculation as a marker of successful revascularisation, however there are no inclusion or exclusion criteria mentioned, nor is there a control group or comparison with limbs that have failed to improve and what changes occur in their microcirculation and associated haemodynamic measures. Two studies used LDF fluxmetry for treatment evaluation after surgery. One found that resting flux values were enhanced following surgery, but this was subject to large overlap between pre and post-operative values and no correlation was found with these measures and ABPI (Wahlberg et al., 1995). The other found that there were no significant differences seen in basal flow, suggesting that this is not a discriminatory test (Delis et al., 2001). Using Xenon washout techniques, Eickhoff demonstrated that post-reconstructive hyperaemia or increased flow at rest manifested only in the presence of an attenuated VAR

pre-operatively (Eickhoff and Engell, 1982b). This represents the most severely ischaemia group and perhaps more passive perfusion until autoregulation is resumed.

Reactive Hyperaemia

Given that reactive hyperaemia can distinguish between healthy individuals and those with PAOD, it seems rational that the same measure could be used to determine successful outcomes following revascularisation. Wahlberg et al carried out a prospective study of 60 consecutive patients undergoing open surgical infrainguinal revascularisation for claudication, rest pain or gangrene (Wahlberg et al., 1995). The reactive hyperaemia (RH) was conducted with the probe on the base of the first toe, with a cuff at the ankle inflated to 250mmHg for a 3 minute occlusion time, the day before surgery and on the first or second post-operative day \pm at day 5 if post-operative values were within 5% of pre-operative measurements. Eight patients who did not have a detectable RH response were excluded. All of these patients had ankle pressures less than 25mmHg and 4 had multi-segment disease.

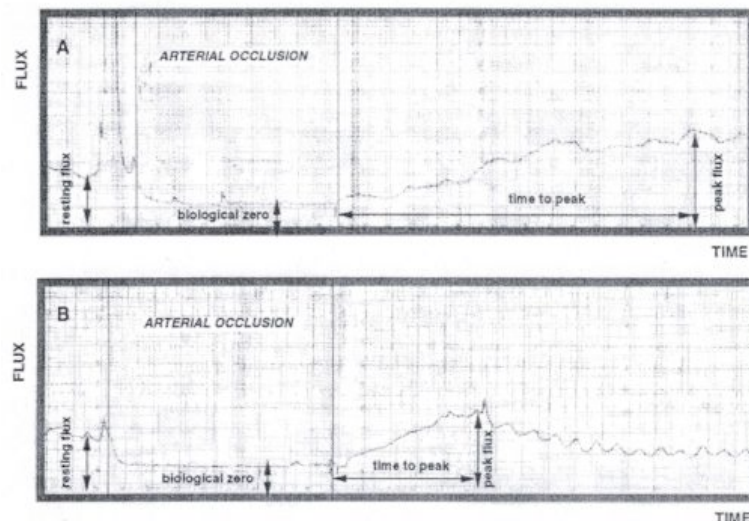


Figure 18: Reactive Hyperaemia curves from a patient with a preoperative ABI of 0.15 before (A) and after (B) infrainguinal reconstruction (Wahlberg et al., 1995).

Following surgery, a significant decrease in time to peak was found ($p < 0.001$) and this correlated with an improvement in ABPI of more than 0.15 ($r = -0.51$). In patients whose ABPI did not increase by more than 0.15, none of the microcirculatory parameters improved, despite some clinical improvement in over 50% of patients in this group. However, on subgroup analysis time to peak was able to distinguish a difference between those who gained clinical improvement, regardless of ABPI ($p < 0.001$). The discrepancy suggests that microcirculatory impairment affects clinical outcome and is a more sensitive indicator than ABPI (Wahlberg et al., 1995).

The cohort itself contained a mixture of PAOD patients and does not relate strictly to CLI, with one third being claudicants. Furthermore the exclusion of patients with no recordable RH meant that the most 'critical' of the group were omitted. In addition, there is no long-term follow up of these patients to see if their time to peak, in spite of an improved ABI was predictive of graft failure.

An earlier study from 1988 identified nine patients from within their cohort who had undergone surgical reconstruction; six aorto-femoral bypasses & three femoro-popliteal bypasses. They concluded that post reconstructive reactive hyperaemia values, including time to peak, improved in all limbs and in fact 'normalised' in reconstructed single level disease (Van den Brande and Welch, 1988). It is not clear whether these differences are attributable to the presence of multi-level disease but only single level reconstruction being performed in certain cases. Similar to previous studies in this area, there is an assortment of disease severity and also anatomical location and they have not compared their findings to any

haemodynamic measures of success. In addition, the timing of the post-operative studies have not been clarified and hence whether this is a short-term or longer-term change.

Local Vasoconstrictor Mechanisms and Oedema

Eickhoff and colleagues undertook a lot of work in this area in the 1980's. They used the Xenon washout technique in ischaemic limbs undergoing revascularisation procedures above and below the inguinal ligament, to describe the changes in local vasoconstrictor responses (Eickhoff and Engell, 1982a). The table below summarises their findings, divided by the level of reconstruction and the pre-operative presence or absence of vasoconstrictor response. All patients are reported to have had clinical improvement and an associated rise in distal pressures and normalisation of autoregulation.

		VAR			
Grp	Procedure	Pre-op	Early post-op: Day 10	Late post-op: 3-6months	Hyperaemia
A	Infrainguinal	Absent	Present: NS	Present: Sig.	Significant
B	Infrainguinal	Present	No change	No change	NS
C	Suprainguinal	Absent	Present: NS	Present: Sig.	Significant
D	Suprainguinal	Present	Absent: Sig.	Present: Sig.	None

Table 11: Summary of findings by Eickhoff and colleagues on the return of VAR and post-operative hyperaemia (elevated resting blood flow). Groups A to D delineate the presence or absence of pre-operative VAR and whether the procedure was performed above or below the inguinal ligament. NS - Not significant, Sig - Significant.

Those with a preserved vasoconstrictor response present at the outset had higher pre-operative ankle and toe pressures and therefore by definition, were not critically ischaemic. Some of the patients in Group A who showed absent responses, were only suffering intermittent claudication and their pre-operative ankle and toe pressures ranged from 25-80mmHg and 0-53mmHg respectively.

The post-operative hyperaemia however, correlated with a low or absent pre-operative VAR and was therefore greatest in those with the most severe ischaemia at the outset and the lowest distal blood pressure (Eickhoff and Engell, 1982a, Jelnes, 1986). Subsequently, those that displayed the greatest change in VAR post-operatively demonstrated the greatest hyperaemic response. This supports the concept of loss of normal small vessel function either from ischaemic damage or atrophy, as the cause for hyperaemia although it has not been correlated directly with limb oedema per se. Overall, the study suggests that revascularisation prompts normalisation of the auto-regulative mechanism of vasoconstriction in this group of patients, which is probably carried out by the arterioles (Eickhoff, 1985, Eickhoff and Engell, 1982a).

In a subsequent study by the same group, postoperative oedema developed after 12/13 infrainguinal procedures and only 1/17 suprainguinal reconstructions (Eickhoff, 1985). Whilst late post-operative improvements in VAR and hyperaemia remained commonplace to the most severe disease (rest pain vs. claudication), postoperative oedema appeared to develop independently to these and appeared more related to the procedural type.

Furthermore, when the groups when analysed by symptoms irrespective of type of reconstruction, the VAR in the rest pain group normalised by the early postoperative period

i.e. within 10 days despite persistent hyperaemia. They dispute pre-capillary resistance as the cause of oedema, as this appears to normalise within 10 days. Instead, they consider lymphatic damage at the popliteal level as a cause and the hyperaemic response to be related to small artery rather arteriolar damage (Eickhoff, 1985). Of course, whilst flow and ischaemia are improved with revascularisation and reperfusion, it does not alter the atherosclerosis in the vascular bed beyond the distal anastomosis (Delis et al., 2001). Others feel that the hyperaemia post-operatively is just a reflection of the normalisation of blood flow and not a true hyperaemia (Jelnes, 1986).

Delis et al also found that loss of autoregulation is not a permanent feature and can be restored following surgical revascularisation. They used laser Doppler fluxmetry to demonstrate the percentage decrease in skin blood flow on sitting was not significantly different between normal and patients', within six days following successful infra-inguinal bypass grafting for severe ischaemia (Delis et al., 2001). In fact, the skin flux during sitting was significantly higher in claudicants compared to the post-operative group ($p < 0.001$). There is however no indication as to what patients VAR's were pre-operatively or whether they were troubled with lower limb oedema as a consequence of vasoparalysis, at day six post-operatively.

The hypothesis that the vasoconstrictor impairment is functional and flow-dependent, rather than as a result of structural damage, is supported by an improvement in VAR at day three following aorto-bifemoral bypass. This was however for non-critical ischaemia, and VAR was present in all patients pre-operatively and improvement was seen by a reduction in sitting blood-flow from 27% to 45% post-operatively. This improvement persisted at 45% 28 days later (Ubbink et al., 1999). This may not be representative of the CLI population where

ischaemic damage and/or atrophy of cells have been reported (Hillier et al., 1999, Coats and Hillier, 2000).

Those patients who displayed oedema post-operatively did not demonstrate return of their VAR until 3-6 months following surgery, which at that time corresponded to improvement in oedema. An attenuated VAR pre-operatively was also correlated with post-reconstructive hyperaemia, or the increase in flow at rest (Eickhoff and Engell, 1982b). Hyperaemia was therefore correlated with severity of ischaemia. This would reinforce the role of arteriolar tone in the development of oedema.

Oedema

After successful infra-inguinal surgical revascularisation lower limb oedema is ubiquitous, irrespective of pre-operative oedema. It occurs within the first 48 hours and is both troublesome for patient mobility and wound healing. The distribution and nature of the oedema is distal and pitting and can last from several weeks to months post-operatively (Porter et al., 1972).

This phenomenon is reasoned to be due to an abrupt increase in arterial inflow and systemic transmural pressure in the region of >70mmHg into an atrophied system that has lost its ability to autoregulate, having become chronically accustomed to low pressures (Eickhoff, 1985, Eickhoff and Engell, 1982a). The reduction in capillary resistance is maintained and there is a high transcapillary filtration rate and subsequent accumulation of interstitial fluid, in keeping with Starlings law. This can further compromise tissue nutrition (Coats, 2003, Coats and Wadsworth, 2005).

However, oedema formation and post-reconstructive hyperaemia have not always been inextricably linked and oedema formation was not correlated with clinical severity of symptoms, VAR or ankle pressures, only with the type of surgery, in the study mentioned above by Eickhoff (Eickhoff and Engell, 1982b). Post-reconstructive hyperaemia and oedema developed after all femoro-popliteal bypasses, but only after a limited number of aorto-bifemoral bypasses (Coats, 2003, Eickhoff and Engell, 1982b, Porter et al., 1972). Similar to previous studies, the vasoconstrictor response was absent in the most severe cases until three to six months later but this did not equate to post-operative oedema. The difficulty of evaluation comes again though where a mixture of disease severity and different anatomical procedures within a small cohort muddies the water of interpretation.

Alternatively, lymphatic disruption has been suggested as a contributing factor to oedema following inguinal and popliteal dissection where major lymphatics can be found (Eickhoff and Engell, 1982b). Lymphangiograms have demonstrated disruption to superficial inguinal lymphatics with contrast extravasation following surgery for femoro-popliteal bypass grafting. The magnitude of disruption has been correlated with the extent of the oedema, particularly if the medial superficial nodes are traumatised. Equally, preservation of lymphatic channels appeared to minimise oedema (Porter et al., 1972). In the event of early graft failure, oedema is not observed. This could be because vast transudation of fluid does not occur because capillary pressure does not reach a critical level and so fluid can still be reabsorbed by lymphatics, albeit if the system has been disrupted. Furthermore, intact lymphatics are able to absorb the increase in filtrate achieved by successful supra-inguinal reconstruction (Eickhoff and Engell, 1982b).

The fact that it is so unusual to experience similar findings following recanalisation with endovascular treatment would support this theory. However, very often the disease severity is not as significant in this patient population as endovascular techniques are less likely to be successful in long segment, severely calcified disease. It seems unprecedented though to have similar magnitudes of lower limb oedema with other forms of femoral dissection, such aorto-bifemoral bypass grafting and insertion of cardiac catheters. Post-operative venous thrombosis and hypoalbuminaemia are not generally acknowledged as causes of unilateral oedema in this situation (Khiabani et al., 1999b, Porter et al., 1972).

Endovascular Techniques

Laser Doppler Fluxmetry and Reactive Hyperaemia

This technique has been used, predominantly in claudicants, who underwent 21 PTA and/or mechanical recanalisation for symptom relief of ilio-femoral disease and were compared to 16 controls that underwent diagnostic arteriography alone (Bongard et al., 1994). The primary aim was to see if endovascular treatment resulted in impaired microvascular haemodynamics and tissue oxygenation as a result of infraclinical microemboli, despite an improvement in the macrocirculation.

Both groups had a pre-intervention ABPI of 0.7. In accordance with angiographic improvement, all patients experienced an improvement in toe and ankle indices 4-6hours following intervention, which remained stable one month later. However, this did not correlate with some of the microcirculatory measures in the treatment arm. In the PTA group, tissue oxygenation ($TcpO_2$) decreased immediately after the procedure compared to pre-operative levels and to the control group (46mmHg (29-67) and 40mmHg (10-65) vs.

46mmHg (26-64) and 46mmHg (29-65), $p=0.0004$), without a corresponding reduction in resting rCBV and rLDF readings. A nutritional index calculated from these latter two readings (rCBV/rLDF) suggesting that there was a reduction in nutritional blood flow following PTA, correlating with a reduction in $TcpO_2$ readings. Whilst the skin blood flow had not decreased overall, rather the blood flow had been redistributed to sub-papillary plexi, consistent with the hypothesis of subclinical micro emboli from the procedure (Bongard et al., 1994). These features did begin to show improvement at one month. Of note, there was no clinical oedema observed in these patients.

These findings are from patients with claudication and therefore cannot be generalised to the CLI population. Furthermore, the study was mainly adopted for looking at complications rather than looking at distal perfusion benefits per se.

Leonardo et al examined 209 limbs, healthy and PAOD, to record their post-occlusive hyperaemia after a three-minute cuff occlusion. Within this group, four limbs with single-level stenosis were treated with PTA and their PORH flow patterns returned to the same range as healthy subjects with time to peak flux improving from 150.0 ± 47.2 to 55.0 ± 7.0 , see figure 19 below. They all had simultaneous resolution of their symptoms although it is not clear over what time course this occurred (Leonardo et al., 1987).

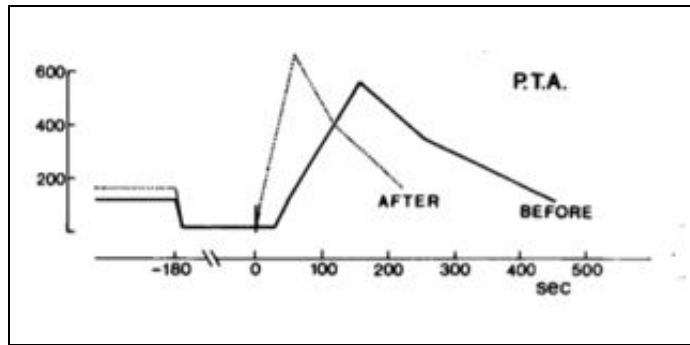


Figure 19: Effects of percutaneous transluminal angioplasty (PTA) on the average pattern of the cutaneous post-ischaemic hyperaemia test curve (Leonardo et al., 1987).

The subject group who underwent intervention is undoubtedly very small and they were suffering with moderate single-level disease as opposed to critical ischaemia. The angiographic techniques for dealing with complex disease was not available in 1987 when this paper was published and the indications and technology are far more advanced now. In the previous paper with a cohort of similar disease severity, a one-minute cuff occlusion did not elicit an improvement in PORH response after a haemodynamically and angiographically successful PTA, with mean time to peak reactive hyperaemia not changing (10 vs. 9 vs. 12 sec before, after and 1 month). (Bongard et al., 1994) These time to peak hyperaemias are very short and are not representative of critical ischaemia.

Local Vasoconstrictor Mechanisms

In a study of 14 patients undergoing femoro-popliteal angioplasty for stable claudication, percutaneous revascularisation significantly improved VAR ($55.5\% \pm 21.2$ from $33.4\% \pm 20.2$), measured by LDF with a probe secured at the tip of the big toe. The change occurred due to a significant reduction in orthostatic blood flow, horizontal blood flow remained unchanged.

The changes persisted at 6 months follow-up although they did not return to the same level as healthy age and sex-matched volunteers ($68.4\% \pm 20.5\%$) (Husmann et al., 2006).

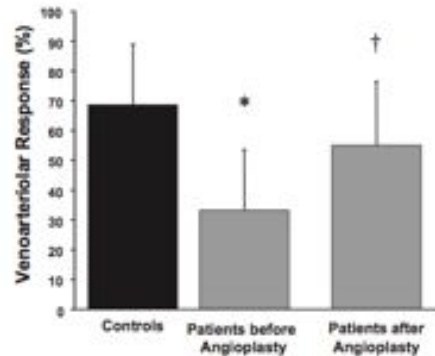


Figure 20: Vasocutaneous autoregulation in controls and in patients with peripheral arterial disease. Decrease in skin blood flow on sitting (VAR) in healthy controls compared to patients with stable intermittent claudication before and after femoro-popliteal angioplasty. * $P < .05$ versus controls; † $P < .05$ before versus after angioplasty (Husmann et al., 2006).

The ABPI's similarly increased immediately after the intervention (0.71 (0.52-0.85) to 0.89 (0.72-1.10)) and remained unchanged during the follow-up, in a similar fashion to VAR. It supports the hypothesis that local vasoregulation is pressure and/or flow sensitive to maintain local tissue perfusion (Husmann et al., 2006). Baseline characteristics in a similar group of patients with non-critical disease also demonstrated a highly significant correlation between VAR and post-exercise ABPI (Ota et al., 2005).

Oedema

Anecdotally, patients who undergo PTA do not tend to experience oedema after the procedure and is rarely objectively measured in endovascular studies. Ray et al specifically measured changes in lower limb volume, calculated from calf girth measurements, one day and one week following balloon angioplasty in 37 patients undergoing intervention for disabling claudication (n=27), rest pain (n=7) and ulceration (n=3). The calf volume of the treated limb

did not increase regardless of anatomical location or severity of disease at one day or one week. However, there was a reduction in the volume of the control leg immediately following PTA, suggesting potential fluid redistribution, as this finding was no longer evident one week later (Ray et al., 1997b).

Diabetes

Limb salvage and long term patient survival are reduced in the diabetic population compared to those without diabetes (Conte, 2010b). Patients are often younger with co-existing coronary artery disease and renal disease. Furthermore, intervention more commonly involves infra popliteal and infra-malleolar vessels and the use of short segment grafts and if ulcers are present they tend to heal more slowly than patients without diabetes (Akbari et al., 2000, Coats, 2003, Lepantalo et al., 2011). However, there is very little data available on any potential changes observed in the microcirculation following revascularisation procedures, particularly with regard to diabetes.

Functional ischaemia has been shown to improve, although not to the levels of healthy controls, in a study of 13 diabetic patients following femoro-distal bypass using iontophoresis and transcutaneous oxygen pressure (Arora et al., 2002). Four-six weeks following surgery all patients had palpable graft pulses. The vasodilatory response to heating and acetylcholine both improved from pre-intervention to post-intervention ($p < 0.05$) but were comparable to patients with neuropathic disease, not healthy controls. C-fibre nociception failed to show any improvement following revascularisation (Arora et al., 2002). This is in keeping with work by Veves who used $TcPO_2$ to demonstrate that reversing hypoxia by revascularising extremities of diabetic patients halts the progress of neuropathy, but does not affect

neuropathic damage already present (Akbari et al., 1997, Veves et al., 1996). The persistent endothelial dysfunction may explain why some patients have persistent or recurrent tissue loss despite a running graft and improvement in the macrocirculation.

1.6 Conclusions

Critical limb ischaemia is characterised by critically low perfusion pressure to lower limb tissues, resulting in loss of small vessel reactivity and autoregulation such that it is unable to regulate its nutritive blood supply. Poor oxygenation, tissue loss, ulceration and ultimately limb loss ensue.

There are two key therapeutic strategies available to manage CLI; open surgical bypass or endovascular recanalisation. To date, the vast majority of trials have appraised these treatments individually, using conventional clinical outcomes including resolution of symptoms, prevention of amputation and/or vessel patency, as predictors of long-term success. Physiological improvements in haemodynamic markers such as APBI have not been shown to have any predictive value in assessment of outcome, with increases in toe pressures variably showing a relationship with patency in the short and longer-term. Changes occurring in the microcirculation are generally under utilised as an outcome measure.

From a surgical perspective, reactive hyperaemia time to peak has been shown to normalise after treatment and correlate with clinical outcome, but not ABPI, in an unselected group of PAOD patients (Van den Brande and Welch, 1988, Wahlberg et al., 1995). In some studies however, patients with the most severe CLI were excluded. Outcomes of other parameters

used in measuring reactive hyperaemia have been generally mixed and unreliable. Auto-regulative control processes such as VAR have improved within days following successful surgery, above and below the inguinal ligament and is associated with a significant hyperaemia at rest (Delis et al., 2001, Eickhoff and Engell, 1982a). Whether the presence or absence of vasomotor control is linked to the development of post-operative oedema has not yet been established.

Such information is only occasionally included in endovascular series and the majority is related to functional, rather than critical ischaemia. There is a reported discrepancy between vessel patency and limb salvage, which would seem to confound macrovascular measures but associated changes in the microcirculation are seldom described. Improvement in reactive hyperaemia has been shown after PTA, but this was in single level disease (Leonardo et al., 1987). Similarly in claudicants, local vasoconstrictor mechanisms improved towards the levels of healthy volunteers following femoro-popliteal angioplasty (Husmann et al., 2006). A relationship with oedema has never been identified following PTA, regardless of severity of disease and flowmotion and corresponding changes in frequencies has not been formally assessed in this setting.

It is evident that there is a paucity of data on the effects of treatment on skin circulation and studies that do measure microcirculatory outcomes are limited and do not present a comprehensive picture, either to compare within treatment or to correlate with clinical outcomes. The BASIL trial went some way to comparing limb salvage and survival in CLI but the two treatment modalities were not compared with respect to their perfusion benefits. Other studies have used a homogenous patient population, with no focus on CLI. Certainly,

no studies have directly compared treatment effects on basal perfusion, reactive hyperaemia, VAR, flowmotion and oedema. Consequently, it remains unclear whether successful revascularisation, by either technique, reverses endothelial dysfunction and the microvascular disease process and perhaps those patients who have a poor outcome despite apparently satisfactory revascularisation, have persistent microvascular dysfunction.

Aim and Hypotheses

2.1 Aim

The aim of this study was to compare open surgical bypass and endovascular treatment in CLI using conventional clinical outcomes (limb salvage, patency, symptom relief), haemodynamic measures (ABPI, toe and skin pressures), microcirculatory measures (resting flux, RH, VAR, flowmotion) and resultant oedema and tissue hardness.

2.2 Hypotheses

It is hypothesised that successful open surgery will lead to superior outcomes compared to endovascular techniques with regard to:

1. Clinical -
 - Resolution of symptoms and higher patency rates

2. Haemodynamic -
 - A greater increase in ankle, toe and skin pressures

3. Microcirculation –

- There will be a greater reduction in reactive hyperaemia time to peak. Consequently one would expect to see other microcirculatory parameters improving with a greater increase in resting basal flux, improved regulation of local postural vasoconstrictor mechanisms and an associated increase in flowmotion related to myogenic and sympathetic control mechanisms

4. Oedema –

- A bigger rise in tissue hardness associated with greater unilateral lower limb oedema

METHODS

3.1 Study Subjects

Forty patients participated in the study. All were undergoing treatment for ischaemic rest pain, tissue necrosis or gangrene of the lower limb. Patients were recruited from Selly Oak Hospital Vascular Department and identified through inpatient or outpatient referral to the vascular team. In addition, site-specific application was approved by local Research and Design committees, to study patients from Birmingham Heartlands Hospital Vascular Department.

Patients, who fulfilled the criteria of Fontaine stage III or IV disease, with multi-level atherosclerotic arterial disease requiring infra-inguinal revascularisation with either arterial bypass surgery (ABS) or percutaneous transluminal angioplasty (PTA), were invited to take part. Patients who had multiple co-morbidities contributing towards lower limb pathology together with single level arterial disease were assessed on the basis of toe pressures <30mmHg, or <50mmHg in the presence of ulceration or gangrene, in accordance with the Trans-Atlantic Society Committee (TASC) definition of critical limb ischaemia (CLI). The exclusion criteria were clinical evidence of deep venous insufficiency, acute limb ischaemia and major limb amputation. In addition, patients who could not tolerate cuff insufflations around the calf were excluded for practical reasons. Patients also requiring concomitant treatment of iliac inflow disease were not excluded. Patients were not retested if they required repeat intervention to the same limb for recurrent symptoms.

Medical history was documented, including data on concomitant illnesses, previous angioplasties or vascular surgeries, for all study participants. Height and weight were recorded using a portable stand-on scale, with an attached height-measuring stand. Body mass index was calculated by the individual's body mass in kilograms divided by the square of his or her height (kg/m^2).

The TASC classification of inflow and femoro-popliteal disease has been used to stratify patient disease and its pattern. Pre-intervention imaging to assess the anatomy of disease was always with Duplex ultrasound in the first instance, by one of two dedicated vascular technicians in the outpatient department. Their findings were recorded on standardised diagrams as seen in Figure 21. Duplex reports were corroborated with subsequent CT or MR angiography, particularly in patients where iliac vessels could not be imaged due to overlying bowel gas, or extensive calcification made it challenging to see infrageniculate vessels on Duplex.

As an adjunct to TASC, the Nicolson-Simms-Brown index (NSB) was devised as a numerical classification of disease severity in the lower limb. It is derived from the degree and length of stenosis as determined by pre-intervention imaging and therefore the higher the number, the more severe the disease.

Table 12 below demonstrates NSB calculation. The columns labelled grade and segment are completed with values from 0-4 dependent on the grade of the lesion and whether it involves all or some of the specified vessel. The sum of each row is added up and multiplied by a factor of 1 to 3 depending on the anatomical impact of the disease, to give a maximum total of

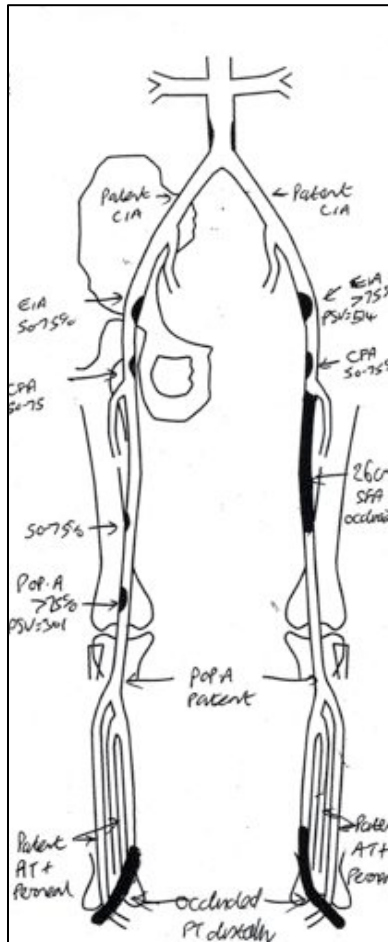
108. The higher the NSB index the greater the burden of disease. An example is shown in Figure 21 below, using the standardised proforma from lower limb ultrasonography.

Nicolson-Simms-Brown Classification: Global index of severity of chronically ischaemic lower limbs

Vessel	Grade	Severity	Multiply	Total	Max Score
CIA			3	0	18
EIA			3	0	18
CFA			2	0	12
SFA			2	0	12
Pop			2	0	12
TPT			1	0	6
AT			1	0	6
PT			1	0	6
Pe			1	0	6
				0	108

KEY	Grade	Severity
0	Patent	0 None
1	Stenotic <50%	1 Partial
2	50-75%	2 Complete
3	75-99%	
4	Occluded	

Table 12: The Nicolson-Simms-Brown Classification to classify severity of CLI by grade and segment, giving a total score of up to 108. The greater the total number, the more severe the disease. CIA – common iliac artery, EIA – external iliac artery, CFA – common femoral artery, PFA – profunda femoris artery, SFA – superficial femoral artery, Pop – popliteal artery, TPT – tibioperoneal trunk, AT – anterior tibial, PT – posterior tibial, Pe – peroneal.



Vessel	Grade	Severity	Multiply	Total
CIA	0	0	3	0
EIA	3	1	3	12
CFA	2	1	2	6
SFA	4	1	2	10
Pop	0	0	2	0
TPT	0	0	1	0
AT	0	0	1	0
PT	4	1	1	5
Pe	0	0	1	0
				33

Figure 21: A schematic representation of a lower limb ultrasound scan used to calculate the NSB index. Using the table above, the NSB of the left leg in this patient was calculated as 33. CIA – common iliac artery, EIA – external iliac artery, CFA – common femoral artery, PFA – profunda femoris artery, SFA – superficial femoral artery, Pop – popliteal artery, TPT – tibioperoneal trunk, AT – anterior tibial, PT – posterior tibial, Pe – peroneal.

The study was approved by the ‘Black Country Research and Ethics Committee’ and performed in accordance with the principles of the Declaration of Helsinki. Each patient gave written informed consent before any study-related procedure was conducted. (See Appendix A)

3.2 Protocol

The principal aim of this observational study was to ascertain whether there was a quantifiable difference in skin blood pressure, flow and vascular reactivity with the extent and method of revascularisation of the affected limb.

Revascularisation of an ischaemic limb can be achieved by open surgical or percutaneous endovascular techniques. Surgery uses autologous vein or a prosthetic graft to bypass the affected segment/s of diseased vessel, providing a new conduit free from disease. Endovascular techniques recanalise diseased vessels in-situ to improve flow to the extremities. Surgical techniques are reported to be more durable in the longer term in comparison to endovascular techniques, as canalised vessels have a tendency to re-stenose after a period of time. In order to compare the efficacy of these treatments their outcomes need to be objectively measured and compared in the short and longer-term.

Patients were assigned to different treatment groups, on clinical grounds and were not randomised. This was based upon pattern and extent of disease and whether it was amenable to endovascular or surgical treatment, patient age and co-morbidities and patient preference. In cases of bilateral limb ischaemia, the most severely affected limb (leg one) was treated first. Table 13 below demonstrates the methods of assessment used to ascertain the primary, secondary and clinical endpoints.

	Method of Assessment
Primary endpoint	❖ Reactive hyperaemia (RH) - change in time to peak (ΔT_p) using laser Doppler flowmetry
Secondary endpoints	❖ Absolute toe pressures & TBPI ❖ Skin LDF pressures ❖ Lower limb anthropometric volume ❖ Tissue depth ❖ Tissue tension (Durometry) ❖ Laser Doppler flowmetry – basal flux and spectral analysis of basal flux (Fourier transformation) ❖ Postural Vasoconstriction (PVC) ❖ Neurothesiometer: Vibration perception & detection thresholds (VPT/VDT)
Clinical endpoints	❖ Absolute ankle pressures & ABPI ❖ Patency ❖ Symptom relief – complete/partial/none ❖ Further intervention/surgery

Table 13: Primary, secondary and clinical study endpoints and their method of assessment

All measurements were taken in the treatment limb and also in leg two, the contralateral limb. Each patient was tested on three separate occasions at discrete time points in relation to the intervention:

- Pre-intervention (within 72hours)
- Post- intervention (within 72hours)
- Six weeks post-intervention

In addition, a small sub-group (n=7) were tested at a six-month follow up visit.

All measurements were made at the three-time points specified, except the measurements involving cuff insufflations around the calf (reactive hyperaemia, ankle and skin pressures), which were not performed immediately post-operatively to avoid inadvertent damage to a newly canalised vessel or graft.

Testing procedures

The subjects lay supine in an examining room at Selly Oak Hospital, or as close to supine as possible for those that found it difficult medically to lie flat. Shoes and socks were removed and the lower limbs exposed from the distal femur with all dressings taken down. The temperature was kept as stable as possible at $22.2 \pm 1.3^{\circ}\text{C}$ because of the importance of ambient temperature on skin blood flow. Direct sunlight was avoided and noise and interruptions were kept to a minimum. No medications, including vasoactive drugs, were stopped but patients were requested not to smoke. Resting heart rate was recorded manually. Measurements that did not involve the laser Doppler were performed first, allowing time for the patient to acclimatise to the environment. All measurements were performed in both legs unless specifically stated; leg one being the most symptomatic.

Anthropometry

Anatomical reference points were marked with a permanent marker pen at the outset to allow repeated measures from the same location. These were 2.5cm proximal to the centre/most prominent part of the medial malleolus and 5cm distal to the tibial tuberosity.



Figure 22: Reference markings made with a permanent marker 2.5cm proximal to the medial malleolus and 5cm distal to the tibial tuberosity

Intervention-related changes in lower limb volume were calculated from anthropometric measurements of the limb segment. A tape measure was used to measure circumference, starting at the ankle 2.5cm proximal to the centre/most prominent part of the medial malleolus and 5cm distal to the tibial tuberosity. The section length between these two points was recorded. In addition, forefoot circumference was measured around the first and fifth metatarsal heads. Lower limb segment volume was determined using the truncated-cone method (Fuller et al., 1999) as follows:

$$V_s = L/12\pi(C_1^2 + C_1 C_2 + C_2^2)$$

V_s - Segment volume, $C_1 + C_2$ - measured circumference at either end of segment of length (L)

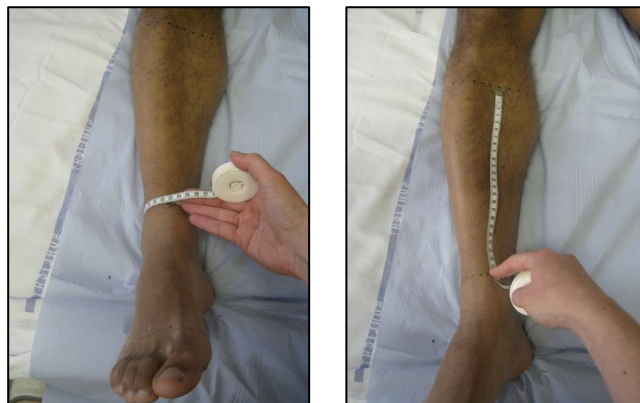


Figure 23: Measuring the ankle circumference at the pre-defined point 2.5cm proximal to medial malleolus and the length of the limb between the two markings.

Tissue Depth

Tissue depth was used as an indirect measure of the distribution of tissue oedema, which may be present in the lower limb subcutaneous tissues following intervention and to corroborate with lower limb volume. A portable ultrasound machine, *Sonosite 180* with a convex probe

was used to measure tissue depth from the skin surface to the fascia in the lower limbs (Figure 24).

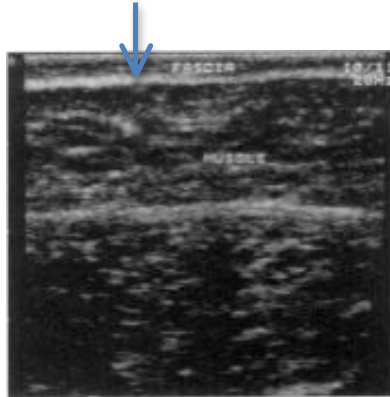


Figure 24: Transverse section demonstrating fascia and underlying muscle of lower leg. Arrow indicates the point of measurement at the fascial surface from the skin surface.

The probe was held perpendicular to the skin. Firstly, in the plane 2.5cm proximal to the medial malleolus, the tissue depth was taken just anterior to the medial malleolus. Secondly, in the plane 5cm distal to the tibial tuberosity over the *tibialis anterior* the tissue depth was measured just lateral to the tibia. Finally, tissue depth in the forefoot was taken over the metatarsal heads.



(A)



(B)

Figure 25: Measurement of tissue depth in the lower limb using a portable ultrasound machine. Depths taken (A) 2.5cm proximal and anterior to the medial malleolus and (B) lateral and 5cm distal to the tibial tuberosity.

Durometry

A Rex Durometer Hand Model 1600, Type 00 (Rex Gauge Company Inc., Buffalo Grove, IL, USA) was used to assess tissue tension in the lower limbs. Within the context of medicine, experience with the Durometer has predominantly been in dermatological conditions such as scleroderma to monitor disease progression and also in the assessment of skin changes in relation to venous disease (Choh et al., 2010, Kissin et al., 2006). It has not previously been used to assess tissue ‘hardness’, caused by tissue oedema in relation to lower limb revascularisation.

Durometry should be performed over areas of soft tissue, not bone, with the instrument being gently supported to allow the weight of the instrument (170 gram) to press against the skin. Ulcerated areas should be avoided if possible. With the patient supine, the Durometer was rested perpendicular against the skin in two places:

- In the plane 2.5cm proximal to the centre/most prominent part of the medial malleolus, lateral to the *extensor hallucis longus (EHL)* with the patients leg slightly internally rotated to keep the Durometer perpendicular
- In the plane 5cm distal to the tibial tuberosity, over *tibialis anterior (TA)* with the patients leg slightly internally rotated to keep the Durometer perpendicular

With the patient sitting and feet resting flat on the floor, Durometry was measured in the first web space of the foot. This was done at the end of the testing so as not to disturb the supine rest period.

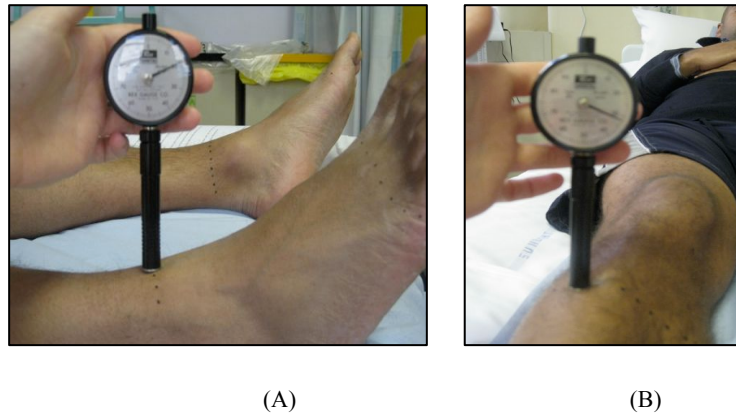


Figure 26: Measurements taken with the Durometer at (A) the ankle, 2.5cm proximal to medial malleolus and (B) the calf, 5cm distal to the tibial tuberosity with the leg internally rotated to keep the Durometer perpendicular

Measurement of arterial pressures and pressure indices

Pressures were measured in both limbs using a manual non-mercury sphygmomanometer and a hand-held 8 MHz Doppler probe. Insonation of the right upper limb was over the radial artery for brachial systolic pressure and the posterior tibial (PT), peroneal (PN) and dorsalis pedis (DP) arteries for ankle pressures. The individual pressures of each of the respective arteries were recorded. In addition, ABPI was calculated for each of the three ankle arteries.

A Vascular Assist® portable laboratory (Huntleigh Healthcare, UK) was used to measure toe pressures in the great hallux of each foot, using the phlethysmography probe. The second toe was used in cases where the first toe was severely ulcerated. Three measurements were taken from each limb and the average reading was used to calculate the TBPI.



Figure 27: Measurement of toe pressure at the hallux with the patient supine, using the Vascular Assist®

Laser Doppler Flowmetry

Principles

The Doppler effect was a term coined in 1844 by Johann Christian Doppler, through experimentation with the pitch of a moving trumpet and a static observer. The principles of ‘Doppler shift’ were swiftly applied to astronomy and later to fields such as medicine and engineering, with the development of laser technologies and fibre-optics in the 1970’s. The advent of laser Doppler flowmetry (LDF) has enabled the study of microvascular function in humans in many different tissues including skin (Shepherd and O\berg, 1989).

Laser Doppler blood flowmetry utilises the principles of Doppler shift to continuously record and measure the dynamics of blood flow, but uses coherent laser light instead of sound. The visible and infrared light emitted by the instrument is monochromatic, so it has very narrow range of wavelengths or extremely high frequencies (Fullerton et al., 2002, Shepherd and O\berg, 1989). It is directed to a limited volume of tissue via an optical fibre, or directly mounted onto a probe. As light enters the tissue, the interactions between photons and moving red blood cells produce a change in frequency, creating the Doppler shift. The magnitude of the shift depends on the velocity of the cells.

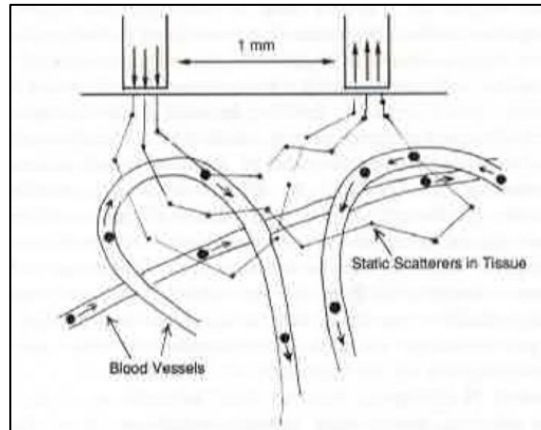


Figure 28: Schematic diagram of light defusing through vascularised tissue from a laser source and back scattered light leaving to return back to a photo detector (Shepherd and O\berg, 1989).

In a true sampling area, the vessels will be of varying size, function, tension, tonicity and micro-anatomical orientation relative to the incident and reflected laser light (Fullerton et al., 2002). The system is however insensitive to the direction of movement by the random spread of blood cell velocities and hence scattered light, so the Doppler shifts attained are distributed on a wide bandwidth (Fullerton et al., 2002, Tenland et al., 1983, Vongsavan and Matthews, 1993). One or more afferent optical fibres, running in parallel within a single probe, collect the light and transmit it back to the monitor where it is converted into an electric signal. This ‘flow signal’ is the average frequency shift normalised with respect to the unshifted signal from the stationary tissue (Johnson et al., 1984, Kvernebo, 1988).

The speed of light is a constant. Therefore, if light strikes a stationary object and is reflected directly back to a detector, the received light will have the same frequency as the emitted light (Shepherd and O\berg, 1989). Only a minor portion of about 3-7% is reflected back (Fullerton et al., 2002). However, the returning light will impart a Doppler shift if it is

reflected back from a moving object such as a red blood cell. The output signal achieved is linearly related to the product of the red blood cell (RBC) velocity and concentrations i.e. flow. Additional frequencies and greater amplitudes are generated when there are multiple collisions, as a result of a greater number of cells scattering the light (Cracowski et al., 2006).

For the blood flow to influence the laser and its output, the photons must penetrate to the depth of tissue blood flow and also return from it. Most biological structures will scatter incident light so only surface layers tend to be easily penetrated anyway. The penetration of laser light into tissues alters depending on its wavelength. Scattering of light in the dermis varies inversely with light wavelength; therefore longer wavelengths penetrate the dermis to a greater extent than shorter wavelengths (Berardesca et al., 2002). The vast majority of the wavelengths are absorbed by components of the dermis and epidermis are much less than those used in laser-Doppler i.e. 660-800nm (Shepherd and O'berg, 1989). Melanin, however, does afford some protection to laser light and reduces its penetration at wavelengths of up to 1200nm. Haemoglobin tends to have its peak absorption around 600nm.

There are different methods to determine the depth of LDF measurement, which vary with the approaches used and on different tissues and their optical properties. In skin, studies involving the increase of blood flow to skeletal muscle by moderate exercise or occlusion techniques do not result in a comparable increase on LDF. This demonstrates that the laser-Doppler is specific to superficial tissues and is not affected by blood flow to deeper tissues such as muscle, when applied to the skin. (Saumet et al., 1988, Shepherd and O'berg, 1989) Although there is no critical depth of detection of flow, the greatest penetration and measurement depth is likely to be no more than 2.0mm with maximum sensitivity around 0.6mm (Saumet et al.,

1988). Although the thickness of skin differs with anatomical location, it is in the range of 1-3.5mm, and this is still deep enough to measure flow in nutritive capillary loops and in the subpapillary arterio-venous plexi of the dermis, without contribution from deeper tissues (Abularrage et al., 2005, Bircher et al., 1994, Saumet et al., 1988).

In the single-probe technique, the sampling size on the skin is around 1-2mm² depending on probe geometry, and is therefore highly sensitive to variations in dermal perfusion. Flow values obtained should be regarded as relative to that particular region and not extrapolated to absolute values (Fullerton et al., 2002). Relative perfusion values representing total blood flow are therefore expressed as arbitrary perfusion units (APU), rather than absolute units. This is as a consequence of the heterogeneity of the sample, such that cutaneous blood flow cannot be measured relative to the volume or weight of tissue, rather to the product of the average concentration and velocities of moving red blood cells in that tissue sample volume. Furthermore, the sensitivity to a particular flow rate within a vessel varies according to the distance of the vessel in the capillary bed from the probe tip (Cracowski et al., 2006, Fullerton et al., 2002, Vongsavan and Matthews, 1993).

$$\text{PERFUSION OR FLUX} = \text{NUMBER OF RBC's} \times \text{MEAN VELOCITY OF RBC's}$$

The LDF signal (in volts) should therefore be linearly related to the volume-velocity product of the blood, assuming proportionality between RBC number and blood volume. This will in turn, depend on the haematocrit of the sample i.e. the number of moving red blood cells in that sample and the volume of capillaries present (Fullerton et al., 2002, Johnson et al., 1984). Platelets and leucocytes are suggested to have little impact on the flux derived as they

contribute relatively little volume overall (Tenland et al., 1983). Flow is not age-dependent and there are no major differences between sex and racial groups. There is however, considerable regional variation due to the lack of uniformity and regional comparisons must be made with prudence (Bircher et al., 1994).

Reproducibility

Natural temporal and spatial variations exist in skin blood flow, therefore 'normal' values, are difficult to describe. The structural differences in skin blood vessel architecture and the dynamic nature of vascular function provide explanation for regional variations in blood flow (Abularrage et al., 2005). However, absolute flux levels can provide a broad overview of perfusion levels in any one area.

Baseline blood flow on cuff occlusion, or biological zero, is highly reproducible, with a coefficient of variation of 6% (Tenland et al., 1983). The flux does not actually return to zero and is not influenced by perfusion and or limb position (Wahlberg et al., 1990). It is due to non-blood-flow related movements of muscle cells and vessels walls (vasomotion), stagnant red blood cells and other molecular movement (Van den Brande and Welch, 1988).

The coefficient of variation of baseline cutaneous blood flow is much higher than provocation tests such as post-occlusive reactive hyperaemia (Bircher et al., 1994). This is due to the site-specific nature of LDF. For example, areas such as the forehead, which are known to be vascularly dense, have higher baseline flux than other areas such as the sternum. The magnitudes of finger and toe pulp LDF readings are similarly reflective of high flow from their AV anastomoses and the density of vessels (Jepsen and Gaehtgens, 1995, Schmidt et al.,

1993). The site-to-site diversity in morphology of the skin microvasculature is reflected in the difficulty in conducting longitudinal LDF studies, due to site-specificity of sampling areas with the single-probe technique. To obtain repeated measures, prudent orientation and localisation of the probe is essential (Tenland et al., 1983). To eliminate spatial variability, Lamah and colleagues used micro tattooing and capillaroscopy to look at capillary numbers in the dorsum of the foot over the course of one month. They found very little temporal difference between counts ($2.4\% \pm 1.82$) although this did not measure capillary flow per se (Lamah et al., 2001).

One of the major drawbacks is the unprovoked intra-individual variation between sites and even the same site in the same individual after hours, days or weeks (Fullerton et al., 2002, Tenland et al., 1983). This is however, a reflection of the in homogeneity of the skin microvasculature. Using a multiple measurement probe can help with standardising results. In the present study, a multiple measurement probe was used at the pulp of the great toes.

Technique

Standardisation of equipment, procedures and measuring conditions must be adhered to, to reduce the risk of errors being introduced:

Reduce motion artefacts: equipment

The pattern of reflection of coherent light is, in part, dependent on the properties of the reflecting surface. If the surface is stationary this ‘speckle’ pattern will not vary. As soon as the reflecting surface, the laser or the fibre optics leading the laser light to the surface are

moved the speckle pattern is also changed, causing movement artefact and subsequent error in blood flow signal (Shepherd and O\berg, 1989).

In an effort to reduce this, a number of variables can be controlled including:

- Reduced fibre size: reducing the diameter of the fibres reduces the motion artefact
- Mechanical fibre dampening: prevents the fibres from moving too fast or going into mechanical oscillations. There are a number of variables including, stabilising the fibres in mechanically dead material, the method of coupling between the distal fibre end and the tissue and the end-surface quality.
- Movements at the measured site: this can include tissue movements due to muscles or pressure waves in the arterial tree causing pulsating tissue movements. This is often indistinguishable from the part of the signal generated by the flux of RBC's. Nevertheless, mechanically restraining tissues can in itself impair blood flow (Shepherd and O\berg, 1989). In this study probes were repositioned in the same location at each time point by concise measurements and for the halluces, centring the probe in the middle of the toe pulp.

Reduce motion artefacts: participants

The laser Doppler is very motion sensitive. Even with probes firmly adherent to the skin surface, movement artefact cannot be eliminated and non-physiological readings can be formed from even the most discrete movements. Tissue structures besides red blood cells and movement of the fibre-optic cables can create Doppler shift and should be kept to a minimum to avoid creating signals, which cannot be distinguished from red cell flux (Vongsavan and Matthews, 1993). Ideally, long studies should be avoided where possible. Clearly the heart-

synchronous LDF signal conducted into the distal tissues under analysis cannot be eradicated (Berardesca et al., 2002). Once the recording is underway, a stable baseline can be achieved in 2-3mins (Sundberg, 1984). Patients were provided with a good explanation of the study process so they were aware of what was expected and should increase compliance and reduced movement.

Standardise and calibrate

In order to maintain accuracy of measurements methods should be calibrated at regular intervals, by comparing them to a standard (Shepherd and O\berg, 1989). Laser Doppler fluxmetry creates a unique difficulty with calibration since flow is spatially variable, it cannot be calibrated in absolute terms. Comparison to other methods has been attempted and generally, there is good alliance but obtaining a unique 'calibration factor' poses a complex problem, largely due to the site-specific nature of the LDF signal (Berardesca et al., 2002). In studies assessing a change in the flux from baseline after a physical intervention, the patient is able to act as their own control, which can avoid the need for calibration and can in fact make results more reliable (Berardesca et al., 2002).

Standardising the laser-Doppler ensures it retains its reproducibility, as flux does not reach absolute zero when there is no perfusion present. Instead, Brownian motion of macromolecules arising from the interstitial space contributes to the remaining signal when red blood cell flow is absent (Cracowski et al., 2006). 'True zero' will only be achieved when the probe is placed against non-living material where no movements occur, so there will be no Doppler shift of the scattered light (Berardesca et al., 2002). There are several techniques used for standardisation and setting the sensitivity of the instrument, but of relevance to this

study is the use of a particle suspension as the reference level. This is a concentrated colloidal suspension of latex spheres, specific to the manufacturer that is used to ensure that the probes are detecting Brownian motion only, giving a standard motility of 250PU (Shepherd and Oberg, 1989, Vongsavan and Matthews, 1993).

Light Source Considerations

The light source, commonly helium-neon (He-Ne) should be stabilised to prevent a high noise to signal ratio. The noise can be misinterpreted as blood flow motion, frequently through a variation in the temperature and thermal expansion of the laser cavity.

Ambient Light

Interference may be introduced through ambient light, and light bulbs and fluorescent lighting are particularly noteworthy due to their fluctuation in intensity at mains frequency (Vongsavan and Matthews, 1993). Although it is suggested that normal, constant levels of background lighting should not interfere with readings (Vongsavan and Matthews, 1993). By attaching the probes with an adhesive tape this in itself provided an element of protection from external light. This was applied gently to the skin, as pressure of the probe and tape application may reduce blood flow to the area concerned (Berardesca et al., 2002).

Room temperatures

Due to the skins normal thermoregulatory function, neutral room temperatures should be maintained to minimise variability from stimulating the microvasculature in extremes of temperature. This is particularly true of the extremities where the greatest numbers of AV shunts are present and flow can vary as much as a factor of 100-200 (Shepherd and Oberg,

1989). Stable readings can be achieved with ambient temperatures between 20 and 25°C (Bircher et al., 1994). In this study, the mean ambient temperature was $22.2 \pm 1.3^\circ\text{C}$ and was maintained by using the same examination room as much as possible. Where patients were not mobile enough to be moved to the examination room e.g. immediately post-operatively, studies were conducted at the bedside. Temperatures did not vary greatly between locations.

Position

The subject position should be controlled. They should be rested supine with the test-site at the relative level of the heart, so the microvasculature is not affected by orthostasis. There should be 20-30 minutes allowed for acclimatisation where the test-site is uncovered (Berardesca et al., 2002). Relaxed experimental settings are also required to prevent neurogenic alterations occurring, which can influence the cutaneous vasculature (Orlandi et al., 1988). A small number of patients were unable to lie supine due to medical conditions such as congestive heart failure or chronic obstructive airways disease. In this situation patients were raised to the lowest tolerable position, which was never greater than 30 degrees.

Vasoactive Substances

This category includes prescribed medication such as beta-blockers and calcium channel blockers that affect vascular tone, in addition to caffeine, nicotine and spicy food which should be avoided for 24 hours prior to testing. (Berardesca et al., 2002). The patients' vasoactive medications were not stopped in this study unless it was clinically indicated. Of the 40 patients, 26 were on 1 or more vasoactive medications including beta-blockers, alpha-blockers, calcium channel blockers and/or angiotensin converting enzyme inhibitors (ACEi). The proportions in each group are displayed in Table 18. This may affect peripheral vascular

tone and hence flow, detected by laser Doppler flowmetry readings. Patients were requested not to smoke prior to testing.

Study protocol for LDF

In this study, the laser Doppler was used to measure basal skin blood flow and to quantify relative changes in blood flow to challenges perturbing the microcirculation. These were a) reactive hyperaemia; b) postural vasoconstriction induced by leg dependency or by sitting.

A 4-channel LDF monitor (MoorLab, Moor Instruments Ltd, UK) was used for simultaneous recordings of skin blood perfusion at a sampling rate of 25Hz, across four standardised measurement sites:

- i) Pulp of great toe, leg 2
- ii) Pulp of great toe, leg 1
- iii) Forefoot, lateral to *EHL*, leg 1
- iv) 10cm distal to tibial tuberosity over *tibialis anterior*, leg 1

A standard calibration (flux standard) of all the probes was made prior to the recordings using the recommended solution.



Figure 29: The 4-channel Moorlab laser Doppler Flowmetry monitor

The light is delivered to each probe via a flexible optical fibre. Probe-holder mounts were secured to the skin with a double-sided adhesive disc and further secured with tape over the probe to the skin. The skin was shaved or dry skin detached as necessary. The MoorLab© output i.e. the product of the number of cells and their mean velocity, is represented by ‘flux’. Red cell flux was expressed as arbitrary perfusion units (APU). The Moorlab was interfaced to a laptop on Moor software, sampling at 25Hz. Recordings from the 4 different skin regions were displayed in real time on the computer screen and flux data was saved in timed sections relating to basal skin perfusion, and to baseline and challenge responses (for reactive hyperaemia and postural vasoconstriction).

Real-time data recorded in Moorlab was analysed either by dedicated software for Fast-Fourier transform analysis (Nevrokard) to calculate the spectral components of basal skin blood flow, or exported to an excel document for assessment of the microvascular responses (*see ‘data analysis’*).

Basal skin blood flow

This was recorded for a period of ten minutes, with patients resting supine. The data from all four channels was then saved as a text file at 10Hz in readiness to analyse the power spectral content of different frequency bands.

Two main techniques exist for analysing the spectral content of a signal by transformation into a frequency domain (cycles per second) from a time domain.

1. Wavelet transformation: this takes into account the time component by using an adjustable window length (Saumet et al., 1986).
2. Fast-Fourier transformation (FFT): analysis of longer segments allows better resolution of low frequency oscillations (Kvernmo et al., 2003). This was the technique of choice for this study.

In this study FFT was used and analysis was performed with Nevrokard software, utilising the Hamming window. The power spectral density was recorded for 5 frequencies, corresponding to oscillations in endothelial, neurogenic and myogenic activity, respiratory rate and heart rate (Table 14). The absolute power (AP) of the signal was also recorded so that relative contributions for each frequency interval could be calculated.

Interval	Frequency (Hz)	Physiological Origin
I	0.009-0.02	Endothelial
II	0.02-0.06	Neurogenic
III	0.06-0.15	Myogenic
IV	0.15-0.6	Respiratory
V	0.6-2.0	Heart rate

Table 14: Frequency intervals (Avery et al., 2009, Kvernmo et al., 1999, Tikhonova et al., 2010)

Postural Vasoconstriction (PVC)

To allow new data to be saved at a rate of 1Hz, a new window was opened in the Moor Software. Flux recordings were continued for two minutes with patients in the supine position with legs horizontal for baseline analysis (HBF). Three further sub-intervals were recorded in continuity, reflecting different postural manoeuvres:

1. After two minutes, leg one was passively moved to rest in the dependent position, with the foot resting flat on a stool at the side of the bed and the knee and hip flexed to 90°. The patient remained supine such that the hydrostatic pressure load at the foot, approximately 30cm distance below the level of the heart, was 23 mmHg. Four minutes of flux during dependency was then recorded (LBF).
2. Passive return of leg one to the supine position for two minutes of basal flux recording.
3. Patients were then positioned upright sitting, with both feet dependent flat on the stool. There was around 60-70 cm distance between the probe and the level of the heart, increasing hydrostatic pressure at the foot by ~ 55 mmHg (de Graaff et al., 2003). Sitting flux (SBF) was recorded for four minutes before patients returned to the horizontal position.



(A)



(B)



(C)

Figure 30: Demonstrating the patient and probe positioning when recording the PVC from (A) horizontal, (B) leg drop/dependency and (C) sitting. In dependent and sitting positions the foot should be supported to allow a 90° bend at the knee joint.

10 min Basal	perfusion	FFT	2 min Basal	4 min Leg dependency	2 min Basal	4 min Sitting
■ ■ ■ ■ ■ ■ ■ ■ ■ ■			■ ■	■ ■ ■ ■	■ ■	■ ■ ■ ■

Figure 31: Timeline of flux recordings for basal flux and postural vasoconstriction. Red boxes represent individual minutes used in the analysis; unfilled boxes are stabilisation periods where data is discarded.

Distortion of the flux recordings due to changes in position or erroneous movements were marked onto the data sheet at the time of the study. The data for each second of flux for each of the probes were exported to Microsoft Excel as a text file. For analysis, the first two minutes of both dependency and sitting recordings were discarded as a stabilisation period and flux values over the following two minutes were averaged to calculate LBF or SBF

respectively. Any erroneous values as a result of movement artefact were preferentially noted at the time and if required, a further continuous two-minute recording was made. If values were noted to be outliers at the time of analysis in Excel, they were substituted with a value calculated from the average of 10 prior values. Care was taken not to remove physiological values.

Once fluxes were calculated for the timed sections of each manoeuvre, the following algorithm was used to determine the PVC at each site:

PVC dependency	= LBF/HBFx100
PVC sitting	= SBF/HBFx100

LBF – leg drop/dependent blood flow, HBF – horizontal blood flow, SBF – sitting blood flow

Therefore, a fall in blood flow on dependency or sitting – postural vasoconstriction - was shown as a percentage decrease in flux relative to baseline; a rise in flux on dependency or sitting was expressed as a percentage increase.

Reactive Hyperaemia

On return to the supine position with legs horizontal, after testing for postural vasoconstriction, a sphygmomanometer cuff was positioned around each calf, proximal to the shin probe on leg one. The patient was left to rest to allow stabilisation of the LDF recordings and two minutes of baseline flux was recorded from the toe probes. The cuff on leg two was first inflated to 50mmHg above brachial systolic pressure and held for 2 minutes. At this

point, the cuff was rapidly deflated and flux recorded for a further 2 minutes. This exercise was then repeated on leg one.

If patients had severely calcified vessels, the cuff was still inflated to 50mmHg above systolic pressure and held until the laser flowmetry display dropped to biological zero for the appropriate channel/s. The stopwatch was started at the point at which biological zero was achieved and held for two minutes as described. Typically, it would take approximately 30seconds to achieve baseline flux in poorly compressible arteries.

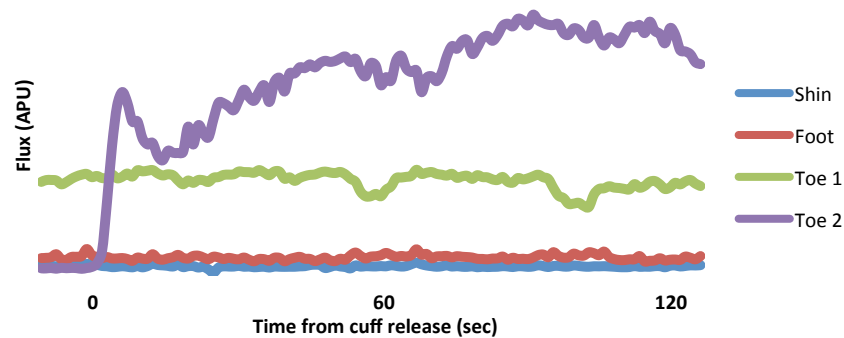


Figure 32: Typical reactive hyperaemia recording from toe two. Recordings from leg one are included and confirm that flow in this limb does not alter with occlusive testing in leg two. Time '0' represents cuff insufflation.

Data was exported into Microsoft Excel at 1Hz for analysis, to calculate the time to peak hyperaemia (T_p), magnitude of the peak (M_p) and area under the curve (AUC) for each channel/skin probe for each patient.

Analyses were made relative to cuff release at 0secs, with cuff occlusion occurring between -120 and 0secs. The basal flux two minutes prior to RH cuff occlusion (t-180 to -120secs) was

averaged to provide a baseline value, taken to represent 100%. Thereafter, actual flux values were recorded per second and calculated as a percentage of the baseline. Actual flux values were also averaged over ten second periods and expressed as % of baseline. This is demonstrated by the Excel Figure 33 below.

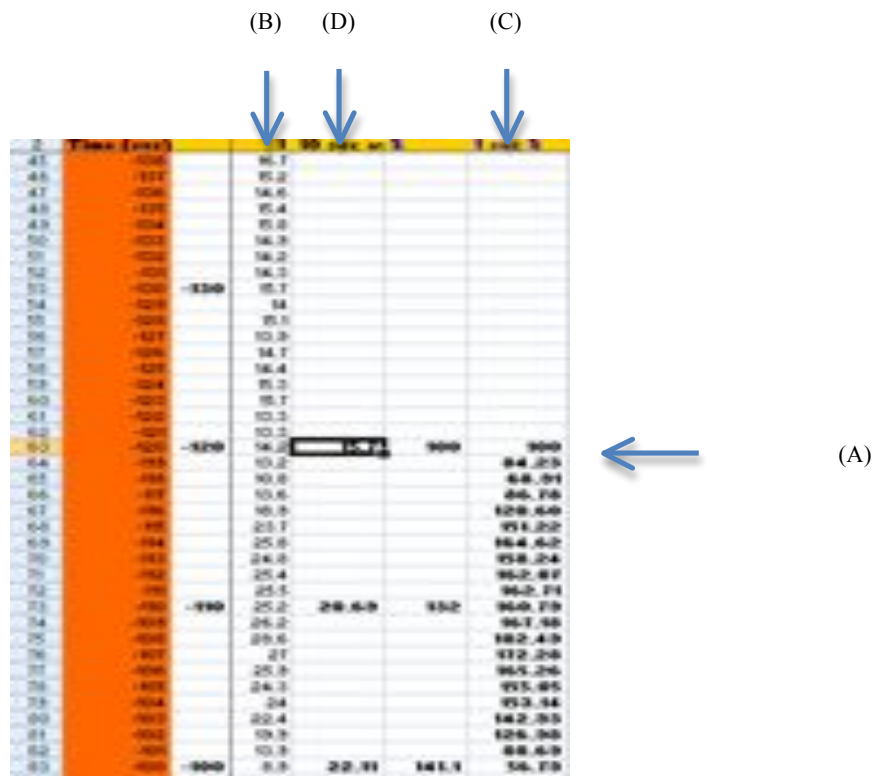


Figure 33: An example of ‘patient 1’ actual flux recording against time in seconds and subsequent analysis. (A) Average baseline flux over two minutes prior to cuff occlusion (B) Flux values per second (C) Flux values per second as a percentage of baseline (D) Actual flux values over 10sec intervals.

Flux values per second were plotted against time for the entire time to include baseline (2 min), cuff occlusion (2 min) and reactive hyperaemia on cuff release (2 min). A plot was drawn of each patient’s flux against time in seconds to enable the time to peak, magnitude of the peak and percentage change from baseline to be visualised. Any outlying values were removed by eye. For any patient whose flux was continuing to climb at the end of the recording, T_p was documented as 120secs.

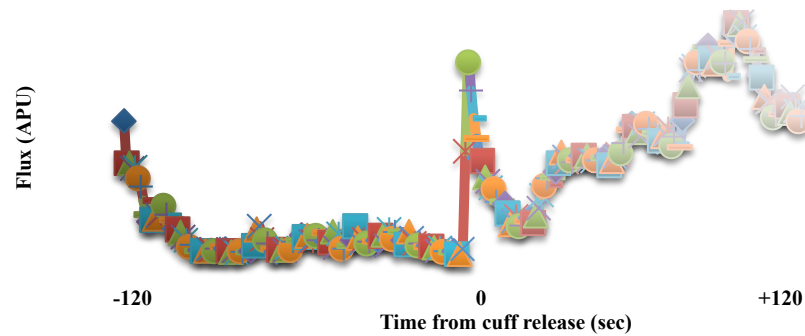


Figure 34: An example of an XY scatter plot per second, for a patient's reactive hyperaemia from t -120s (cuff occlusion) to t +120s to visualise the time and magnitude of the peak and to identify any outlying values.

Calculation of area under the curve was made by the trapezium method (Matthews et al., 1990) using the 10-second averages (Figure 35):

- 1) The change in flux relative to baseline was calculated for each 10 sec average.
- 2) The increase above baseline was divided by two
- 3) Using linear interpolation between 10 sec time points, the difference between fluxes was divided by two and added to the first flux value.
- 4) All values were totalled to give the total area under the curve

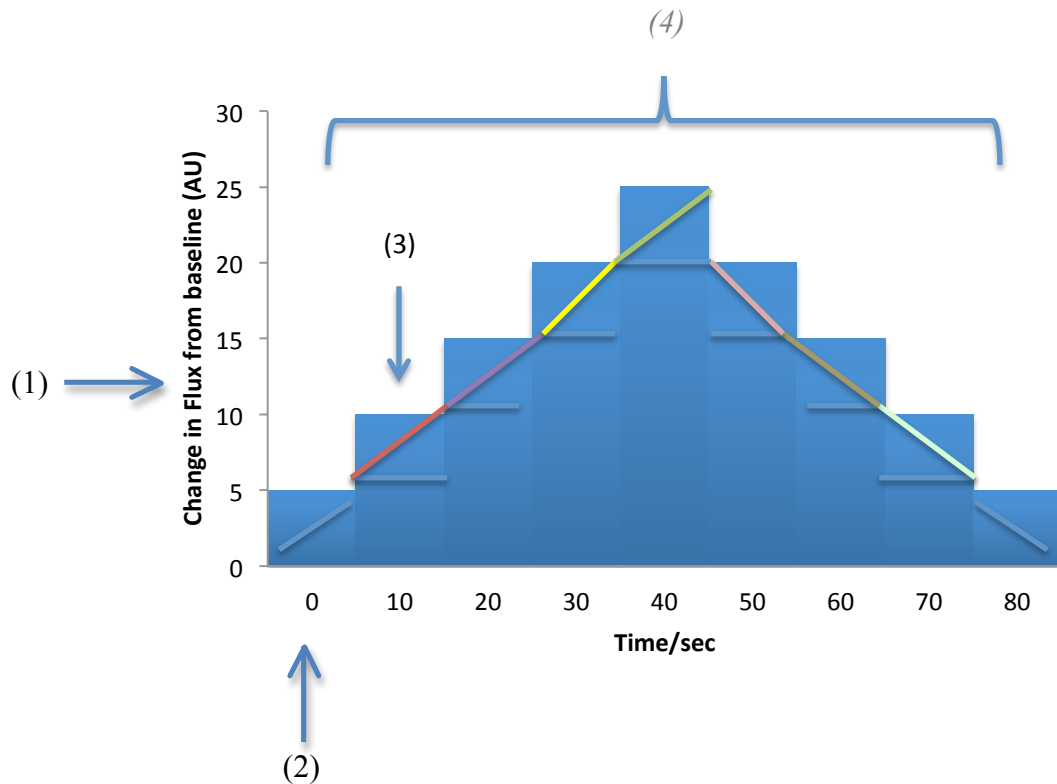


Figure 35: A schematic representation of calculating area under the curve using 10sec averages of the change in flux relative to baseline (1). The increase above baseline was divided by two (2). Using linear interpolation between 10 sec time points, the difference between fluxes was divided by two and added to the first flux value (3). All values were totalled to give the total area under the curve (4).

Skin LDF Pressures

A method for measuring skin LDF pressure was implemented using the laser Doppler, utilising a similar technique described by Castronuovo et al for measuring skin perfusion pressure (Castronuovo et al., 1997). However, instead of the laser Doppler probes being secured under a cuff at the toe, the cuff remained at the ankle with the LDF probes attached to the toe pulps of leg 1 and 2.

The cuff was inflated to 20mmHg above brachial systolic blood pressure to achieve a near zero flux. The cuff was then deflated in 10mmHg stepwise decrements every 5 seconds to a

pressure of 50mmHg. Deflation then proceeded in 5mmHg decrements every 15seconds until laser Doppler output decreased for two consecutive pressure values. The pressure at which this first occurs was recorded (Castronuovo et al., 1997).

Neurothesiometer

The Neurothesiometer is an electromagnetic device used to quantitatively measure sensitivity to increasing or decreasing amplitudes by the delivery of voltage (O'Neill et al., 2006). It is commonly used in the diabetic population to assess peripheral neuropathy and the risk of ulceration, as the vibration threshold reflects the functional integrity of the sensory fibres (Goldberg and Lindblom, 1979). The outcome measures are vibration perception threshold (VPT) and vibration detection threshold (VDT). The VPT is the stimulus amplitude at which sensation is felt, as voltage is increased. Likewise, the VDT is the stimulus amplitude at which sensation disappears, as voltage decreases (O'Neill et al., 2006).

In this study, patients remained in a supine position with the foot to be tested in an everted position, with the knee slightly flexed. This allowed the Neurothesiometer (Horwell Scientific, London, UK) to bear down perpendicular to the medial aspect of the MTH, under its own weight. The Neurothesiometer was applied to the patients' right hand as a suprathreshold stimulus in a symptom free region, so they were able to recognise the 'normal' sensation first. With the patients' eyes closed the Neurothesiometer was then applied to the first metatarsal head (MTH) of each foot and the method of limits (Dimitrakoudis and Bril, 2002) was employed to assess the VPT and VDT. The voltage was dialled at approximately 1V/second, but with higher VPTs the intensity of the stimulus was increased to within the VPT range immediately and then slowed to 1V/second for accurate measurements. Three

readings were taken for each and the average voltage was recorded. The first reading was omitted and repeated if it was clearly erroneous.

The MTH was used as a testing site as it was found to be more sensitive than the pulp of the toe, particularly for VDT, during the pilot study (*See section 3.3*). There is no clear guidance in the literature as to where the ideal location is to place the head of the probe.

3.3 Pilot study

Pilot testing was done to determine logistic factors influencing the final protocol. It focused on the use of the Neurothesiometer, the Durometer and the measurement of tissue depth with the Sonosite 180. The day-to-day reproducibility of these techniques was evaluated in 7 healthy volunteers (2 male and 5 female, aged 23-51 years, median 35 years), on both legs. All parameters were assessed during the course of two afternoons, one week apart.

Neurothesiometer

The volunteers were tested using VPT at two different anatomical sites, the pulp of the great toe and the medial aspect of the metatarsal head. There was a lower threshold on the MTH than the pulp of the toe for both VPT (3.2 vs. 3.7) and VDT (3.9 vs. 4.2) and anecdotally volunteers reported that it was easier to detect a distinct presence or absence of stimulation over the MTH compared with the toe pulp, presumably because the stimulus was transferred to the underlying bone at the MTH. This was reflected in the lower standard deviation at the MTH compared to the toe pulp (VPT 0.8 vs. 1.05 and VDT 0.7 vs. 0.9 respectively).

Overall VDT was generally less distinct than VPT and Goldberg also found that the variability was significantly less for VPT than VDT. In spite of this the thresholds were indisputably coherent and the threshold gap (VPT-VDT) tended to be constant for each site (Goldberg and Lindblom, 1979). It is not possible to comment on false negatives and sensitivity, as all volunteers were healthy with no evidence of peripheral neuropathy but based on these findings it was elected to test vibration perception at the MTH.

It may be that the relatively large surface area of the bone at the MTH provides a better surface for detection of vibration than the pulp of the toe, which may dampen the signal. Furthermore, Armstrong et al considered the value of testing at multiple sites on the foot and ankle and felt it was of no considerable practical benefit compared with single site testing (Armstrong et al., 1998). It is also worthwhile to note that some patients included in the study had digit amputations and testing at the MTH allowed consistency of the testing site throughout the study. At both locations, the neurothesiometer was found to be reproducible on a test-retest within acceptable limits of <10% for biological variables. Data are calculated based on Hopkins definition of typical error for a test – retest (Hopkins, 2000).

Location		Mean \pm SD AUs	Typical error
MTH	VPT	3.2 \pm 0.7	0.5
	VDT	3.7 \pm 0.8	0.6
Toe Pulp	VPT	3.9 \pm 1.3	0.9
	VDT	4.2 \pm 1.1	0.8

Table 15: The reproducibility of the Neurothesiometer in healthy subjects on a test-retest measurement at two different sites for VPT and VDT, expressed as typical error (% mean). Data was included from measurements on both legs so n = 14 (7 individuals) for each site

Durometer

The data in the literature are limited with regards to the medical use of the Durometer and certainly assessing tissue hardness in the context of revascularisation is a novel approach. Lower limb hardness and oedema is potentially subject to change over time in healthy individuals, due to changes in tissue osmotic pressure. Variability of lower limb Durometry was assessed one week apart at the three described locations at the calf, ankle and foot. Within the three locations the Durometer was found to be reproducible on a test-retest.

Location	Mean \pm SD AUs	Typical error
Calf	36.1 \pm 2.7	1.9
Ankle	38.8 \pm 3.1	2.2
Foot	16.1 \pm 2.3	1.6

Table 16: The reproducibility of Durometry in healthy subjects on a test-retest measurement at three different sites, expressed as typical error (% mean). Data was included from measurements on both legs so n = 14 (7 individuals) for each site

Tissue Depth

Tissue depth was shown to be greatest at the ankle and lowest at the foot, consistent with the presence of subcutaneous tissue. This was consistent between legs and did not vary over the course of time.

Location	Mean \pm SD	Typical error
Calf	0.32 \pm 0.02	0.03
Ankle	0.41 \pm 0.06	0.05
Foot	0.25 \pm 0.03	0.02

Table 17: The reproducibility of tissue depth using ultrasound in healthy subjects on a test-retest measurement at three different sites, expressed as typical error (% mean). Data was included from measurements on both legs so n = 14 (7 individuals) for each site

The within-subject variations for all measurements are within acceptable range for typical error, confirming that they are precise and reliable tests.

3.4 Data reduction and Statistical analysis

Patient numbers and power calculations

The skin toe pulp reactive hyperaemia was selected as the primary outcome measure of microcirculation pre and post-treatment. Based on published data showing that time to peak for RH in CLI patients reduces following successful treatment, a power calculation was carried out using the statistical program ‘G power’. Data was used from Wahlberg et al, 1995 and Ostergren et al, 1988 and the following assumptions were made:

- a) A baseline Tp for CLI of 126seconds (Wahlberg et al., 1995)
- b) A standard deviation of 81sec (Ostergren et al., 1988)
- c) An expected decrease in Tp following treatment of 39sec, giving a post-treatment mean Tp of 87sec and a SD of 27sec. (Wahlberg et al., 1995)

This calculates that a total sample size of 38 provides 95% power at the 5% significance level.

Patient descriptors

Demographic data descriptive of the clinical status of the patient population, including presence / absence of risk factors, are presented as numbers, proportions or percentages falling into different categories of classification. Physical characteristics at baseline are presented as mean values \pm standard deviations (SD) to demonstrate variance. Measured

variables – pressures, fluxes, anthropometric measurements – are presented as mean values \pm standard errors (SE).

Statistical tests

Test for normality of data

All numerical data for the two treatment groups, Open and Endovascular, were subjected to a Kolmogorov-Smirnov test of normality, with the resultant p value required to be > 0.05 for a normal distribution to be assumed. All variables were normally distributed apart from the time to peak of reactive hyperaemia. Therefore parametric tests were used to make comparisons between and within groups and time points for all data except the time to peak values (sec) which were log transformed and then subjected to parametric tests, as described below.

Comparisons pre-intervention within and between treatment groups

Pre-intervention, data for the Open and Endovascular groups were compared by unpaired t-test, assuming unequal variances.

Comparisons between leg 1 and leg 2 for anthropometric measures (limb volumes, circumferences, tissue depths), Durometry readings, ankle, toe and skin pressures, skin perfusion fluxes (basal; oscillatory frequency power) and microcirculatory responses (reactive hyperaemia time to peak, magnitude, AUC; postural vasoconstriction) were made by paired t-test.

Changes in flux during reactive hyperaemia or postural vasoconstriction were analysed by paired t-test to determine if they were significant. Derived values of time to peak flux, % increase in flux for reactive hyperaemia, and % change in flux for postural vasoconstriction (leg dependency or sitting) were then compared between open and endovascular groups by unpaired t-test.

Anthropometric measures and postural vasoconstriction data were made at different sites on the limb and where appropriate, regional comparisons were made between sites by performing a two-factor Anova, with treatment group and site as factors. Leg volumes were analysed in a two-factor Anova with treatment group and gender as factors.

Relationships between variables such as tissue depth and Durometry reading, between ankle or toe pressures and the NSB index of disease severity were analysed by correlation and calculation of Pearson's product moment coefficient.

In pilot studies on a small number of control healthy subjects, typical errors of measurement of tissue depth and Durometry measurements were estimated from the standard deviation of the difference scores of test-re-test data (Hopkins, 2000).

Comparison of treatment effects within and between groups

Anthropometric data, ankle pressures, basal skin fluxes and postural vasoconstrictor responses were tested pre-, post- and six weeks after intervention. Repeated measures Anova was used to look at differences between interventions over time.

All statistical analyses were performed using Statview v5.0 (SAS Institute Inc, NC, USA). A p-value <0.05 was considered as statistically significant. In selected cases, borderline significances are also shown. All data will be presented as mean \pm SE.

On occasion, measured variables could not be obtained because patients could not participate in all testing sessions following intervention due to graft failure, amputation or mortality. This reduced the n numbers below 40, particularly for repeated measures analysis, and lowered the power of statistical comparison. This will be highlighted as appropriate.

RESULTS

4.1 Study cohort

Table 18 provides demographic data on the patient study group as a whole and for the groups divided according to treatment intervention. The most noticeable difference between the groups were the numbers, 30 receiving open surgery and 10 receiving endovascular treatment. This arose because patients were allocated to the most clinically appropriate intervention and during the course of the study there were more patients deemed suitable for open surgery than endovascular intervention. Furthermore, some patients declined to take part in the study, either when they were initially approached or failed to return following initial testing pre-intervention. More of these patients were in the endovascular group (open 6: endovascular 11). Any significant differences in characteristics between the treatment groups that are pertinent to likely treatment effects will be pointed out.

	ALL PATIENTS (n=40)		OPEN (n=30)		ENDOVASCULAR (n=10)	
Number	40	(100)	30	(75)	10	(25)
Age (yrs)	72	(r52-92)	70	(r 52-89)	76	(r 59-92)
Gender (M:F)	26:14	(65:35)	21:9	(70:30)	5:5	(50:50)
BMI (kg/m ²)	27	(r16-44)	27	(r16-44)	25.9	(r19-30)
Resting heart rate (bpm)	72±3		69±3		80 ± 6	
Brachial systolic blood pressure (mmHg)	151±4		154±3		142 ± 10	
TASC (femoro-popliteal):						
A	0	(0)	0	(0)	0	(0)
B	10	(27)	3	(10)	7	(70)
C	9	(23)	7	(23)	2	(20)
D	21	(53)	20	(66)	1	(10)
Inflow disease	9	(23)	9	(30)	0	(0)
Fontaine:						
III	23	(58)	19	(63)	4	(40)
IV	17	(43)	11	(37)	6	(60)
Risk Factors:						
Cholesterol	33	(83)	27	(90)	6	(60)
Hypertension	32	(80)	24	(80)	8	(80)
Smoker (current or ex)	36	(90)	29	(97)	7	(70)
Diabetes	16	(40)	12	(40)	4	(40)
IHD	19	(48)	13	(43)	3	(30)
TIA or CVA	8	(20)	8	(27)	0	(0)
Dialysis	4	(10)	2	(7)	2	(20)
Medications:						
Beta blocker	10	(25)	8	(27)	2	(20)
Ca ²⁺ channel antagonist	13	(33)	10	(33)	3	(30)
Vasodilators	5	(13)	3	(10)	2	(20)
ACEi/AT2 blockers	18	(45)	13	(43)	5	(50)
Diuretics	19	(48)	15	(50)	4	(40)
Aspirin/Clopidogrel	36	(90)	29	(97)	7	(70)

Table 18: Demographics of the patient study group as a whole and divided into the two intervention groups. Figures in parentheses represent the proportions within each group of the specified factor. IHD – Ischaemic heart disease, TIA – Transient Ischaemic Attack, CVA – Cerebrovascular Accident. Values are reported as mean ± SE.

Physical characteristics

The mean age of all study participants, 72 ± 11(SD) years, included a small number of very elderly patients but the age ranges of the two treatment groups were similar (Table 18). Overall there was a preponderance of male over female patients (26:14), reflecting the

distribution of vascular disease in this age of population. With the disparity in numbers between the treatment groups, this M:F ratio was maintained in the open group but was equal in the endovascular group. Any potential influence of gender balance on measured variables will be indicated where appropriate. For example, although the height ($170 \pm 2\text{cm}$, mean \pm SEM) and weight ($80 \pm 3.7\text{ kg}$) for the open group tended to be greater than for the endovascular group (height $160 \pm 3\text{cm}$, weight $68 \pm 4.1\text{kg}$) because of more men in the former, values of BMI for the two groups were similar (open: 27 ± 1.1 , endovascular: $25 \pm 1.0\text{ kg/m}^2$).

Disease classification

There were however, evident differences in disease status between the two groups at the outset. When disease was categorised using the Fontaine classification, there were proportionately more open patients fulfilling Fontaine III criteria and more endovascular patients with Fontaine IV disease.

Likewise, with the TASC classification there was a difference across the categories with >65% of open patients in class D and 70% endovascular patients in class B. There was no correlation between increasing TASC classification, which is defined by disease morphology and increasing Fontaine classification, which is based upon patient symptoms. For example, a patient with peripheral ulceration satisfying Fontaine criteria IV may have less anatomical disease present than a patient with rest pain, Fontaine III. This may be due to other co-existing conditions that predispose ulceration, despite a lower vascular disease burden.

A new descriptive index of disease severity based on length and degree of stenosis, the NSB, was used to discriminate better between the groups. For all patients, the numerical values obtained increased with an increasing femoro-popliteal segment TASC classification (p0.03 ANOVA). Similarly, when inflow disease was present in the iliac vessels, a significantly higher NSB index was recorded (inflow disease 55.9 ± 4.2 AU, no inflow disease 31.7 ± 2.2 , $p < 0.0002$).

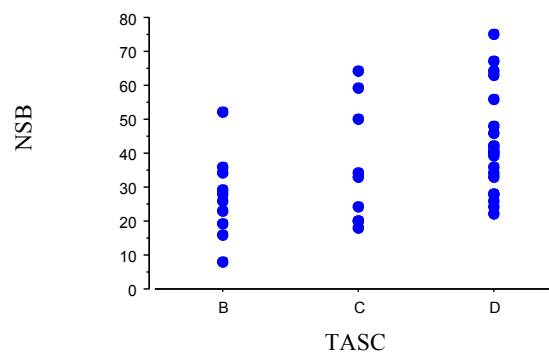


Figure 36: Femoro-popliteal segment TASC classification and its relationship to the NSB index of the most symptomatic limb

Thus, the NSB index correctly matched with disease severity. It also distinguished the difference in disease status between the treatment groups, being significantly greater in the open group (40.1 ± 3.2 AU) compared to the endovascular group (25.7 ± 3.2 AU) reflecting the anatomical severity of the disease in the former.

With regard to other factors impacting upon critical limb ischaemia and disease status, there were more patients with hypercholesterolemia in the open group and a higher proportion of smokers, current or ex. There were similar numbers of hypertensives and diabetics in each group but a larger proportion of patients with renal failure requiring dialysis in the

endovascular group. There were more patients in the open group with symptomatic disease in other vascular beds, including CVAs and ischaemic heart disease (Table 18).

So, overall disease severity was greater in the open group with a higher TASC classification and a greater percentage of patients with inflow disease. This also correlated with greater symptomatic systemic vascular disease in this group.

4.2 Haemodynamic Evaluation

Pre-intervention, brachial systolic blood pressures were slightly higher in the open compared to the endovascular group (154 ± 3 vs. 142 ± 10 mmHg, NS) but this difference was not significant. Both groups had similar consumption of vasoactive medications for hypertension. More patients were on anti-platelet agents in the open group (97%) compared to the endovascular patients (70%), which may be due to the higher incidence of IHD in this group (Table 18). Likewise, resting heart rates in the two groups were not different (open 69 ± 3 , endovascular 80 ± 6 beats.min⁻¹, NS). Overall, the cardiovascular status of these two groups was similar.

Ankle Pressures

Ankle-brachial pressure index (ABPI) is widely accepted as a useful tool to screen for the presence of haemodynamic disease in the lower extremity arteries. Pressures were measured at the posterior tibial (PT), dorsalis pedis (DP) and peroneal (PN) arteries of both legs and the ABPI was calculated for each individual ankle vessel.

In leg one (the worst affected and scheduled for intervention), absolute ankle pressures for all patients were significantly lower in the open group compared to the endovascular group for the PT and PN vessels, and DP values also tended to be lower in the open group (Figure 37a). However, the variance of pressure measurements within the endovascular group especially was large due to the presence of several abnormally high readings, greater than systolic pressure, as a result of vessel calcification. As this renders ABPIs uninterpretable, these outliers were removed and figure 37b shows that whilst the open group still tended to have lower ankle pressures, there were no statistical differences between the two groups.

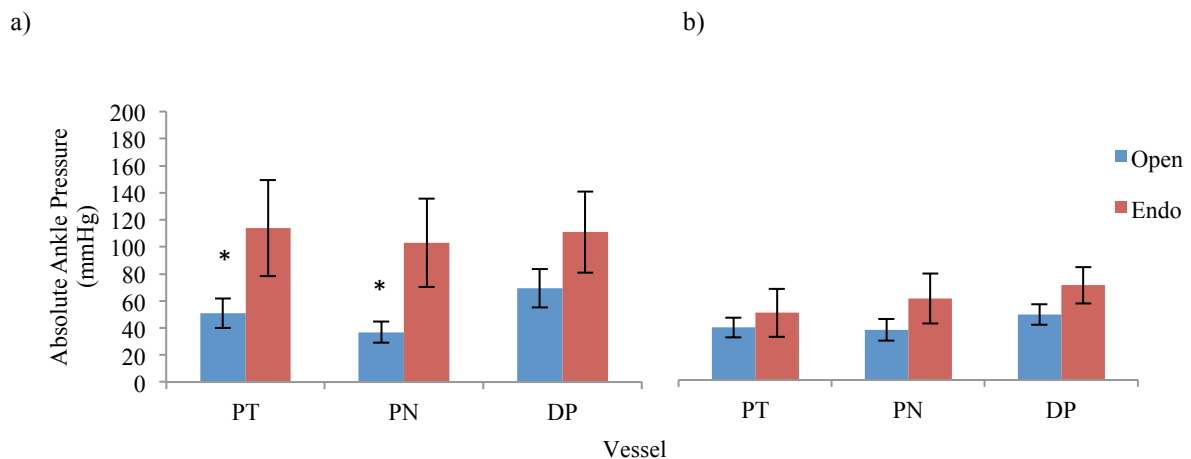


Figure 37: Absolute ankle pressure in leg one pre-intervention, divided by open and endovascular a) in all patients and b) after removing outliers. Values are mean \pm SE. Posterior tibial (PT), Peroneal (PN) and Dorsalis pedis (DP) vessels. * $p < 0.05$ between groups.

The impact of variable ankle pressures on ABPIs is shown in Table 19 below. Inclusion of all patient values resulted in significantly higher ABPIs in the endovascular than open group (Table 19a) whereas removal of the outliers abolishes this difference (Table 19b). The pressure in the dorsalis pedis vessel tended to be higher than the PT and PN vessels in both groups, but this was not significant.

All			Outliers removed	
Index	Open (n=30)	Endo (n=10)	Open (n=28)	Endo (n=7)
PT	0.35 ± 0.8	0.93 ± 0.34 *	0.25 ± 0.05	0.30 ± 0.11 (NS)
PN	0.23 ± 0.05	0.81 ± 0.31 *	0.23 ± 0.05	0.39 ± 0.12 (NS)
DP	0.45 ± 0.09	0.86 ± 0.29 (NS)	0.30 ± 0.05	0.45 ± 0.08 (NS)

Table 19: Average pre-intervention ABPI for each vessel in leg 1 for open and endovascular groups for all patients and after removal of outliers. Values are mean ± SE. Artery abbreviations as in Figure 37. * p<0.05, NS non-significant between groups.

The same applied to ankle pressure measurements made on the contralateral leg (leg 2, least affected and remaining untreated), as vessel calcification was usually present bilaterally. Absolute pressures (Figure 38a) and indices (Table 20a) were higher in the open than endovascular group when including all patients but the difference was abolished on removal of the outliers, except for the peroneal artery index which remained significantly higher in the endovascular group. This is often the worst affected ankle vessel of the 3 in PVD, particularly in diabetic patients. The greatest effect of removing outliers was seen in the endovascular group, as there were proportionately more patients with falsely elevated ABPIs in this group.

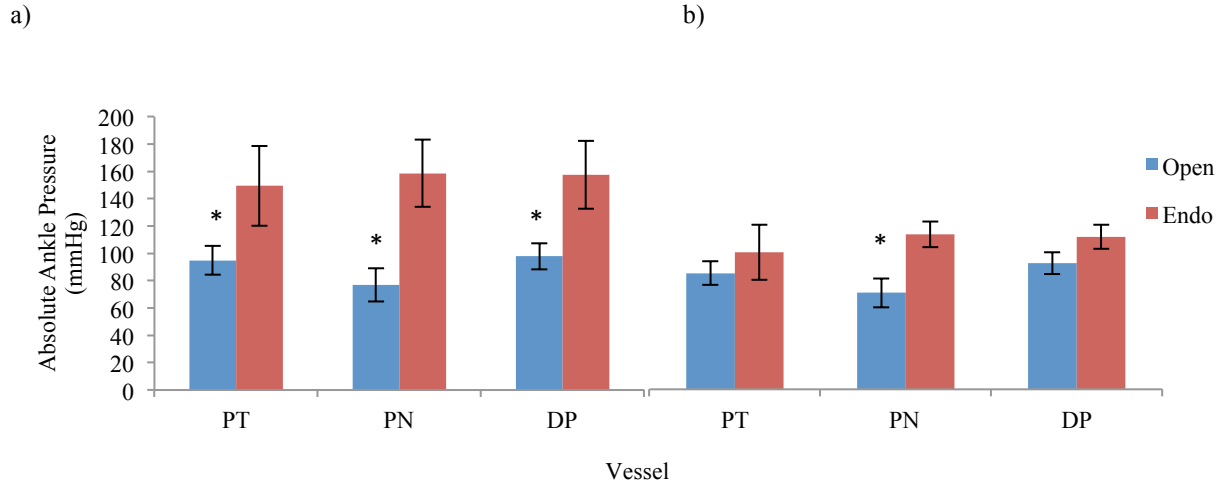


Figure 38: Absolute ankle pressure in leg two pre-intervention, divided by open and endovascular a) in all patients and b) after removing outliers. Values are mean \pm SE. Artery abbreviations as in Figure 37. * $p < 0.05$ between groups.

Index	All		Outliers removed	
	Open (n=30)	Endo (n=10)	Open (n=28)	Endo (n=7)
PT	0.62 ± 0.07	1.19 ± 0.29 *	0.55 ± 0.05	0.67 ± 0.14 (NS)
PN	0.50 ± 0.08	1.25 ± 0.27 *	0.45 ± 0.07	0.75 ± 0.07 *
DP	0.65 ± 0.07	1.23 ± 0.27 *	0.60 ± 0.05	0.74 ± 0.07 (NS)

Table 20: Average pre-intervention ABPI for each vessel in leg two, divided by open and endovascular for all patients and after removal of outliers. Values are mean \pm SE. Artery abbreviations as in Figure 37. * $p < 0.05$, NS non-significant between groups.

Comparison of Figures 37a and 38a shows that ankle pressures in leg one were significantly less than the respective pressures in leg two pre-intervention, reflecting the most symptomatic leg in both groups.

Toe Pressures

Although small vessels in the foot may be calcified in patients with CLI, it is rare for pedal vessels to be significantly calcified and absolute toe pressure measurements therefore remain a reliable indicator of the severity of disease, even in patients with non-compressible ABPIs (Dattilo and Casserly, 2011). Consequently, all patient data were included.

Figure 39 shows that there was no significant difference between the absolute toe pressures between groups in either leg one or leg two. In leg one, the mean values were 15 ± 4 mmHg in the open group and 24 ± 7 mmHg in the endovascular group. As such they fall below 30 mmHg, consistent with a diagnosis of CLI, whereas for leg two, the values were above this level (open 55 ± 6 mmHg; endovascular 65 ± 12 mmHg). Likewise, there were no significant differences in toe pressure indices between groups in either leg one (open 0.10 ± 0.02 ; endovascular 0.15 ± 0.05) or leg two (open 0.39 ± 0.04 ; endovascular 0.47 ± 0.07).

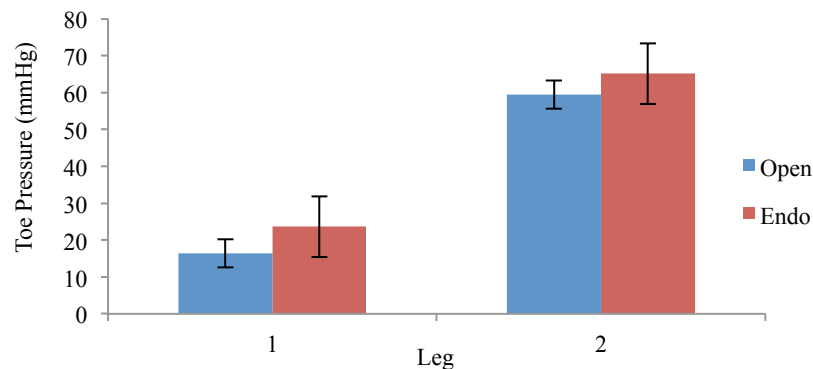


Figure 39: Average toe pressures in leg one and two pre-intervention, divided by open (n=30) and endovascular (n=10).

Values are mean \pm SE. Mark sigs * $p < 0.0001$ between legs.

Skin LDF Pressures

Measurements were made using the laser probe on the great toes of respective legs. A rise in LDF above baseline, with reducing cuff pressure around the calf, indicated return of perfusion. Both groups had similar pressures at the outset in leg one and leg two. The pressure in leg two was significantly greater than in leg one for both groups, as expected.

Pressure (mmHg)	Open (n=30)	Endo (n=9)	
Leg 1	47.8 ± 5.4	54.0 ± 10.0	(NS)
Leg 2	94.6 ± 5.9 *	91.8 ± 15.0 *	(NS)

Table 21: Average pre-intervention skin LDF pressures (mmHg) for leg one and two, for open and endovascular groups.

Values are mean ± SE. NS non-significant between group; * $p < 0.05$ between legs

Ankle, toe and skin pressures in leg one were significantly less than the respective pressures in leg two pre-intervention, reflecting the most symptomatic leg in both groups.

4.3 Assessment of lower limb oedema

Lower limb anthropometry

Measurements were made of the volume of the lower limb segment (knee to ankle) to provide baseline data from which to assess whether treatment affects limb swelling and oedema. The mean limb volumes of leg one and two were significantly different between groups at baseline, with the open group having larger average volumes. Since the open group included a higher proportion of males than in the endovascular group, and males in general have larger limb size than females, a two-factor Anova was carried out to examine the effects of gender and group on volume. This showed that it was gender, the preponderance of men in the open group that significantly influenced volume ($p=0.02$) rather than treatment group *per se* ($p=0.10$).

Limb Volume (ml)	Open (n=30)	Endo (n=10)
Leg 1	1620 \pm 0.07	1302 \pm 0.12 *
Leg 2	1620 \pm 0.08	1330 \pm 0.10 *

Table 22: Average pre-intervention limb volume (Lq) for leg 1 and 2, for open and endovascular groups. Values are reported as mean \pm SE. * $p<0.05$ between groups.

As an assessment of oedema due to CLI, leg one volume (ml) was graded with TASC classification, higher mean values occurring with TASC D (1630) > C (1490) > B (1410). (Figure 40) The same was also true for leg two, but there was no significant difference between the legs. There were similar proportions of males and females in each TASC group (Females A0%, B29%, C29%, D43% vs. males A0%, B23%, C19%, D58%)

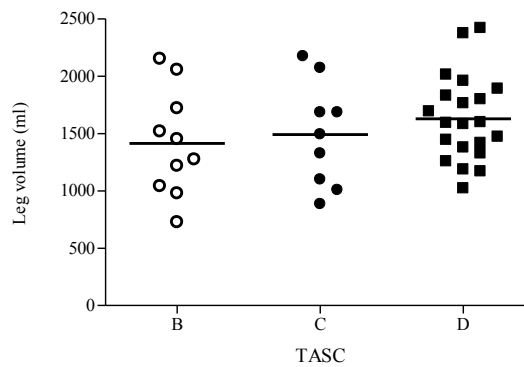


Figure 40: Point chart of average lower limb volume (ml) in leg one pre-intervention compared to TASC classification.

There was no correlation between and lower limb volume and foot circumference pre-intervention and the disease severity, as indicated by NSB index (Figure 41).

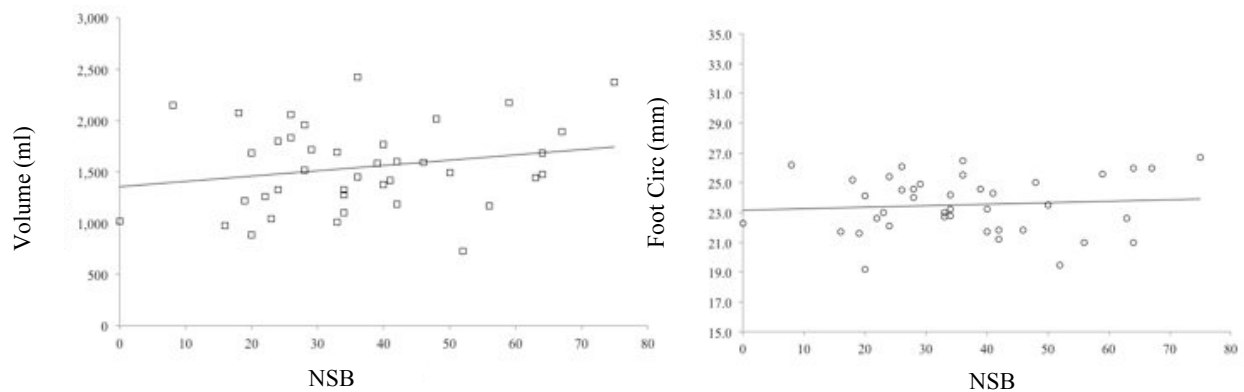


Figure 41: Pre-intervention a) limb volume of leg one and b) foot circumference and their relationship to the NSB index of the most symptomatic limb.

Tissue oedema was also evaluated in the forefoot by circumferential measurements. At baseline there were no group differences in circumference of the forefoot in leg one (Open 23.6 ± 0.3 , Endo 23.5 ± 0.7 cm, NS) or leg 2 (Open 23.7 ± 0.4 , Endo 23.2 ± 0.7 cm, NS). Although this contrasts with findings for lower limb segment volume, it is not surprising

given that tissue composition at the forefoot is less dependent on subcutaneous adipose and hence less subject to gender influences.

However, ultrasound measurement of tissue depth from skin surface to fascia, to assess subcutaneous oedema, did not detect any baseline differences between groups at any site in either leg one or leg two. When subdivided by gender, there was a tendency for females to have greater tissue depths than males at all 3 regions but this was not statistically significant. There was no difference in tissue depth between leg one and two in any region pre-intervention.

Tissue depth (cm)	Open (n=30)		Endo (n=10)	
	Leg 1	Leg 2	Leg 1	Leg 2
Tibial tuberosity	0.36±0.04	0.34±0.04	0.30±0.04	0.32±0.04
Medial malleolus	0.65±0.07	0.59±0.08	0.63±0.11	0.54±0.10
Forefoot	0.57±0.07	0.51±0.06	0.55±0.09	0.48±0.08

Table 23: Average pre-intervention tissue depth (cm) for leg one and two, divided by open and endovascular. Values are mean ± SE.

Consequently, the relationship between tissue depth at calf and ankle and the leg volume for all patients (not subdivided into open and endovascular groups) was not very close pre-intervention, as shown by figure 42a below. The correlation coefficients are 0.43 and 0.32 respectively, although there is a relationship between the two variables, $p < 0.001$. Foot circumference against tissue depth also demonstrated a relationship, $p < 0.001$ although this was not a strong correlation $r^2 0.2$. (Figure 42b)

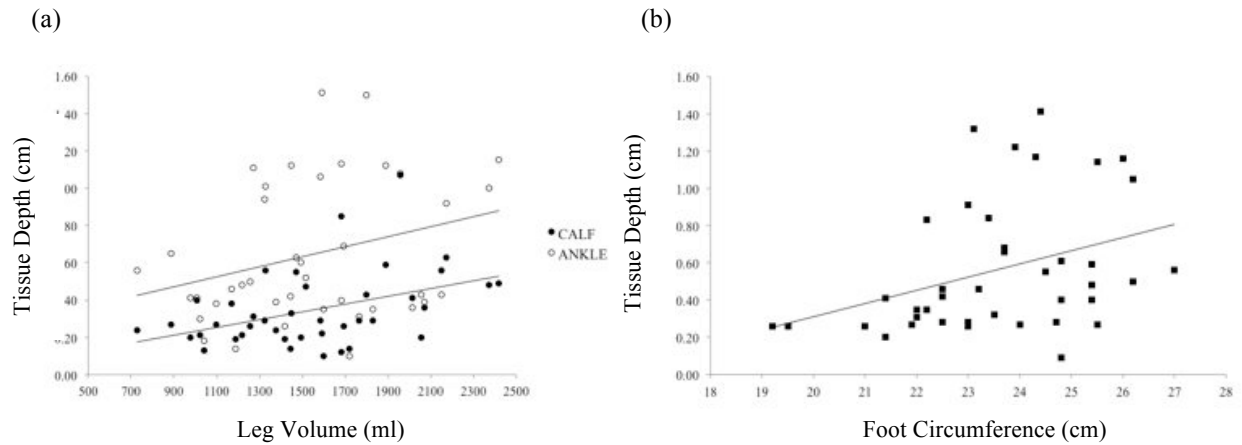


Figure 42: Tissue depth at the calf and ankle against leg volume for all patients (a) and tissue depth in the forefoot against for circumference (b). Values are mean \pm SE. Artery abbreviations as in Figure 37.

There was an initial difference in lower limb volume between groups, which is gender related and was not dependent on differences in subcutaneous fat. There was a tendency for higher TASC classifications to have greater limb volume.

Durometry

Durometry measurements have been shown to be a sensitive indicator of tissue hardness and can demonstrate changes over time (Choh et al., 2010, Kissin et al., 2006).

Tissue hardness was greatest at the ankle and least at the forefoot but there were no differences between the treatment groups in Durometry values at any of the 3 defined locations in either leg. There were no differences in tissue hardness between the genders or between leg one and leg two at the calf and the ankle. However, forefoot tissue hardness in

leg one was significantly greater than in leg two. As tissue depth in this region was similar between the legs, this indicates greater ‘turgor’ in the more symptomatic leg.

Durometry	Open (n=30)		Endo (n=10)	
	Leg 1	Leg 2	Leg 1	Leg 2
Tibial tuberosity	39.1±1.05	37.0±5.70	36.1±2.03	37.0±2.28
Medial malleolus	45.1±1.40	44.6±1.54	43.9±2.46	40.1±2.13
Forefoot	29.8±2.01	26.3±1.76 †	27.2±3.09	23.6±3.23 ††

Table 24: Average pre-intervention tissue Durometry for leg one and two, for open and endovascular groups. Values are mean ± SE. †p<0.05, †† p<0.01 between leg one and leg two.

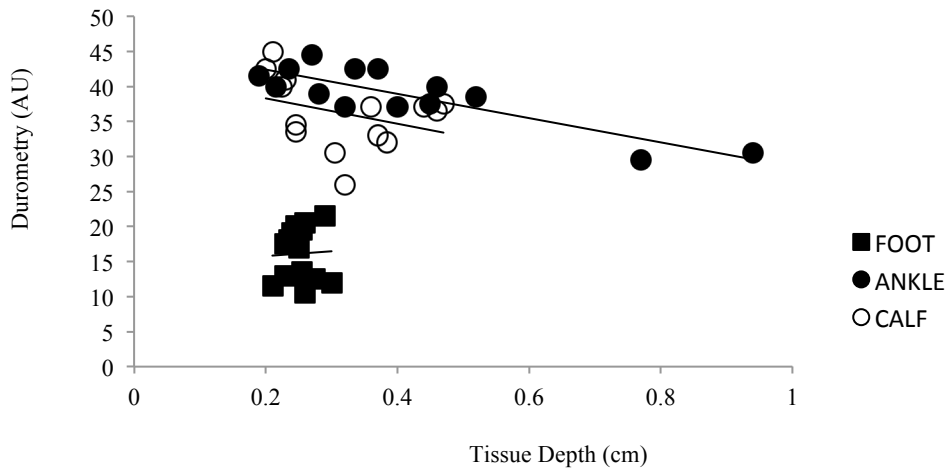
Tissue hardness was similar between the two groups at all three points. The forefoot in the symptomatic limb displays greater skin turgor than the contralateral foot.

Overall, anthropometrical measurements showed a greater limb volume in patients with more severe disease and in the male population. This was not related to an increase in subcutaneous tissue depth or an increase in tissue hardness in the limb. At the forefoot, circumference and tissue depth were not increased but skin hardness is greater in the symptomatic limb.

Healthy volunteers

Using data from our 4 healthy control subjects, Durometry was inversely related to tissue depth at the ankle and the calf. (Figure 43) That is, the greater tissue depth, the less the tissue hardness. Eight data points have been used in total from the mean of 2 measurements for each person and using separate data from each leg. This goes against the notion that Durometry

increases with tissue depth, but this will depend on what the tissue depth is due to i.e. soft fat or hard oedema.



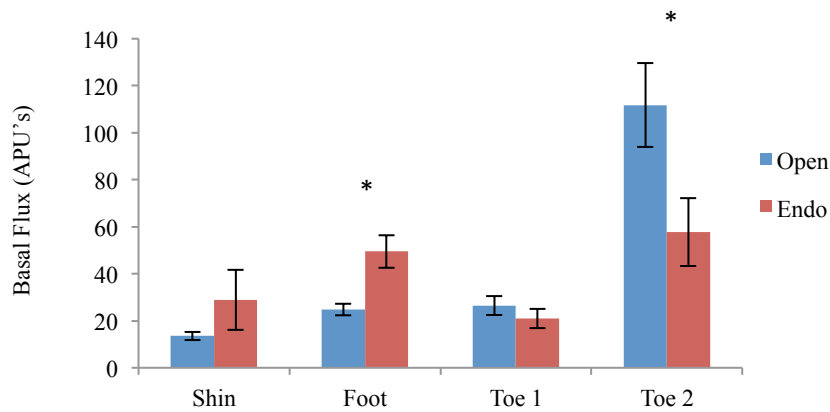


Figure 44: Basal flux for shin, foot and toes for each open (n=30) and endovascular (n=10). Values are means \pm SE. *Foot p0.004, toe 2 p0.02.

In terms of actual flux, values tended to be lower prior to cuff inflation in the endovascular than the open group, and this was the case for both legs (Table 25).

Reactive Hyperaemia

The ability of the skin microcirculation of the toes to display reactive hyperaemia, following a two-minute arterial occlusion, was assessed using Laser Doppler flowmetry. As described in 'methods' section, Laser Doppler fluxes are measured in arbitrary units rather than absolute values because of the variability of tissue volume and micro vessel numbers detected by the probe. Reactive hyperaemia was therefore quantitatively measured using three parameters derived from the flux signal:

1. Time to peak (Tp)
2. Magnitude of the peak (Mag)
3. Area under the curve (AUC)

Actual flux units and/or percentage change in flux from baseline express a change in flux.

The figure below demonstrates the reactive hyperaemia curve from the four healthy control subjects and the shape of a typical response following cuff occlusion, up to 50seconds post cuff release. The average time to peak was 32.8 ± 4.1 sec, with a peak percentage change in magnitude of flux $372.2 \pm 96.9\%$.

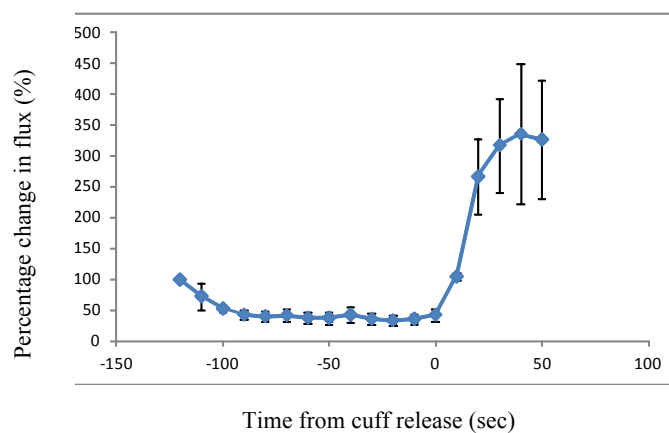


Figure 45: Time course of reactive hyperaemia as a percentage change in flux from baseline for healthy controls. Values are means \pm SE for 10s average values. Arterial occlusion commenced at $t = -120$ s and the cuff was released at $t = 0$ s. Brownian motion prevents the flux going to absolute zero when the cuff is inflated.

With regards to the treatment groups, Figure 46 illustrates the time course of the flux signal in Toe 1 for both groups pre-intervention, expressed as actual flux (Figure 46a) and as % change from baseline (Figure 46b).

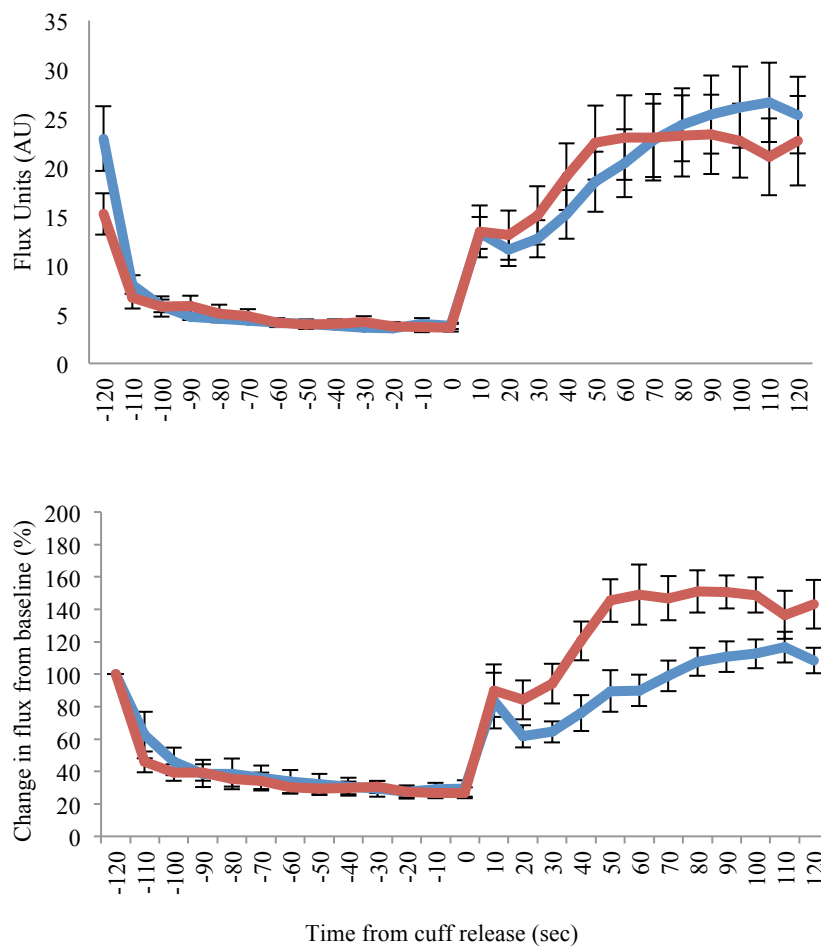


Figure 46: Time course of (a) actual flux and (b) flux expressed as % of baseline for open group ($n = 29$, blue line) and endovascular group ($n = 9$, red line) for the toe of the most symptomatic leg. Values are means \pm SE for 10s averaged values. Arterial occlusion commenced at $t = -120$ s and the cuff was released at $t = 0$ s. Brownian motion prevents the flux going to absolute zero when the cuff is inflated.

In comparison to the control subjects, neither group showed any great degree of hyperaemia in toe 1, and flux values of many individuals hardly reached baseline levels by the end of the 2 min post-occlusion observation period (Figure 46a). The peak magnitude of toe one post-occlusion response was not different between the groups whether expressed as actual flux or relative to baseline (Table 25), but it was significantly lower than that in toe two in the open group, consistent with being the limb with worse perfusion. The AUC was significantly different between groups at the outset, with the open group not achieving a flux above baseline and hence a negative AUC in leg 1. (Figure 47) There were no significant differences between the groups in leg 2.

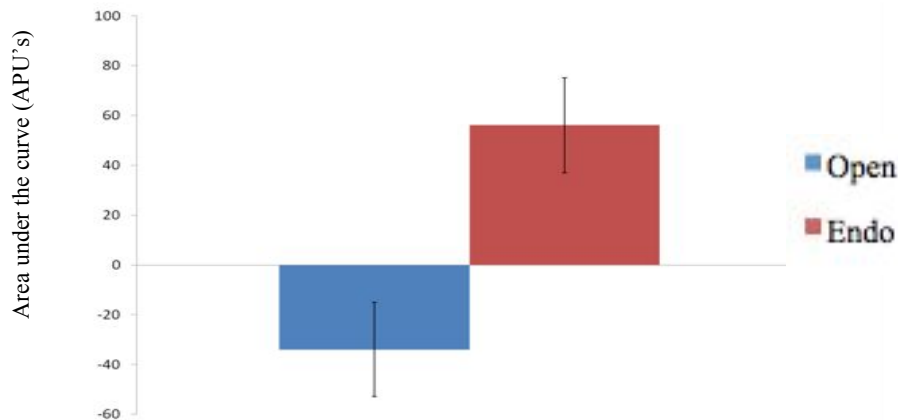


Figure 47: Area under the curve for reactive hyperaemia in leg 1 pre-intervention, in open (blue) and endovascular group (red). Values are means \pm SE.

	Open (n= 29)		Endo (n=9)	
	Toe 1	Toe 2	Toe 1	Toe 2
Baseline flux (APUs)	22.9 \pm 3.3	95.5 \pm 15.9† <i>p</i> <0.001	15.2 \pm 2.1	50.1 \pm 13.0† <i>p</i> 0.03
Peak flux magnitude (APUs)	32.0 \pm 4.5	159.8 \pm 19.2† <i>p</i> <0.001	29.6 \pm 4.8	108.5 \pm 21.3† <i>p</i> 0.03
Peak flux magnitude (% baseline)	150.0 \pm 14.2	306.7 \pm 80.0† <i>p</i> 0.05	196.8 \pm 20.1	264.4 \pm 60.5
Time to peak magnitude (sec)	100 \pm 4* <i>p</i> 0.003	82 \pm 5	69 \pm 9	63 \pm 12
Area under the curve (APU's)	-34.0 \pm 18.9* <i>p</i> 0.01	251.0 \pm 76.2† <i>p</i> 0.0007	56.1 \pm 19.0	179.3 \pm 84.5

Table 25: Average pre-intervention LDF recordings for toe one and two, for open and endovascular groups. Actual flux “units” are recorded as ‘Arbitrary Perfusion Units’ (APU’s) for baseline flux and peak flux magnitude. The percentage values are relative to baseline, which is 100%. Values are mean \pm SE. †p value between toe 1 and toe 2. * p value between open and endovascular.

Pre-intervention there was a significant difference between groups in toe 1 average time to peak due to a slower response in the open than endovascular group (Table 25) most likely because of the poorer perfusion pressures in the former. These data contrast markedly with reactive hyperaemias in the control group of 4 healthy volunteers with normal pressures (ABPI 1.06 \pm 0.05) in whom peak magnitude reached 372 \pm 96.9% of baseline and times to peak

were 32.8 ± 4.1 sec. Although this is not age-matched data, it is consistent with data in the literature of age-matched individuals (36.1 ± 4.25) (Van den Brande and Welch, 1988).

It was of interest to note that in toe two of both groups in the present study, times to peak were similar to those in toe one despite toe pressures being significantly higher in leg two.

In CLI, the hyperaemic response was flatter, delayed and prolonged compared to the rapid rise in flow seen in healthy individuals. This was most marked in the symptomatic leg, compared to the contralateral limb. The response in leg two however was also dampened in contrast to a healthy limb.

Vasoconstrictor mechanisms

In CLI peripheral microvessels show a compensatory dilatation and a loss of local regulatory control mechanisms, characterised by impaired myogenic and sympathetic neural constriction. The normal postural vasoconstrictor response to leg dependency (myogenic) or sitting (neurogenic/sympathetic) is usually absent.

Figure 48a shows the changes in skin perfusion during leg dependency for 3 sites on the most symptomatic leg one – shin, foot dorsum and toe. Constriction was only evident in the endovascular group where flow decreased by 15% (NS), 26% ($p=0.02$) and 17% (NS) for the three sites respectively. In the open group, constriction was only evident in the foot (10% decrease, $p=0.05$) while flow increased slightly at the shin and toe. Thus the open group showed greater impairment of the myogenic constrictor response than the endovascular group, consistent with their worse pressures and basal perfusion.

Figure 48b shows the changes in skin perfusion when patients assumed the sitting position from lying supine. In this situation, the hydrostatic pressure load would be expected to increase by ~ 40-60mmHg in a normal circulation and elicit a constrictor response at the toe (~50% flow reduction, De Graaf et al. 2003).

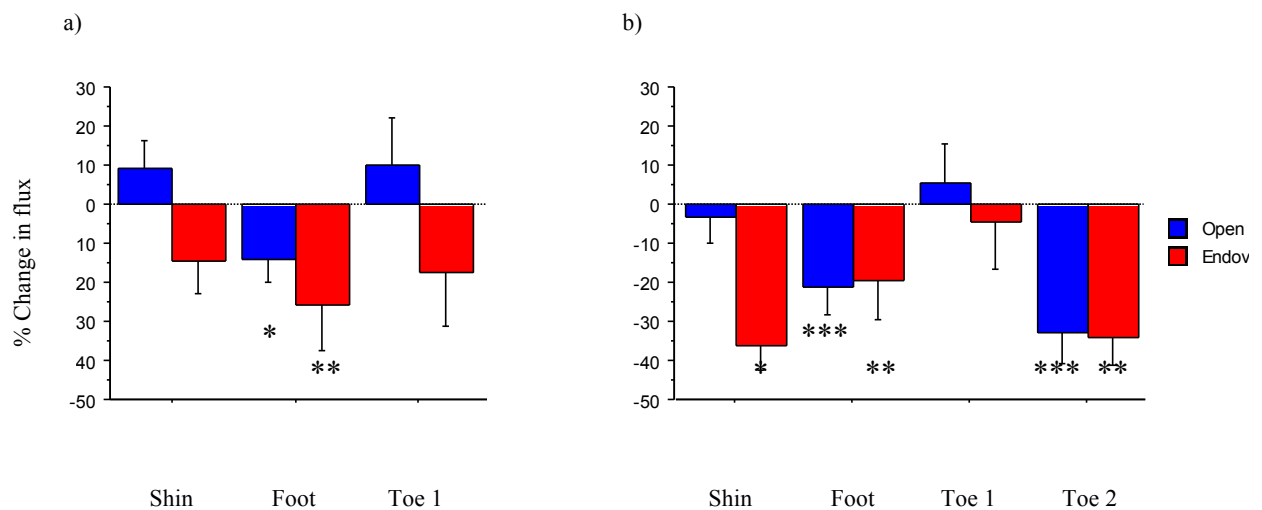


Figure 48: Change in skin perfusion from horizontal to (a) leg dependency in shin, foot and toe 1 and (b) sitting in shin, foot, toe 1 and toe 2 for each open (n=30) and endovascular (n=10). Horizontal flux is 0% with an increase in flux seen as a rise above baseline and constriction or reduction in flux is seen as a fall below baseline. Values are means \pm SE. *p<0.05, **p<0.02 ***p<0.003.

On moving to a sitting position, the decrease in skin perfusion in the endovascular group was graded according to the site. It was greatest at the shin (40%), less at the foot (20%) and absent at the toe of leg 1 (5%). In the open group, there was no change in perfusion at the shin, a modest decrease at the forefoot and no change at the toe. For the toe of leg two, significant constriction was seen on sitting (~40%) and there was no difference between the treatment groups. Thus, postural alteration demonstrated greater impairment in the VAR of the open group in comparison to those undergoing endovascular treatment given their more

pronounced reduction in flow with sitting. This was consistent with greater compromise of the microcirculation in the former.

Skin perfusion did not reduce in line with a postural vasoconstrictor response in CLI. The endovascular group kept a modest response compared to the open group. Postural vasoconstriction was most evident at the foot.

Frequency analysis of microvascular oscillations

A Fast Fourier Transform power spectral analysis of the Laser Doppler flux signal from toe one was carried out to examine whether the microvascular controls relating to myogenic, neurogenic and endothelial mechanisms would be altered by treatment.

The total power pre-intervention was not different between the two groups (open: 206.2 ± 28.9 nu endovascular: 159.9 ± 20.8 nu, NS) and therefore the power ascribed to the five different frequency bands is expressed as a proportion of the total. Both groups displayed similar distributions of power in each band at the outset (Figure 49). The majority of power was displayed from the heart rate and respiration. Frequencies of myogenic origin showed least power.

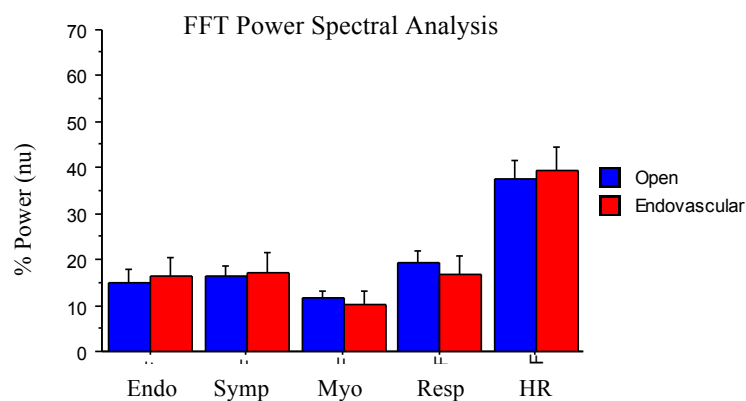


Figure 49: Fast Fourier Transform power spectral analysis of Laser Doppler flux, expressed as % of total power for open group (n = 30, blue) and endovascular group (n = 10, red) for the toe of the most symptomatic leg. Values are means \pm SE. The frequency and origin ascribed to the bandwidths are displayed below.

Origin	Band	Frequency	% Power
Endothelial	VVLF	0.009-0.02	O 14.8 \pm 2.9 E 16.3 \pm 4.2
Neurogenic	VVLF	0.02-0.06	O 16.4 \pm 2.3 E 17.3 \pm 4.2
Myogenic	VLF	0.06-0.15	O 11.7 \pm 1.4 E 10.3 \pm 2.8
Respiratory	LF	0.15-0.6	O 19.3 \pm 2.6 E 16.9 \pm 3.8
Heart rate	HF	0.6-2.0	O 37.7 \pm 3.8 E 39.2 \pm 5.4

It is not possible to comment on whether the distribution is representative of any impairment in CLI as there is no comparable data examining the toe in CLI with FFT and age-matched controls. However, below is the control subject data from the pilot study (non age-matched) expressed as percentage power (Figure 50). Compared to the CLI patients there is a similar power ascribed to endothelial and neurogenic frequencies but myogenic and respiratory frequencies are considerably lower, with the difference being ascribed to a greater percentage power of heart rate. Presumably this reflects the elastic nature of healthy vessels and the unobstructed transmission of flow to the peripheries.

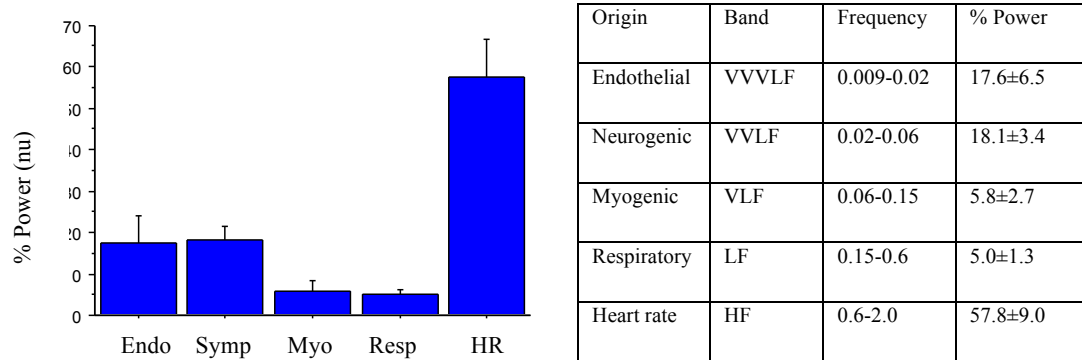


Figure 50: Fast Fourier Transform power spectral analysis of Laser Doppler flux, expressed as % of total power for the healthy control group. Values are means \pm SE. The frequency and origin ascribed to the bandwidths are displayed above.

The actual power attributed to each of the microvascular controls is shown below (Figure 51).

There were no significant differences between the groups for any of the power bands.

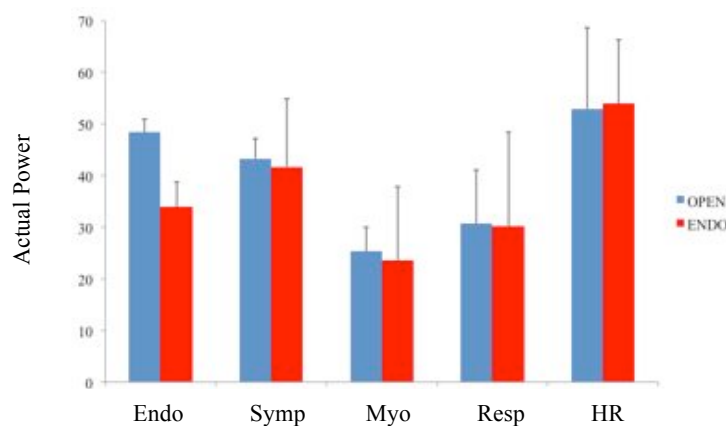


Figure 51: Fast Fourier Transform power spectral analysis of Laser Doppler flux, expressed as actual power for open group (n = 30, blue) and endovascular group (n = 10, red) for the toe of the most symptomatic leg. Values are means \pm SE.

4.5 Treatment effects

Clinical Outcomes at six-weeks after Intervention

Out of 36 patients tested at six weeks, 21 (58%) of vessels remained patent. The proportion of patency was considerably higher in the open (73%) than in the endovascular group (20%). This was linked with the presence of tissue necrosis, which occurred in only a third of the open group but in two thirds of the endovascular group (Figure 52a). Patency did not associate with symptom relief, which was similar in both groups (Figure 52b). The success of the open intervention was also evidenced by the necessity for re-intervention/operation, which was required in 5 out of 26 in the open group, with 2 patients requiring operative reintervention twice following initial surgery. All but one of the endovascular group required repeat endovascular reinterventions. There were 5 wound infections in the open group.

Within 30 days of intervention, 3 patients in the open group died of cardiac causes one of which died following limb amputation after an unsuccessful bypass procedure; they had NSB scores ranging from 24 to 64. There were no immediate deaths in the endovascular group. One further patient in the open group underwent amputation; they had an NSB score of 56.

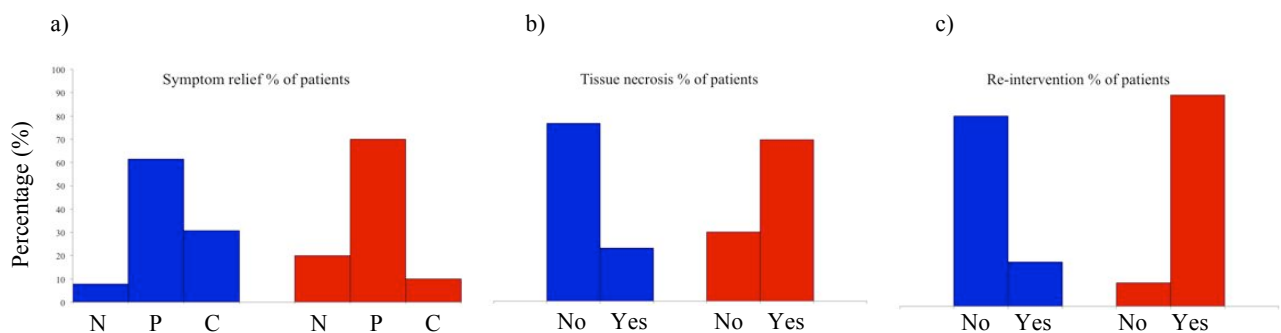


Figure 52. Percentage of patients within open (blue) and endovascular (red) groups a) experiencing none (N), partial (P) or complete (C) symptom relief, b) with presence of tissue necrosis at six weeks, c) requiring re-intervention.

Consistent with patency, the vast majority of patients in the open group became ‘unclassified’ (U) by femoro-popliteal TASC criteria at six-weeks, as radiological evidence of significant disease was no longer present. A small number of patients in this group had minor stenoses in their graft and four patients had significant occlusions at six-weeks. On the other hand, in the endovascular group the distribution of classification changed from predominantly ‘B’ pre-intervention to a mixture from A to D inclusive and 2 patients with no evidence of disease radiologically (Figure 53).

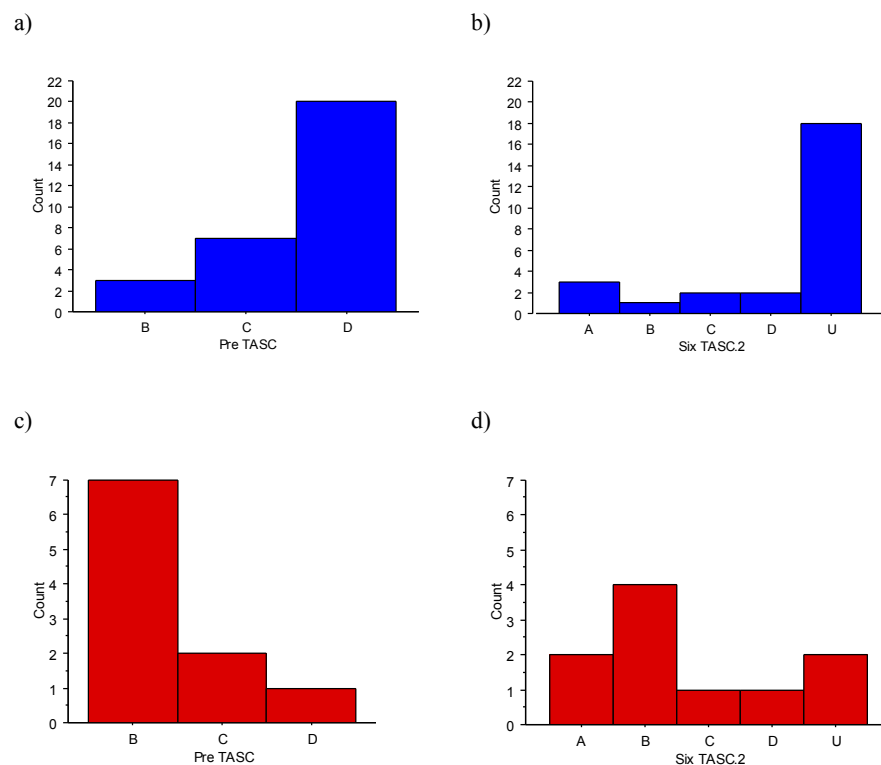


Figure 53: TASC classification by absolute numbers in the open group (blue) a) pre-intervention and b) at six weeks and in the endovascular group (red) c) pre-intervention and d) at six weeks.

NSB Index

Supporting the evidence that the surgical invention was successful at improving disease status was the highly significant decline in the novel NSB index from pre-intervention to six-weeks in this group with a reduction of 25 ± 3 AU ($p < 0.0001$). In the endovascular group the reduction of 7 ± 3 AU was less marked ($p < 0.03$), but the pre-intervention score was already lower than in the open group.

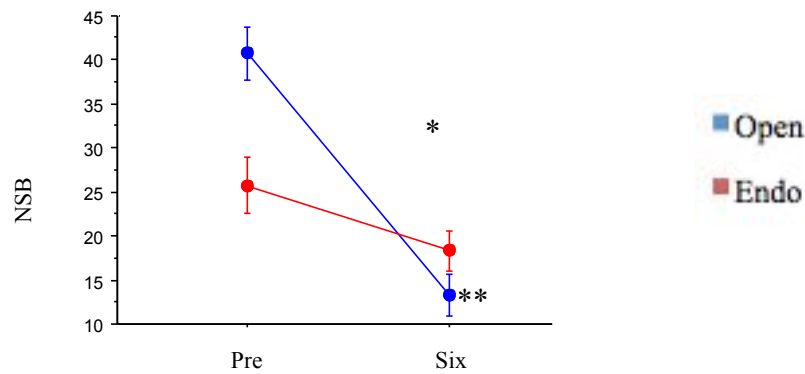


Figure 54: Mean \pm SE values for NSB index pre-intervention and at six-weeks post-intervention for open (n=26) and endovascular (n=10). ** $p < 0.0001$, * $p < 0.05$.

Haemodynamic evaluation after treatment

Ankle pressures

Figure 55 shows ankle pressures for the PT, DP and PN arteries pre-intervention and 6 weeks later. Both groups showed significant increases, the magnitude being similar with either intervention for each artery (PT: Open 70 ± 12 , Endo 76 ± 25 mmHg; DP: Open 53 ± 3 , Endo 46 ± 22 mmHg; PN: Open 71 ± 11 , Endo 56 ± 27 mmHg) with no significant difference

between treatment effects. However, there was greater variation in the pressure increase achieved in the endovascular than open group, as demonstrated by the large variance.

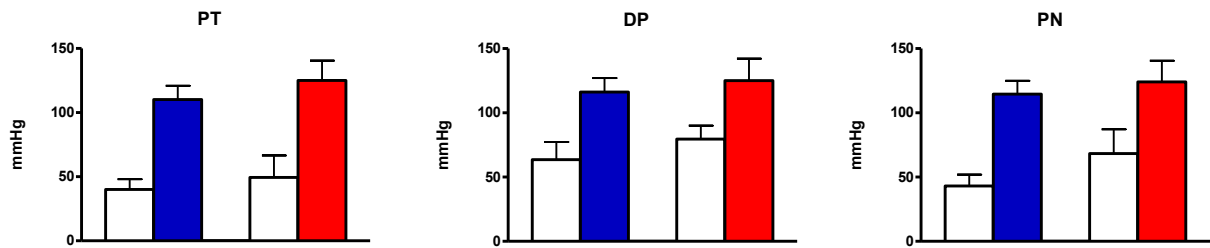


Figure 55. Ankle pressures \pm SE for posterior tibial (PT), dorsalis pedis (DP) and peroneal (PN) arteries pre-intervention (open columns) and six weeks after surgery (open: blue and endo; red). Values are means \pm SE.

Systolic blood pressures decreased within 72 h of intervention, most likely due to residual effects of surgery and medication, but had recovered to pre-intervention values by 6 weeks (Figure 56 a and b).

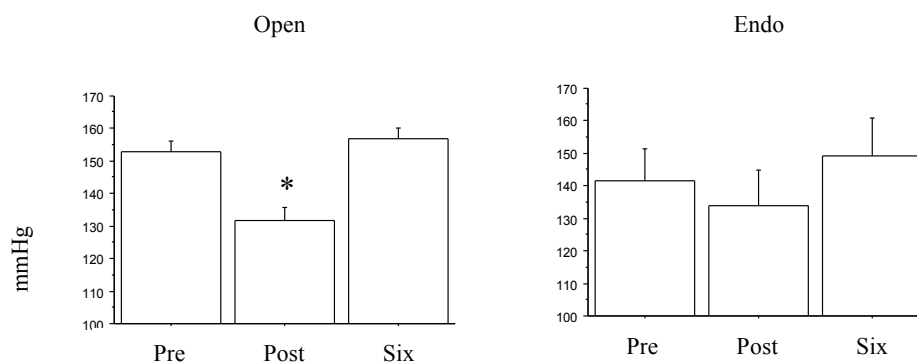


Figure 56. Brachial systolic blood pressure \pm SE at the three-time intervals; pre-intervention, post-intervention and at six weeks for a) open and b) endovascular groups. Values are means \pm SE. * $p < 0.05$ over time.

In keeping with absolute pressures, the ankle pressure indices have increased from pre-intervention to six-weeks in both intervention groups (Table 26).

	Open (n = 25)		Endo (n = 7)	
	Pre	Six weeks	Pre	Six weeks
PT Pressure	40.0 ±7.97	110.2 ±10.7	49.4 ±17.7	125.1 ±15.4
Index	0.26 ±0.05	0.69 ±0.07 †	0.30 ±0.11	0.80 ±0.08 †
DP Pressure	63.5 ±13.8	116.2 ±10.9	79.6 ±10.4	125.1 ±17.1
Index	0.40 ±0.08	0.73 ±0.07 †	0.51 ±0.06	0.79 ±0.09 †
PN Pressure	43.0 ±8.9	114.6 ±10.3	68.3 ±18.9	124.0 ±16.6
Index	0.27 ±0.06	0.72 ±0.06 †	0.44 ±0.13	0.78 ± 0.08 †

Table 26: Absolute ankle pressure and indices by vessel for leg 1 open and endovascular, pre-intervention and at six weeks.

Values are means ± SE. †p value p<0.001 between pre-intervention and six weeks.

In leg 2, ankle pressures and indices (Table 27) showed no significant change over time.

	Open (n = 25)		Endo (n = 7)	
	Pre	Six weeks	Pre	Six weeks
PT Pressure	93.8 ±11.1	115.3 ±11.9	100.3 ±20.2	126.9 ±13.4
Index	0.61 ±0.08	0.73 ±0.08	0.67 ±0.14	0.81 ±0.09
DP Pressure	96.0 ±11.2	121.0 ±11.3	111.6 ±8.7	120.9 ±15.3
Index	0.63 ±0.08	0.76 ±0.08	0.74 ±0.07	0.75 ±0.06
PN Pressure	80.3 ±13.6	102.0 ±14.1	113.4 ±9.3	128.3 ±13.9
Index	0.53 ±0.09	0.65 ±0.09	0.75 ±0.07	0.82 ±0.07

Table 27: Absolute ankle pressures and indices by vessel for leg 2 open and endovascular, pre-intervention and at six weeks.

Values are means ± SE.

Toe Pressures

Figure 57 below illustrates the actual (a) toe pressures (b) ankle pressures recorded at the toe, recorded in leg 1 over time. Both groups showed increased pressures after intervention but whereas in the open group, toe pressures were elevated immediately post- and increased further after six weeks (p<0.05), increases in the endovascular group were not evident until after six weeks and were not deemed significant.

After six weeks, the magnitude of increase in the toe pressure ($38.5 \pm 4.6\text{mmHg}$) in the open group was significantly greater than in the endovascular group ($17.5 \pm 6.7\text{mmHg}$). The magnitude of increase in the ankle pressure recorded at the toe was similar in the two groups (open $46.9 \pm 5.3\text{mmHg}$, endovascular $56.3 \pm 4.3\text{mmHg}$).

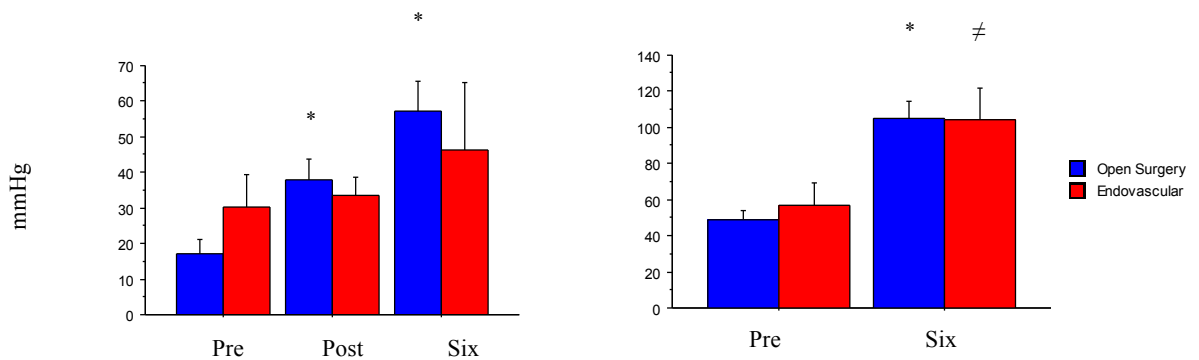


Figure 57: a) toe pressures pre-, post- and six weeks after intervention in open (blue) n=25 and endovascular (red) n=9 groups; b) ankle pressure recorded at the toe pre-intervention and six-weeks. Values are means \pm SE. *p<0.05, # p < 0.1 > 0.05 over time.

No significant changes were observed in toe pressures in leg two in either group but the slight drop immediately post-intervention mirrored that in systolic blood pressure, indicating a systemic effect. Likewise, skin LDF pressures of leg two were unaltered.

	Open (n = 24)			Endo (n = 7)		
	Pre	Post	Six weeks	Pre	Post	Six weeks
Toe 2	59.4 \pm 6.6	42.4 \pm 7.1	57.1 \pm 5.9	65.1 \pm 11.6	54.7 \pm 11.5	67.2 \pm 9.6
Skin Pressure Toe 2	95.1 \pm 6.0		101.2 \pm 6.7	97.1 \pm 17.7		115.4 \pm 21.1

Table 28: Absolute toe pressures for leg 2 open and endovascular pre-, post- and six weeks. Skin pressures in leg 2 pre-intervention and at six weeks. Values are means \pm SE.

Relationship of pressure changes to intervention clinical outcome

Increases in ankle pressure above pre-intervention values tended to be greater in patients with patent than non-patent vessels (Table 29). This was however, significant only for the posterior tibial artery in the open group, although this was the commonest site of distal anastomosis in the crural vessels for bypass. Increases in toe pressures were also significantly higher in those with patent than non-patent vessels for all patients in the open group.

	ALL PTS		Open only		Endo only	
Patent	Yes	No	Yes	No	Yes	No
Patency	21 / 36	15 / 36	19 / 26	7 / 26	2 / 10	8 / 10
Δ PT mmHg	84±11 (21)	37±16 (15) **	84±12 (19)	23±22 (7) **	81±53 (2)	50±23 (8)
Δ DP mmHg	60±14 (21)	42±21 (15)	62±15 (19)	21±26 (7)	47±19 (2)	62±32 (8)
Δ PN mmHg	79±12 (21)	69±23 (15)	80±41 (19)	73±34 (7)	69±41 (2)	65±34 (8)
Δ Average ankle mmHg	74±8 (21)	49±16 (15) ≠	75±9 (19)	39±21 (7) *	66±38 (2)	59±25 (8)
Δ Toe 1 mmHg	54±8 (19)	6±9 (15) ***	52±8 (18)	5±12 (7) **	96 (1)	8±15 (8)
Δ Skin mmHg	75±8 (21)	11±14 (14) ***	79±7 (19)	-13±18 (7) **	39±45 (2)	34±18 (7)

Table 29. Changes in ankle, toe and skin pressures in relation to patency at six weeks. ≠ $p < 0.1 > 0.05$, * $p < 0.05$, ** = $p < 0.01$, *** $p < 0.0001$ for patent vs. non-patent.

Toe 1 pressure and skin LDF pressure in toe 1 correlated significantly with a change in NSB from pre-six weeks for the open group. A similar but much weaker relationship was observed in the endovascular group due to low numbers.

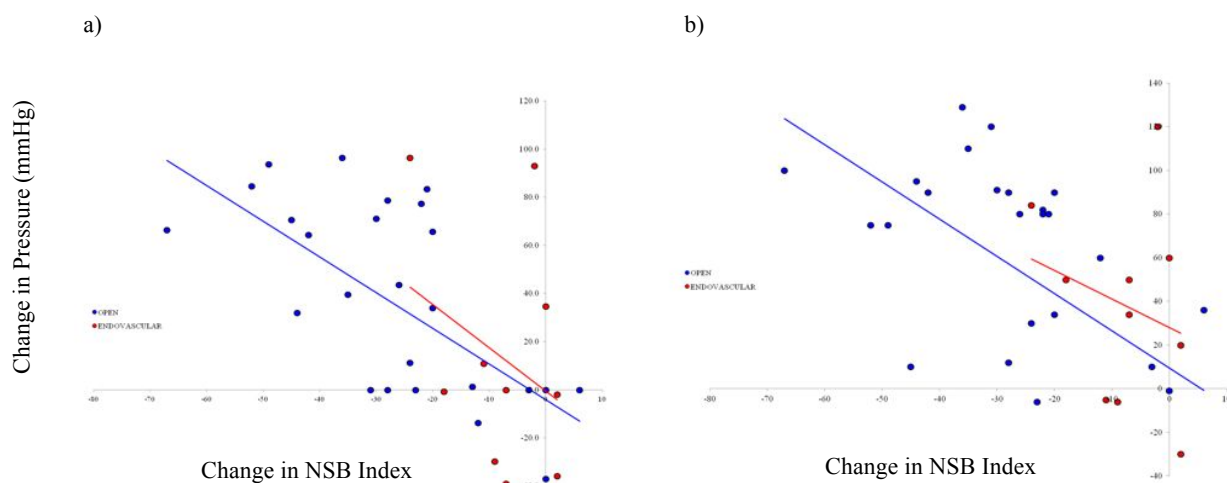


Figure 58. Change in a) toe pressure and b) skin LDF pressure at the toe in the open group (blue) and endovascular (red) group, against change in NSB index value from pre-intervention to six weeks.

Open surgery gave better outcome than endovascular in terms of patency at six weeks with a concomitant rise in ankle and toe pressures. Toe and skin pressures related to NSB index better than conventional ankle pressures.

Oedema, limb volumes and Durometry

In the open group, leg one volume increased immediately post-intervention, as did ankle tissue depth. After six weeks, increased leg one volume was associated with greater tissue depth at both ankle and calf, indicative of oedema. This response was delayed in the endovascular group as limb volume and ankle tissue depth increased only after six weeks, but this was not significant. Overall limb 1 volume increased by a mean of 11.9% from pre-six weeks. The mean difference in limb volume between leg one and two at six weeks was 0.13L, which is also an 11.9% increase in the mean volume of leg one compared to leg 2.

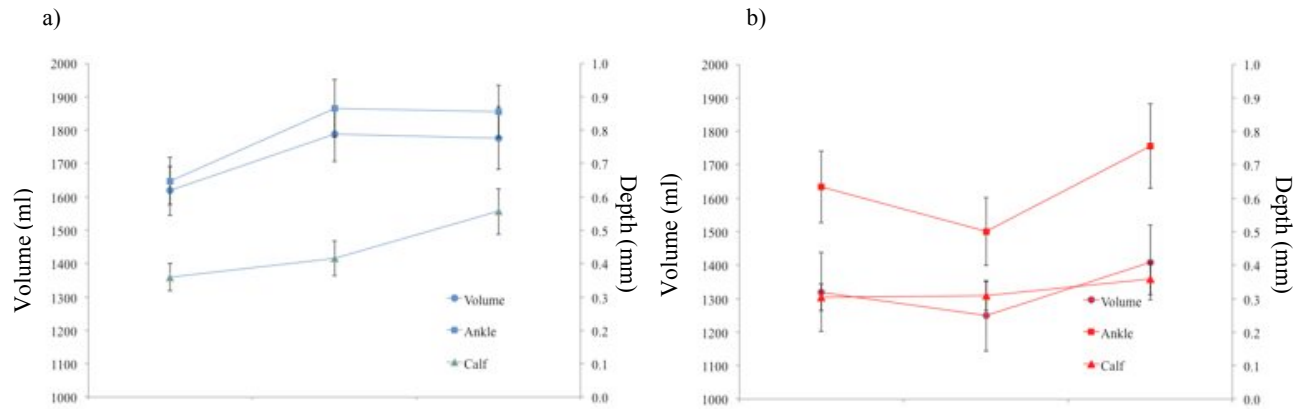


Figure 59: Change in leg volume in a) the open group (blue) and b) endovascular group (red), against change in tissue depth from pre-, to post- to six-weeks. Values are means \pm SE.

Foot circumference increased immediately post-intervention in the open group without change in tissue depth, suggesting circumferential fluid accumulation/oedema. After six weeks this was normalised. There was no significant change in either measure in the endovascular group.

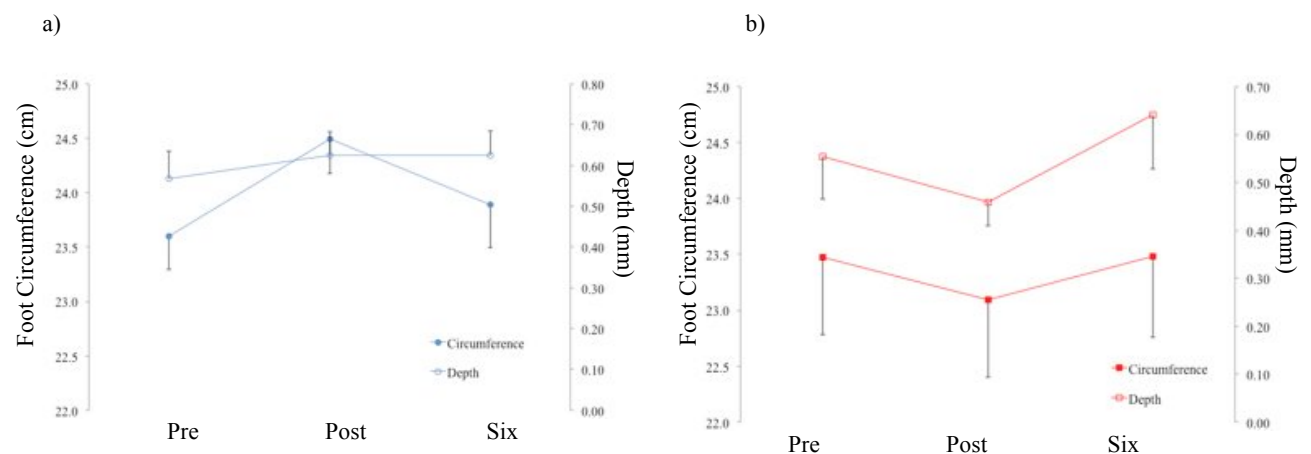


Figure 60. Change in foot circumference in a) the open group (blue) and b) endovascular group (red), against change in tissue depth from pre-, to post- to six-weeks. Values are means \pm SE.

In the open group increases in ankle and calf tissue depth at six weeks and in foot circumference post-intervention were associated with higher values of Durometry. There were no significant changes in Durometry over time in the endovascular group.

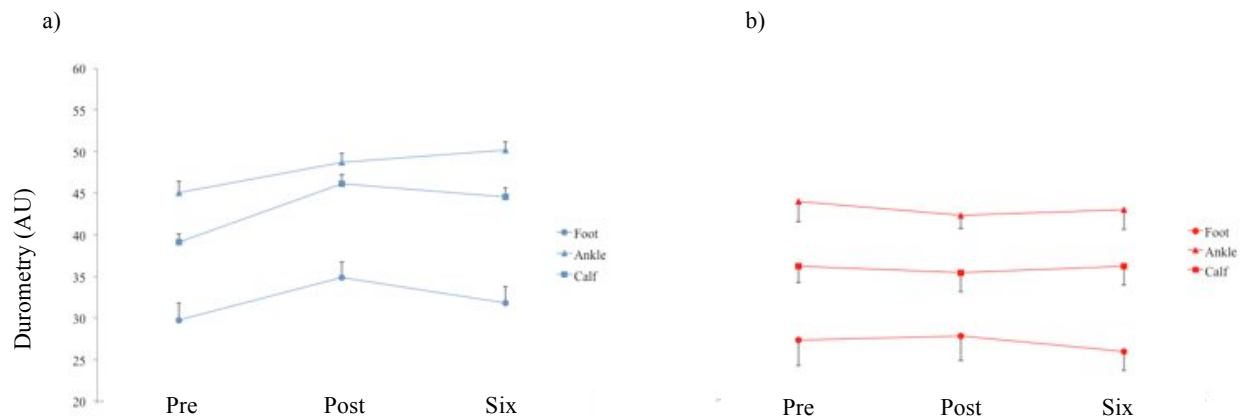


Figure 61. Change in Durometry in a) the open group and b) endovascular group from pre-, to post- to six-weeks. Values are means \pm SE.

Irrespective of intervention group, a relationship between tissue depth and Durometry reading was only evident for the foot ($p < 0.001$), with a correlation coefficient of 0.2 and 0.49 pre-intervention and six-weeks respectively. There was no relationship at the ankle ($p = 0.07$) or calf ($p = 0.52$), suggesting the most distal tissues accumulate fluid and have increasing tissue tension/hardness. Perhaps this is due to the foot being a smaller soft tissue compartment and having greater hydrostatic forces. In the open group at the calf and the ankle, the tissues get harder but do not change significantly in depth.

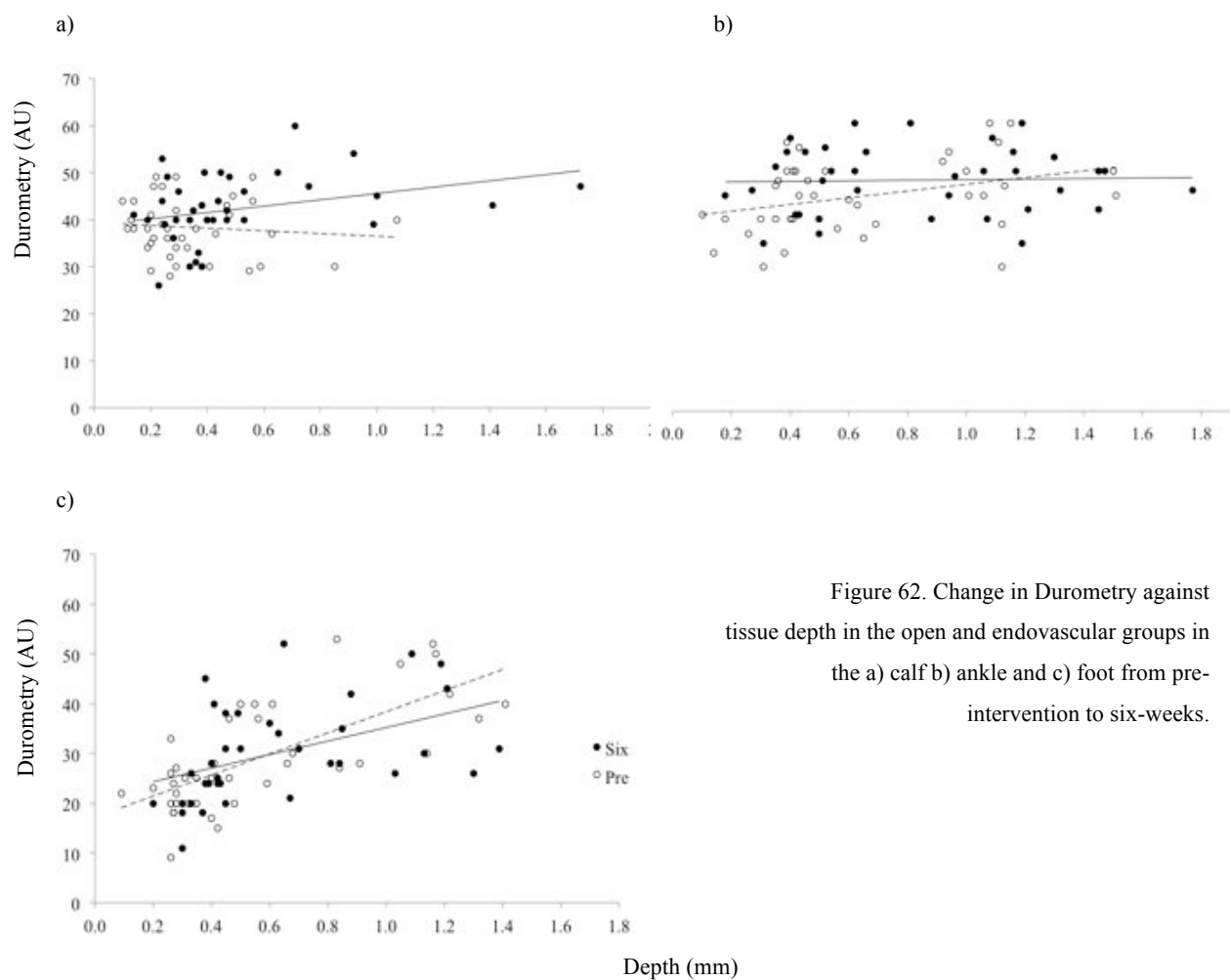


Figure 62. Change in Durometry against tissue depth in the open and endovascular groups in the a) calf b) ankle and c) foot from pre-intervention to six-weeks.

For Leg 2, volume, foot circumference, tissue depths and Durometry at all regions did not change over time (Table 30).

	Time	Open	Endovascular
Leg 2 volume (mls)	Pre	1623 ± 81	1332 ± 104
	Post	1618 ± 82	1276 ± 101
	Six	1628 ± 82	1324 ± 102
Leg 2 foot circumference (cms)	Pre	23.7 ± 0.4	23.2 ± 0.7
	Post	23.9 ± 0.4	23.0 ± 0.6
	Six	23.4 ± 0.4	23.2 ± 0.6
Leg 2 foot depth (mms)	Pre	0.52 ± 0.05	0.48 ± 0.08
	Post	0.50 ± 0.05	0.59 ± 0.07
	Six	0.47 ± 0.06	0.51 ± 0.10
Leg 2 ankle depth (mms)	Pre	0.59 ± 0.07	0.54 ± 0.10
	Post	0.59 ± 0.07	0.49 ± 0.09
	Six	0.57 ± 0.07	0.58 ± 0.10
Leg 2 calf depth (mms)	Pre	0.33 ± 0.04	0.32 ± 0.04
	Post	0.33 ± 0.04	0.31 ± 0.04
	Six	0.37 ± 0.05	0.33 ± 0.04
Leg 2 foot Durometry (AUs)	Pre	26.3 ± 1.8	23.6 ± 3.2
	Post	27.2 ± 1.6	25.7 ± 3.4
	Six	25.9 ± 1.9	22.4 ± 2.4
Leg 2 ankle Durometry (AUs)	Pre	44.6 ± 1.5	40.1 ± 2.1
	Post	43.8 ± 1.1	41.3 ± 1.6
	Six	42.7 ± 1.3	40.3 ± 2.4
Leg 2 calf Durometry (AUs)	Pre	39.1 ± 1.0	37.0 ± 2.3
	Post	42.6 ± 1.0	37.4 ± 2.0
	Six	39.1 ± 0.9	37.7 ± 1.9

Table 30. Change in leg volume, foot circumference, tissue depths and Durometry in leg 2 from pre-, to post- to six-weeks.
Values are means ±SE.

Comparing anthropometrical measures against patency between pre-intervention and six, there was only a significant relationship between patency and change in Durometry at the foot, p0.02 suggesting that increasing tissue hardness at the foot may be predictive of vessel patency.

Overall, oedema was more apparent in the open group than the endovascular group. Post-operative onset of unilateral limb oedema and increased Durometry at the foot could be indicative of early success of operative intervention.

Skin microvascular perfusion

Basal flux

Laser flowmetry is not an absolute measure of skin blood flow; it is defined in arbitrary flux units, and is usually used to examine changing flows in response to provocations that elicit microvascular reactivity. However, it can also indicate trends for changes in levels of basal perfusion and on that basis, comparisons were made between different regions of skin on the leg and foot before and after the two treatment interventions. These are shown in the Figure below as absolute flux for shin, foot and toe of leg one (a, b & c). Figure 63 shows the absolute flux and the change in flux for the toe of leg two.

In leg one, all three regions tended to show increased perfusion immediately post-intervention, although this was not significant in the endovascular group, nor at the foot in the open group. It was most noticeable in the toe of the open group ($p<0.05$). After six weeks, perfusion had returned to pre- levels in the toe, with slightly higher flux in the shin and foot of the open group only.

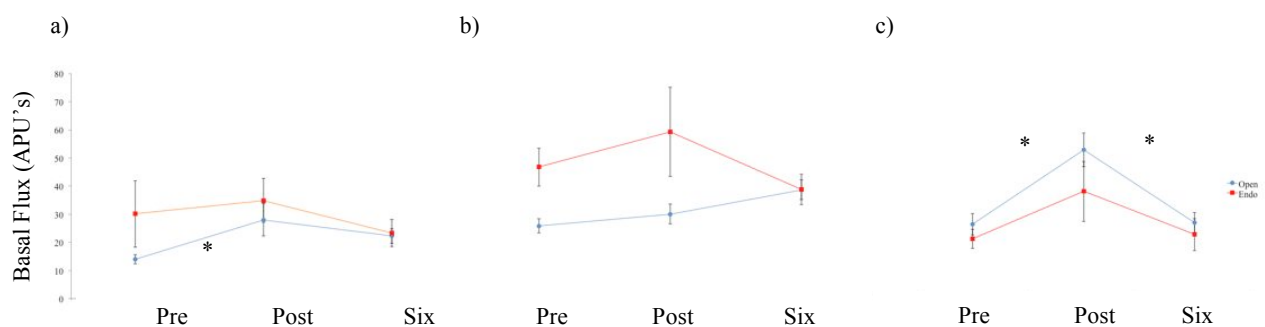


Figure 63: Change in basal perfusion in the open group (blue) and endovascular group (red) at pre-intervention, post-intervention and at six-weeks at a) shin, b) foot, c) toe 1. * $p<0.05$ over time. Values are means \pm SE.

In leg two, toe perfusion decreased considerably in the open group from pre- to post-intervention but this was not significant ($p=0.06$) and changes in endovascular were non-significant.

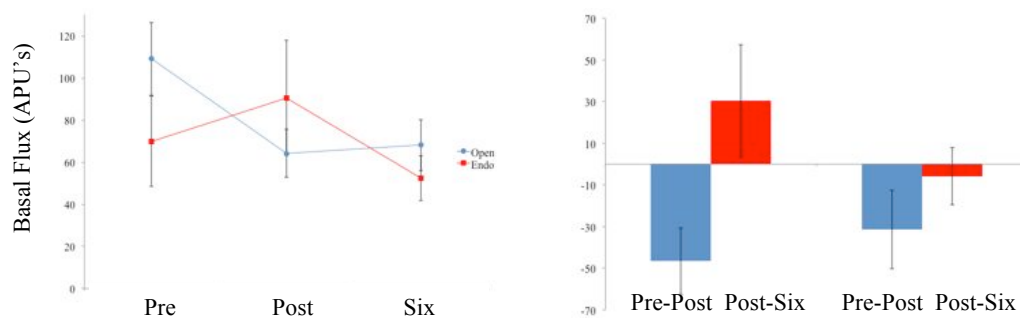


Figure 64 a) Actual flux in the open group (blue) and endovascular group (red) at pre-intervention, post-intervention and at six-weeks in toe 2 and b) change in basal perfusion in the open group (blue) and endovascular group (red) from pre-post intervention and from -intervention and at six-weeks in toe 2

Overall, there was a transient improvement in the shin and toe 1 perfusion that did not persist beyond the immediate post-intervention period and returned to pre-intervention levels by six weeks. In toe 2 the open group showed a non-significant decrease in basal flux, which may have been due to the effect of steal, consistent with the literature (Saucy et al., 2006).

Reactive Hyperaemia

Prior to intervention, the reactive hyperaemic response in leg 1 in both open and endovascular groups was very poor, in many cases with failure to attain basal perfusion levels during the 2-minute post-occlusion period. Six weeks after intervention, there was a substantial improvement in response in the open group but not in the endovascular group (Figure 65 a and b). This change was evident in the average time to peak response, which, although

significantly longer in the open group at the outset, had significantly shortened after six weeks by 41 ± 5 sec from pre-intervention in this group (Table 31). Contrary to this, endovascular time to peak did not change over this time period (14 ± 10 sec, NS). Whereas the time course of the reactive hyperaemia was improved in the open but not the endovascular group, the magnitude of the peak flow, either in actual flux units or as a percentage change at peak, did not demonstrate consistent changes. In toe one of the open group, it increased following treatment, almost reaching significance ($p=0.07$) but there was no change in peak magnitude in the endovascular group.

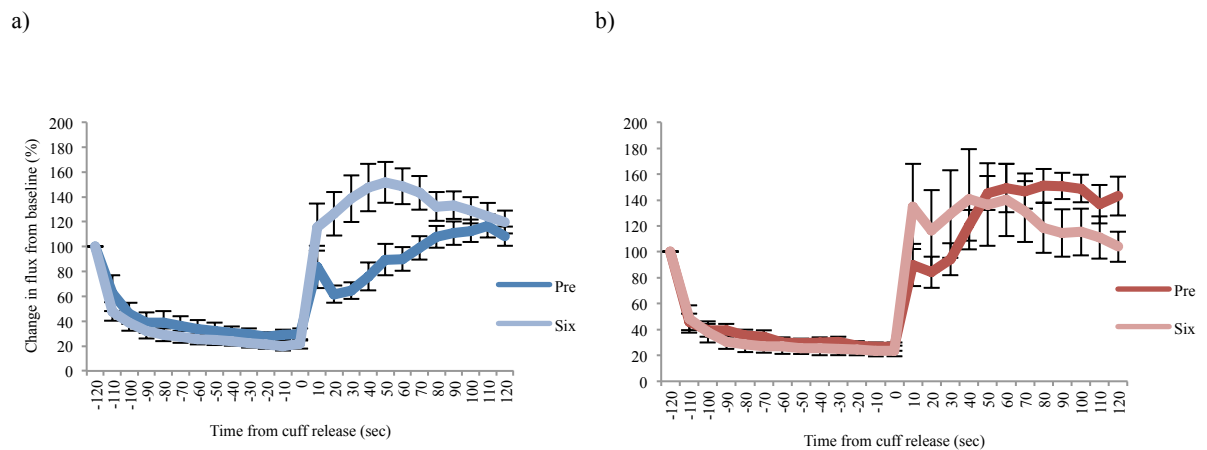


Figure 65: Average percentage change in flux from baseline during reactive hyperaemia 1 for a) open and b) endovascular patients', pre and six weeks post intervention. Values are means \pm SE.

Side	Variable	Intervention	Pre-intervention	Six-weeks
Toe 1	Time to peak hyperaemia (sec)	Open	100 ± 4	59 ± 7 **
		Endo	69 ± 9	55 ± 10
Toe 1	Magnitude of hyperaemia (% increase in flux)	Open	150.0 ± 14.2	204.9 ± 20.7 \neq
		Endo	196.8 ± 20.1	194.6 ± 45.4
Toe 1	Area under the curve (APU's)	Open	-34.0 ± 18.9	342.1 ± 39.7 **
		Endo	56.1 ± 19.0	284 ± 72.6 *

Table 31: Average time to peak, magnitude of hyperaemia and area under the curve in toe one for open and endovascular.

* $p < 0.05$, ** $p < 0.001$, $\neq p < 0.1 > 0.05$. Values are means \pm SE.

It was interesting to note that intervention in leg 1 also had an effect on the reactive hyperaemia response of leg two in the open group. The magnitude of the response (3-fold increase in perfusion from baseline compared to <1-fold in leg 1) was unaffected, but the time to peak was significantly shortened by 20 ± 5 sec, as seen in leg 1. This is illustrated in Figure 66 and Table 32. There were no changes in the endovascular group.

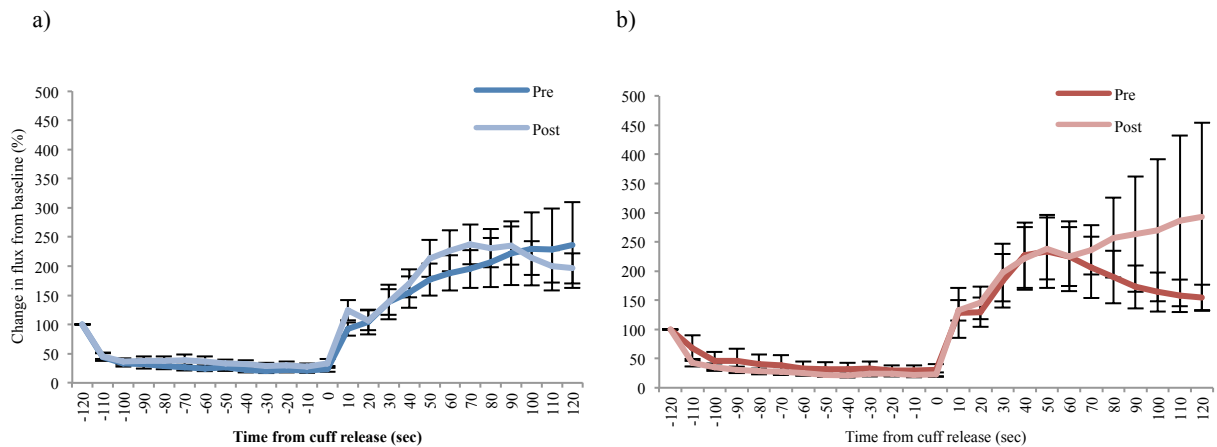


Figure 66: Average percentage change in flux from baseline during reactive hyperaemia 2 for a) open and b) endovascular patients', pre and six weeks post intervention. Values are means \pm SE.

Side	Variable	Intervention	Pre-intervention	Six-weeks
Toe 2	Time to peak hyperaemia (sec)	Open	82 ± 5	$62 \pm 5.5^*$
		Endo	63 ± 12	57 ± 9
Toe 2	Magnitude of hyperaemia (% increase in flux)	Open	306.7 ± 80.0	329.2 ± 41.1
		Endo	264.4 ± 60.5	438.8 ± 154.2
Toe 2	Area under the curve (APU's)	Open	247.8 ± 90.8	283.0 ± 55.1
		Endo	179.3 ± 84.5	237.6 ± 62.9

Table 32: Average time to peak and magnitude of hyperaemia in toe two for open and endovascular. * $p < 0.05$. Values are means \pm SE.

The area under the curve was the only parameter that significantly changed in the endovascular group from pre-intervention to six-weeks ($p=0.01$). Whilst the change was not as great as in the open group ($p<0.001$), they had significantly better hyperaemia at baseline. There was no change in the AUC in leg 2.

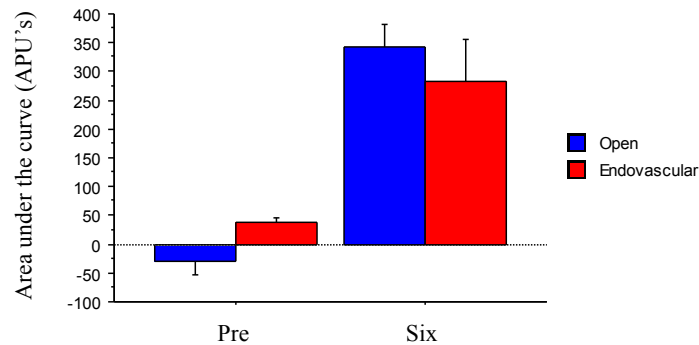


Figure 67: Average change in area under the curve during reactive hyperaemia for open $n=24$ (blue) and endovascular $n=8$ (red) patients', pre and six weeks post intervention. Values are means \pm SE.

The change in time to peak in leg one correlated well with the change in toe pressure between pre-intervention and six-weeks, particularly in the open group ($p=0.04$). Similarly, time to peak shortened with an improvement in NSB over the six weeks, although this correlation was not significant in the open group ($p=0.11$) and the changes in NSB were less marked in the endovascular cohort. (Figure 68) There was no correlation between change in time to peak and average change in ankle pressures ($p=0.2$).

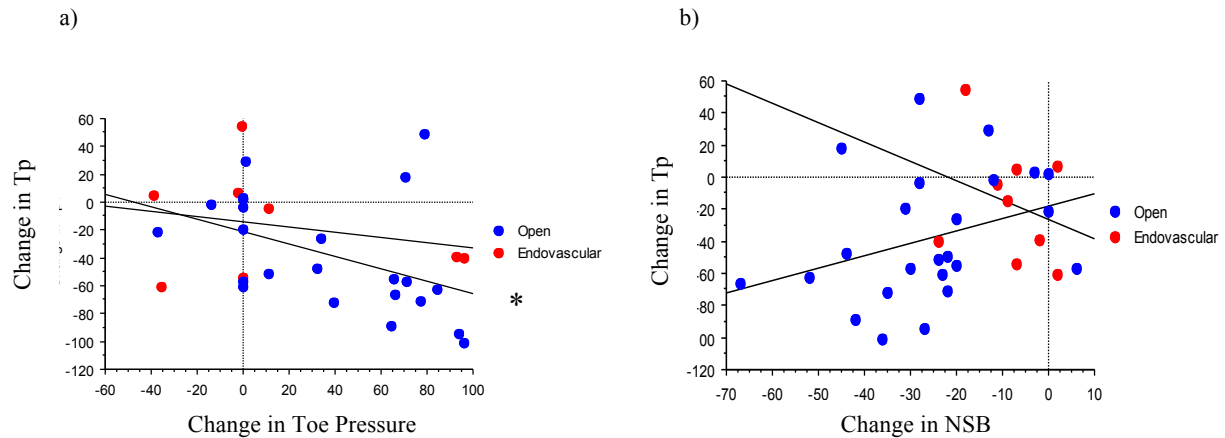


Figure 68: Relationship between change in time to peak and a) change in toe pressures and b) change in NSB between pre and six-weeks for toe 1, divided by open and endovascular. * $p < 0.05$.

Following open surgery, the time course of reactive hyperaemia was improved. This was not seen in the endovascular group, although their AUC had significantly improved and their time to peak was better at the outset. This is associated with a concomitant improvement in NSB and toe pressure, particularly in the open group.

Postural vasoconstriction & local microvascular reactivity

In healthy individuals assumption of upright posture induces a reduction in skin perfusion of around 60%, a vasoconstriction that is lost in CLI (Eickhoff and Henriksen, 1985). This is due to combination of myogenic constriction and a local sympathetic neural veno-arteriolar constrictor response. Lowering the leg of the supine patient below heart level tested the myogenic component of postural vasoconstriction response. This increases the hydrostatic pressure load by less than 25 mmHg, which is the threshold for triggering the local sympathetic neural veno-arteriolar response (reference). The VAR response was tested by

measuring skin perfusion on assumption of the sitting position, in which the hydrostatic pressure load exceeds the 25mmHg threshold.

Leg dependency

Pre-intervention, all regions – shin, foot and toe of leg one, toe of leg two – failed to show postural vasoconstriction to leg dependency (Table 33). Immediately post-intervention, responses had not changed. However, after six weeks, vasoconstriction had been restored to some degree in the shin and the foot in the open group while there was no improvement in the endovascular group (Figure 69). In toe 1 of the open group there had been some improvement in vasoconstriction in the open group, although this was not significant. In toe two of the untreated leg, absence of vasoconstriction pre-intervention was converted to some constrictor response after six weeks in both open and endovascular groups however this did not reach statistical significance.

SITE		PRE-INTERVENTION	POST-INTERVENTION	SIX-WEEKS
Shin	Open	111.8 ± 8.4	101.1 ± 8.5	81.6 ± 3.0*(pre-six)
	Endo	88.3 ± 8.4	97.6 ± 9.5	85.5 ± 8.5
Foot	Open	81.2 ± 6.1	93.3 ± 10.0	60.6 ± 2.8*(pre & post-six)
	Endo	79.4 ± 11.5	83.8 ± 8.6	78.2 ± 5.8
Toe 1	Open	104.7 ± 13.2	122.6 ± 16.3	84.3 ± 9.1≠ (pre-six)
	Endo	87.1 ± 14.8	97.0 ± 7.4	90.8 ± 11.0
Toe 2	Open	101.7 ± 3.6	113.3 ± 8.6	92.3 ± 5.0
	Endo	112.2 ± 11.7	112.6 ± 13.3	82.9 ± 7.4

Table 33: Flux on leg dependency in open and endovascular groups' from pre-, to post-intervention to six weeks. Expressed as a change from baseline (=100). Values are means ±SE. *p<0.05, ≠ p < 0.1 > 0.05 over time.

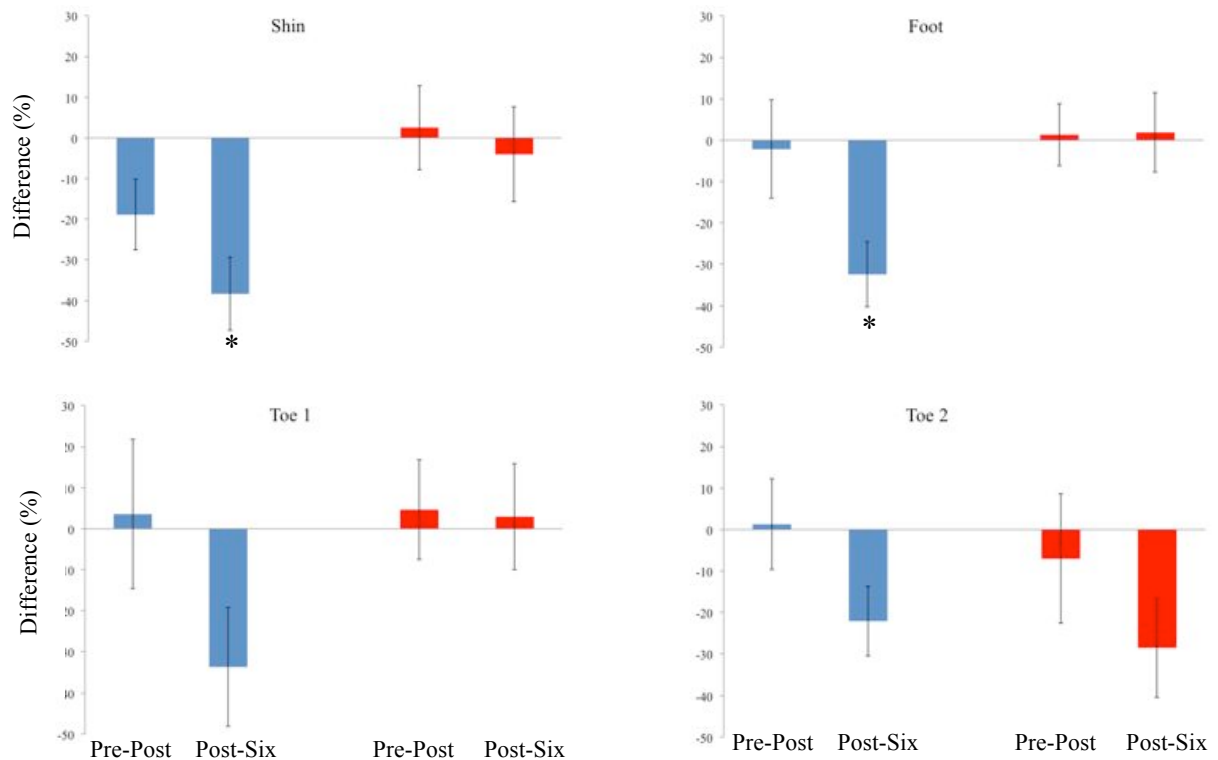


Figure 69: Change in myogenic PVC with leg drop from pre- to post-intervention and from post- to six weeks in the open and endovascular groups by location (shin, foot, toe 1, toe 2). Values are means \pm SE. * $p < 0.05$ over time.

Sitting

Pre-intervention, sitting induced VAR-mediated vasoconstriction in the foot of both open and endovascular groups, and in the shin of the endovascular group. Six weeks after intervention, the VAR was restored in all regions of the treated leg in the open group but in the endovascular group remained static in the foot, absent at the toe and was considerably further impaired in the shin. In toe two of the untreated leg, the vasoconstrictor response seen at baseline was unchanged after intervention.

SITE		PRE-INTERVENTION	POST-INTERVENTION	SIX-WEEKS
Shin	Open	98.3 ± 7.4	125.5 ± 13.8	80.7 ± 8.3*(post-six)
	Endo	70.2 ± 5.9	87.4 ± 14.9	125.6 ± 22.2
Foot	Open	72.4 ± 6.8	96.3 ± 8.5	52.2 ± 2.3*(pre & post-six)
	Endo	75.8 ± 10.8	80.2 ± 15.7	78.6 ± 8.0
Toe 1	Open	99.9 ± 11.0	117.5 ± 16.2	80.3 ± 7.8*(post-six)
	Endo	96.6 ± 16.4	95.4 ± 16.4	100.3 ± 18.0
Toe 2	Open	62.6 ± 8.4	121.9 ± 39.4	69.0 ± 6.6
	Endo	68.3 ± 8.4	60.9 ± 19.0	79.9 ± 20.9

Table 34: Flux on sitting in open and endovascular groups from pre-, to post-intervention to six weeks. Expressed as a change from baseline (=100). Values are means ±SE. *p<0.05.

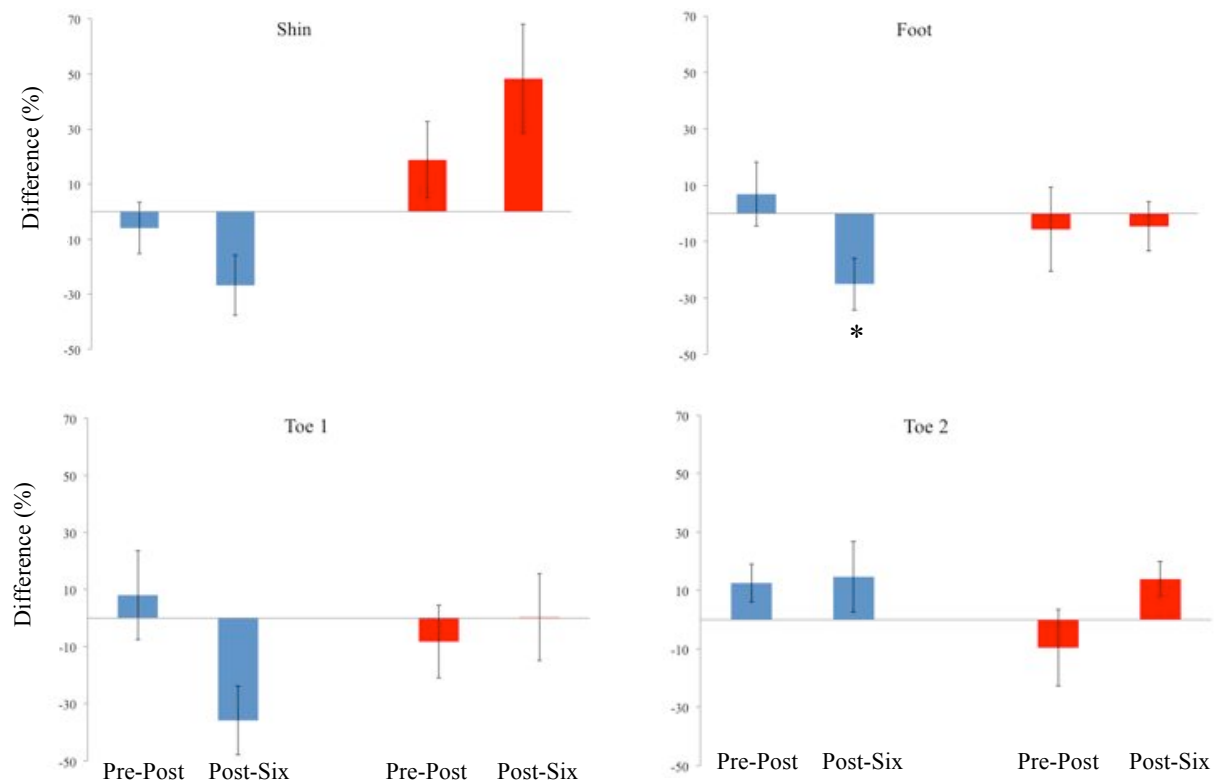


Figure 70: Change in sympathetic VAR with sitting from pre- to post-intervention and from post- to six weeks in the open and endovascular groups by location (shin, foot, toe 1, toe 2). Values are means ±SE. *p<0.05 over time.

The hydrostatic pressure increase elicited during leg dependence was less than the threshold for VAR and therefore the response was myogenic. Subsequently, one may expect to see a

relationship where the greater the percentage-change in myogenic FFT, the greater the negative difference constriction on dependency. The only region that showed a relationship was in toe 1, although this was weak (R^2 0.11). All other regions did not show any significant relationships. On changing position to sitting, hydrostatic pressure should be greater than the threshold for VAR and subsequently elicit a change in sympathetic frequencies, as seen on FFT. There were no correlations seen between sympathetic frequencies and VAR at any location.

FFT analysis

The actual power ascribed to each of the microvascular controls did not alter significantly following treatment. The power ascribed to heart rate and respiratory frequencies remained unchanged in both the open and endovascular groups. There was a tendency for myogenic, sympathetic and endothelial frequencies to reduce in the open group but these were not significant (endothelial 0.08). The open group had a higher power in myogenic frequency post-operatively and at six weeks compared to the endovascular group.

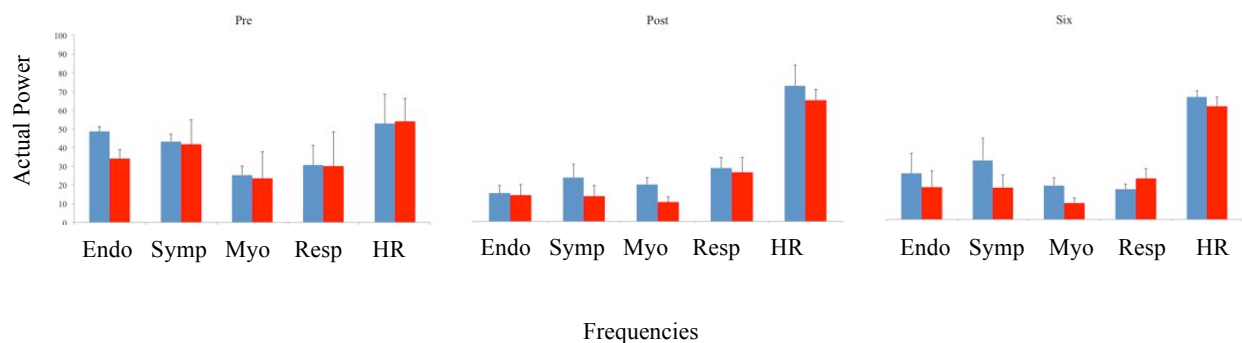


Figure 71: Actual power in the 5 frequency bands pre-, post- and six weeks after intervention, divided by open (blue) and endovascular (red). Values are means \pm SE.

The most noticeable change when comparing the FFT as a percentage of total power was the increase in heart rate from pre-post and post-six weeks, although this only reached significance in the open group (Table 35). In-keeping with the literature, this is likely to reflect better transmission of cardiac output along the length of less disease vessels (Rossi et al., 2005, Rossi et al., 2008).

FREQUENCY		PRE	POST	SIX-WEEKS
Endothelial	Open	16.0 ± 3.2	7.6 ± 1.2* (pre-post)	9.9 ± 2.1
	Endo	16.2 ± 4.7	9.2 ± 2.8	10.3 ± 4.1
Sympathetic	Open	17.5 ± 2.6	11.4 ± 2.0	13.6 ± 2.4
	Endo	17.6 ± 4.7	8.5 ± 2.4	11.3 ± 3.7
Myogenic	Open	11.1 ± 1.5	11.0 ± 2.0	10.2 ± 1.6
	Endo	10.8 ± 3.0	7.2 ± 1.4	7.0 ± 1.3
Respiratory	Open	18.0 ± 2.6	16.4 ± 2.1	11.6 ± 1.8* (pre-six)
	Endo	17.8 ± 4.1	18.8 ± 4.8	18.0 ± 3.6
Heart Rate	Open	37.4 ± 4.2	53.7 ± 4.5* (pre-post)	54.7 ± 4.8* (pre-six)
	Endo	37.7 ± 5.8	56.3 ± 7.1	53.3 ± 8.2

Table 35: Fast Fourier Transform power spectral analysis of Laser Doppler flux, expressed as % of total power for open group (n = 26) and endovascular group (n = 9) from pre-, to post-intervention to six weeks in toe 1. Values are means ± SE.

*p<0.05.

In summary, both surgical and endovascular treatment for critical limb ischaemia elicited a change in haemodynamic, anthropometrical and microvascular measures to a differing degree. Whilst symptoms were similarly improved across both groups, vessel patency was superior in the open group, which was reflected in the lower reintervention rate. The anatomical severity of the disease, described by the NSB correlated with an improvement in toe and skin perfusion pressures in the open group only, despite an increase at six weeks in

these outcomes in the endovascular group. Ankle pressures and indices improved by a similar magnitude in both groups, although this was only related to patency in the open group.

Measures relating to limb oedema show that this is more apparent in the open group, with increasing leg volume and tissue depth immediately post-intervention and at six weeks, with a transient increase in foot circumference. Tissue depth tended to be associated with an increase in Durometry but this difference was only significant at the foot. The endovascular group did not exhibit any significant change in anthropometry or Durometry.

From a microvascular perspective, there was only a transient improvement seen in the shin and toe 1 perfusion in the open group that did not persist beyond the immediate post-intervention. In toe 2 the non-significant decrease in basal flux in the open group may have been due to the effect of steal. What was much clearer was the significant decrease in time to peak and the increase in area under the curve with reactive hyperaemia in the open group. The magnitude of the peak was not helpful in delineating a change. The VAR showed evidence of vasoconstriction only in the open group with restoration in the shin and foot on leg dependency and in toe 1 only with assuming the sitting position. Toe 2 exhibited no change. This showed a degree of correlation with myogenic control, which was greater in the open than the endovascular group.

Whilst, endovascular intervention provides symptom relief at least in the short term, it did not provoke the same microvascular restoration that surgery appeared to and therefore may not offer a longer-term solution to improving the microcirculation.

4.5 Six-month follow up data

Seven patients were followed up after 6 months, 6 from the open group and 1 after endovascular treatment. (3 further endovascular patients were offered 6 month follow up; one had died of unrelated causes and 2 declined). The patient demographics were similar to the study cohort overall, bar a smaller percentage of six-month follow up patients with hypertension. (Table 36)

Variable	All Patients	Follow-up	Range	SE
Age (yrs)	72	70	59-82	3.3
Gender M:F (%)	65:35	71:29		
BMI (kg/m ²)	27	29.3	23.2-43.7	2.7
Cholesterol (%)	83	86		
Smoker: Y or ex (%)	90	100		
Hypertension (%)	80	57		
Diabetes (%)	40	29		
Dialysis (%)	10	14		

Table 36: Patient demographics for the six-month follow up group compared to the total study cohort.

Two out of seven patients (1 open, 1 endovascular) had further intervention during the time period. Of the remaining 5 patients, 4 whose grafts were patent six weeks after intervention were still patent after six months and were still pain free, unclassified according to TASC and reduced from Fontaine 3 or 4 pre-intervention to 1.

NSB and Pressures

The improvement in NSB index at six weeks was maintained at six months (Figure 72). Despite this, there was regression of the increases in DP and PN artery ankle pressures in the treated leg with values at six months returning to pre-intervention levels (Figure 73). Likewise, toe and skin pressures in leg 1 reverted to pre-intervention levels.

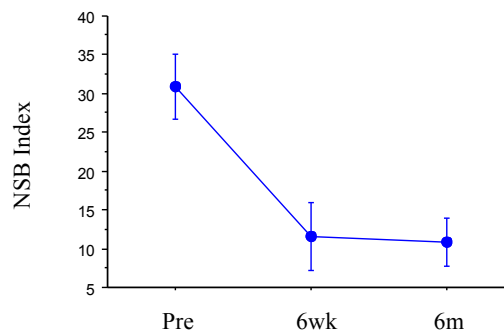


Figure 72: Change in NSB index from pre-intervention, six weeks to six months a) for individual patients and b) overall.

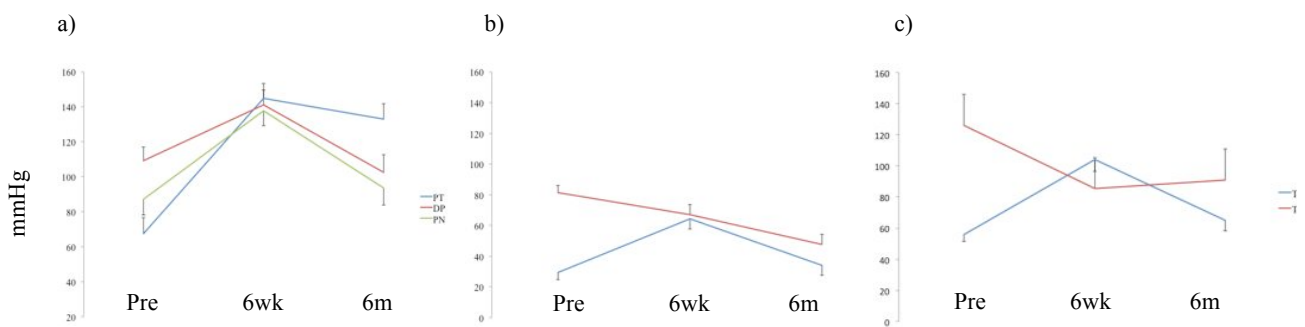


Figure 73: Change in a) ankle pressures in leg 1 b) toe pressures and c) skin LDF pressures recorded at the toe in both legs from pre-intervention, six-weeks and six months. Values are means \pm SE.

Pressures in toe 2 (untreated leg) did not show significant changes from 6 weeks to six months.

Anthropometry and Oedema

In the treated leg, the increase in limb volume after six weeks was associated with greater tissue depths at the ankle and calf indicative of tissue oedema. Some changes had begun to recover by six months, with a significant decrease in calf tissue depth ($p < 0.05$), implying resolution of oedema. By six months, ankle tissue depth remained unchanged from post-

intervention. In the foot, tissue depths and circumference were not significantly different from pre-intervention values. Volume and tissue depths in leg 2 did not change.

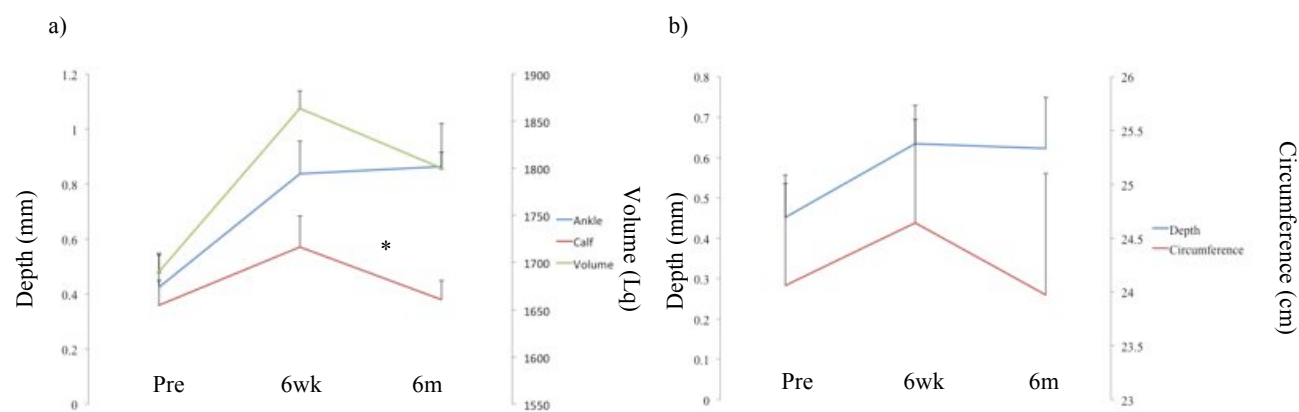


Figure 74: Change in a) limb volume and tissue depth at the ankle and calf in leg 1 b) foot circumference and tissue depth in leg 1, pre-intervention, six-weeks and six months. Values are means \pm SE.

Durometry at the calf did not change significantly between six weeks and six months. The tissue depth appeared to improve prior to an improvement in Durometry with tissues still remaining hard, but this did not worsen.

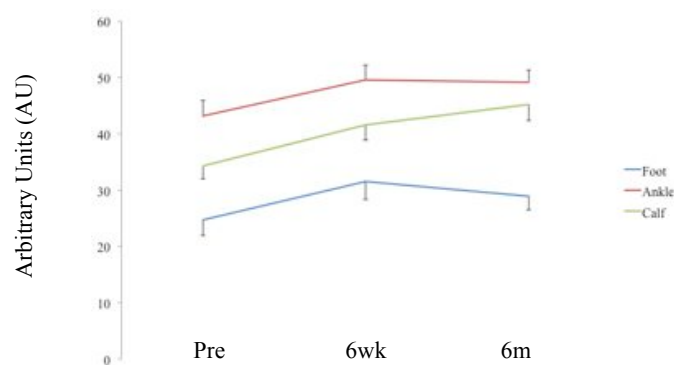


Figure 75: Change in Durometry in a) leg 1 and b) leg 2, from 1- pre-intervention, 2- six-weeks and 3 - six months. Values are means \pm SE.

The Durometry in the calf of leg 2 did increase at six months in this cohort ($p < 0.05$), this was unrelated to leg volume and tissue depths, which were not altered from six weeks to six months. It is unclear for the cause of an isolated rise in calf Durometry.

Skin Microvascular Perfusion and Reactivity

Basal Skin Perfusion

The basal skin perfusion did not change significantly from six weeks to six months in either leg 1 or leg 2. In fact, despite the temporary post-operative increase seen at the foot and the toe in the open group (see treatment effects), the basal flux did not change significantly from pre-intervention to six-weeks and six-months and suffered large variability.

Reactive Hyperaemia

The time to peak of the reactive hyperaemia response, which at six weeks had decreased significantly from pre-intervention, worsened by six months and was no longer significantly shorter in either limb.

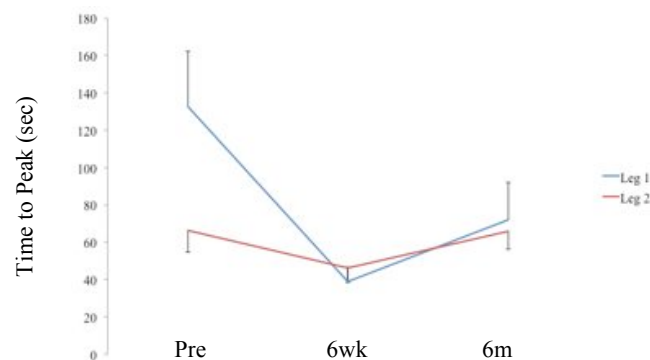


Figure 76: Change in reactive hyperaemia time to peak in leg 1 and leg 2, from 1- pre-intervention, 2- six-weeks and 3 - six months. Values are means \pm SE.

Postural Vasoconstriction

Interestingly, the recovery of myogenic vasoconstriction in the treated leg during limb dependency continued at six months at the toe but reverted in the foot (p0.05). Veno-arteriolar constriction during sitting was unchanged between six weeks and six months at the toe and the foot, and there were no changes in the untreated leg two toe responses.

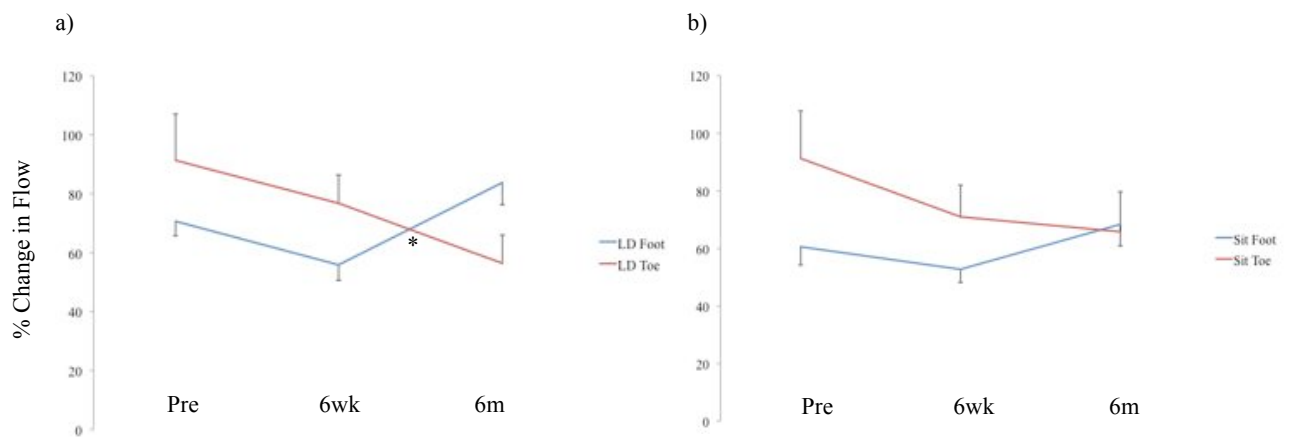


Figure 77: Leg 1 percentage change in flux a) on leg dependency and b) on sitting at the shin, foot and toe 1- pre-intervention, 2- six-weeks and 3 - six months. Values are means \pm SE.

FFT

In contrast to the data at six weeks, there were some considerable changes in flowmotion by six months from pre-intervention. All of the lowest frequencies (endothelial, sympathetic and myogenic) significantly reduced their actual power over this time (p0.05). Respiratory rate and heart rate remained unchanged.

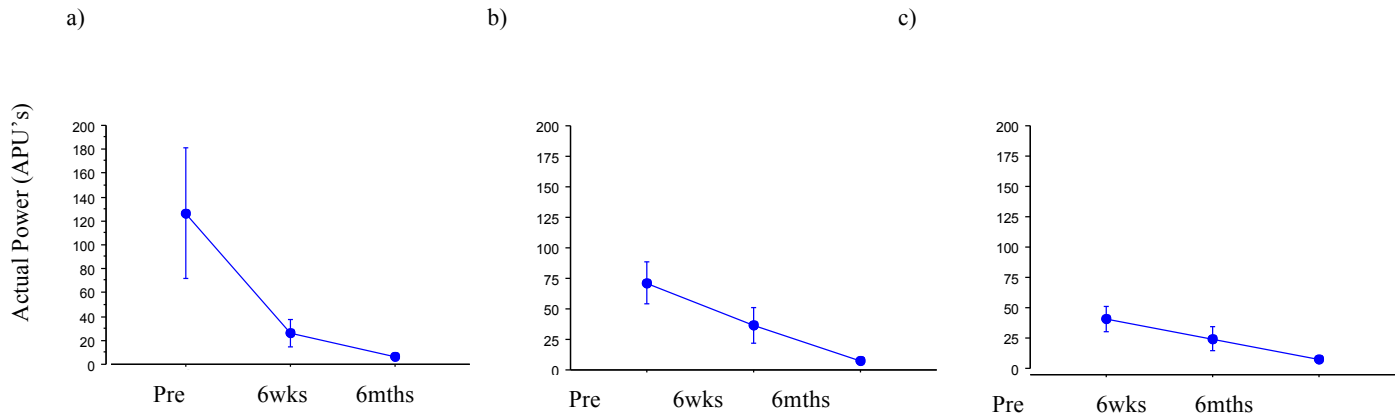


Figure 78: Actual Power of a) endothelial, b) sympathetic and c) myogenic frequencies from pre-intervention, to six-weeks and six-months. *p0.05. Values are means \pm SE.

Frequency	Pre	Six Weeks	Six Months
Respiratory	34.7 \pm 9.8	16.2 \pm 4.8	31.5 \pm 8.0
Heart Rate	58.62 \pm 4.9	65.5 \pm 6.2	58.5 \pm 5.3

Table 37: Actual Power of respiratory and heart rate frequencies from pre-intervention, to six-weeks and six-months. Values are means \pm SE

A small number of patients were followed up at six months, predominantly from the open group. Despite maintenance of the NSB index, all measured pressures had reduced again by 6 months and rather surprisingly the time to peak hyperaemia also reduced to pre-intervention levels. Oedema appears to be resolving by this stage although the tissues in the calf still remained tense. Alongside this, myogenic vasoconstriction continued to improve in all but the foot and sympathetic constriction had not deteriorated from six weeks, but equally showed no signs of improvement. Changes in flowmotion, corresponding to endothelial,

sympathetic and myogenic frequencies under basal conditions had continued to reduce over time and did not appear to relate to improvements in PVC. The microcirculation has suffered a long period of atrophy prior to treatment and its function does not return to normal levels and further declines by six-months, evidenced by the poor haemodynamic and microcirculatory parameters.

4.6 Diabetes data

Pre-intervention

There were 12 diabetics in the cohort who underwent open surgery. They were a mixture of insulin dependent (4), tablet (2) and diet controlled (6). The male to female preponderance was 5:7. They were compared to 18 non-diabetics who had also undergone surgical revascularisation. Unless mentioned, comparisons are based on 12 diabetics and 18 non-diabetics.

There was no difference between the NSB scores between diabetics and non-diabetics at the outset (39.9 ± 4 vs. 41.7 ± 4 respectively), but there were significantly more diabetics with class IV disease (50%) compared to those without diabetes (28%).

Pressures

As anticipated, there was no difference in the average toe pressures between groups (17 ± 6 vs. 14 ± 5). APBI was not significantly higher in the diabetic population, despite the expected consequences of mediocalcinosis (Figure 79).

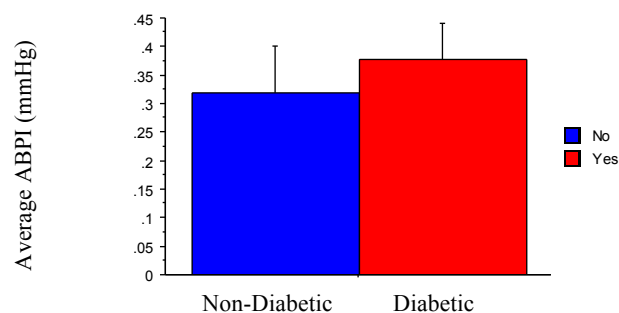


Figure 79: Average pre-intervention ankle brachial pressure index divided into diabetic (red) or non-diabetic (blue) groups.

Values are means \pm SE. $p=0.12$

Neuropathy

Neuropathy was assessed using a neurothesiometer on the metatarsal head of the great toe. Both VPT and VDT were assessed. If patients could not feel the vibration at 50V this was assigned the value of 50 to allow quantitative comparison. There was no significant difference between diabetic and non-diabetic groups. In addition, there were no significant differences between leg 1 and leg 2 readings.

Neurothesiometer	Diabetic (n=8)	Non-Diabetic (n=10)
VPT (v)	31.4 \pm 5.3	28.9 \pm 5.3
VDT (v)	29.4 \pm 5.7	26.4 \pm 5.5

Table 38: Neurothesiometer values for vibration perception and detection thresholds in diabetics and non-diabetics pre-intervention. Values are means \pm SE

Microcirculation

Basal Flux

The basal flux was not shown to be different from non-diabetic patients at baseline across any of the four sites, despite diabetics usually having greater total blood flow due to autonomic neuropathy and the presence of significant AV shunts.

Reactive Hyperaemia

Reactive Hyperaemia time to peak, magnitude of peak and AUC were no different between the groups at the outset (Figure 80).

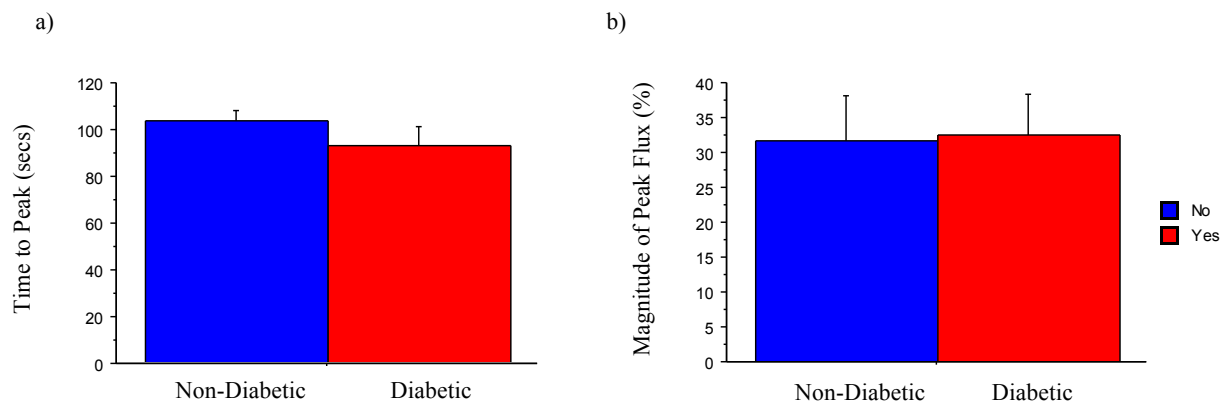


Figure 80: Reactive hyperaemia a) time to peak and b) magnitude of the peak in diabetics n=11 (red) and non-diabetics n=18, (blue) groups. Values are means \pm SE.

Postural Vasoconstriction

Myogenic constriction does not show any attenuation in the diabetic group pre-intervention (Table 39). There is a tendency for flow to increase on dependency at the toe more than non-diabetics but there was greater variation in flow as seen by larger SE's.

Site	Diabetic	Non-Diabetic
Shin	111.8 ± 12.3	107.8 ± 8.6
Foot	82.1 ± 6.9	88.6 ± 9.1
Toe 1	122.4 ± 18.9	101.5 ± 15.7
Toe 2	104.2 ± 5.6	103.6 ± 4.8

Table 39: Percentage change in flow with leg dependency in diabetics and non-diabetics pre-intervention in leg 1 and 2.

Values are means ± SE

Testing VAR did not elicit a difference in sympathetic constriction at any site compared to non-diabetics but there was a similar tendency for increased flux in toe 1 (Table 40).

Site	Diabetic	Non-Diabetic
Shin	96.1 ± 10.0	96.7 ± 8.7
Foot	72.0 ± 8.0	83.3 ± 10.1
Toe 1	121.9 ± 20.0	94.4 ± 9.7
Toe 2	56.3 ± 8.1	74.2 ± 12.0

Table 40: Percentage change in flow with sitting in diabetics and non-diabetics pre-intervention in leg 1 and 2. Values are

means ± SE

Flowmotion

There was no correlation between sympathetic FFT and VAR at the toe. Similarly, FFT pre-intervention was similar between the two groups across all frequency bands (Figure 81).

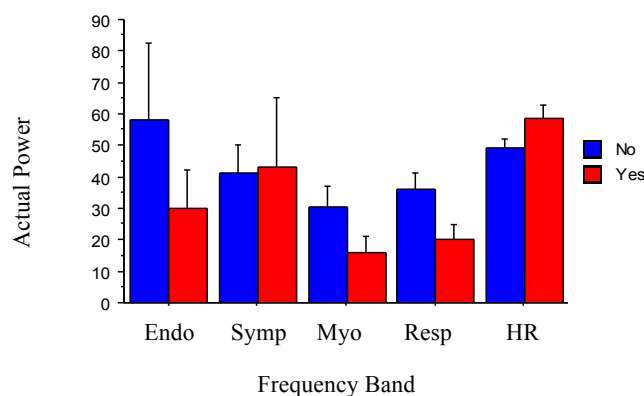


Figure 81: FFT analysis pre-intervention in diabetics (red) and non-diabetic (blue) groups. Values are means ± SE.

It was previously suggested that systemic pressure had an influence on endothelial activity in the diabetic population. There was no correlation identified between ABPI and endothelial activity on FFT.

Treatment Effects

In the six weeks following intervention, there were two deaths in the diabetic group and one amputation, two of which had had redo-surgery prior to these events. There were also two further diabetic patients requiring major surgical reintervention for graft failure. In the non-diabetic group there was also one death, one amputation and 3 patients requiring redo-surgery during this time period.

The average change in NSB was the same between diabetics and non-diabetics (24 ± 4 vs. 26 ± 5), suggesting that resolution of disease was similar. Consequently, symptom relief was also comparable across the groups (Table 41).

Symptom Relief	Diabetic (n=10)	Non-Diabetic (n=16)
Complete	6 (60%)	10 (63%)
Partial	3 (30%)	5 (31%)
None	1 (10%)	1 (6%)

Table 41: Symptom resolution in diabetics and non-diabetics from pre-intervention to six weeks in leg 1 in absolute numbers and percentages.

Pressures

The change in pressure measurements between pre-intervention and six-weeks was not statistically different between groups (Table 42).

Variable	Diabetic (n=9-10)	Non-Diabetic (n=16)
Δ Ankle Pressure (mmHg)	52.4 ± 7.1	73.5 ± 13.7
Δ Toe Pressure (mmHg)	24.2 ± 10.7	46.6 ± 10.5
Δ Skin LDF Pressure (mmHg)	54.9 ± 19.9	54.3 ± 12.7

Table 42: Average change in ankle, toe and skin pressures in diabetics and non-diabetics from pre-intervention to six weeks in leg 1. Values are means ±SE

Neurothesiometer

There was an improvement in neurothesiometer VPT and VDT readings from pre-intervention to six-weeks (21.0±3.8 and 19.1±3.7) but this was not dependent on diabetic status. Furthermore, an improvement in neurothesiometry did not correlate with patency. Readings from leg 2 changed by less than 1 volt on average after treatment.

Microcirculation

Basal Flux

The basal flux did not change over time at the shin or the foot in leg one, nor was it different between groups. At the toe, there was a significant increase in post-intervention flux at the toe in non-diabetics but this was not significantly different between groups.

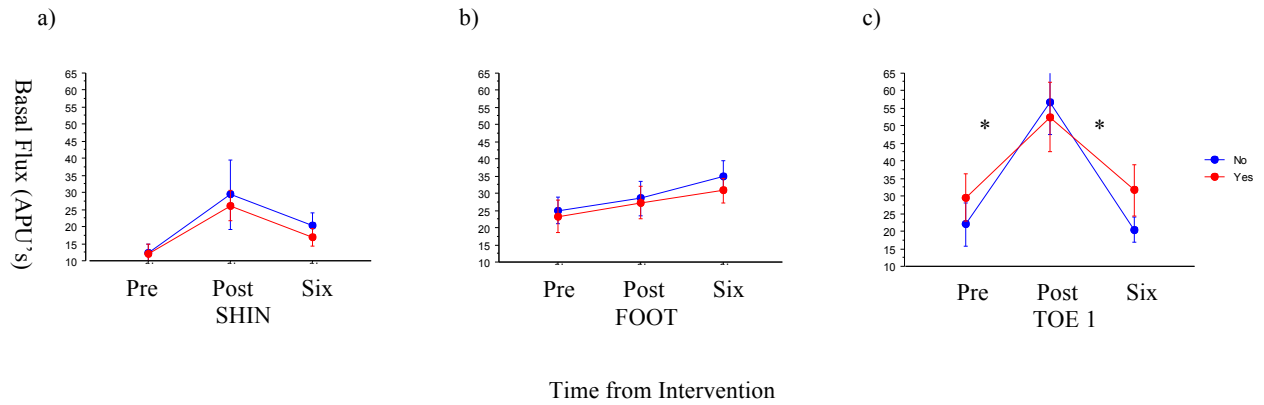


Figure 82: The change in basal flux (APU's) over time in the a) shin, b) foot and c) toe 1 divided by diabetics n=9 (red) and non-diabetics n=15 (blue). Values are means \pm SE.

In toe 2, there was a tendency for basal flux to be higher in the diabetic group ($p=0.07$). By six-weeks the flux was similar in both groups (Figure 83).

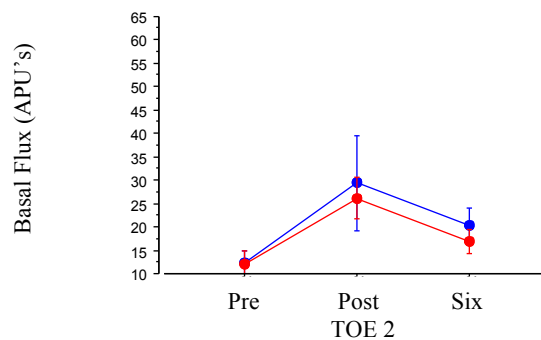


Figure 83: The change in basal flux (APU's) over time in toe 2 divided by diabetics n=13 (red) and non-diabetics n=20 (blue). Values are means \pm SE.

Reactive Hyperaemia

The reactive hyperaemia response measured by change in the time to peak was not affected by diabetes ($p=0.09$). There was a trend however for the diabetics to have a slower time to peak compared to non-diabetics ($\Delta 20.1 \pm 14.6$ vs. 48.7 ± 9.0). The change in AUC was the same between the two groups (Figure 84).

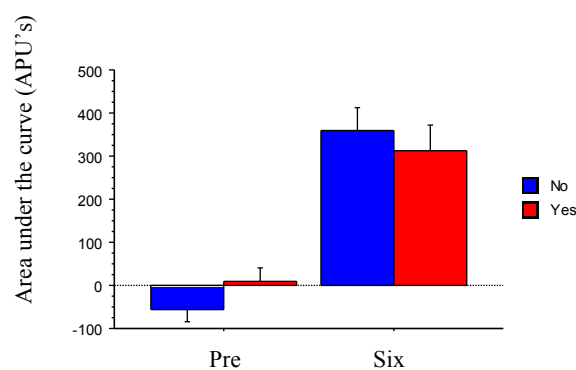


Figure 84: The change in area under the curve over time in diabetics $n=9$ (red) and non-diabetics $n=15$ (blue). Values are means \pm SE.

Postural Vasoconstriction

Myogenic constriction improved from pre-intervention to six-weeks at the shin and from post-intervention to six-weeks at the foot in non-diabetics ($p=0.05$), but there was no significant difference between the two groups overall. Whilst there was no significant change in flux over time at the toe, there was a tendency for the diabetic group to have a smaller myogenic response to dependency at the toe ($p=0.06$).

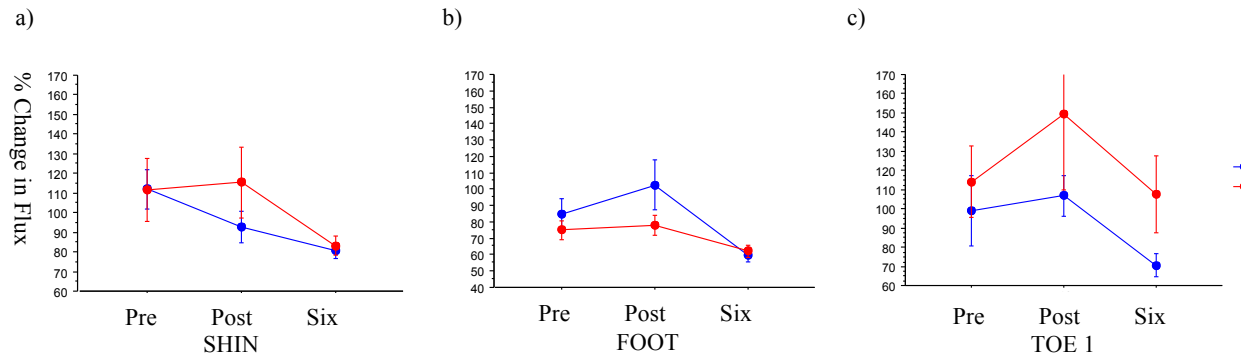


Figure 85: The change in flux with leg dependency over time in the a) shin, b) foot and c) toe of leg one, divided by diabetics n=9 (red) and non-diabetics n=15 (blue). Values are means \pm SE.

Sympathetic vasoconstriction was evident from post-intervention to six-weeks in both groups in the foot ($p < 0.05$). At the shin, these changes were only seen in the diabetic group, contrary to the findings with leg dependency. In toe 1, the diabetic group started off with a higher flux pre-intervention, but neither group improved over time. The changes did not differ significantly between groups at any location.

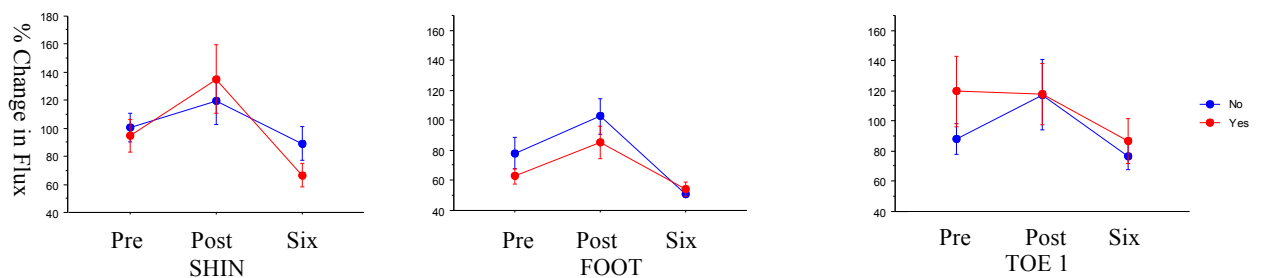


Figure 86: The change in flux with sitting over time in the a) shin, b) foot and c) toe of leg one, divided by diabetics n=9 (red) and non-diabetics n=15 (blue). Values are means \pm SE.

Flowmotion

Flowmotion remained unchanged for both groups from pre-intervention to six weeks. Endothelial activity did not change over time but there were large variances, particularly in the non-diabetic group. (Figure 87)

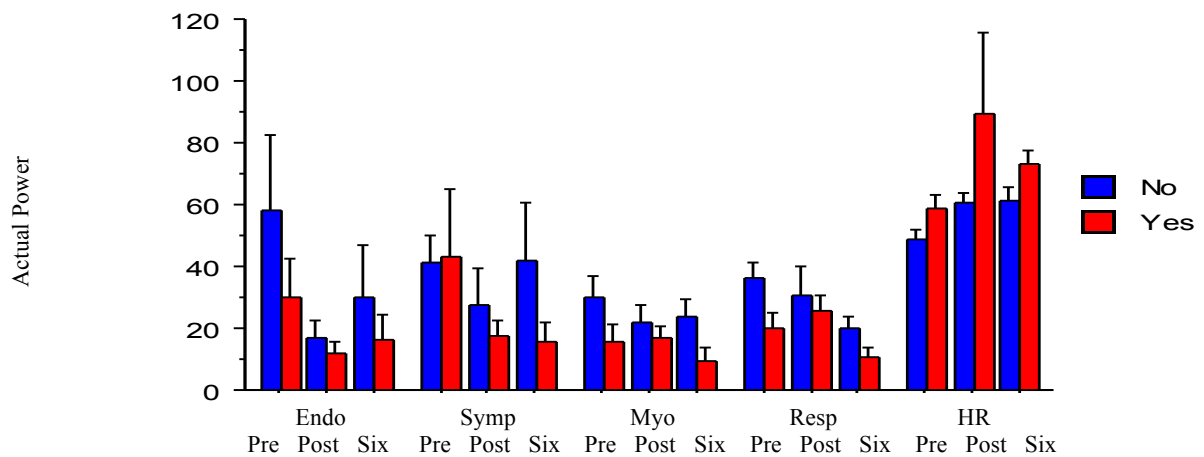


Figure 87: Change in actual flux over time for the 5 frequency bands corresponding to endothelial, sympathetic, myogenic, respiratory and heart rate, divided by diabetics (red) and non-diabetics (blue). Values are mean \pm SE.

The diabetic cohort had similar severity of disease anatomically to the non-diabetics but suffered with more tissue necrosis. The ankle, toe and skin pressures were not different between groups and changes were similar following treatment. Neuropathy was evident in both groups and equal in both legs. Improvements were no more apparent in diabetics. The basal flux and FFT did not change over time with treatment. Reactive hyperaemia time to peak shortened with treatment with a non-significant trend for diabetics to have a longer time to peak. There were no evident differences in postural vasoconstriction between groups. Overall, this small cohort of open surgical diabetic patients does not show significant differences in outcome measures to those without diabetes.

DISCUSSION

This study set out to show that microvascular control mechanisms would be enhanced following open surgery, to a greater extent than is seen with endovascular treatment and that this would be supplemented with a concomitant improvement in clinical and haemodynamic parameters.

I have objectively shown that skin microvascular perfusion and reactivity is improved after open bypass surgery and patients undergoing endovascular recanalisation do not display these findings to the same degree. This was demonstrated in a number of ways:

- **Basal flux:** a transient increase in basal perfusion post-operatively in the open group only, most significantly at the toe pulps.
- **Reactive hyperaemia:** a significant shortening of the time to peak from pre-intervention to six-weeks and an almost significant increase in peak magnitude, seen only in the open group. There was also an improvement noted in the hyperaemic response of toe 2 in the surgical group, which was not seen in the endovascular cohort.
- **Postural Vasoconstriction:** the myogenic component of PVC improved significantly at six weeks at the shin and the foot in the open group, but not in the endovascular group. There had been some improvement at the toe in the surgical group but this was not significant. The sympathetically mediated VAR remained impaired in the

endovascular group at six weeks but was restored in all regions of the treated limb in the open group. Vasoconstriction in the contralateral limb remained unchanged in both groups.

- **Flowmotion at rest:** Significant increases are seen both post-intervention and at six weeks in the heart rate frequency in the open group only, reflecting improved peripheral transmission of cardiac output.

The microvascular changes observed in the open group correlated with greater vessel patency (73%) and improvements in both NSB and TASC criteria. Interestingly, the magnitude of improvement in ankle pressures was similar between both groups. Equally, they both displayed significant increases in toe pressures by six weeks, but the magnitude of improvement was significantly greater in the open group, with both parameters improving within 72hrs following intervention. The magnitude of increase in skin pressure recorded at the toe with the laser Doppler was similar between the two groups.

Oedema was inferred through increases in lower limb volume and tissue depth at the calf and ankle at six weeks in the open group. There were no significant changes seen in the endovascular cohort. Interestingly, foot circumference increased significantly immediately following surgery as well but had returned to pre-intervention levels by six weeks. The Durometry or tissue hardness had increased in association with tissue depth immediately post-operatively in the open group and correlated well with vessel patency ($p0.02$). Endovascular intervention did not elicit these changes.

The Literature

Clinical and Macrocirculatory Outcomes

From a clinical perspective regardless of intervention group, the vast majority of patients had gained partial or complete resolution of their symptoms by six weeks (85%). The CLI population is a very morbid group and is highly associated with cardiovascular events, even greater than symptomatic coronary artery disease (Conte, 2010c). Their 30 day mortality rate in this study was 7.5% overall, with all deaths cardiac-related in the open group. This is in contrast to the BASIL trial where mortality between the two groups was equal at 30 days. That said there were unrelated deaths in the endovascular group by six months that equalized the mortality by group.

Surgical revascularisation was performed with autologous vein in all cases, which has consistently been shown in the literature as the best available conduit. This was invariably LSV but on some occasion supplementary arm vein was utilized. Twelve bypasses were to crural vessels of which, some were complex composite sequential bypasses to more than one outflow vessel, with 10 below knee and 4 above knee infra-inguinal grafts. All had common femoral endarterectomies and 2 had concomitant iliac procedures performed. Patency at 6 weeks was 73%, with 5 patients requiring major reinterventions for revisions, extensions or surgical thrombectomy in the immediate post-operative period. Two patients had amputations within 30 days. Approximately 25-30% of autologous vein grafts will suffer stenosis in the first year but there is no data specifically quoting patency rates after six-weeks and many will exclude grafts that stenose within the first 30 days (Dick et al., 2011).

The majority of endovascular procedures were sub-intimal balloon angioplasties \pm stent insertion, performed on both SFA and crural vessels with high immediate technical success but six-week patency rates were 20%, similar to the patency rates reported at 1 year for CLI-related occlusions (Kandarpa et al., 2001). The low rates are a consequence of having diffuse, calcified occlusive disease with compromised distal vasculature. This would correlate with Met who found a significant discrepancy between vessel patency (50%), limb salvage rates (80-90%) and clinical success (50-70%) in functional and critical limb ischaemia patients, demonstrating that patency does not correlate with symptom relief (Met et al., 2008). This also extends to 5 years where there is an almost 60% difference in primary patency rates and limb salvage (Kudo et al., 2005). Our vessel patency was significantly lower than this but our limb salvage and symptom relief were comparable despite being solely based on CLI patients. This again highlights how challenging this group is to treat given that outcomes have not changed significantly, despite significant advances in endovascular technologies since Met's paper. Furthermore, most of the endovascular patients had class IV disease, which has an inferior outcome to class III disease for infrapopliteal angioplasty (Dorros et al., 2001). The number of segments treated and the most distal vessel treated are also known to significantly affect primary and secondary patency rates, but not clinical outcome or limb salvage (Kudo et al., 2005).

Ankle Pressure/ABPI

Ray et al suggested that ABPI is not considered to be a sensitive indicator of predicting success and patency/clinical outcome in the longer term (Ray et al., 1997a). This is consistent with our findings that there is no correlation between conventional ABPI and patency, despite an improvement in distal pressures from pre-intervention to six weeks. Consequently it

affords no prediction for graft closures within six weeks of intervention. Similarly we were unable to demonstrate a correlation between ABPI and change in the NSB index indicating the severity of any residual disease following treatment. It certainly does not deduce those who will need an amputation and those who will not. It has been quoted that ABPI is expected to increase by 0.15 following open or endovascular intervention, usually within 7 days but can be up to 1 month (Husmann et al., 2006, Jelnes, 1986). In both intervention groups the ABPI rose by a similar magnitude and this was by more than a mean of 0.15 in each vessel by six weeks. Nevertheless, it does not relate to outcome.

ABPI does not provide information on the nutritive blood supply in the microcirculation and highlights that outcome cannot be predicted solely by macrocirculatory parameters if there is a discrepancy between macro- and microcirculatory function. Bongard demonstrated this incongruity by measuring a fall in TcPO₂ and LDF after angioplasty, but an increase in the ABPI (Bongard et al., 1994).

Toe and Skin LDF Pressures

Toe and skin LDF pressures reflect the perfusion pressure in the smaller vessels of the foot and seem to be a much better indicator of patency by six weeks ($p < 0.01$). Saucy et al found that successful surgical revascularisation was associated with a rise in toe pressures from 21-40mmHg immediately post-operatively and similar values were found in this study, with a continued increase in the open group at six-weeks (Saucy et al., 2006). Ray did not find toe systolic pressures of < 30 mmHg to predict clinical improvement, having just 55% sensitivity and 27% specificity. However, this was at six months following revascularisation and we found a similar trend with toe pressures dropping to near pre-intervention levels at this stage,

even with 86% patency (29% having had reintervention to achieve secondary patency). The mean six-month value was above the critical level of 30mmHg, which has been found to predict foot viability and no patients had clinically critically ischaemic limbs at this stage. It seems that the window to predict patency in relation to pressure changes is prior to six months. This may be a reflection of persistent underlying microcirculatory dysfunction, even with initial post-operative normalization of arterial pressures.

Previously, skin perfusion pressures have been shown to correlate highly with toe systolic pressures in the limbs of diabetics and non-diabetics (Tsai et al., 2000). In our study, as well as correlating with patency (change in skin LDF pressure 78 ± 8 vs. 11 ± 14 mmHg), skin LDF pressures were also shown to correlate with a change in the NSB index over the same time period. This is in keeping with SPP being able to detect the severity of disease, although this was as a diagnostic tool rather than at follow up from an intervention (Castronuovo et al., 1997). At absolute pressures below 30mmHg, ulceration has been shown to be significantly less likely to heal than those with a perfusion pressure greater than this. From our data, a change in skin LDF <30 mmHg appears predictive of non-patency although numbers are small and this is a relative change rather than an absolute change. Perfusion pressures following treatment are greatly under utilized particularly in view of its predictive value in diagnosing CLI and predicting limb salvage and ulcer healing. A Japanese group measured SPP before and after endovascular recanalisation but improved SPP values are compared to the presence of a wound blush and not patency per se and therefore it is difficult to interpret and compare in this context. Given that wound blush was predictive of limb salvage it would suggest that SPP is also related to limb salvage and could be a useful marker but the study was not powered with this in mind (Utsunomiya et al., 2012).

Microcirculation

Basal Flux

Resting or basal flux using LDF is reduced in severe ischaemia at the foot and in the toe (Bongard and Fagrell, 1990, Saucy et al., 2006). Following surgical intervention, this parameter has been shown to increase considerably, although it is subject to noticeable overlap (Wahlberg et al., 1995). Laser Doppler Imaging similarly displays a global increase in flow ten days following surgery, with a simultaneous reduction in flow on the untreated side (Saucy et al., 2006). Our cohort exhibited similar findings with an increase in basal perfusion by day 3 (post-intervention) and a non-significant decrease in the basal flux of toe 2 ($p=0.06$). By six-weeks basal perfusion in toe 1 had returned to pre-intervention levels but remained elevated at the foot and the shin. Toe 2 maintained its post-operative flux at six-weeks. Lamah et al suggested that in CLI whilst there are fewer capillaries present anatomically, a much higher proportion of these are perfused. This may explain why the flux returns to baseline levels by six weeks, if the capillary numbers return to 'normal' and flux remain constant.

Reactive Hyperaemia

Reactive hyperaemia offers a quantitative global assessment of the microvascular function of a limb, with the time to peak providing the commonest and most discriminatory method of assessment. It has been shown to correlate with disease severity because the pressure lost across a stenosis or occlusion causes a delay in the time to peak hyperaemia, or it may even be abolished all together in severe disease. The configuration of the traces themselves can be characteristic of certain disease patterns and there are many parameters described in an attempt to quantify them for comparison. For example, 'double-humped' curves were

described by Kvernebo who suggested that this was due to the presence of a significant arterial pressure gradient, which became more pronounced with more significant gradients. They measured the f_{diff} (the difference between the flux value of the first peak and the minimum flux value between the two curves) to try to assign a value to it for comparison, but this was not shown to have any discriminatory value (Line et al., 1996). In our experience, these types of curves are typically seen in diabetics, dialysis dependent patients or the very elderly with severe mediocalcinosis where the vessels are stiff and non-compliant giving rise to sudden movement on release of the cuff, which is detected by the Laser Doppler as an initial artifact peak and a subsequent peak related to flow transmission. This artifact was not used as the reading for time to peak in this study. (Figure 88 a and b) Furthermore, the period of occlusion prior to cuff release at time 0 shows that it is very difficult to compress these vessels and you would expect to see a disproportionately high ABPI in these patients.

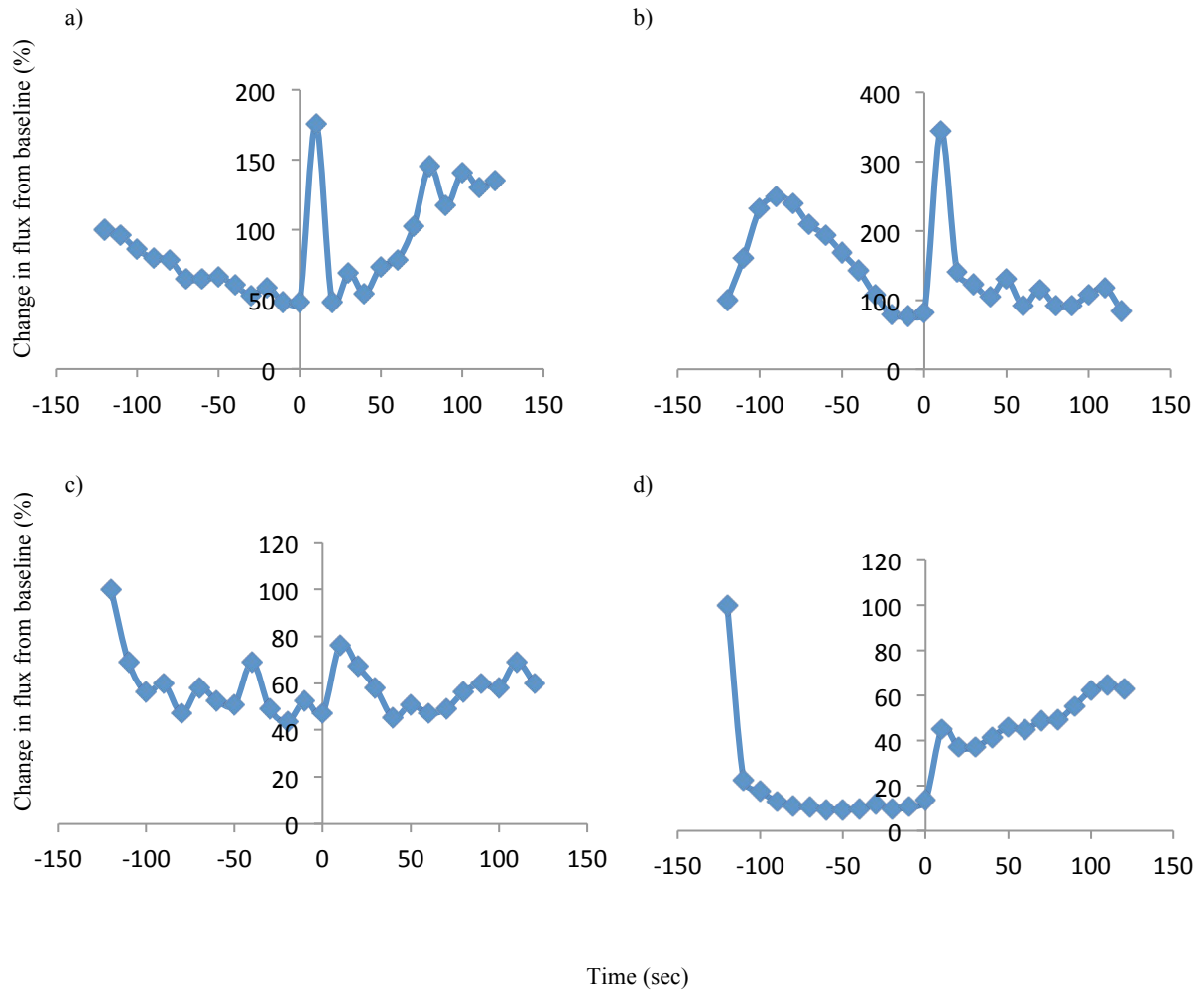


Figure 88: Examples of individual reactive hyperaemia curves from the toe of the most symptomatic leg.
a) Diabetic patient, b) dialysis dependent diabetic, c) severe ischaemia with calcified disease, d) severe ischaemia with no peak above baseline. Values are means \pm SE for 10s averaged values. Arterial occlusion commenced at t = -120s and the cuff was released at t = 0s.

In many cases basal perfusion was not attained during the two-minute post-occlusion period. Figure 88 c and d demonstrate patients with particularly severe disease where a peak hyperaemia is not achieved, as they do not go above baseline. Patient c had extensive disease and subsequently died. Patient d later required an amputation. In a study by Ubbink et al, absent reactive hyperaemia was one variable used in predicting amputation in addition to capillary density values of $<20\text{mm}^2$, $\text{TcpO}_2 <30\text{mmHg}$, giving a specificity of 87% and

predictive value of 73% for amputation (Ubbink et al., 1999). In a healthy patient there is a smooth ephemeral rise to a peak within 50seconds and this was seen in our control patients.

Because reactive hyperaemia represents the vascular resistance of the limb as a whole, the time to peak is not affected by the positioning of the probe above or below the stenosis. However, the positioning of the probe in relation to the cuff has been shown to affect the time to peak and consequently we ensured this was standardized for every hyperaemia tested (Line et al., 1996). The site of measurement was always from the toe, which would also have provided higher peak flux values than other sites due to the high number of AV anastomoses (Kvernebo et al., 1989). In terms of attempting to distinguish curve patterns based on anatomical location of disease, this was not possible in the small numbers present in each group. Furthermore, many of the studies use high pressure ~300mmHg for 3minutes occlusion time. In this study we used a 200mmHg for 2 minutes, which allowed successful discrimination without putting the patient through further discomfort from increasing ischaemic times.

Following treatment, the time to peak in the open group changed significantly ($p<0.001$), consistent with Wahlberg's findings, but this was not seen in the endovascular group. The six-week time to peak was similar between the two groups (59 ± 7 open vs. 55 ± 10 endovascular) but the initial times to peak were significantly different from the outset (100 ± 4 open vs. 69 ± 9 endovascular), suggesting more severe disease in the open group. The magnitude of the peak did not change significantly over time, consistent with findings in the literature, which have not found this parameter to be highly discriminatory. The area under the curve is not a commonly reported measure for comparison of hyperaemic responses. In

this study there was a significant rise confirming that flow rose above baseline, which was not seen in the open group pre-intervention and rarely in the endovascular group. It reflected time to peak in the open group but was the only measure in the endovascular group that changed significantly. It seems to detect subtler, insidious rises in flow above baseline than a one-off peak reading. However, this time of response is not the 'normal' reactivity of a vessel, which is better reflected by a combination of a sharp rise and a large area under the curve.

Interestingly, the time to peak in toe 2 of the open group was also significantly shortened. Whilst this has not been specifically noted by previous studies, other parameters suggestive of improved flow have been shown in the contralateral limb, such as an increase in basal flux and improved postural vasoconstriction. This may be as a consequence of systemic increases in vasodilator metabolites or a heightened response from local reflexes such as myogenic or sympathetic pathways.

A significant relationship was found between the change in time to peak and the change in toe pressures in the open group ($p<0.04$). Although many authors have concluded that reactive hyperaemia is more discriminatory than ABPI and can distinguish clinical improvement regardless of ABPI, the time to peak has not been correlated with changes in toe pressures before. It would seem logical that this would be more consistent given that it takes account of small vessels changes in addition to the macrocirculation. The results achieved following endovascular treatment have varied with regards to time to peak but have not tended to involve critically ischaemic patients. Leonardo reports a significant improvement in time to peak flux returning to 55 ± 7 sec with single-level PTA, whereas Bongard did not elicit an improvement in response at all despite haemodynamically and angiographically successful

procedures (Bongard et al., 1994, Leonardo et al., 1987). It is difficult to comment given that the study populations are different, but our endovascular group had the same mean time to peak at six weeks (55 ± 10 sec) as Leonardo's group, although this was not significantly different from the outset, but the majority of the endovascular patients did not achieve patency by his time (80%).

Postural Vasoconstriction

Patients suffering with CLI have regional passive dilatation of distal small vessels in an attempt to compensate for poor flow. Subsequently there is greater perfusion during dependency compared with the supine position, with flow increasing by 28% in dependency in limbs that suffer with rest pain (Eickhoff and Engell, 1982b). This increased flow is greatest at pulp of toe compared with the foot and/or the ankle predominantly due to the presence of AV shunts and also its most distal positioning (Khiabani et al., 2000a).

Delis found that VAR with sitting improved within 6 days following surgery for severe ischaemia. In our surgical cohort, whilst there were improvements in VAR seen at all three locations, they were only significant from post-intervention to six weeks at the shin and toe and from pre-intervention to six-weeks at the foot and hence there was no improvement in the immediate post-operative period. Similarly after leg dependency and initiation of the myogenic reflex, constriction improved at the foot and the shin, with the toe showing a non-significant tendency to improve over this time. There was no real change identified immediately post-operatively. Eickhoff and colleagues showed that a low or absent pre-operative VAR was only significantly improved 3-6months after surgery if clinical improvement was evident (Eickhoff and Engell, 1982a), which is more in keeping with the

findings from our cohort. They suggested that the time taken to show improvement would be more suggestive of loss of normal small vessel function from ischaemic damage or atrophy, rather than as a functional consequence that is flow dependent and hence more likely to recover quicker (Eickhoff and Engell, 1982a).

Return of vasoconstriction was not seen in the endovascular group. In a small study of claudicants that had undergone angioplasty, orthostatic blood flow at the toe improved equating to an overall improvement in VAR. However, there was already some evidence of postural vasoconstriction at the outset making it difficult to compare the two groups, as this was not evident in our cohort pre-intervention. In contrast to the findings following surgery, it has been proposed that the change in VAR are pressure and flow sensitive due to their correlation with an increase in ABPI (Husmann et al., 2006). Our endovascular group did show some improvement in their ABPI of >0.15 but there was no associated improvement in VAR and therefore does not support this suggestion. It is likely that patients with functional ischaemia do not have loss of small vessel function and therefore postural vasoconstriction may recover more quickly as a consequence of increased pressure and flow.

Flowmotion

The rhythmical variation in blood flow is composed of oscillations of differing frequencies and amplitudes. Over time these oscillatory wave patterns have evolved to describe specific mechanisms of regulatory control processes. However, the description and frequency of the bands, the method of analysis and the units reported differ, making it difficult to directly compare like for like.

Following periods of ischaemic stress in claudicants the power bands ascribed to endothelial, sympathetic and myogenic control were blunted compared to controls although flowmotion was preserved, if not amplified, in the same group under resting conditions using wavelet analysis (Rossi et al., 2005, Rossi et al., 2008). This was not however the case in CLI, where the same frequency bands were found to be reduced at rest (Anvar et al., 2000b). On comparison of our pre-intervention data with our healthy controls (not age-matched) a similar percentage power was seen for endothelial and neurogenic frequencies, although myogenic was lower in the control group. This was attributed to a reduction in frequency associated with heart rate in the CLI group, which was also seen by Rossi, even in functional ischaemia, as a consequence of reduced transmission of cardiac output to the peripheries.

Unfortunately there are no studies that have used FFT to examine the distribution of frequencies at the toe in CLI or to compare changes following a treatment intervention. This study showed that microvascular controls do alter after treatment with more power ascribed to heart rate, particularly in the open group, and respiratory frequencies remaining unchanged in both groups, as expected. In the lower frequency ranges, there is a tendency for endothelial, sympathetic and myogenic to fall but this is not significant. It may be that basal conditions are not sufficient to highlight changes in flowmotion and it requires a period of physiological demand to demonstrate differences. This study did not identify a significant correlation between a change in postural vasoconstriction and FFT myogenic or sympathetic power. Consequently, it is difficult to know if flowmotion has a role in improving PVC after treatment, or whether the relationship is not evident from FFT readings that have been taken during basal conditions, rather than under conditions of stress.

Oedema

There is controversy around the cause for post-surgical oedema and how intimately resolution of oedema may be associated with the return of PVC. The phenomenon of post-intervention oedema is seen in 65-100% of successful femoro-popliteal bypass reconstructions (Eickhoff and Engell, 1982b), but is not encountered in endovascular patients and consequently it is rarely objectively recorded in this group. In our experience oedema is not seen in the endovascular group, as measured by limb volume and tissue depth. Ray also found that calf volume of the endovascularly treated limb did not increase regardless of disease severity at day one or one week following intervention (Ray et al., 1997b).

The surgical group remains thought provoking and certainly in our surgical cohort, limb volume and tissue depth increased in the immediate post-operative period and persisted at this level at six weeks. Calf tissue depth did increase further by six weeks but this did not impact on limb volume. Autoregulation of postural vasoconstriction in this group did not show signs of improvement until six weeks, by which point oedema had plateaued. It would be consistent with a picture of persistent vasomotor paralysis, together with an increase in systemic arterial pressure favoring oedema formation early post-operatively.

Counter arguments to the cause of oedema formation have been related to surgical trauma and inguinal lymph node disruption. All patients in the surgical cohort underwent surgery at the inguinal region but lymph node disruption was not assessed in this study. It has been suggested that the magnitude of disruption is correlated with the extent of oedema, although some oedema was still present even if lymph nodes were preserved (Porter et al., 1972). Equally, if there is early surgical graft failure oedema is not observed, which is counter-

intuitive, however they suggest that this may be due to such low capillary perfusion pressures that excess filtrate can be absorbed adequately even in atrophic beds. Eickhoff also studied PVC in surgical patients and whilst they too did not see the return of autoregulation until 3-6 months, they did not find this correlated with the presence or resolution of limb oedema, suggesting that this appears independently to PVC post-surgery (Eickhoff and Engell, 1982b).

Durometry

Durometry was initially described for its use in industry but it has been used in medical specialties to assess tissue tension. This has predominantly been in the field of dermatology (Kissin) but it has also been reported in identification of skin changes in venous disease (Choh). It has not been used in critical limb ischaemia and hence there is no data in this area.

We found the increase in ankle and calf tissue depth at six weeks was also associated with an increase in Durometry, in contrast to healthy controls that displayed lower levels of tissue tension with increasing tissue depth. Whereas the controls have healthy, compliant and elastic tissues with supple subcutaneous tissues, the vascular patients typically have pitting oedema with 'water logged' skin, which is much less compliant and has evidently higher readings of tissue tension.

Interestingly, it was just the tissue depth of the foot compartment that correlated with Durometry readings. It is likely that smaller compartments become 'harder' because fluid accumulates within the fascial planes, which are more rigid in smaller areas and subject to increasing hardness with much more ease. This also had a relationship with patency ($p < 0.02$), where higher Durometry readings correlated with patency. It is difficult to identify a cut-off

value that identifies patent from non-patent, as anecdotally patients who had the most severe disease at the outset seemed to have tensor lower limb tissue compartments. Nevertheless, it may be interesting to prospectively look at whether foot Durometry could be predictive of successful revascularisation in the early post-operative period.

Six-Month Data

Unfortunately by six months, pressure indices and reactive hyperaemia had reduced to pre-intervention levels, although this did not correlate with a decline in patency and NSB index. The patients' symptoms had not deteriorated and therefore one can assume that the transient rise in pressure was sufficient to deal with the metabolic demands of the tissue. Beyond the immediate post-operative period there is no comparison in the literature on which to relate our finding of a return of pre-intervention time to peak.

The vasoconstrictive mechanisms had maintained some improvement, alongside resolving oedema. Even at six months this has still not returned to even pre-intervention levels. Eickhoff suggested that constrictive mechanisms should be present within 3-6mths following surgery in those where it was previously absent (Eickhoff and Engell, 1982a). Whilst we have not demonstrated normalization, there has been ongoing improvement in this time. The timings of improvement are contrary to Delis who demonstrated normalization of VAR within 6 days of surgery (Delis et al., 2001). They do not however, comment on pre-operative VAR to assess existing pre-operative function. Our findings do not show early return of VAR and hence is not in accordance with the theory that VAR is purely a physiological mechanism related to flow. Furthermore power in endothelial, sympathetic and myogenic frequencies have a declining pattern and do not show signs of improvement by this time.

The outcomes at this stage following treatment are not favorable towards the microcirculation regaining normal function.

Diabetes

Diabetic patients are commonplace in vascular clinics, carrying significant risk for lower limb amputation. Many studies in the literature exclude diabetic patients due to their complex microvasculature but we felt they were worth special consideration and separate analysis.

Diabetic feet are particularly prone to wounds from minor trauma and this was evidenced by the propensity to present with ulceration as opposed to rest pain (50% vs. 28%). Peripheral neuropathy (DPN) is present in over a quarter of patients with diabetes (Parry, 1999) and is a causal factor for ulceration. In some cases this may mean that there is relatively less macrovascular compromise to cause symptoms and signs consistent with CLI, but in this cohort the NSB was the same in both groups suggesting that the anatomical profile of disease was equally severe in each group. Neuropathy was clearly seen in the diabetic cohort with mean VPT and VDTs of approximately 30volts, with scores of >25v known to significantly increase the risk of developing an ulcer (Abbott et al., 1998, Young et al., 1994). In the non-diabetic group mean VPT and VDT were not significantly different from the diabetic group suggesting that ischaemic neuropathy plays a large role in these patients. Neuropathy scores also improved equally between groups with treatment suggesting that there is no sensory regeneration in diabetic neuropathy over and above that related to ischaemia. Equally, if there had been an improvement in autonomic neuropathy then you perhaps would have expected to see basal flux reduce significantly in the diabetic group, which did not occur. It does reduce at the toe from post-intervention, but no more so than in the non-diabetic group. It may be too

early at six-weeks to expect significant changes in neurological function. Toe two in the diabetics showed a persistently elevated basal flux compared to that in non-diabetics, perhaps consistent with systemic disturbance in microcirculatory flow. Typically the literature does not suggest there is any difference in resting basal flow between patients with diabetes and PVD (Forst et al., 1998).

The diabetic circulation cannot initiate hyperaemic responses to increasing metabolic requirements such as infection or necrosis, partly because they already have an over perfused skin circulation due to neuropathic changes. As a result there was a trend for a reduced reactive hyperaemia time to peak (20.1 ± 14.6 vs. 48.7 ± 9.0) but this was not significant, perhaps because of the small numbers involved. Jaffer found that the time to peak was significantly impaired compared to controls, but there was no comparison made following treatment. The coexistence of diabetes and peripheral vascular disease have not been shown to further impair reactive hyperaemia (Schramm et al., 2006), which is shown from the pre-intervention being similar, but following treatment an evident difference remains.

Sympathetic vasoconstriction or VAR is reported to be attenuated in diabetics. (Belcaro et al., 1992, Cacciatori et al., 1997) We found no significant differences in vasoconstriction, at any of the locations, although there was a tendency for diabetics to have an inferior myogenic response at the toe ($p=0.06$). Changes were not evident during sitting between groups either, suggesting that diabetes does not cause further deterioration in PVC over and above CLI. Flow increased by a mean of 23% from horizontal in the diabetic group. This is also consistent with failure for sympathetic and myogenic vasomotion to improve with treatment.

There is little data on diabetic microcirculatory changes following treatment, not least because they tend to be excluded from such studies. TcPO₂ is the most commonly used parameter that has identified changes after intervention and found that vasodilatory responses do not return to those of a healthy individual, suggesting persistent endothelial dysfunction. This conclusion is compatible with our findings, with a functioning graft or recanalised vessel not being sufficient. This is reflected by a further 3 limb amputations in the diabetic group compared to one in the non-diabetic group by 12 months.

Limitations

The natural history of CLI is distinct from the broader PAOD group and consequently it is important to consider them as separate entities for data comparison, although this can be challenging. Existing literature is often retrospective, comparing one single treatment and utilizes inconsistent definitions giving rise to broad cohorts, not specifically ‘critical’ patients. Certainly much of the work that has been used to compare the outcomes from this study has included data from non-critical ischaemia, which are not representative of outcomes for CLI.

In addition, it is paramount to delineate a causal link between symptoms and vascular status, but the interplay of patient co-morbidities can heavily influence vascular status, making inclusion/exclusion of critical status difficult. Whilst the BASIL trial has been a cornerstone in this field as the only RCT, it exemplifies the challenges of cohort definitions and trial designs. Given this spectrum of ‘CLI patients’, Wolfe attempted to further stratify critical ischaemia into high and low risk groups, essentially representative of what is clinically critical and severe. They found that only 5% of patients in the high-risk group survived with an intact limb unless they had a surgical intervention, compared to 27% in the lower risk group (Wolfe

and Wyatt, 1997). Whilst the outcomes are very poor in both there is a clear distinction and this is a very useful tool to improve sensitivity and specificity of outcome even within the umbrella term of CLI.

Recently, the Society for Vascular Surgery has undertaken an initiative to define set measures and outcomes from interventions ‘objective performance goals’ (OPG’s) to try and stratify future research and allow comparability to better appraise interventions and their outcomes. These have largely been based on regression models of available data for evaluation of non-randomised trials for comparison of new treatments (Goodney et al., 2011). Whilst this has not been widely applied, initial experience using the Vascular Study Group of New England (VSGNE) data to validate the approach has shown OPG’s to be representative of safety and efficacy outcomes. The 1039 lower extremity bypass operations for CLI, using autogenous vein, were divided into high clinical risk (age >80yrs and tissue loss) and high anatomical risk (infrapopliteal target artery) and conduit high risk (lack of single segment greater saphenous vein). The primary efficacy endpoint of freedom from any major adverse limb event or post-operative death within the first year was similar between the two datasets (77% OPG, vs. 74% VSGNE) suggesting that the OPG’s should be used as a standard format to compare outcomes from trials (Goodney et al., 2011). Whilst essential to improve methodology, large patient numbers will be needed in trials to adequately adjust for subgroup analysis.

Furthermore, there is no single outcome measure that defines the failure or success of a procedure and the timing and severity of representation will be different (Conte et al., 2009). Statistically, analysis of time-to-event of composite end points considers treatment efficacy based on the aggregate of multiple important outcome measures (Subherwal et al., 2012).

Whilst there are a number of advantages with this commonplace technique, Conte and colleagues suggested that it is not providing a comprehensive picture predominantly because only the initial event during the trial is considered for statistical analysis and does not assess the cumulative burden of multiple recurrences or procedures on the patient (Conte et al., 2009, Subherwal et al., 2012). An innovative approach is the 'global rank method' of analysis, which permits consideration and capture of all recurrent events rather than just the initial one, by using a hierarchical method of scoring the outcome events (Subherwal et al., 2012). By analysing the study outcomes in this way, the results should encompass a more comprehensive picture of both favorable and unfavorable outcomes.

Outside of generic CLI methodology difficulties, the greatest challenge with this study was the patient numbers, predominantly the disparity between numbers in each intervention group. Power calculations calculated that a sample size of 38 would provide approximately 95% power at the 5% significance level for the primary endpoint Tp reactive hyperaemia. Whilst total numbers were 40 this was further reduced on occasions if measured variables could not be obtained in all testing sessions, due to patient drop out in the event of amputation or mortality, particularly for repeated measures analysis. Whilst this has been highlighted appropriately throughout the study, it does make data analysis difficult and conclusions guarded. Greater than 50 patients appeared very achievable at the outset based on the work performed by the unit on an annual basis. However, there were a large number of patients particularly in the endovascular cohort that declined to take part. The main reason for this was not wanting to return to the hospital for repeat testing if this was not part of their routine clinical follow up, particularly as many of them already had extensive co-morbidities and poor mobility. Surgical patients were already inpatients during the post-operative phase and

were therefore happier to comply with the trial design. This adversely affected the number of patients in each group (open vs. endovascular) and hence they were not equally shared between the two treatment modalities.

The trial was an observational study. The BASIL study had already highlighted the challenges and complexities associated with an RCT, particularly in this field, and their study population was just 10% of the total number of critically ischaemic patients treated during this time as they were required to be in equipoise regarding the best treatment. This study could not have been further limited by smaller patient numbers and was designed to observe the changes that occurred in the microcirculation with different treatments in day-to-day vascular practice and not necessarily those that were in treatment equipoise.

The follow up from this study was six weeks, which was the routine follow up at this hospital following a surgical or endovascular treatment. A smaller cohort was followed up for six months with the aim of assessing longer-term outcomes. Ideally follow up would be for a minimum of one year and in the light of BASIL two years would have been preferable, but this was not feasible in the time allowed for the study.

Supplementing current clinical practice with assessment of the microcirculation would need to offer additional insight into patient outcomes. Based on the findings from this study, reactive hyperaemia would be the best measure to use as it offers an instant snapshot of the status of the limb with a quantitative Tp output. It also provides additional information through 'eyeballing' the shape of the curve, which is difficult to quantify but does appear to have some discriminatory value and needs further analysis in future work. The test itself is non-

invasive, quick to perform and practical in a clinical setting. It is much less time-consuming than other methods such as TcPO₂. It has been shown to be a valid assessment of the global status of the limb and changes are evident by the typical post-operative surgical review of six-weeks. Unlike some other microcirculatory measures such as baseline flux and FFT it gives a robust distinction and is not subject to significant overlap. Furthermore, measuring Durometry in the immediate post-operative period, particularly in the foot, appears to offer a quick, indirect measure of perfusion. Its association with patency requires further research to see if this could be a valid tool.

This study has prospectively assessed CLI patients at a single centre undergoing treatment for infrainguinal disease. It has clearly defined patient demographics and treatment approaches. It has highlighted shortcomings in the available literature with regards to the lack of microcirculatory outcome measures and direct comparisons between treatments. It has also used novel techniques of measuring oedema and reperfusion changes that can be quickly, cheaply and non-invasively applied to a clinical setting. It represents a useful analysis of microcirculatory changes, which can be applied to future work.

Word count: 47,664

APPENDIX A

Reperfusion as A Marker of Success Of distal Revascularisation (RAMSOR study)

Consent Form (Version 0.2, 5th January 2009)

Authorisation and Signatures

I, _____, have been invited to participate in a study (The RAMSOR study), looking at blood vessel reactivity and skin perfusion in patients who require treatment in the form of surgery or endovascular techniques for chronic critical limb ischaemia, under the direction of Mr Simms, in which I voluntarily consent to take part.

- a. The implications of my voluntary participation in this study, its nature, the duration, the purpose, the methods and means by which it is to be conducted, and the hazards which may be expected have all been explained to me by Miss Anna Nicolson. ☐
- b. I have read and understood all the written materials that have been provided to me describing this study and its potential risks and benefits. ☐
- c. I have been given an opportunity to ask any questions I wish concerning the procedures, and all such questions have been answered to my complete satisfaction. ☐
- d. I understand that my participation in this study is voluntary and that I am free to withdraw at any time, without giving reasons, and without my medical care or legal rights being affected. ☐
- e. I agree to allow authorised personnel to examine my medical records and understand that this will be done in the strictest confidence. ☐
- f. I agree to allow authorised personnel to inform my GP about my participation in this study. ☐

Date _____

Patient signature

Patient name (Printed)

Doctor signature

Doctor name (Printed)

APPENDIX B

Reperfusion as A Marker of Success Of distal Revascularisation: 'The RAMSOR study'

Pre-intervention Protocol (Version 0.4, 19th December 2008)

Date _____ ID _____ Patient
undergoing: Open surgery Endovascular

Inclusion and Exclusion Criteria

CCI fulfilling TASC criteria (Fontaine stage III/IV)	YES/NO
Iliac/femoral/popliteal/crural disease	YES/NO
Amputee	YES/NO
Evidence of chronic venous insufficiency on duplex	YES/NO
Clinical evidence of motor or sensory neuropathy	YES/NO

Patient Demographics

ID	–	Diabetic	YES/NO
D.O.B	–	Hypertensive	YES/NO
Gender	–	Hypercholesterolaemia	YES/NO
BMI (kg/m ²)	–	Smoker	YES/NO/EX
TASC Classification–		Tissue loss	YES/NO
Fontaine stage	–	Level of occlusion	I/F/P/C
Mean stenosis	–	Numerical index	

Room temperature (°C) _____ Resting heart rate (bpm) _____
NB Leg 1 = study limb Leg 2 = contralateral limb

Patient lying supine and rested.

Check patient can rest leg over edge of couch comfortably with minimal movement.

Anthropometries (lower limb volume & mean muscle mass):

Measurements (cm) using a standard tape measure in leg 1: L/R

- Forefoot circumference (1st–5th Metatarsals) _____
- Circumference 2.5cm above medial malleolus (MM) _____
- Circumference 5cm below tibial tuberosity (TT) _____
- Section length between MM & 5cm below TT _____

Measurements (cm) using a standard tape measure in **leg 2**: L/R

- Forefoot circumference (1st–5th Metatarsals) _____
- Circumference 2.5cm above medial malleolus (MM) _____
- Circumference 5cm below tibial tuberosity (TT) _____
- Section length between MM & 5cm below TT _____

Tissue Tension/Durometry (arbitrary comparative scale)

Hold perpendicular to skin. Average of four readings over soft tissue.

Tissue 'hardness' in **leg 1**:

- Dorsum of forefoot medial to EHL _____
- 2.5cm above and medial to medial malleolus _____
- 5cm below an medial to tibial tuberosity _____

Tissue 'hardness' **leg 2**:

- Dorsum of forefoot medial to EHL _____
- 2.5cm above and medial to medial malleolus _____
- 5cm below an medial to tibial tuberosity _____

Tissue depth using Sonosite 180 Ultrasound machine (cm)

Depth of tissue down to fascia in **leg 1**:

- Middle of dorsum of forefoot _____
- 2.5cm above medial malleolus _____
- 5cm below tibial tuberosity _____

Depth of tissue down to fascia in **leg 2**:

- Middle of dorsum of forefoot _____
- 2.5cm above medial malleolus _____
- 5cm below tibial tuberosity _____

Systolic brachial blood pressure (mmHg) _____

Absolute ankle pressure (mmHg)

Use the highest reading in each leg for ankle brachial index calculation

- **Leg 1** posterior tibial _____ dorsalis pedis _____
- **Leg 2** posterior tibial _____ dorsalis pedis _____

Ankle brachial index (ratio)

-**Leg 1** _____ -**Leg 2** _____

APPENDIX C

Reperfusion as A Marker of Success Of distal Revascularisation: 'The RAMSOR study'

Post-intervention Protocol (Version0.3, 6^h January 2009)

Date _____ ID _____ Patient
undergone: Open surgery Endovascular

Room temperature (centigrade) _____

Patient lying supine and rested. Check patient can rest leg over edge of couch comfortably with minimal movement.

Anthropometries (lower limb volume & mean muscle mass):

Measurements (cm) using a standard tape measure in **leg 1**: L/R

- Forefoot circumference (1st–5th Metatarsals) _____
- Circumference 2.5cm above medial malleolus (MM) _____
- Circumference 5cm below tibial tuberosity (TT) _____
- Section length between MM & 5cm below TT _____

Measurements (cm) using a standard tape measure in **leg 2**: L/R

- Forefoot circumference (1st–5th Metatarsals) _____
- Circumference 2.5cm above medial malleolus (MM) _____
- Circumference 5cm below tibial tuberosity (TT) _____
- Section length between MM & 5cm below TT _____

Tissue Tension/Durometry (arbitrary comparative scale)

Hold perpendicular to skin. Average of four readings over soft tissue.

Tissue 'hardness' in **leg 1**:

- Dorsum of forefoot medial to EHL _____
- 2.5cm above and medial to medial malleolus _____
- 5cm below an medial to tibial tuberosity _____

Tissue 'hardness' **leg 2**:

- Dorsum of forefoot medial to EHL _____
- 2.5cm above and medial to medial malleolus _____
- 5cm below an medial to tibial tuberosity _____

Tissue depth using Sonosite 180 Ultrasound machine (cm)

Depth of tissue down to fascia in **leg 1** :

- Middle of dorsum of forefoot _____
- 2.5cm above medial malleolus _____
- 5cm below tibial tuberosity _____

Depth of tissue down to fascia in **leg 2**:

- Middle of dorsum of forefoot _____
- 2.5cm above medial malleolus _____
- 5cm below tibial tuberosity _____

Systolic brachial blood pressure (mmHg) _____

Absolute toe pressure with vascular assist device (mmHg)

-Leg 1 _____ -Leg 2 _____

Toe brachial index (ratio)

-Leg 1 _____ -Leg 2 _____

Skin perfusion pressure with vascular assist device (mmHg)

- Leg 1 _____
- Leg 2 _____

Laser Doppler Fluxmetry (arbitrary comparative scale)

Ensure smooth surface and shave hair as necessary & attach probes with adhesive sticker and tape. Ensure probes are in correct channels.

Three probes on leg 1:

- Pulp of great toe (multisite probe)
- Middle of dorsum of forefoot
- 15cm below tibial tuberosity

One probe on leg 2:

- Pulp of great toe (multisite probe)

Patient lying supine and rested

Baseline reading 10 minutes

Save data for FFT analysis

Patient to stay lying supine

Baseline reading 2 minutes

Drop leg over the edge of the couch **Leg drop** – 4mins reading

Patient to sit upright with foot resting on level

Sitting – 4mins reading

VAR = (HBF-SBF)/HBF x 100

APPENDIX D

Reperfusion as A Marker of Success Of distal Revascularisation: ‘The RAMSOR study’

Six Weeks Post-intervention Protocol (Version 0.3, 6^h January 2009)

Date _____ ID _____

Patient undergone: Open surgery Endovascular

Room temperature (centigrade) _____

Patient lying supine and rested. Check patient can rest leg over edge of couch comfortably with minimal movement.

Anthropometries (lower limb volume & mean muscle mass):

Measurements (cm) using a standard tape measure in **leg 1**: L/R

- Forefoot circumference (1st–5th Metatarsals) _____
- Circumference 2.5cm above medial malleolus (MM) _____
- Circumference 5cm below tibial tuberosity (TT) _____
- Section length between MM & 5cm below TT _____

Measurements (cm) using a standard tape measure in **leg 2**: L/R

- Forefoot circumference (1st–5th Metatarsals) _____
- Circumference 2.5cm above medial malleolus (MM) _____
- Circumference 5cm below tibial tuberosity (TT) _____
- Section length between MM & 5cm below TT _____

Tissue Tension/Durometry (arbitrary comparative scale)

Hold perpendicular to skin. Average of four readings over soft tissue.

Tissue ‘hardness’ in **leg 1**:

- Dorsum of forefoot medial to EHL _____
- 2.5cm above and medial to medial malleolus _____
- 5cm below an medial to tibial tuberosity _____

Tissue ‘hardness’ **leg 2**:

- Dorsum of forefoot medial to EHL _____
- 2.5cm above and medial to medial malleolus _____
- 5cm below an medial to tibial tuberosity _____

Tissue depth using Sonosite 180 Ultrasound machine (cm)

Depth of tissue down to fascia in **leg 1**:

- Middle of dorsum of forefoot _____
- 2.5cm above medial malleolus _____
- 5cm below tibial tuberosity _____

Depth of tissue down to fascia in **leg 2**:

- Middle of dorsum of forefoot _____
- 2.5cm above medial malleolus _____
- 5cm below tibial tuberosity _____

Systolic brachial blood pressure (mmHg) _____

Absolute ankle pressure (mmHg)

Use the highest reading in each leg for ankle brachial index calculation

- Leg 1 posterior tibial _____ dorsalis pedis _____
- Leg 2 posterior tibial _____ dorsalis pedis _____

Ankle brachial index (ratio)

-Leg 1 _____ -Leg 2 _____

Absolute toe pressure with vascular assist device (mmHg)

-Leg 1 _____ -Leg 2 _____

Toe brachial index (ratio)

-Leg 1 _____ -Leg 2 _____

Skin perfusion pressure with vascular assist device (mmHg)

- Leg 1 _____
- Leg 2 _____

Laser Doppler Fluxmetry (arbitrary comparative scale)

Ensure smooth surface and shave hair as necessary & attach probes with adhesive sticker and tape. Ensure probes are in correct channels.

Three probes on leg 1:

- Pulp of great toe (multisite probe)
- Middle of dorsum of forefoot
- 15cm below tibial tuberosity

One probe on leg 2:

- Pulp of great toe (multisite probe)

Patient lying supine and rested

Baseline reading 10 minutes

Save data for FFT analysis

Patient to stay lying supine

Baseline reading 2 minutes

Drop leg over the edge of the couch **Leg drop – 4mins reading**

Patient to sit upright with foot resting on level

Sitting – 4mins reading

VAR = (HBF-SBF)/HBF x 100

Patient to lie supine and attach pneumatic cuff around ankle of leg 1

Baseline reading 2 minutes

Reactive hyperaemia (RH): 2 minute occlusion with pneumatic cuff around ankle, 50mmHg above systolic brachial pressure _____

Measured as the time to peak flux (sec) after release of cuff.

Attach pneumatic cuff around ankle of leg 2

Baseline reading 2 minutes and repeat RH in leg2

USS – Patent vessel/graft

YES/NO

Further intervention/surgery required

YES/NO

Symptom relief –

Complete

Partial

None

APPENDIX E


Reperfusion as A Marker of Success Of distal Revascularisation

The RAMSOR study (Version 0.4, 25th November 2009)

Researchers:

Miss A.M Nicolson	Vascular Research Fellow
Mr. M.H Simms	Consultant Vascular Surgeon
Dr. M.D Brown	Reader in Cardiovascular Physiology

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Introduction

Thank you for reading this leaflet. This hospital is undertaking a study to observe the changes in tissue perfusion (skin blood flow) and blood vessel reactivity, in patients with chronic critical ischaemia (CCI) of their lower limb (leg) and observe the effect treatment (surgical or x-ray guided method) has on these.

This is an invitation to patients such as you to consider joining this study. If there is anything you do not understand, or you have any other questions, your doctor or research investigator will be able to discuss this with you or you can contact us directly (see below for details).

Background Information

What is Chronic Critical Ischaemia?

CCI is a condition resulting from narrowing or blockage of the major leg vessels (arteries) that supply oxygen-rich blood to the legs. When this blood supply is severely reduced for a period of time, the tissues such as skin become ischaemic (oxygen starved) and can become gangrenous.

What are its symptoms?

Patients develop pain in their foot when they are resting, particularly in bed at night time. Ulcers and gangrene of the tissues can develop and do not heal because the blood supply is poor.

How is it treated?

Treatment is aimed at relieving the pain and healing the ulcers. In order to do this the blood supply in the legs needs to be improved ('Revascularisation'). Also factors that we know cause, and worsen this condition should be improved such as smoking, high cholesterol and high blood pressure.

What is Revascularisation?

Revascularisation refers to the process of improving the blood supply (flow) to the affected limb by either increasing flow in the native artery using endovascular techniques like angioplasty (balloon inflation to stretch the arteries) or surgically bypassing the blocked artery.

How do I know which Revascularisation Procedure is right for me?

The choice of procedure (surgery or endovascular) is determined on an individual basis depending on the type, size and position of the blockage/s in the artery and the general health of the patient. Your doctor will discuss your treatment options with you.

How does revascularisation affect blood flow in the skin and vessel reactivity?

By improving the blood flow in the big arteries in the leg, the blood flow in the skin of the foot and vessel reactivity should also improve and therefore reduce pain and improve ulcer healing. It is not clear from studies if the improvement in blood supply is directly linked to the extent of revascularisation.

The RAMSOR Study

What is the research study about?

This is an observational study of patients who require intervention (surgery or endovascular) to treat their CCI. It is looking at whether or not skin blood flow and vessel reactivity improve, and by how much, after treatment and comparing this to the type of blockage that you have in your blood vessels.

It is being performed at Selly Oak Hospital or Birmingham Heartlands Hospital where you will be having your treatment.

What will I have to do?

To carry out the study we need individuals like you, who are having a revascularisation procedure. We would require you to undergo measurements on three separate occasions during your treatment at hospital:

1. On the day before or the day of your treatment.
2. On the day after your treatment.
3. Six weeks after your treatment, at your routine clinic appointment.

The measurements will take approximately one hour and will be:

- *Blood pressure* in your arm, ankle and toe, using a pneumatic blood pressure cuff.
- *Limb circumference* using a tape measure.
- *Limb 'hardness'* using a gauge that rests on the skin.

- *Limb tissue depth* using an ultrasound (jelly) scan.

- *Skin blood flow* using a laser Doppler. This will involve putting four small sticky probes onto your legs, below your knee. This will be connected to a computer to allow measurements to be continuously visualised. The blood flow will be measured with you in the lying and sitting position. There will also be a blood pressure cuff inflated around the ankle for a two-minute period to measure vessel reactivity on visit 1 and 3. This may result in a feeling of fullness in the foot but should not cause pain. This test will not damage your blood flow.

All of the measurements will be taken whilst you are lying comfortably on a couch/bed, with the exception of part of the laser Doppler test where you will be required to sit for ten minutes.

There will be no tests requiring any physical exertion and no invasive tests such as blood taking will be performed.

What are the benefits?

Although this study may be of no personal benefit to you, we hope to gain a better understanding of what happens to patients' skin blood flow when they have a revascularisation procedure. We hope this will aid doctor's decision-making in future management of this condition.

What are the risks?

There are no adverse risks to taking part in this study. The only burden is the time taken to participate in the study.

What if I do not want to take part?

If you decide not to take part in the study this will not affect any treatment you will receive.

What happens to the information?

The identity of the volunteers in the study will be kept strictly confidential. Only after statistical analysis will we be able to say if we have identified differences between different groups of patients.

What happens at the end of the study?

We hope to publish the results in medical journals to enable other doctors to learn from our findings. This may help the treatment of others in the future. You will receive a copy of the result and your identity will be anonymous in these publications.

What happens if I have more questions or I do not understand something?

If you would like further information, please speak to one of the doctors or the independent person listed below.

What happens now if I decide to take part?

If you decide to take part, please let one of the doctors looking after you know. Alternatively, the lead researcher will contact you after you have had chance to read this information to see if you have any questions and if you are willing to take part. You will then be asked to sign a form to say you consent to taking part in the RADAR study.

What happens if I change my mind during the study?

You can withdraw from the study at any time and this will not affect your future management in any way. If you want to withdraw, please speak to one of the doctors listed below.

Your decision

You may want to think a little bit longer or discuss it with a relative before deciding whether or not you will take part in the study. If you decide to join we will inform your GP and send them information about the study.

Investigators

Miss Anna Nicolson

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Mr Malcolm Simms

Consultant Vascular Surgeon
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Tel: 0121 627 8534

Independent Person

**Patient Advice and Liason Service (PALS)
Officer**

Selly Oak Hospital



Heartlands Hospital



APPENDIX F

Vascular Department 'S block',
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B29 6JD

Dear Dr _____,

Reperfusion as A Marker of Success Of distal Revascularisation
(The RAMSOR study)

I am writing with regards to your patient _____, who has agreed to take part in the above named study, being conducted in the Vascular Department at Selly Oak Hospital.

It is an observational study of all patients undergoing treatment for chronic critical limb ischaemia of the lower limb, to assess tissue perfusion and vessel reactivity before and after treatment and see if this correlates with the anatomical extent of revascularisation. This will predominantly be measured using Laser Doppler studies (non-invasive measurements) on three separate occasions, over a six week period.

Your patient has been given information on the study and is happy to proceed. Their participation will not affect their treatment in any way.

If you would like any further information on the study please feel free to contact me.

Kind Regards,

Miss Anna Nicolson

(MBChB MRCSEd)

Vascular Research Fellow to Mr M. Simms

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