Short term Physiological Changes secondary to Exercise in Intermittent Claudication

Subtitle: Short Term Physiological Changes in Claudication

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Being a thesis submitted for the degree of Doctor of Medicine
University of Hull, University of York
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December 2013

❖ Abstract

Background: In patients with intermittent claudication (IC), supervised exercise programmes (SEP) improve walking distance and quality of life (QoL); however the mechanisms by which these benefits are achieved remain unclear.

Endothelial dysfunction is recognised as a trigger of the athero-inflammatory cascade and subsequent cardiovascular disease. In health, training improves cardiorespiratory physiology, inflammation and endothelial function. Changes in cardiorespiratory physiology, inflammatory markers and endothelial function are contradictory in IC.

Objectives: This thesis aimed to assess the impact of SEP on cardiopulmonary physiology, endothelial function and athero-inflammatory markers in patients with IC.

Methods: Following local research ethics committee and R & D approval, patients with IC were recruited from outpatient clinic. After providing informed written consent, patients underwent baseline assessment on two separate days.

Session 1: participants completed a constant load treadmill test with pre and post exercise ankle brachial pressure indices.

Session 2: measured QoL, endothelial function (EndoPAT2000, Itamar, Israel), venepuncture and a cardiopulmonary exercise test (CPET) using cycle ergometry. Participants then underwent a 12 week period of SEP which consisted of circuit training, with re-assessments at six and twelve weeks. The primary outcome measure was a 1.5ml/kg/min improvement in peak VO_2 after six weeks of exercise. Secondary outcomes included changes in endothelial function, quality of life, walking distance and inflammatory markers at both six and twelve weeks.

Results: No significant improvements in CPET measurements, endothelial function or inflammation were demonstrated at any time point. Traditional markers of walking ability and QoL demonstrated an improvement by 12 weeks.

Conclusions: The underlying mechanism through which exercise improves walking distance remains un-identified. Further work regarding the changes at the cellular level within the muscle is of importance.

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❖ **Acknowledgments**

I would like to thank the following people who have been instrumental in helping guide me through this MD; Professor Chetter, Patrick Coughlin, Leigh Madden and Dr Adrian Midgley. The time and energy that they have invested over these three years has been invaluable.

A special thanks is also included for those who have helped me to progress and develop not only this MD but future projects; Professor Naseem, Sean Bennet, Ian Scott, Ann Lowry, Rebecca Vince and Professor Carroll.

This MD would have not been possible without the help and advice from the therapies centre (Angela Green, Liz Minnich and Nicola Gilchrist), my colleagues with the exercise programme (Hashem Barakat, Tom Wallace, Lucy Green, Anna Firth, Emma Clarke, Junaid Khan, Dan Carradice, Panos Souroullas, Sandip Nandra, Joesph El-Sheika, Austin Smithies and Romesh Sarvandan.) and the continued support from the vascular consultants without which, the SEP could not have run (Professor Chetter, Mr Akomolafe, Mr Renwick, Mr Johnson, Mr Kapur and Professor McCollum).

In addition, I would like to thank Josie Hatfield for running through the treadmill testing protocols with me, and testing patients on my behalf. Jenny Bryce and Carole Tennison for assistance in identifying patients, Barbara McCloy, Debbie McDonald and Claire Acey for administrative assistance; and Daniel Carradice and Fayyaz Mazari for statistical assistance.

We have been fortunate to receive funding from the Yorkshire Vascular and Surgical Research Fund and the Centre for Cardiovascular and Metabolic Research in order to purchase essential equipment.

Finally, on a personal note, I would like to thank my parents, my brother Amit, my husband Tristan and my friends (Rachel, Anita, Chicken, Dan, Ian, Jude, Dan and Katie) for their help and support through the highs and lows associated with research.

❖ **Authors Declaration**

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

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

A motto that has remained of importance throughout the last five decades for patients with peripheral arterial disease is to …

"Stop smoking, keep walking"

(Housley 1988)

With the increasingly sedentary lifestyle that our ageing population is adopting, patients and colleagues should take up this edict alike.

1. INTRODUCTION

1.1. Overview

This thesis aims to provide insight into the underlying short term physiological changes which occur secondary to exercise training in patients with peripheral arterial disease; specifically claudication.

The first chapter of this thesis shall explore the PAD, its aetiology, risk factors, diagnosis and management. As part of management I shall endeavour to outline what is known about exercise within the role of claudication treatment. The second chapter, on exercise research shall outline how the concepts of frailty, quality of life and cardiopulmonary exercise have impacted on research and how this has shaped the methodology of this project.

1.2. Peripheral arterial disease (PAD)

Peripheral arterial disease (PAD) encompasses a broad spectrum of atherosclerotic disease; including aneurysms and stenoses affecting all non-coronary vessels e.g. lower limb stenoses (Hirsch 2006). When referring to the term PAD from this point forward, it will be confined to the spectrum of disease that affects the lower limb; asymptomatic, intermittent claudication, rest pain, ulceration or limb loss.

1.2.1. Background

Claudication is the main symptom of PAD and comes from the Latin claudicare; ‘to limp or be lame (Dumesnil 2011).’ The Roman Emperor Tiberius Nero Claudius Germanicus was born in 10BC and named Claudius due to his limp.

The medical term was coined in the 1831 by a French veterinarian after observations on the effect of repeated exercise in horses (Bollinger et al. 2000).

Vascular ‘intermittent’ claudication classically presents as muscular leg pain, precipitated by exercise and quickly relieved by rest. The distance walked prior

to the onset of claudication tends to be relatively constant and reproducible. At the onset of pain a visible change in an individual's gait is observed where after an antalgic gait predominates (King et al. 2012; Mockford et al. 2010)

The pain occurs due to muscle ischaemia secondary to inadequate blood flow. The distribution of the pain may indicate the arterial segment affected by PAD i.e. buttock, thigh and calf claudication suggests aorto-iliac disease, whereas isolated calf claudication suggests stenosis in the femoro-popliteal section (Widmer et al. 1964).

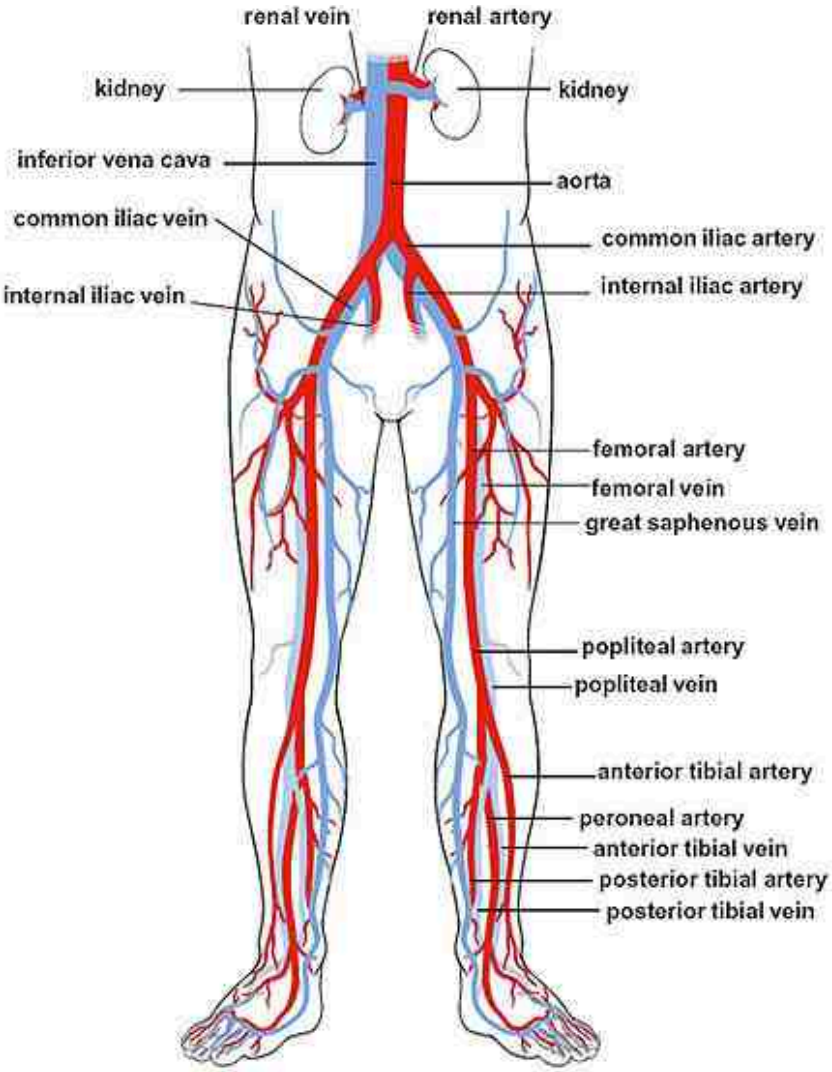

Figure 1 Arterial Circulation of the lower limb

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The reduction in blood flow is secondary to arteriosclerosis. This is a disease whereby fat and calcium deposit into the vessel wall resulting in narrowed lumen. (See Figure 2)

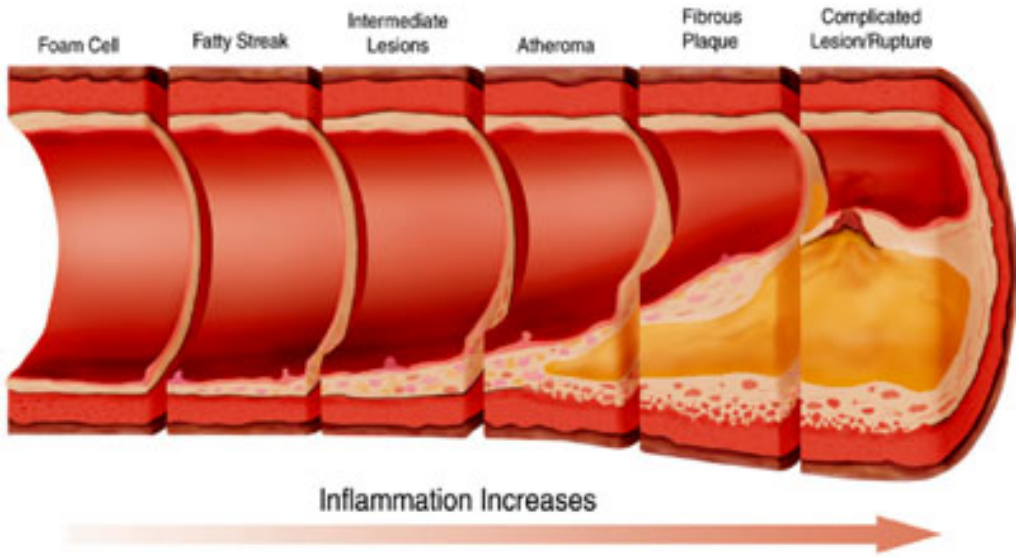

Figure 2 The internal narrowing of a vessel secondary to arteriosclerosis

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1.2.1.1. Incidence & Prevalence

Peripheral arterial disease involving one or more major vessels of the lower limb is common in the western world. The prevalence of PAD increases with age as evidenced by the National Health and Nutritional Examination Survey (NHANES). This US based survey from the Centre for Disease Control (CDC) reported the prevalence of PAD to be 2.5% in the 50-59 year group to 14.5% in the over 70 year group (Widmer et al. 1964; Selvin & Erlinger 2004; Criqui 2001) (See Figure 3). A difference between the genders has also been identified, with a male predisposition demonstrated. In those over 50 years of age, 2-7% of men and 1-2% of women are likely to suffer from IC (Criqui 2001). The ratio of men to women suffering from IC and PAD does however vary (Norgren et al. 2006; Widmer et al. 1964).

The natural incidence of PAD is difficult to assess as 10-50% of claudicants have often not consulted their GP, and a large proportion are asymptomatic. Screening studies have demonstrated that the background prevalence of symptomatic intermittent claudication is 4.5%, asymptomatic disease as defined by an abnormal ankle brachial pressure indices (ABPI<0.9) equated to a further 24.6% (Fowkes et al. 1991). This equates to a total of 29.1% of the population being positive for peripheral arterial disease. This was the same prevalence as the PARTNERS (PAD Awareness, Risk, and Treatment: New Resources for Survival) study (Hirsch et al. 2001). From these studies, the ratio of symptomatic to asymptomatic disease is approximately 1:3 or 4 and is independent of age.

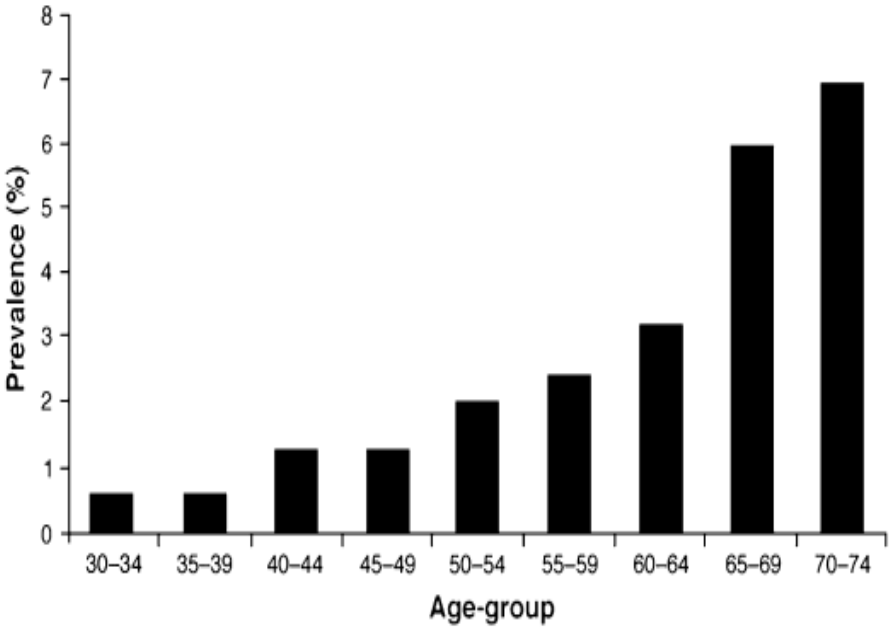

Figure 3 Prevalence of IC (symptomatic PAD) in large population based Studies

Reproduced from TASCII 2007 Elsevier with permission (Hirsch et al. 2006)

NHANES autopsies of unselected adults identified that 15% of men and 5% of women were asymptomatic with a greater than 50% stenosis of a lower limb artery (Selvin & Erlinger 2004). In addition, 20-30% were asymptomatic with a complete occlusion of at least one coronary. The morbidity (19%) and mortality (30%) associated with known peripheral arterial disease accounted for 34% of all circulatory deaths in 2010 (Department of Health 2010). As the prevalence of this condition (both symptomatic and asymptomatic) increases in our ageing population, this will result in a higher burden on the NHS and society due to reduced mobility, depression, and the increasingly complex medical demands of this elderly cohort of cardiovascular patients.

1.2.1.2. Pathophysiology

The pathophysiology of PAD is due to arteriosclerosis, which is defined as a hardening of the arterial wall and encompasses both atherosclerosis and other causes such as fibrosis i.e. arteriosclerosis obliterans. Atherosclerosis is the primary aetiology underlying development of PAD and is secondary to the deposition of lipids within the intima leading to hardening of the arterial wall.

The initiating factor in atherosclerosis is felt to be an insult to the vascular endothelium leading to inflammation and formation of a fatty streak (Kaperonis et al. 2006; Libby 2002; Libby 2012; Stary et al. 1995). Triggers for this insult include a high fat laden diet, which results in expression of both vascular cellular and intercellular adhesion molecules (VCAM-1 and ICAM-1) by the vascular intima. The expression of theses leukocyte attracting molecules occurs at branch points within the arteries, a place of altered laminar flow (Libby 2002). VCAM-1 and ICAM-1 attract monocytes and T-lymphocytes, which then penetrate the intima. The monocytes then initiate an inflammatory cascade, as macrophages they ingest lipid deposits resulting in foam cells. Necrosis of the foam cells leads to cellular debris, calcium deposition, and degradation of collagen, resulting in the formation of a ‘fibrolipid plaque’ (Paffen & Demaat 2006).

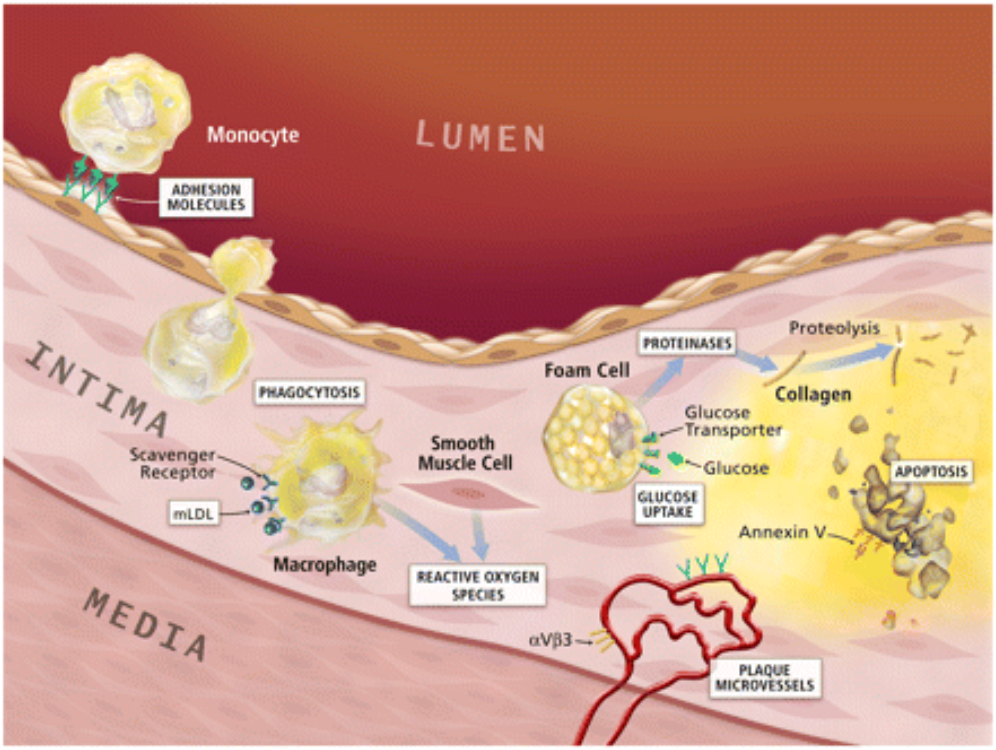

Figure 4 Diagram of Atherosclerotic Progression

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The arterial luminal diameter decreases with plaque progression, creating a stenosis, which alters the blood flow, resulting in turbulent rather than laminar flow. Once the stenosis exceeds 50-70% of the lumen diameter, impaired flow and reduced blood supply follow and clinically apparent symptoms occur (Gjønnæss et al. 2006).

Poiseuille's law explains the physics underlying the change in flow. This states that the vessel wall radius to the power of four (r^4) is indirectly proportional to the change in pressure along the length of that vessel (see Figure 5). I.e. the smaller the radius becomes, the larger the change in pressure drop across the vessel, assuming flow is constant (How 1996).

Therefore in the presence of a stenosis, the vessel radius decreases, increasing the pressure gradient across the stenotic region. Vessels may have more than one stenosis, and the impact on blood flow is additive when lesions are located sequentially within the same artery

Poiseuille's Law states that flow (Q) is related to the lumen's radius (r), length (L) of the vessel and viscosity (ṅ) of the fluid and pressure gradient along the vessel (P):

$$Q = \frac{\pi r^4 P}{8 L \dot{n}}$$

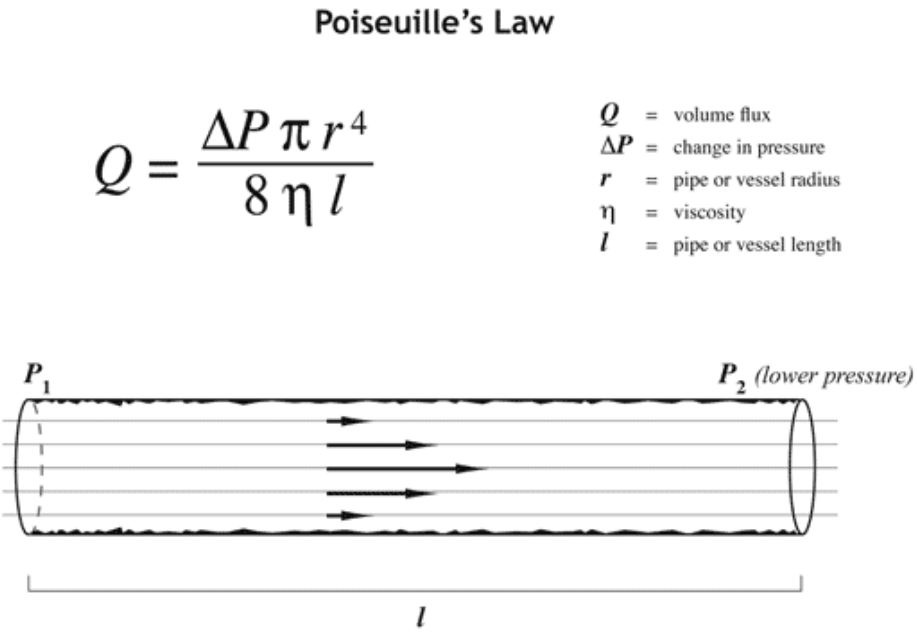


Figure 5 Diagram illustrating Poiseuille's Law

As the lumen narrows further, the pressure gradient increases proportional to the blood flow. The increased flow leads to turbulence and increased shear stress on the vessel wall, which can in turn result in plaque rupture and subsequent embolism. The sub-endothelium and lipid core is then exposed leading to thrombosis formation, and partial or complete acute arterial occlusion (Wheeler & Brenner 1995).

1.2.1.3. Endothelial dysfunction

The endothelium, cells lining vascular vessels, are the site at which initial dysfunction results in subsequent atherosclerosis (Schmieder 2006; Wight et al. 1997). This organ is the site at which numerous mechanisms occur; angiogenesis (formation of new blood vessels), vascular permeability, regulation of thermoregulation and blood pressure (vasodilation and vasoconstriction) and fluid balance (Yuan & Rigor 2010).

The main compound associated with endothelial dysfunction is nitric oxide (NO). A reduction in NO is often reported in the presence of dysfunction and may occur secondary to a lower activity of the enzyme endothelial NO synthase (eNOS) (Kojda et al. 2001; Vita & Hamburg 2010). As the eNOS changes its function from oxidative to reducing, this induces a change from NO production to that of pro-athero-inflammatory reactive oxygen species (ROS) (Galkina & Ley 2009; Kim et al. 2013).

An increased concentration of ROS has been associated with the following disease states; hypertension, hypercholesterolaemia, renal failure, diabetes, hyperhomocysteinaemia and ageing or premature menopause (Bonetti 2002; Deanfield et al. 2007; Virdis & Taddei 2012; Friedewald et al. 2008).

The ROS are associated with the uptake of lipids into macrophages and the initial formation of foam cells. This starts a cascade resulting in the atherosclerotic plaque forming, smooth muscle hypertrophy and collagen deposition into the vessel wall (Friedewald et al. 2008). Vascular endothelial

ROS may be identified from a variety of locations; NO synthases, a dysfunctional mitochondrial respiratory chain, platelets and lipoxygenases. Reactive oxygen species may be neutralised by anti-oxidants, however their production can exceed that of their neutralisation. The resultant concentrated ROS result in damage to DNA, lipids, membranes and proteins (Galkina & Ley 2009).

The ability to measure this phenomenon may allow early detection of a modifiable risk factor and reduce the subsequent cardiovascular morbidity and mortality associated with the dysfunction (Kojda & Hambrecht 2005; Friedewald et al. 2008).

1.2.2. Risk Factors for Peripheral Arterial Disease

Several risk factors are associated with an increased risk of atherosclerosis and development of peripheral arterial disease. A genetic predisposition and family history are important alongside environmental factors.

1.2.2.1. *Ethnicity*

Non-Caucasians are quoted to have a higher prevalence of PAD (Selvin & Erlinger 2004; Allison et al. 2006), especially distal arterial disease. In 2011, 55 million (87%) of the UK population were described as Caucasian (National Records of Scotland / Northern Ireland Statistics and Research Agency & Office for National Statistics 2013). The Office of National Statistics regional demographics for the population of Kingston upon Hull (see Figure **6**), identified that in 2011 the proportion of Caucasian residents was 94.0% (241,037) (Office of National Statistics 2011; Spicer 2013).

1.2.2.2. Population by broad ethnic group 2011

2011 Census: Ethnic group	All categories: Ethnic Group Persons Number	White	Asian / Asian British -Indian -Pakistani -Bangladeshi -Chinese -Other	Black / African / Caribbean / Black British	Mixed / Multiple Ethnic Groups
Yorkshire & the Humber	5,283,733	4,687,578	385,964	80,345	40,910
East Riding of Yorkshire	334,179	327,562	2,961	598	530
Kingston upon Hull, City of	256,406	241,037	6,471	2,996	2,164

Figure 6 Office of National Statistics Regional Demographics for Kingston upon Hull for ethnicity (Office of National Statistics 2011)

The values for Yorkshire and Humber are include the following separate locations: East Riding of Yorkshire, Kingston upon Hull (Humberside), North East Lincolnshire, North Lincolnshire, York, North Yorkshire, South Yorkshire and West Yorkshire.

1.2.2.3. Hypercholesterolaemia and hypertriglyceridemia

Hypercholesterolaemia is a well-known risk factor for the development of atherosclerosis and subsequent vascular disease. Current practice requires that all patients suspected of PAD are screened for dyslipidaemia and commenced on treatment appropriately (Hirsch et al. 2001; Hirsch 2006).

Statins are a well-established treatment for hypercholesterolaemia in PAD (Cassar, Coull, et al. 2003b), the assessment for hypercholesterolaemia is a mandatory part of any diabetic treatment regimen (Escobar et al. 2011). A 2003 randomised control trial (Collins et al. 2003) of the use of simvastatin in healthy individuals and diabetics (n= 5963) demonstrated a significant reduction in coronary mortality, non-fatal MI, stroke (CVA) or any major vascular event. The odds ratio for any vascular event was in favour of treatment with simvastatin in comparison to placebo (OR =0.76, 95% CI 0.72-0.81, $p<0.0001$). A recent meta-analysis of twenty-two trials to identify whether benefit was present in the use of statins for those with low levels of LDL demonstrated an improvement in stroke rate, major coronary event rate and overall rate of any major vascular event at five years (Mihaylova et al. 2012). Whilst evidence exists to support the early use of statins, questions remain over whether this should be widened to include everyone over the age of 50 (Ebrahim & Casas 2012).

Evidence exists from numerous studies supporting the use of statins for improvement of walking distance in patients with claudication and as a way of reducing all cause and cardiac mortality. The evidence behind each statin is further discussed in section 1.2.4.3.

1.2.2.4. Smoking

Smoking is associated with and attributed to be a causal agent leading to both atherosclerosis (Jonas et al. 1992; Tzoulaki et al. 2007) and carcinogenesis. Smoking is associated with endothelial dysfunction (Esen et al. 2004; Celermajer

et al. 1996; Celermajer et al. 1993) and increased inflammation, which are reversible on cessation of smoking (Bakhru & Erlinger 2005; Celermajer et al. 1993).

In the context of PAD; since 1911 smoking has been associated with a three-fold increase in the number of people with PAD compared to non-smokers (Erb 1911; Simoni et al. 1994). The relative risk for the development of PAD in current smokers compared to non smokers was 1.87 (95% CI 1.36–2.57) (Wattanakit et al. 2005).

A prospective longitudinal study of 1950 men in Japan demonstrated that ex-smokers had a reduced level of the inflammatory cytokine CRP (Ohsawa et al. 2005) when compared to current smokers. This was also supported by the NHANES III study which advocated a five-year period of abstinence to reduce CRP (Bakhru & Erlinger 2005). A case control study of male claudicants (n=102) and non claudicant controls (n=99) identified that ex smokers (RR = 7) were 7 times more likely than non smokers to develop PAD, and it was16 times more likely in current smokers when compared to non smokers ($p<0.001$) (Cole et al. 1993). The paper by Cole attributed 76% of subsequent PAD development on smoking. A five year period of abstinence was associated with a reduction in the risk of stroke back to the same level as in non-smokers (Jonas et al. 1992; Wolf et al. 1988).

1.2.2.5. *Diabetes mellitus*

Diabetes mellitus (DM) is associated with numerous aspects of the atherosclerotic pathway. Hyperglycaemia is associated with the production of advanced glycation end-products (AGE) and their receptors (RAGE). AGE in turn is associated with increased sequestration of NO resulting in endothelial dysfunction. AGE also induces ROS, interleukin-6 and other pro-inflammatory markers (Libby 2002). The combination of hyperglycaemia with endothelial dysfunction results in a marked micro and macrovascular disease (Huysman & Mathieu 2009).

The impact of poor glycaemic control can be demonstrated by the increased risk of PAD associated with it; a 1% elevation in $HbA1_c$ is associated with a 26% increase in developing PAD (Selvin et al. 2004).

The presence of DM is seen with more aggressive forms of PAD (National Diabetes Information Clearing house 2010); and associated with a hazard ratio of 2.20 (95% CI 1.52–3.18), $p<0.001$ for all cause mortality, in patients with CLI undergoing a second limb amputation (Abola et al. 2012). Awareness has been raised about the high proportion of amputations occurring in the diabetic PAD cohort (Moxey et al. 2011). The Quality Improvement Framework produced by the Vascular Society aims to improve the perioperative mortality of those with PAD requiring an amputation through the use of a multidisciplinary approach to care (Moxey et al. 2012). This has already been demonstrated to lead to a reduction in amputation rates in those with DM (Schaper et al. 2012; Krysa et al. 2012).

1.2.2.6. Hypertension

Hypertension (HTN) is associated with alterations of the arterial intima and functional alterations of the endothelium that are similar to the changes observed in hypercholesterolemia and established atherosclerosis (Wight et al. 1997). The pathophysiology of HTN is hypothesised to relate to the development of shear stress on already compromised arterial walls (Lahoz & Mostaza 2007). Endothelial dysfunction, a feature of hypertension, hyperlipidaemia, and atherosclerosis is known to contribute to a pro-thrombotic and athero-inflammatory state associated with the initiation of atherosclerosis (Deanfield et al. 2007). This is aggravated by further shear stress on turbulent flow within vessel walls leading to a positive feedback. This vicious cycle promotes further acceleration of atherosclerotic vascular disease and an increased incidence of clinical complications (Bonetti 2002) such as peripheral arterial disease.

1.2.2.7. Obesity

Adipose tissue is associated with insulin resistance and advanced glycation end-products (AGE) (Gaens et al. 2013). The receptors for AGE (RAGE) are associated with a higher degree of calcification (Wendt et al. 2006). AGE is associated with obesity, metabolic syndrome and hypertension, making it a risk factor for PAD. The increasing incidence of obesity within the UK (Spanier et al. 2006) and worldwide (Swinburn et al. 2011) along with the presence of AGE within processed foods, makes the combination of high importance in patients who develop PAD (Vlassara & Striker 2011).

1.2.2.8. Biomarkers

The risk factors above are well regarded within the literature and basic medical teaching. Further risk stratification of patients and use of haemo-rheological factors to predict prognosis and outcome have included the following markers listed below;

Inflammatory markers: C reactive protein (CRP) (Packard et al. 2000; Paffen & Demaat 2006; Cooke & Wilson 2010; Ridker 2003; Bo et al. 2008)

IL-6 (Fernandez-Real et al. 2001)

TNF alpha

Elevated fibrinogen levels (Paraskevas, Baker, et al. 2008a; Tzoulaki et al. 2007)

1.2.3. Diagnosis of Peripheral Arterial Disease

1.2.3.1. *Symptoms*

In humans, when the metabolic demands of the muscle exceed blood flow, claudication symptoms ensue. Symptoms include an ache, numbness, cold foot. Claudication symptoms alone lack sufficient sensitivity for the reliable diagnosis of PAD, as patients with asymptomatic PAD are clearly not identified. Differential diagnoses for claudication include spinal claudication, popliteal entrapment syndrome, diabetic neuropathy or alcoholic neuropathy (Norgren et al. 2006).

1.2.3.2. *Ankle Brachial Pressure Index (ABPI)*

In normal extremities with the effect of gravity eliminated, the mean blood pressure drop from the heart to the ankles is no more than a few millimetres of mercury. The measured systolic pressure distally in the feet is higher than that in the arm due to the reduction in vessel diameter (the change in pressure is proportional to radius^4). If the length of the vessel increases, the change in the radius does so to the power of four. As the flow and viscosity remain constant, there is little change in pressure across the length of the limb unless a stenosis is encountered. In an atherosclerotic limb, each stenotic arterial segment narrows the vessel lumen. The flow changes from laminar to turbulent resulting in a loss of energy and reduction in pressure. In addition, the elasticity of the artery is reduced, increasing resistance and reducing the radius of the artery over a short length. Therefore there is a reduction in the perfusion pressure experienced by distal muscle groups resulting in claudication symptoms (Klabunde 2001).

$$\text{Flow} = \frac{\text{change in pressure} \times r^4}{\text{Viscosity} \times \text{Length}}$$

The ratio of the systolic pressures measured in the arteries at the ankle (posterior tibial or dorsalis pedis arteries) and the brachial artery is termed the ankle brachial pressure index (ABPI). An ankle brachial pressure index is calculated by taking the blood pressure at both brachial arteries, and both posterior tibial (PT) and dorsalis pedal (DP) arteries. The highest brachial pressure reading is used as the denominator, and the highest of the either the DP or PT is used as the numerator to calculate the ABPI of that lower limb (Hirsch 2006). The same is repeated for the other side (see Figure 7).

A reduction in ankle blood pressure leading to a ratio of less than 0.9 is the haemodynamic definition of PAD (Rooke et al. 2012). This threshold value is associated with significant lower limb arterial stenosis, and may be used as a screening tool to identify those who are asymptomatic within the community (Hirsch et al. 2001).

The resting ankle blood pressure in an individual with peripheral arterial disease is generally less than that of a healthy person. Once physical activity starts, heart rate and blood pressure increase, however calcification of major vessels and loss of elastic recoil results in a reduction in flow to the affected lower limb and may result in symptoms.

In health the ABPI is expected to lie between 0.9 to 1.3 at rest and remains greater than 0.9 following exercise(Yao et al. 1969; Al-Qaisi et al. 2009). The ABPI is a sensitive and specific instrument to identify PAD. ABPI's were compared to colour duplex and identified to be most sensitive under the threshold of 0.6. Overall, ABPI had an 83% agreement with duplex (kappa 0.66). Post-exercise, this agreement reduced to 69% (kappa 0.37) (Allen et al. 1996). In comparison to arteriograms, ABPI has been demonstrated to have a 76% sensitivity for detecting arteriogram positive stenoses attributed to PAD when the ABPI is <0.9, and 90% specificity(Guo et al. 2008).

As both peripheral and cardiovascular disease are outcomes of the same pathology, it is of no surprise that an ABPI less than 0.90 is associated with a

three to six fold risk of cardiovascular mortality(Carman & Fernandez 2000; Ankle Brachial Index Collaboration 2008).

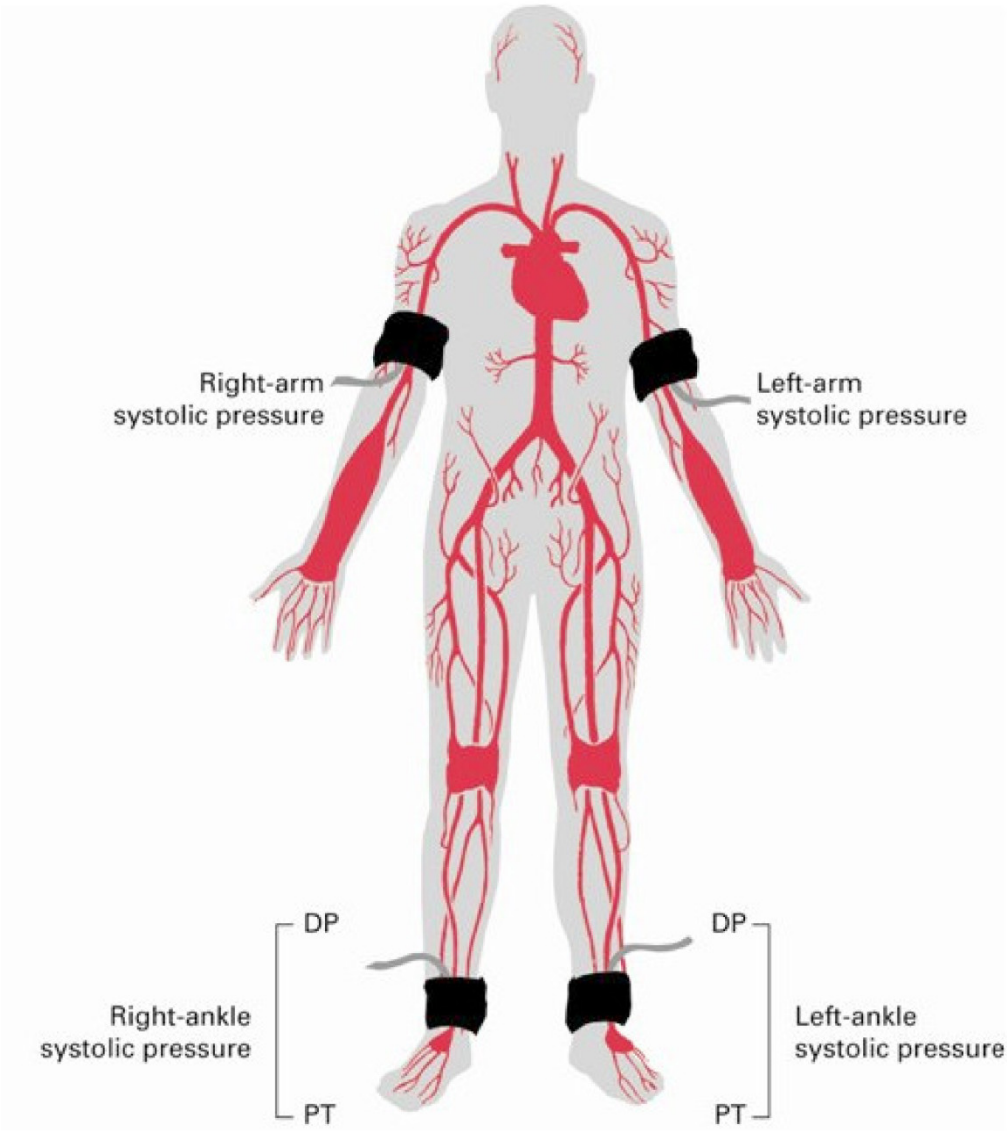


Figure 7 Diagram Illustrating the Calculation of the Ankle Brachial Pressure Index (ABPI)

1.2.3.3. Treadmill test

On occasions where resting ABPI's are normal, the use of stress testing may allow identification of a reduction in arterial flow secondary to exercise. Patients who do not demonstrate a decrease with exercise may be considered as highly unlikely to be suffering from claudication and other differential diagnoses may need to be explored.

Individual's undergo an ABPI pre and post a constant load treadmill test. The International Society of CardioVascular Surgery (ISCVS) recommends the following constant load protocol which is routine in our unit; 2.5km per hour, using a 10% grade until claudication pain occurs (or a maximum of 5 minutes) (Mazari, Carradice, et al. 2010a). A reduction in ABPI can then identify whether an individual is suffering from PAD or another lower limb pathology mimicking claudication.

Rutherford (See Table 1) has used this protocol to grade claudication severity, allowing standardisation of claudication severity worldwide (Rutherford et al. 1997). An alternative tool for classification of claudication is the Fontaine grade (see Table 2). This thesis will utilise the Rutherford grading system.

1.2.3.4. Imaging

Additional methods for diagnosis of lower limb arterial insufficiency include Duplex ultrasound, magnetic resonance angiogram (MRA), CT angiogram (CTA) or percutaneous transluminal angiography (PTA).

Duplex ultrasound is especially good for quantifying flow in the infra-inguinal region. Above the inguinal ligament, ultrasound is less effective at assessing for stenoses, therefore MRA or CTA are of benefit in these circumstances.

Table 1 Summary of Rutherford Grade categorisation of lower limb Peripheral Arterial Disease

Rutherford Grade	Clinical Changes
0	Asymptomatic
I	Mild Claudication: Able to complete five minutes in a constant load treadmill test
II	Moderate Claudication: Unable to complete five minutes on a constant load treadmill test. Ankle pressure greater than 50mmHg post exercise
III	Severe Claudication: Unable to complete five minutes on a constant load treadmill test. Ankle pressure less than 50mmHg post exercise
IV	Ischaemic Rest Pain
V	Tissue Loss: digits only
VI	Tissue Loss beyond digits

Table 2 Summary of Fontaine Grade categorisation of lower limb Peripheral Arterial Disease

Fontaine Grade	Clinical Changes
I	Asymptomatic
IIa IIb	Claudication pain > 200m Claudication pain <200m
III	Rest pain
IV	Tissue loss

More recently, graded load treadmill protocols have been used to determine walking time rather than distance (Brass et al. 2007). These prevent a ceiling effect as the individual walks until they fatigue with increasing levels of stress. However, an amalgamation of studies and meta-analyses of smaller claudication studies is limited by the numerous treadmill protocols used within research (Degischer et al. 2002). Only when standardized protocols are agreed, can different treadmill protocols be compared to assess the effects and efficacy of different therapies. The use of treadmill assessments in claudication has been controversial due to the associated learning curve, use of handrails (Gardner et al. 1991) and the option of other testing modalities such as the 6 minute walk which are deemed to be more realistic (Zwierska et al. 2004).

1.2.4. Management

Management of PAD is based on three important outcomes; symptom control, reducing the rate of disease progression through lifestyle modification [see section 1.2.4.2] and improvement of quality of life [see section1.6].

Symptom control:

The primary complaint of claudicants is pain resulting in a reduction in walking distance and speed, and subsequent loss of physical function, independence and embarrassment. Treadmill walking time or distance is used as a reproducible measure of symptom control and way to assess Rutherford grade improvement as per the International Society for Cardiovascular Surgery (ISCVS) criteria (Rutherford et al. 1997).

Lifestyle modification is required to reduce the progression on to subsequent cardiovascular morbidity and mortality. A low ABPI was associated with a three to four fold increased risk of having a cardiovascular event over the next ten years when compared to those with a normal ABPI (see Table 3). This data was identified from a meta-analysis of 16 population-based cohorts. It included

Table 3 Summary of Morbidity and Mortality associated with a low ABPI (Ankle Brachial Index Collaboration 2008)

	Hazard Ratio (95% CI)	
ABPI < 0.9 compared with ABPI between 1.11-1.40	Men	Women
All cause Mortality	3.33 (2.74-4.06)	2.71 (2.03-3.62)
Cardiovascular Mortality	4.21 (3.29- 5.39)	3.46 (2.36-5.08)
Major Coronary events	2.97 (2.33-3.78)	3.05 (2.25-4.15)

approximately 50,000 participants who had no prior history of coronary heart disease (Ankle Brachial Index Collaboration 2008).

The 10 year minor and major amputation rate was 10%, whilst 18% of individuals underwent a surgical revascularisation. In addition, they identified a decline in both ABPI (0.014/year) and walking distance (9.2yards/year) (Aquino et al. 2001).

Quality of life can be measured through the use of both generic and disease specific questionnaires; the Medical Outcomes Short Form 36 (SF- 36) (Ware & Sherbourne 1992) or the Kings College VascuQoL (de Vries et al. 2005) questionnaire respectively [see section 11.1]. Numerous disease specific questionnaires exist for claudication; walking impairment questionnaire (WIQ) (Regensteiner et al. 1996; Wullink et al. 2001; Nicolaï, Kruidenier, et al. 2009b), intermittent claudication questionnaire (ICQ) (Chong et al. 2002; Ketenci et al. 2009; Kirchberger et al. 2012), Claudication scale (Egberg et al. 2010) and VascuQoL. Our unit has continued to use the VascuQoL (Mehta et al. 2006; Mazari, Gulati, et al. 2010b; Mazari, Carradice, et al. 2010a).

The complete assessment of treatment outcomes in claudicants requires the use of both clinical (Cassar, Coull, et al. 2003b) and patient-based parameters (Chetter, Dolan, et al. 1997a; Chetter, Spark, et al. 1997b).

Current clinical strategies for improving claudication take one of four options, conservative management including (1) pharmacotherapy, (2) supervised (Watson et al. 2008; Bendermacher et al. 2006) or unsupervised exercise, (3) percutaneous transluminal angioplasty (PTA) (Nylænde et al. 2007; Hobbs &

Bradbury 2006) with or without stents and (4) finally surgery (Lensvelt et al. 2011; Nordanstig et al. 2011).

1.2.4.1. *Active Risk Factor Modification*

At 5 years after symptom onset, patients with intermittent claudication have a 10-15% mortality rate and 20% risk of a non-fatal heart attack or stroke (Hirsch 2006). Given this significant risk of concomitant cardiovascular morbidity and mortality in patients with intermittent claudication, they require optimal risk factor management for their atherosclerotic disease (Cassar, Coull, et al. 2003b; Cassar, Belch, et al. 2003a; Wilson et al. 2007). This includes diagnosis, dietary advice and management of co-morbidities (Hatfield et al. 2008) such as smoking cessation, hypertension, hyper-cholesterolaemia, diabetes mellitus, metabolic syndrome; and aggressive secondary prevention using antiplatelet medication and treatment with HMG-CoA reductase inhibitors (statins).

1.2.4.2. *Diet*

A diet restricted in saturated fat (Nestel et al. 2005), cholesterol and salt should occur in conjunction with pharmacotherapy (Giri et al. 2006).

1.2.4.3. *Statins; HMG-CoA reductase inhibitors*

Lowering serum cholesterol and treating hyper-lipidaemia has been associated with a reduction in mortality and coronary morbidity. The statin drug class lower serum total cholesterol, low density lipo-protein (LDL), triglyceride levels and elevate high density lipo-protein (HDL) levels. They work by inhibiting the rate-limiting step of cholesterol synthesis in the liver.

Large multi-centre randomised controlled trials of various statins have demonstrated an improvement in cardiac morbidity and mortality. What follows is a brief summary of these.

Simvastatin; The MRC/ BHF Heart Protection Study (n=14573) included adults with coronary disease; occlusive disease of non-coronary arteries; or treated hypertension and no diagnosis of diabetes and a separate group with diabetes (n=5963) to be randomised to either 40mg of simvastatin or placebo. In both the diabetic and non diabetic group, an improvement in outcome of first major vascular event was noted in all taking simvastatin when compared to placebo (OR 0.76; CI 0.72-0.81, $p<0.0001$). Five year data focused on the cohort with occlusive arterial disease, demonstrated that simvastatin had an absolute risk reduction of 62/1000 (s.e. 8, $p<0.0001$) when compared to placebo for proportion with first major vascular event (Heart Protection Study Collaborative Group 2003).

The Scandinavian Simvastatin Survival Study (4S) (n=4444) included men and women between the ages of 35 to 70 who had coronary heart disease, angina pectoris or previous myocardial infarction and serum cholesterol levels of 213-310 mg/dl (5.5-8.0 mmol/litre). They found a significant 29% relative risk reduction (RRR) in the overall mortality rate (8% died in the statin group vs 12% in the placebo group) and a 42% RRR in the cardiac mortality rate (5% vs 8.5%) after an average of 5.4 years of follow-up (Larsen 1995; Kjekshus & Pedersen 1995; Pedersen et al. 1998) in those on simvastatin. Follow up work also identified that the relative risk of developing new or worsening intermittent claudication was 0.62 (95% CI 0.44-0.88, $p=0.008$) in those on simvastatin (Pedersen et al. 1998).

Pravastatin; has been widely explored through The Cholesterol and Recurrent Events (CARE) study (Pfeffer et al. 1999) (n=4159), the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) trial (The Long-term Intervention

with Pravastatin in Ischaemic disease lipid study group 1998) (n=9014) and the West of Scotland Coronary Prevention Study (WOSCOPS) (Ford et al. 2007) (n=6595). The CARE and LIPID studies enrolled patients who had a previous myocardial infarction within the preceding 3 to 36 months who also had total cholesterol levels and fasting triglyceride level less than preset values. The values in each of these studies were similar. The WOSCOPS study enrolled participants who had abnormal lipid levels but who had not had a previous MI.

Overall, these studies found a 24-29% RRR in non-fatal MI and 30% reduction in cardiovascular death at approximately 5 years follow up.

Rosuvastatin; the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) randomised placebo controlled trial (Mora & Ridker 2006) demonstrated a lower cardiovascular mortality in healthy individuals with low to normal LDL cholesterol randomised to rosuvastatin. This study was terminated early due to the evidence of a significant reduction in cardiovascular morbidity and mortality (Ridker 2009) in the active treatment group. Hs-CRP levels, were significantly lower in the rosuvastatin group (Ridker et al. 2009).

Combined therapy;

Ezetimbe is a part of a class of chemicals known as azetidinones. It acts to lower cholesterol by blocking intestinal absorption of LDL and triglycerides, but increases the absorption of HDL (Patel 2004).

The combination of ezetimibe and statins have been demonstrated to be beneficial in reducing the inflammatory marker CRP in patients with primary hypercholesterolemia (Sager et al. 2003). The sequence of which treatment is instigated first, has affected plaque progression. When a statin was started prior

to ezetimibe, this was associated with plaque acceleration rather than reduced rate of growth (West, Anderson, Meyer, et al. 2011b; Taylor et al. 2012). If the patient was found to be statin naïve and commenced on both, the inclusion of ezetimibe did not have a detrimental effect.

Niacin, also known as nicotinic acid is a B3 vitamin. It is a water-soluble vitamin that is absorbed from the diet and excreted via the kidneys. Niacin has been demonstrated to improve HDL and lower both triglycerides and LDL. The exact mechanism underlying this reduction remains unclear (Ginsberg & Reyes-Soffer 2013).

The use of niacin in conjunction with statin therapy has been advocated in preference to the use of ezetimibe as niacin reduced mean carotid intima media thickness (IMT) (Villines et al. 2010). Further evidence is required to merit a change in management from ezetimibe to niacin(Nicholls 2012; Cybulska & Kłosiewicz Latoszek 2012). A recent RCT using a combination of triple therapy (ezetimibe, niacin and simvastatin) to mono-therapy (simvastatin 40mg) also failed to demonstrate any significant improvement in SFA lumen at 24 months. At 12 months, a reduction in total cholesterol and HDL were observed in the triple therapy group in comparison to the mono-therapy group (Brunner et al. 2013). The use of niacin extended release has recently been deemed to have significant side effects including worsening of diabetes, increased likelihood of diabetes, gastrointestinal complications and bleeding. These side effects have closed the HPS2-THRIVE study early (HPS-THRIVE 2013).

1.2.4.4. Anti-hypertensives

Anti-hypertensives are used as recommended by NICE and the British Hypertension Society (BHS) by General Practitioners (Williams et al. 2004). Current guidance recommends the use of a calcium channel blocker as first line treatment in the over 55 year of age population, and an angiotensin converting enzyme inhibitors (ACE-I) in those under 55 (McCormack et al. 2012).

The recommendation of one type of antihypertensive over others in the management of peripheral arterial disease has not yet occurred (Lane & Lip 2013; McManus et al. 2012). There is growing evidence that ACE-I are implicated in endothelial dysfunction (Kim et al. 2013; Chen et al. 2002) and may have a role in modulating this (Wilmink et al. 1999), therefore the use of ACE-I early in the treatment of PAD may occur as evidence grows (Shahin et al. 2013; Ahimastos et al. 2013; Shahin et al. 2011).

Angiotensin II is a vasoconstrictor that can also instigate intimal inflammation. It induces this through increasing expression of VCAM, IL-6 and other pro-inflammatory cytokines, leading to the production of superoxide anions and smooth muscle cells (Libby 2002). Angiotensin II receptor inhibition is associated with a dose dependent reduction in IL-6 and nF kappa B (Rompe et al. 2010). The use of angiotensin converting enzyme inhibitors has been associated with a reduced relative risk of all cause mortality (5% p=0.032) and cardiovascular mortality (7%, p=0.018) (Ferrari & Boersma 2013).

Beta blockers have been previously prioritised in those with cardiac failure (Thompson 2013), or a recent myocardial infarction, however they have not been advocated as the first line anti-hypertensive in patients with peripheral arterial disease. A previous Cochrane review (Paravastu et al. 2008) has been performed to investigate any detrimental association between the use of beta blockers and walking distance in patients with intermittent claudication. The review identified no association between the two and advocated the use of beta blockers with caution until data from large trials is available.

1.2.4.5. Anti-platelet therapy

Cochrane reviews of antiplatelet therapy identified them to reduce all cause and cardiovascular mortality when compared to placebo (Wong et al. 2011). The optimal antiplatelet was not identified, but a 20% resistance to aspirin has

previously been identified (Karnabatidis et al. 2013). An improved outcome was identified with combined therapy or single therapy such as clopidgrel, picotamide or dypyridamole instead of aspirin (Wong et al. 2011; Jagroop et al. 2004; Robless et al. 2001).

1.2.4.6. Smoking

Smoking cessation has been deemed a highly beneficial treatment strategy in the progression of PAD (Simoni et al. 1994). The use of nicotine replacement therapy, bupropion or varenicline and targeted discussions with patients has made this a key area in the active management of risk factors in patients with PAD (Lu & Creager 2004; Assadian et al. 2006). The role of smoking cessation is to ensure abstinence for five years to allow full benefits to be gained. Both the general practitioner in combination with the vascular surgeon need to encourage uptake of smoking cessation services (Ferguson et al. 2012; Carpenter et al. 2011; Ruff 2010). Current guidelines recommend that smokers or ex smokers should be asked about their tobacco usage at every visit and be counselled or referred for smoking cessation at each possible visit. All smokers should be advised to stop and consider the treatment options available (Anderson et al. 2013).

Unfortunately uptake of services is mixed and continued abstinence past six months or one year is also variable. It is felt that a continued effort from the medical profession to ask and encourage abstinence at each encounter will aid patients to quit. Frustratingly, patients suffering from PAD who are abstinent for more than five years, have no improvement in their quality of life (Hoogwegt et al. 2010).

1.2.4.7. Diabetes

A ten-year study of newly diagnosed type two diabetics was conducted to identify the effect of early treatment with metformin or other oral anti-glycaemic

agents in comparison to dietary control (Holman et al. 2008). This study did not demonstrate a significant relative risk reduction of either stroke or peripheral arterial disease between the two groups. In contrast, the Atherosclerosis Risk In Communities (ARIC) study demonstrated that the presence of diabetes increased the relative risk of developing PAD to 1.87 (95% CI 1.36-2.57; when adjusted for age, gender and ethnicity) (Wattanakit et al. 2005).

It remains widely advocated that good glycaemic control is of relevance to the progression of PAD as diabetes is implicated as a risk factor. An increase in HbA1C of 1% is associated with a 28% increase in the incidence of PAD (Adler et al. 2002). In those with established PAD and concomitant DM, there are no specific recommendations for the best medical management of DM (Jude et al. 2009; Papazafiropoulou et al. 2010) other than to ensure tight control between of blood sugars between 4.0 to 7.0mmol/l (National Diabetes Information Clearing house 2010; The Diabetes Control and Complications Trial Research Group 1993). The use of statins within the diabetic cohort has been advocated as this has been demonstrated to reduce cardiovascular risk (Goldberg et al. 2009; Goldberg 2009).

1.2.5. Exercise and Claudication

NICE guidelines propose exercise should be the primary treatment for all patients with claudication (NICE 2012). Evidence clearly demonstrates that a supervised exercise programme (SEP) is superior to best medical therapy and unsupervised exercise in the treatment of claudication (Wind & Koelemay 2007). A Cochrane Review from 2008 (Watson et al. 2008), has shown SEP to increase walking distances, while an earlier meta-analysis has suggested that the most effective SEPs involve exercising to the point of maximal claudication pain (pain threshold), with sessions lasting longer than 30 minutes, three times per week for at least 3 months (Gardner & Poehlman 1995). The mechanism by which exercise improves walking distances remains unknown.

Intermittent claudication has significant deleterious effects on quality of life (QoL) (Chetter, Dolan, et al. 1997a; Mazari, Carradice, et al. 2010a), lower limb function (McDermott, Liu, et al. 2004b) and mobility (McDermott et al. 2007). The poorer baseline functional performance seen in patients with PAD is mirrored by significantly lower activity levels during daily life; which also predicts increased long term mobility loss (McDermott et al. 2007). PAD patients with higher physical activity have reduced mortality and cardiovascular events compared with PAD patients with the lowest physical activity, independent of confounders (McDermott, Tian, et al. 2008c).

TASC and meta-analyses (Wind & Koelemay 2007; Watson et al. 2008; Gardner & Poehlman 1995) advocate walking based programmes for treatment of claudication. A recent systematic review (Parmenter, Raymond, Dinnen, et al. 2011b) of different types of SEP for claudicants concluded that all forms of SEP are beneficial, irrespective of modality.

An exercise hierarchy has been suggested and as follows (Parmenter, Raymond, Dinnen, et al. 2011b);

supervised walking exercise of any intensity

unsupervised walking exercise

all other exercise regimens were seen as third choice.

Improvements in maximum walking distance (MWD) of between 33-151% have been observed with non-walking based SEPs. The improvements are more modest but have been identified from a variety of different programmes e.g. lower extremity aerobic exercise (includes strength, power and aerobic exercise for the lower limbs has improved MWD by 122%); upper and lower body training (MWD 122%) and interestingly arm cycling has demonstrated a modest but significant improvement in MWD of between 33 to 43%.

1.2.5.1. Supervision versus unsupervised exercise programmes

Cochrane and systematic reviews (Watson et al. 2008; Wind & Koelemay 2007; Bendermacher et al. 2006) have produced Level 1/A evidence of the efficacy of exercise for PAD. The question of supervision in this group remains controversial. The American College of Cardiology (ACC) and the American Heart Association (AHA) currently support supervised classes for PAD and cardiac rehabilitation. In 2006, the Cochrane review of supervised versus unsupervised exercise programmes (Bendermacher et al. 2006) demonstrated an increase in walking distances of greater than 150m in those who were supervised.

Gardner *et al's* (Gardner et al. 2011) RCT on standard walking advice, home based exercise or a supervised class found no significant difference between the groups with regards to energy expenditure, number of strides or compliance. The difference between the groups (length of time exercising and cadence) can be attributed to shorter higher intensity training in the class compared to a lower intensity when patients are left unsupported. This RCT of 92 participants (Gardner 2011) expanded on previous work (n=304) (Nicolaï, Teijink, et al. 2010b) which suggested that adequate supervision doesn't need to be hospital based. These two RCTs constitute the only level 1 evidence available to support the use of non-hospital supported programmes.

1.2.5.2. Exercise versus Percutaneous transluminal angioplasty

Whilst exercise is deemed to be the primary modality for treatment of claudication, invasive treatments are also utilised. Stenting and angioplasty of aorto-iliac disease is a well-established treatment, however infra-inguinal disease remains a more difficult disease to treat due to recurrence and disease progression. Two year primary patency rates for a primary percutaneous intervention were 30-58% although secondary patency rates were 64-72% (Cheng et al. 2001; Gordon et al. 2001; Pozzi et al. 2003).

The debate continues as to how well the two modalities compare in PAD.

Comparison of SEP and PTA in the management of claudication secondary to infra-inguinal PAD was first addressed by two trials, one from Oxford (Perkins et al. 1996) the other from Edinburgh (Whyman et al. 1997; Whyman et al. 1996). Unfortunately the results from these two trials are not directly comparable as they studied different interventions. The Edinburgh study group compared angioplasty and medical treatment to medical treatment alone. Medical treatment comprised of risk factor modification; smoking cessation, consumption of a 75mg aspirin daily and exercise advice. At six months, the combination group had a significantly better resolution of symptoms, fewer stenoses and better walking distances when compared to the medical treatment group. At two years, the disparity between the two groups has reduced with no significant difference in walking distances or quality of life being present.

The Oxford study group compared PTA to SEP (twice weekly for six months). The Oxford study, found a greater improvement in both median and maximum walking distances with SEP at 15 months in both the SFA and proximal iliac artery disease distributions when compared to angioplasty alone.

Subsequent RCTs directly comparing SEP to PTA have found recruitment to be problematic. Two large multi-centre trials, the mild to moderate intermittent claudication study (MIMIC) (Greenhalgh et al. 2008) and the exercise versus angioplasty in claudication trial (EXaCT) (Hobbs & Bradbury 2006), have either been underpowered or had to terminate the trial prematurely due to failure to recruit to time or target.

The Oslo Balloon Angioplasty versus conservative treatment (OBACT) investigators who studied both aorto-iliac and infra-inguinal disease, identified a significant improvement in walking distances ($p<0.01$) with PTA and best medical therapy which included exercise, compared to best medical therapy and exercise alone(Nylænde et al. 2007). The MIMIC trial, although underpowered, identified a significant improvement in MWD of 38% (when adjusting for baseline variables) with PTA and SEP versus SEP alone (Hazard ratio 1.38; CI

1.01-1.90, p=0.04) (Greenhalgh et al. 2008). Spronk *et al* randomised patients with aorto-iliac and infra-inguinal disease to either PTA or SEP in a trial powered to detect quality of life (QoL) improvement (Spronk et al. 2009). At 6 and 12 months post intervention, no difference was found between the groups for walking distances or QoL. Mazari *et al* randomised claudicants with infra-inguinal disease to one of three groups: SEP, PTA or SEP and PTA. At one and three months, the SEP and PTA group demonstrated statistically significant improvements in walking distance over the PTA alone group (Mann Whitney U; $p<0.05$) (Mazari, Gulati, et al. 2010b).

The CLEVER trial (Murphy et al. 2009), a multi-centre three armed RCT, compared 1) optimal medical care (with a home exercise advice, diet advice and cilostazol) to 2) SEP and optimal medical care three times a week with a 12 months behavioral input and finally 3) aorto-iliac stent placement and optimal medical care. The allocation of patients was asymmetric with 20% being randomised to home exercise, and 40% allocated to the other two groups. A fourth group combining angioplasty and SEP was discontinued due to slow recruitment and the need to increase numbers recruited to meet the primary outcomes.

In summary, in the treatment of claudication due to infra-inguinal disease, SEP and PTA seem equally efficacious and perhaps the benefit is greatest when two are combined (Frans et al. 2011). In aorto-iliac disease, an improvement in community walking, peak walking time, claudication onset time and quality of life at six months in both SEP and PTA groups when compared to walking advice.

1.2.6. Inflammation

Inflammation is a key stepping stone between the initial endothelial dysfunction (Brevetti et al. 2003), subsequent atherosclerosis and cardiovascular disease. Without this systemic inflammatory response to a local area of endothelial

dysfunction (Brevetti et al. 2008), the subsequent atherosclerosis could remain as a local isolated phenomenon. As atherosclerosis affects micro and macro vasculature, this results in varying manifestations of both cardiac and peripheral vascular disease; angina, claudication, limb loss, renal failure, hypertension, stroke and so on.

Tissue ischaemia reperfusion injury remains a common pathway in exercise, which mediates remodelling of the muscle tissue (Walsh, Gleeson, Shephard, et al. 2011b; Walsh, Gleeson, Pyne, et al. 2011a) in both health and disease (Hansson et al. 2002). In intermittent claudication, it is thought that the remodelling of striated muscle fibres occurs in response to the cycle of ischaemia and reperfusion (Shek & Shephard 1998).

A number of inflammatory mediators from the re-perfused tissue lead to a systemic inflammatory response. Research into inflammation has implicated the following inflammatory markers in the progression of atherosclerosis (McDermott, Guralnik, et al. 2004a; Libby 2002; Hansson et al. 2002; Nylænde et al. 2006) in peripheral arterial disease: C reactive protein (CRP), interleukin 6 (IL-6), urinary N acetyl beta D glucosamide (NAG), micro-albuminuria.

1.2.6.1. C-Reactive protein (CRP)

Background

C reactive protein was first described eighty years ago (Tillett & Francis 1930). As a pentraxin, it has a calcium dependent binding molecule. CRP is an immune response protein and a non-specific inflammatory marker. It is comprised of five 23kDa subunits (Inforzato et al. 2012). CRP is produced by the liver in response to elevated levels of the protein interleukin-6. CRP tends to elevate after an inflammatory event, and is often detectable 6-8 hours after the onset, but takes up to 48 hours to peak (Pepys & Hirschfield 2003).

Atherosclerosis

CRP induces cell adhesion molecule expression and mediates the uptake of LDL into endothelial bound macrophages (Zwaka et al. 2001). Its presence in atherosclerotic lesions occurs as a diffuse layer in the intima, but nowhere else within the vascular endothelium. This demonstrates that CRP plays a part within the atherosclerotic cascade at both the level of the foam cell and in the fibro-elastic layer of the intima ((Ridker 2003; Torzewski et al. 1998) see Figure 8.) Normal CRP levels are quoted to be less than 10mg/l with a median of 0.8mg/l (Pepys & Hirschfield 2003), elevated levels have been associated with arterial disease (Paraskevas, Bessias, et al. 2008b; Ridker et al. 2010) (carotid atherosclerosis, intermittent claudication and critical limb ischaemia) and as a prognostic indicator for the development of diabetes.

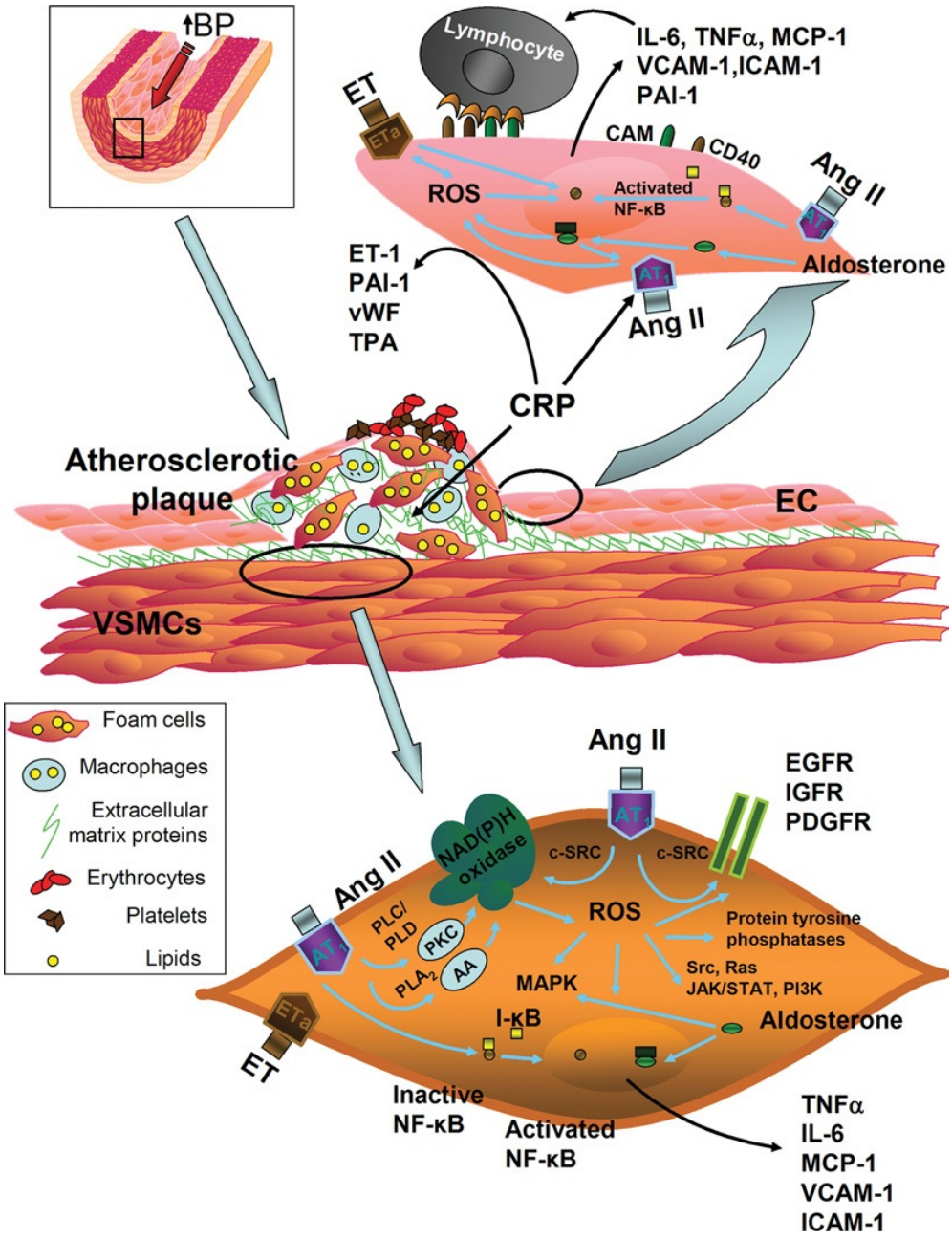


Figure 8 Scheme of C-reactive protein-induced inflammation

Abbreviations: Ang II, angiotensin II; AT1R, angiotensin type 1 receptor; BP, blood pressure; CRP, C-reactive protein; ET-1, endothelin-1; ICAM, intercellular adhesion molecule; NAD(P)H oxidase, nicotinamide adenine dinucleotide phosphate oxidase; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; TPA, tissue plasminogen activator; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor; (-), reduction. Reproduced with permission (Savoia & Schiffrin 2007)

CRP and CVS disease

CRP reflects systemic inflammation (Pepys & Hirschfield 2003). Chronic inflammation plays a role in the pathogenesis and progression of atherosclerosis and CRP may be used in conjunction with other markers to predict future risk of disease progression, morbidity and mortality (Owens et al. 2012; Urbonaviciene et al. 2012). Interventions such as statins (Coppola & Novo 2007) or smoking cessation (Bakhru & Erlinger 2005), which modify cardiovascular risk, have also been demonstrated to reduce CRP.

Baseline CRP levels add to the predictive value of lipid parameters in determining the risk of vascular events in apparently healthy men and women without a history of coronary heart disease (Ridker et al. 2010; Ridker & Glynn 2010). In combination with LDL levels, the Justification for use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER) trial placed those with a CRP > 5.4mg/l as high risk for future events.

A cohort study in patients with established PAD and four year follow up quoted the mean CRP of survivors (4.96mg/l, s.e =+/-0.49mg.l) was significantly lower than that of decedents (7.65mg/l, s.e.=0.98mg/l; p=0.015) (Vidula et al. 2008). A 50% CRP increase from baseline has been shown to have an increased risk of both cardiovascular (HR 1.17 (CI 1.05-1.30) p=0.003) and all-cause mortality (HR 1.14 (CI 1.05-1.24) p=0.003) at four year follow up. All-cause mortality was adjusted for age, sex, diabetes mellitus, smoking, ABPI and number of cardiovascular diseases (Vidula et al. 2008).

A review from 2008 (Paraskevas, Bessias, et al. 2008b) demonstrated an elevated CRP was associated with an increased likelihood of vascular disease and vascular events. CRP has been linked to cardiac, peripheral and carotid atherosclerosis. The level of CRP at baseline has been associated with the risk of progression for carotid atherosclerosis in the subsequent median 7.5 months (Schillinger 2005). The ICARAS trial was a prospective study of asymptomatic patients to screen for carotid flow and disease burden using carotid duplex

ultrasound for quantification of this. The study demonstrated that a CRP > 0.38mg/dl was associated with an increased three fold increased risk of developing carotid atherosclerosis (OR≥ 3.32 (95%CI 1.49-7.39) (Schillinger 2005)). This is supported by the prospective Rotterdam cohort study (van der Meer 2002), which demonstrated a significant linear association between CRP and intima media thickness (IMT) before (β= 0.021, 95% CI 0.013 to 0.029) and after (β= 0.018, 95%CI 0.010 to 0.027) adjustment for risk factors (BMI and diabetes mellitus).

The role of CRP remains contentious as adjustment for other cardiovascular risk factors can remove this variable as a dependent variable in regression models, as demonstrated by Bo *et al's* (Bo et al. 2008). They undertook a single centre prospective observational study to assess how CRP levels were associated with other markers of subclinical atherosclerosis. Participants were sub-stratified by their CRP values (<1.04mg/l, 1.05-1.97mg/l, 1.98-3.40mg/l, >3.40mg/l) and a significant difference was identified throughout all baseline variables: gender (Chi-squared, p=0.002), hypertension (Chi-squared, p<0.001), diabetes (Chi-squared, p=0.010), age (Kruskal Wallis, p<0.001), BMI (Kruskal Wallis, p<0.001), IMT (Kruskal Wallis, p=0.022), ABPI (Kruskal Wallis, p<0.001), total (Kruskal Wallis, p=0.018), and HDL cholesterol levels (Kruskal Wallis, p=0.005), creatinine levels and 10 year cardiovascular risk as calculated through the cuore risk assessment tool (Kruskal Wallis, p<0.001) (Bo et al. 2008). Whilst univariate analysis was of significance, they did not follow up their patients and therefore risk of subsequent events was not established.

hsCRP and intermittent claudication

Numerous studies have associated the level of CRP with an increased progression of atherosclerosis, worsening severity of PAD (van der Meer 2002; Cassar et al. 2005; Schillinger 2005) but not greater walking disability as measured by the walking impairment questionnaire (McDermott, Guralnik, et al. 2004a). Casssar *et al* (Cassar et al. 2005) were able to demonstrate that the CRP

levels not only increased between health and claudication, but elevated further in patients with critical limb ischaemia.

Nylaende *et al's* (Nylænde et al. 2006) study demonstrated no significant correlation (Spearman's rank) between CRP and ABPI ($r=-0.023$, $p>0.05$), angiographic score ($r=0.084$, $p<0.05$) or maximum treadmill walking distance ($r=-0.108$, $p>0.05$), this was also confirmed on multiple regression analysis.

The associations between CRP and vascular disease, namely claudication has led to the measurement of serial levels of this biomarker within our study. We felt that this would allow us to compare results with other published data.

Future predictor

Baseline CRP levels were found to be predictive of future symptomatic peripheral vascular disease in a cohort of healthy men (Ridker et al. 1998). The physician's health study (PHS) was a multi-centre randomised double-blind placebo controlled trial to assess the role of aspirin and β-carotene in the prevention of cardiovascular disease and cancer in men aged between 40-84 years of age. The PHS reported that the baseline levels of those who subsequently developed PAD (1.34mg/ml) were greater than those who did not (0.99mg/ml, $p=0.04$).

Within this study, patients were then sub-stratified according to their CRP to assess the risk associated with development of future PAD when changing between groups. An increase in CRP from <0.55mg/ml to >2.1mg/ml, was associated with a 2.2 relative risk (95% CI 1.1-4.8, $p=0.04$)) of developing future PAD. (This was after adjustment for hypercholesterolaemia, hypertension, diabetes and BMI (Ridker et al. 1998)).

IL-6 and CRP both elevate during acute coronary syndromes and the difference from baseline values is associated with an increased risk of subsequent events (Ridker 2003)

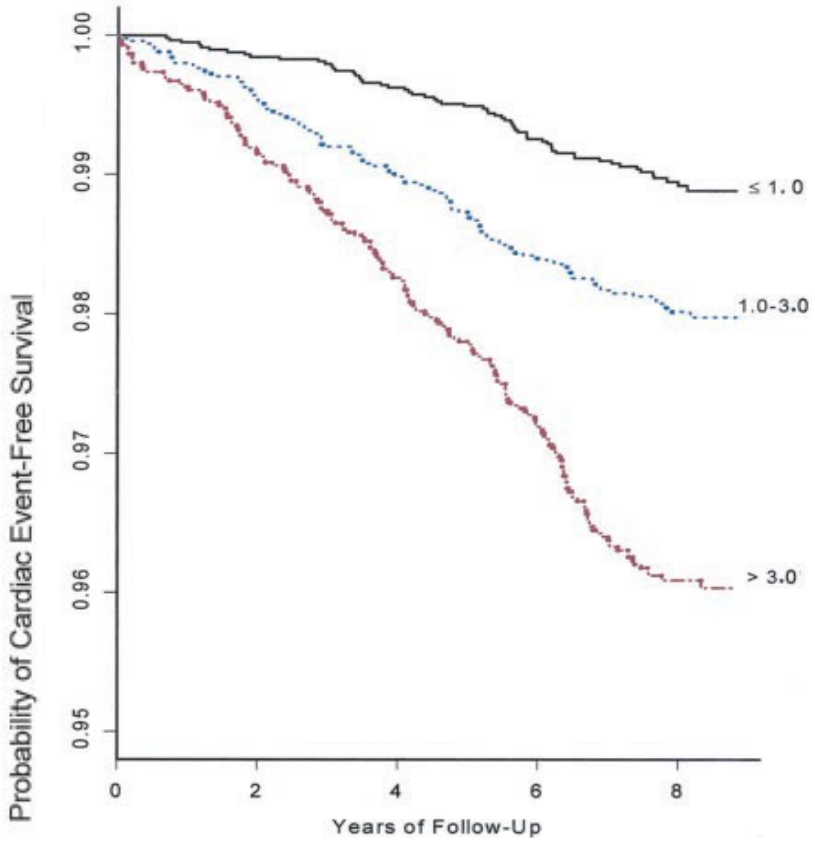


Figure 9 Cardiovascular event-free survival among apparently healthy individuals according to baseline CRP levels

Data are shown using 3 simple clinical cut-points for HsCRP levels <1, 1-3 or >3mg/L. Reproduced with permission from Wolters Kluwer Health (Ridker 2003)

Ameliorating inflammation

In 1997, Tisi *et al* (Tisi et al. 1997) demonstrated that healthy controls had a lower CRP (2.1mg/l, (IQR 1.0-2.8mg/l), n=15) than claudicants (4.7mg/l (IQR 2.2-9.0mg/l), n=67) at baseline (MWU, $p<0.0001$) (see Table 4). The PAD group were randomised to either observation or four weeks of an exercise programme with education on how to maintain this at home. Three months post randomisation, CRP levels were lower in those who exercised, however this returned to baseline by six months. Of note, the CRP levels of the observed group fell to a similar level to that of the healthy controls by twelve months, but this was not deemed to be of significance.

Other risk factors such as smoking cessation (Ohsawa et al. 2005), and the use of hydroxymethylglutaryl coenzyme A (HMG-CoA; statins) reductase inhibitors (Albert et al. 2001) are also associated with a reduction in inflammation. The use of aspirin at 81mg/day led to a significant reduction in serum thromboxane, but not CRP levels (Feldman et al. 2001).

Statins reduce inflammation in patients with a previous MI when compared to placebo (Ridker et al. 1999). From the CARE trial, the mean percentage change in CRP at five years was -18.4% with treatment and +19.4% with placebo. This was supported by the Pravastatin Inflammation CRP evaluation (PRINCE) trial, which also demonstrated a reduction in CRP in both primary and secondary prevention groups for cardiovascular disease at 12 and 24 weeks (Albert et al. 2001). Median CRP change at 12 weeks for the primary prevention group was -0.02mg/dL (-14.7% change) and -0.02mg/dL (-14.3% change) for the secondary prevention group. At 24 weeks, the values had not altered and overall pravastatin use reduced median CRP values by -0.02mg/dL (-13.8% change). In a separate study of patients with primary hypercholesterolaemia, a significant reduction in LDL, and CRP was noted (Ridker et al. 2001) with the use of cerivastatin. The effect was not found to be dose dependent when comparing the 0.4mg and 0.8mg dosage groups.

Table 4 Summary of Tisi et al's work on the effect of exercise on CRP in patients with Intermittent Claudication when compared to their sedentary counterparts (Tisi et al. 1997)

*Demonstrates a significant difference compared to baseline

Treatment for PAD	CRP (mg/l)			
	Baseline	3 months	6 months	12 months
Exercise	5.3 (2.8-10.5)	4.4 (1.8-7.2)*	6.6 (3.4-9.0)	4.8 (2.2-9.3)
Observed	5.6 (2.7-8.9)	4.8 (2.1-9.2)	5.4 (1.8-12.6)	2.0 (0.8-15.0)

1.2.6.2. Interleukin 6

Interleukin 6 (IL-6) is an acute phase cytokine (Hirano 1998) which induces the synthesis of CRP from the liver. This cytokine is comprised of four alpha helices (Muñoz-Cánoves et al. 2013).

Baseline median levels of IL-6 in a healthy cohort from Spain (n=228), demonstrated that levels were higher in men (6.4pg/ml) than women (5.8pg/ml) (Fernandez-Real et al. 2001). IL-6 was influenced by smoking status and fasting insulin resistance index.

As with CRP, this cytokine has been associated with an increased risk of cardiovascular disease (Basso et al. 2002) and its associated risk factors (Tzoulaki et al. 2007; Lim et al. 2004). IL-6 rises 6 hours post MI and was found to be an independent predictor of cardiovascular events. This is further supported by the moderate positive correlation between CRP and IL-6 ($r=0.56$, $p<0.001$) (van der Meer 2002).

Exercise results in an increased level of circulating Il-6 up to three hours after exercise, with an associated rise in IL-1, IL-10 and reduced level of TNF alpha (Scott et al. 2013). Long term, studies have demonstrated no change in IL-6 levels secondary to exercise up to 22 weeks (Loria-Kohen et al. 2013; Karabulut et al. 2013).

IL-6 suffers from diurnal variation with a low level in the morning and elevation at night (Meier-Ewert et al. 2001) in comparison to CRP.

The biomarker of IL-6 was used as supportive evidence for any changes demonstrated in CRP secondary to exercise. It was hoped that any change in CRP secondary to exercise would be backed up by reciprocal changes in IL-6.

1.2.6.3. Tumour Necrosis Factor Alpha

TNF alpha is a trimer of 52kDa (Fiers 2001). The level of circulating TNF-alpha was associated with an increase in age; a mean level of 1.4pg/ml is normal in 18-30 year olds, is 1.7pg/ml in 55-65 year olds and is higher in those over 80 years of age (Bruunsgaard et al. 1999; Bruunsgaard et al. 2000). TNF alpha is a pro-inflammatory cytokine with receptors present on most cells. It is an early mediator of acute inflammation, and is associated with the production of IL-6 (Bruunsgaard et al. 2000).

It is synthesised from adipose tissue, therefore levels are higher in obese individuals (Fiers 2001). Obesity is associated with higher TNF alpha levels, diabetes, insulin resistance, and AGE, all of which increase the pro-atherogenic tendency of the endothelium.

TNF alpha is associated with impaired insulin mediated glucose uptake by the skeletal muscle cells in both animals and humans (Halse et al. 2001).

Exercise precipitates an acute elevation in TNF alpha (Scott et al. 2013), which falls with training (Lee et al. 2013; Loria-Kohen et al. 2013) allowing concentrations to return to baseline, or lower.

In cardiovascular disease, elevated TNF alpha is associated with an increased risk of future myocardial infarction, and present in patients with diabetes, obesity, and smokers (Petersen & Pedersen 2005). A significantly higher mean concentration of TNF alpha (t-test = 2.105, p = 0.036) has been identified in those with PAD (1.14 ± 0.45) when compared to individuals without PAD (1.04 ± 0.45). Although significant, the difference was not of clinical importance as both values lie within the normal range (Gherman et al. 2013).

As TNF alpha was expected to influence IL-6, and follow on to elevate CRP, it was hoped that evidence of a cascade mechanism could be elicited by focusing on one single inflammatory chain. Therefore the three biomarkers focused on within the thesis were TNF alpha, IL-6 and CRP.

The following additional biomarkers have been discussed briefly, but were not included within this thesis. The reason for exclusion was the additional cost for ELISA kits required for three further samples (fibrinogen, CD-40 and soluble VCAM). It was felt that following analysis of the first biomarkers (CRP, IL-6 and TNF alpha), things could be reassessed if time permitted to allow additional biomarkers to be analyzed.

1.2.6.4. Fibrinogen

Fibrinogen is a molecule comprised of three pairs of polypeptide chains (alpha, beta and gamma). Cleavage of the alpha chains in fibrinogen, results in the formation of fibrin, and a subsequent scaffold for further intravascular thrombus (Mosesson 2005). Elevated serum fibrinogen is associated with atherosclerotic risk factors, including smoking, age, and diet (Canseco-Avila et al. 2006).

The ARIC study performed multivariate analysis and have demonstrated that any concentration of fibrinogen greater than 275mg/dl is associated with an increased relative risk of PAD (RR 2.14, CI 95% 1.31-2.51, p=0.003 (Wattanakit et al. 2005)). This was supported by subsequent analysis from the Edinburgh Artery Study, elevated fibrinogen was associated with a significant hazard ratios (HR 1.20 95% CI 1.08-1.33, $p<0.01$), after adjusting for demographics, co-morbidities, and other risk factors (Tzoulaki et al. 2007).

Recent evidence suggests that elevated fibrinogen levels are a strong independent predictor of future cardiovascular events in both apparently healthy patients and patients with a history of cardiovascular disease (Canseco-Avila et al. 2013; Canseco-Avila et al. 2006; Okwuosa et al. 2013). The evidence directly correlating serum fibrinogen levels and increasing severity of PAD is also convincing (Paraskevas, Baker, et al. 2008a; Okwuosa et al. 2013).

1.2.6.5. Soluble vascular cell adhesion molecule-1 (sVCAM-1)

An atherogenic diet induces a change in the vascular endothelium. Vascular cell adhesion molecules (VCAMs) bind monocytes and T lymphocytes to the endothelium, which does not normally occur in health (Libby 2002; Libby et al. 2010).

VCAM-1 expression has been noted to increase over areas of plaque, whilst in Apo-lipoprotein E double knockout (ApoE -/-) mice treated with anti-VCAM-1 antibodies, plaque development was reduced (Park et al. 2013). It is hypothesised that at arterial bifurcations, impairment in vascular nitric oxide (NO) occurs secondary to turbulence. This in turn places these areas at increased risk of plaque formation and subsequent stenosis (Vita & Hamburg 2010; Libby 2002).

A higher level of sVCAM-1 is present in patients with PAD, irrespective of symptoms (Cheng et al. 2012). The elevated levels have been used in conjunction with CRP, glomerular filtration rate, and albumin as a model for predicting mortality post lower extremity bypass in patients with PAD(Owens et al. 2012). Of note, CRP up-regulates both sVCAM and CD40 via different pathways, both of which result in atherosclerosis (Paffen & Demaat 2006).

1.2.6.6. CD40 ligand

CD40 is a trans-membrane protein that is expressed by the endothelium, vascular smooth muscle cells and activated platelets (Vishnevetsky et al. 2004). It is a member of the TNF family. When bound to its protein receptor, this initiates an inflammatory response as part of the atherosclerotic cascade (Nylænde et al. 2006).

CD40 ligand is a 33kD glycoprotein (attached to T lymphocytes) that is activated and induced by binding to CD40 (which is expressed on vascular endothelium) (Paffen & Demaat 2006).

CD40 has been associated with an increased risk of cardiovascular events as well as diabetes mellitus (Lim et al. 2004). In the presence of hyperglycaemia, this ligand has been up regulated, resulting in endothelial activation and subsequent dysfunction secondary to monocyte recruitment to the arterial wall (Lim et al. 2004).

Elevated CD40 is associated with an increased risk of cardiovascular events (Vishnevetsky et al. 2004). In patients with diabetes, risk factor modification has reduced levels of CD40 when compared to healthy controls (Cipollone et al. 2005).

A prospective study examined how inflammatory markers correlated with markers of PAD, such as angiographic score, treadmill distance, ABPI. They demonstrated a weak correlation between CD40L and angiographic score (Spearman's rho =0. 176, $p<0.05$), but no correlation between CD40L and MWD (-0.080, $p>0.05$) or ABPI (-0.061, $p>0.05$) (Nylænde et al. 2006). Multiple regression analysis identified CD40L to be a significant independent variable (OR=0.22; 95% CI 0.10-0.69, $p<0.01$) of disease severity (angiographic score) in patients with PAD.

1.2.7. Assessing Endothelial Dysfunction

The dysfunction seen at the endothelial level may be measured using surrogate measures, namely the effects of nitric oxide on the vessel wall (Celermajer 2008). Within normal vessels, occlusion of the arterial inflow vessel and venous return leads to the accumulation of metabolites secondary to the ischaemia downstream of the artery (Sinoway et al. 1989). Removal of the extrinsic compression leads to an increased blood flow, shear stress on the vascular endothelium leading to vasodilation of the artery and increased erythema downstream to the previously occluded tissue. Vasodilation of the artery occurs in response to the release of nitric oxide, prostacyclin amongst other vasodilators when an intact intimal layer is present (Pohl et al. 1986). This response in the

tissue is known as reactive hyperaemia, and the change at the artery is termed flow-mediated dilatation (FMD).

The degree of endothelial dysfunction has been associated with the amount of nitric oxide released at the vessel wall, which in turn affects the degree of vessel wall dilation. I.e. lower concentrations of NO equates to reduced vasodilatation, and increased endothelial dysfunction (Corretti et al. 2002).

Endothelial dysfunction may be measured by invasive techniques using intra-arterial angiography and non-invasive methods of measuring FMD through brachial arterial ultrasound (BAUS) or capillary vessel changes (through pulse amplitude tonometry) or circulating levels of endothelial derived micro-particles (as measured through flow cytometry). BAUS has become the gold standard in non-invasive assessment of FMD in patients (Deanfield et al. 2007). The method employed for BAUS has been outlined by the International Brachial Artery Reactivity Task Force (Corretti et al. 2002), however debate remains about the precision of the instruments, timing of measurements and electronic equipment used (Bianchini et al. 2006).

The International Brachial Artery Reactivity Task Force state that the expected minimally important statistically significant change in FMD is 1.5-2% (Corretti et al. 2002). An equivalent change to 2% has not been highlighted in the literature for the EndoPAT.

The reactive hyperaemia index as measured by pulse amplitude tonometry (PAT) is the main output measure of the EndoPAT2000. This device measures endothelial dysfunction at a capillary level, rather than arterial (as in the BAUS) (Bonetti et al. 2004). The distal phalynx is sensitive to changes in sympathetic activity, with subsequent changes in vasodilation mediated by NO (Celermajer 2008). The reactive hyperaemia index (RHI) PAT index is calculated using the average amplitude of the PAT signal from the occluded arm and is normalized against the non-occluded arm. This occurs for 3 to 5 minutes pre cuff inflation to provide a baseline and then at one minute intervals following cuff deflation (Bonetti et al. 2004).

The technique has been validated by previous studies (Bonetti et al. 2004; Kuvin et al. 2007). This non-invasive test has been utilised in the Framingham study to predict high mortality cases (Rubinshtein et al. 2010) of cardiovascular disease. This has recently however been contested and placed a question mark over the usefulness of the EndoPAT as a marker of endothelial function in isolation when compared to other tests (Lind 2013; Seager et al. 2013; Müller et al. 2013; Allan et al. 2013).

The use of EndoPAT in the third generation Framingham study demonstrated that women had a higher PAT ratio, and obesity reduced the ratio. A threshold value for what constituted healthy endothelium was not suggested (Hamburg et al. 2008). The first published threshold value for the RHI-PAT was 1.35 as the level under which endothelial dysfunction was present (Bonetti et al. 2004). Coronary endothelial dysfunction was predetermined in the cohort through the use of coronary vasoreactivity using cardiac catheterisation and a change in coronary blood flow of more than 50% in response to acetylcholine. The 1.35 level was determined by receiver operating characteristic (ROC) curves, with a sensitivity of 80% and specificity of 85% for detecting coronary endothelial dysfunction. Of note, the mean score for RHI-PAT in health was 1.78 +/-0.08 (n=39), but was 1.27+/-0.05 in those with established dysfunction. The correlation between the RHI-PAT and invasive coronary blood flow values was moderately significant ($r=0.405$, $p<0.001$) (Bonetti et al. 2004). The threshold value was later raised from 1.35, to 1.67 by the company, Itamar, they quote this increase to improve sensitivity to 82%, however specificity fell to 77% (Anon 2009). This change in the threshold value was recommended by the company, but remains unsupported by any published data. The figure of 1.67 has been quoted in papers to have a 82% sensitivity and 77% specificity for determining coronary endothelial dysfunction (Bonetti et al. 2005; Bonetti et al. 2004). Although initial papers from 2004 stated a value of 1.35 (Bonetti et al. 2004) (80% sensitivity and 85% specificity for determining coronary endothelial

dysfunction), re-analysis of the data prior to submission to the FDA identified that the higher value was more sensitive. The scores come from a 2004 paper in which an increase in capillary blood flow greater than 50% after receipt of intracoronary acetylcholine was deemed normal.

1.2.7.1. PAD and Endothelial Dysfunction

Patients with peripheral arterial disease have demonstrated a lower FMD than healthy controls (Loffredo et al. 2007). In 95% of healthy individuals, the change in vessel diameter as measured with brachial artery ultrasound is expected to be greater than 6.2%. A change in vessel diameter equal to or less than 6.2% has previously been used to denote endothelial dysfunction (Brevetti et al. 2003).

In a cohort of vascular patients (n=267) undergoing elective peripheral vascular operations that were followed up for a year, those who had a post-operative cardiovascular event had a significantly reduced FMD (No events' mean % change in FMD: 6.9% +/-4.6% versus postoperative event groups' mean % change in FMD: 4.5% +/-3.0%, $p<0.001$) (Huang et al. 2007). The authors also divided the cohort into three according to FMD and hyperaemic flow velocity. The top group in each was compared to the lower two pooled, with a high FMD defined as ≥7.9%, and a hyperaemic velocity of ≥105cm/sec. Both higher groups were associated with a better prognosis and early and late total event-free survival when compared to the lower groups (Huang et al. 2007). A second large cohort study of elective vascular surgical patients (n=199) showed a similar threshold of 8.1% as the determinant value between high risk of early (30 day) and late (30 days to one year) survival and total events (Gokce et al. 2003).

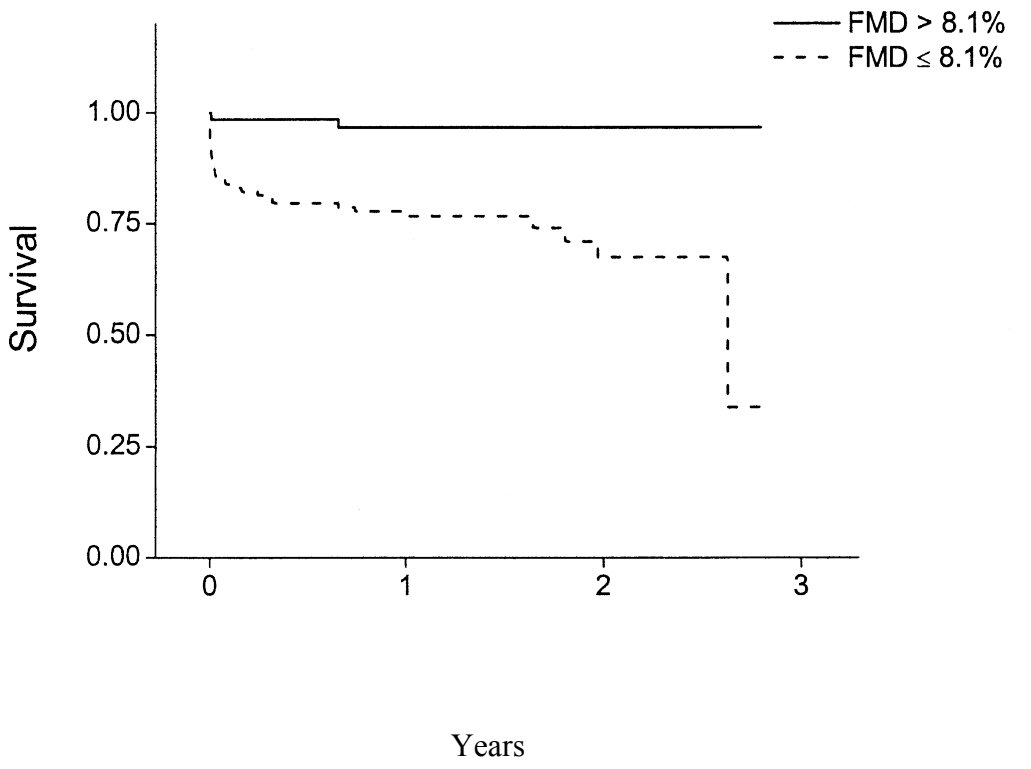


Figure 10 Kaplan Meier plots demonstrating the survival difference between those deemed to be low risk (FMD>8.1%) and those at high risk of subsequent events

Reproduced with permission from Elsevier (Gokce et al. 2003)

In 106 vascular patients undergoing elective abdominal aortic aneurysm repair of supra or infra-inguinal bypass; the mean RHI-PAT score was 2.24+/-0.84 (Chan et al. 2010). This observational study identified a weak, significant correlation between the RHI-PAT and diabetes ($r=-0.21$, $p=0.04$), serum HDL ($r=0.24$, $p<0.05$), bilirubin ($r=0.24$, $p<0.05$) and systolic blood pressure ($r=0.30$, $p<0.05$). This level of 2.24 was greater than expected, as vascular patients often have a degree of concomitant cardiovascular disease. Additional correlation of the EndoPAT scores in this group with coronary angiography would have been of value.

1.2.7.2. Comparison of EndoPAT to BAUS

Endothelial dysfunction can be assessed in numerous ways. The gold standard invasive test, coronary angiography and non-invasive test, BAUS have both been compared to the EndoPAT machine. In three small prospective studies (n=40 to 89), the correlation of EndoPAT to BAUS was mild to moderate (rho=0.3-0.55, $p<0.05$ (Kuvin et al. 2003; Kuvin et al. 2007; Dhindsa et al. 2008)). When reviewing the correlation of these two measures of endothelial dysfunction with the number of cardiovascular risk factors a patient had; BAUS was the more sensitive test (BAUS FMD $r=0.4$, $p=0.0001$, PAT $r=0.3$, $p=0.002$). However, this does not take into account the level of expertise of the sonographer. The EndoPAT RHIPAT score lowered as the number of cardiovascular risk factors increased (See Table 5 and Table 6).

Table 5 Analysis of independent groups with specific risk factors to those without to assess the difference in mean RHI-PAT score (Kuvin et al. 2003)

*denotes that controls were women. ** denotes that controls were premenopausal women.

	Risk Factors mean PAT score (+/- s.e.m)	Control group's mean PAT score (+/- s.e.m)	t Test p
Hypertension	1.40 +/-0.08	1.60+/- 0.07	0.006
Hyperlipidaemia	1.50 +/-0.10	1.70+/- 0.08	0.020
Tobacco use	1.40+/- 0.08	1.60+/-0.08	0.040
Family History of Coronary Artery Disease	1.40+/-0.07	1.8+/- 0.1	0.010
Men	1.50+/-0.07	1.7+/- 0.1*	0.040
Postmenopausal women	1.6+/-0.2	1.9+/- 0.1**	0.010

Table 6 demonstrates the mean RHI-PAT score for participants with differing numbers of cardiovascular risk factors (CRF)

The far right column shows the statistical significance between the mean scores when comparing one risk factor with the presence of 2-4 risk factors or more than four risk factors. (Kuvin et al. 2003)

Number of cardiovascular risk factors **(**CRF)	Number of patients	Mean PAT +/- standard error of the mean (SEM)	1 CRF vs (p)
1	20	1.8+/- 0.1	---
2-4	55	1.5 +/-0.06	0.005
+4	14	1.3 +/-0.09	0.0005

1.2.7.3. Effect of Exercise on Endothelial Function

Exercise is associated with improved endothelial function. This has been demonstrated in animal models. In dogs, exercise twice a day for four to ten weeks improved cardiac output, capillary density and epicardial coronary diameter when compared to non-exercised controls. When coronary artery rings from both groups were treated with acetylcholine, the levels of nitrite production in the exercise group was greater than the non (Sessa et al. 1994).

One mouse model elegantly demonstrated that forced sedentation reduced the vaso-reactivity of the arterial flow (Suvorava et al. 2004), which was then reversed with the re-introduction of a nine week course of exercise. A second study has again demonstrated that endothelial function improves with exercise. It has also taken this a stage further by focusing on the underlying mechanism. The authors demonstrated that dysfunction at the site of the enzyme endothelial nitric oxide synthase (eNOS) was key in regulating endothelial function. Heterozygous mice for the eNOS gene were unable to respond to exercise with an improvement in vasoreactivity (Kojda et al. 2001).

In humans, changes in flow-mediated dilatation have been observed. In patients with chronic heart failure or coronary artery disease, enrolment in an exercise programme (Hambrecht, Wolf, et al. 2000b; Hambrecht, Hilbrich, et al. 2000a), resulted in an improved response to acetylcholine. A four week programme of supervised exercise consisting of 10 minute sessions six times throughout the day were prescribed in patients with coronary artery disease(Hambrecht, Wolf, et al. 2000b). A similar result was also demonstrated in patients with chronic heart failure (pre-exercise of 2.2%+/- 0.2% to 8.8%+/-0.9%, $p<0.001$ (Hambrecht, Hilbrich, et al. 2000a)).

1.2.7.4. The Augmentation Index (AI)

At the commencement of this thesis the recognised output measure from the EndoPAT was the RHI-PAT. A second outcome measure, the augmentation index was also present but undergoing further tests by the company to validate it.

The AI is routinely calculated using validated software such as the SphygmoCor (Atcor, Australia) or the Omron (Kyoto, Japan). Pressure waves alter as the wave moves further from the heart. The central augmentation index is measured by dividing the augmentation pressure by the pulse pressure. A peripheral augmentation index is measured by dividing the late systolic pressure by the early systolic pressure.

The augmentation index has been considered to be a measure of arterial stiffness and has been an increasingly important marker of cardiovascular risk (Nurnberger et al. 2002; Janner et al. 2012). Evidence remains mixed, with numerous studies suggesting that AI should not be used interchangeably with other terms such as arterial stiffness or pulse wave velocity (Jerrard-Dunne et al. 2008; Fantin et al. 2006).

The reliability of the AI has been quoted as an intra-class coefficient of 0.83 when focused in healthy volunteers (McCrea et al. 2012). The only paper to validate the EndoPAT device has again been undertaken in healthy individuals, demonstrating a repeatability of 0.84 (Pearsons rho), and a correlation to the aortic SphygmoCor of 0.79, radial SphygmoCor of 0.88 and radial Omron of 0.78 (Dhindsa et al. 2011).

At the outset of this thesis, this outcome measure was still under investigation by the company. As literature grows, the association of the Augmentation index with arterial stiffness is developing into a new avenue of research. This outcome measure has been included in the thesis, but not focused on in the same depth as its validity and reliability in diseases is yet to be confirmed.

1.3. Exercise

The UK population is currently ageing. The UK Department of Health expects that by 2024, approximately half of the population will be over 50 years of age (Department of Health 2010). Ageing is frequently associated with a sedentary lifestyle, which is an important contributing factor to the decline in physical functional ability in this group. Currently less than 40% of adults in the UK meet the recommended guidelines for physical activity (Pugh 2012).

Physical inactivity is a recognised modifiable risk factor for atherosclerosis and specifically coronary heart disease. Regular exercise has been shown to reduce the risk of coronary heart disease in a number of observational epidemiological studies (Paffenbarger et al. 2001; Blair et al. 1989). The mechanisms for this apparent benefit include an increase in HDL cholesterol, nitric oxide release and a decrease in body weight, insulin resistance, and blood pressure. Most health benefits occur with at least 150 minutes per week of moderate-intensity physical activity, such as brisk walking (Department of Health 2011). Additional benefits occur with more physical activity (Paffenbarger et al. 1993; Garber et al. 2011).

1.3.1. Mortality & morbidity

Increased exercise has an inverse, linear relationship with all-cause mortality (Blair et al. 1989; Paffenbarger et al. 1993; Kokkinos et al. 2010; Lee & Skerrett 2001). This response was independent of gender and observed in all ages, including those over 60 years (Bijnen et al. 1999). An expenditure of 1000kcal/week resulted in a 30% reduction in mortality, whilst 2000kcal/week resulted in a 50% reduction in mortality (Lee & Skerrett 2001).

Longitudinal cohort studies have demonstrated that exercise on prescription reduces cardiovascular mortality (Nam 2011; Paffenbarger et al. 2001) in middle-aged men and at younger ages.

1.3.2. Cardiac & Respiratory Improvement

The effects of regular exercise (training) on an ageing healthy population are primarily cardiac (Singh 2002). An increased cardiac output, VO_2 max and rise in haematocrit occur secondary to training. Conversely blood pressure, heart rate on acute exertion, and resting heart rate may remain stable or lower.

With regards to the effects of training on respiratory function in the healthy aging population, the main improvement is in tidal volume, which stabilizes at approximately 65% of vital capacity (Stanfield & Cocora 2011). In individuals with PAD, haemodynamic changes secondary to exercise are yet to be documented.

1.4. Exercise testing

Currently exercise physiology in claudicants has primarily focused on an improvement in patients walking ability. This has been measured as maximal walking distance and claudication distance on a treadmill (2.5km/hr at 10% incline) or undergoing a 6-minute walk on the flat. The 2008 Cochrane review (Watson et al. 2008) highlighted the necessity for a supervised exercise programme for claudicants, and that such programmes confer a benefit up to two years post completion. Secondary, but important, outcomes of exercise programmes in claudicants includes improvements in physical ability (McDermott, Ades, et al. 2008a), quality of life (Nicolaï, Teijink, et al. 2010b; Verspaget et al. 2009; McDermott, Guralnik, et al. 2008b) and balance (Mockford et al. 2011; Gohil et al. 2012).

1.4.1. Physical functional ability

1.4.1.1. *The Short Physical Performance Battery Score (SPPB)*

The short physical performance battery score has been utilised as a measurement of physical functional ability. The score has been increasingly used as a predictor for future mobility (Volpato et al. 2008; Chiarantini et al. 2010). Vasunilashorn's study

(Vasunilashorn et al. 2009) of 452 patients followed up over three years has shown that those with a score ≤10 had increasingly worse outcomes by three years. It predicted loss of ability to walk 400m in addition to identifying patients at high risk of lower body functional limitations. There has been increasing interest in this score over the last 15 years (Guralnik et al. 1994; Vasunilashorn et al. 2009; Fisher et al. 2009; Volpato et al. 2011); it has been identified as an objective measure of lower-extremity function with a high predictive value for subsequent disability. This score will allow us to identify patients with poor mobility and provide an intervention, which may improve their score and prevent the development of frank disability.

Recent work has demonstrated that components of the SPPB may be used as measures of physical functional ability rather than the whole battery score. The following two components were focused on (Wright et al. 2010; Wennie Huang et al. 2010): The Chair Stance Time and Semi-Tandem Stance.

1.4.1.2. The Timed up and Go Test (TUG)

The TUG test is a second commonly used measure of physical functional ability. It has been shown to be a reliable (Steffen et al. 2002) and valid test for quantifying functional mobility as well as assessing a patient's ability to turn (Podsiadlo & Richardson 1991). The major limitation of this test however is that the chair height is standardised, not adjusted according to the individuals leg length. (The seat height is adjusted for cycle ergometry because of lower limb biomechanics, however the same does not occur for the TUG or CST.)

This test is repeated three times and the best score may be used in the analysis (Bohannon 2006; Schaubert & Bohannon 2005). An age appropriate threshold may be used (see Table 7) or a cut off of 13.5 seconds has been associated with a 87% sensitivity and 87% specificity for identifying those who are prone to falls (Shumway Cook et al. 2000).

Table 7 Normative reference Values by Age for the Timed up and Go test

Reproduced with permission (Bohannon 2006)

Age Group	Time (seconds)	95% CI
60-69	8.1	7.1-9.0
70-79	9.2	8.2-10.2
80-99	11.3	10.0-12.7

None of these markers reflect an improvement in overall patient cardiovascular physiology. Any physiological improvements secondary to SEP remain limited (Stewart et al. 2008; Oka et al. 2005). Identifying changes in cardiopulmonary exercise outcomes including, anaerobic threshold (AT) and oxygen consumption at maximal exertion (peak VO_2), may provide some insight into the cardio-respiratory effects of exercise training to pain threshold in claudicants.

1.4.2. Cardiopulmonary Exercise testing (CPET)

Anaesthetists and sports physiologists utilise cardiopulmonary exercise testing (CPET) (Forman et al. 2010). CPET measures gas exchange data and breath-by-breath analysis of physiological changes that occur during exercise. Exercise physiologists and the ACSM have utilised CPET to train athletes and assess what is normal in health for the past 60 years.

The role of CPET has extended to cardiac rehabilitation where it superseded cardiac treadmill stress tests for patients with heart failure (Wang & Dai 1992; Scardovi et al. 2007; Franco 2011), post MI (Dickstein et al. 1988; Bigi et al. 2001; Che et al. 2008) or respiratory conditions (Orens et al. 1995; Miyoshi et al. 1990; Holverda et al. 2008). CPET has now become the modality of choice for assessing progress, and determining who is safe to undertake strenuous activities. Surgery is a large physiological strain for the body; therefore identifying how an individual copes under strain has been the key determinant for utilising CPET in pre-assessment. Anaesthetists use anaerobic thresholds, peak oxygen consumption (peak VO_2) alongside other markers to risk stratify patients prior to major surgery (Thompson et al. 2011a; Weisman 2001; Smith et al. 2009).

Cardiopulmonary exercise testing is a safe, reproducible, standardised test, which allows better quantification of an individuals' ability. The literature suggests that the risk of death when undergoing CPET is 2-5/100,000 exercise tests performed (Ross 2003). It measures subject's cardiac and respiratory function, maximal oxygen uptake (peak VO_2) (Keteyian et al. 2010; Albouaini et al. 2007) and anaerobic

threshold (AT). The American Thoracic Society and American College of Chest Physicians 2003 consensus document (Ross 2003) advocates the use of a reference range against which individuals should be compared. Their systematic review from 1970 to 2003 produced a CPET reference range, based on an individual's gender, age, weight and the type of ergometer used.

For cycle ergometry

In men, Max VO_2 = Weight in kg.[50.75-0.372(age in years)]

In women, Max VO_2 = (Weight in kg+43)[22.78-0.17(age in years)]

Overall, peak VO_2 attained should be greater than 84% of that predicted, and AT should be between 40-80% of the predicted max VO_2. The blood pressure should not exceed 220/90, and the respiratory rate should be less than 60 breaths per minute. The minute ventilation (VE/VT) should remain less than 34 at the time of AT.

1.4.2.1. VO_2 peak

The peak pulmonary oxygen uptake is defined as the peak or maximum amount of oxygen that an individual can transport and utilise during maximal exercise. The maximum VO_2 is defined as the upmost capacity that a healthy individual can perform, whilst peak VO_2 is typically used when the VO_2 max criteria have not been satisfied or when an ergometer other than the treadmill, has been used. It is often used in clinical populations where the test is symptom limited and the maximum VO_2 achieved is not indicative of the capacity of the oxygen transport and utilisation. Peak VO_2 differs to maximal testing as the test may terminate due to 85% of maximal heart rate being achieved, shortness of breath, chest pain, or medical concerns over ECG changes. In health, these aspects do not often cause a limitation to the CPET, making it a maximal test. When a steady state is achieved, despite an increasing workload, this means that maximal VO_2 has been attained.

In health, men under 45 years and women under 55 years of age may undergo a CPET without a doctor present. For those with cardiovascular disease, guidelines have been produced by the ACSM (Kohl et al. 1990). CPET (n=24,332) was established to be safe with one in a thousand found to be abnormal. In men with cardiovascular disease, the likelihood of an abnormal treadmill result increased with the number of cardiovascular risk factors.

VO_2 can be estimated through equations or quantified through a maximal physical test; Uth Sorenson Overgaard Pedersen equation, Cooper test equation, ACSM equation. Whilst differing equations exist, the most accurate option has remained contested. Equations used to determine maximum VO_2 are highly variable, when compared to actual values attained on testing. At times they have been 40-60% lower and in other cases were so high that they were non-physiological; this was identified to be more marked in disease states (Climstein et al. 1993; Yano et al. 2003; Peterson et al. 2003; Malek et al. 2004). The optimal way to identify peak or maximum VO_2 is therefore by CPET.

1.4.2.2. Anaerobic threshold (AT)

Exercise leads to increased utilisation of oxygen and increased blood supply to the exercising muscle. All metabolic pathways function continuously, however the relative contribution of the anaerobic component may change over time. As the cellular metabolic rate exceeds that of the oxygen carrying capacity of the blood, the body is postulated to increase its anaerobic metabolism. The rate of lactic acid production then begins to exceed the rate at which it may be cleared, leading to its accumulation. This excess lactate is known as the anaerobic threshold and occurs when the metabolic demands can no longer be met by the body's aerobic metabolism. Anaerobic energy is generated by two mechanisms, the utilisation of muscle stores of high-energy phosphates (ATP and creatine phosphate), or glycolysis. There is a limited supply of high-energy phosphates stored within the muscle. These are rapidly utilised when activity exceeds approximately thirty

seconds, making this a temporary energy providing solution. Glycolysis remains a more long-term option, which occurs in both anaerobic and aerobic metabolism.

The change in metabolism from aerobic to anaerobic can be measured by either invasive or non-invasive techniques. Invasive techniques include the serial venous blood lactate levels during exercise. These blood samples have been taken at the ante-cubital fossa, femoral vein or as finger prick analysis. Numerous ways of quantifying the increase in lactate levels exist. The accumulation of lactic acid is deemed to be the most sensitive method of assessing the onset of the lactate threshold, for which AT is a surrogate marker. Non-invasive methods use breath-by-breath analysis to identify the change in the rate and depth of breathing, levels of carbon dioxide expired and oxygen inspired. This is performed using a tightfitting facemask or mouthpiece and continuous sampling of the amount of carbon dioxide and oxygen expired. The AT can be visualised from the graphical data as the break-point in the relationship between VO2 and VCO2, i.e. where the lines cross over (See Figure 11).

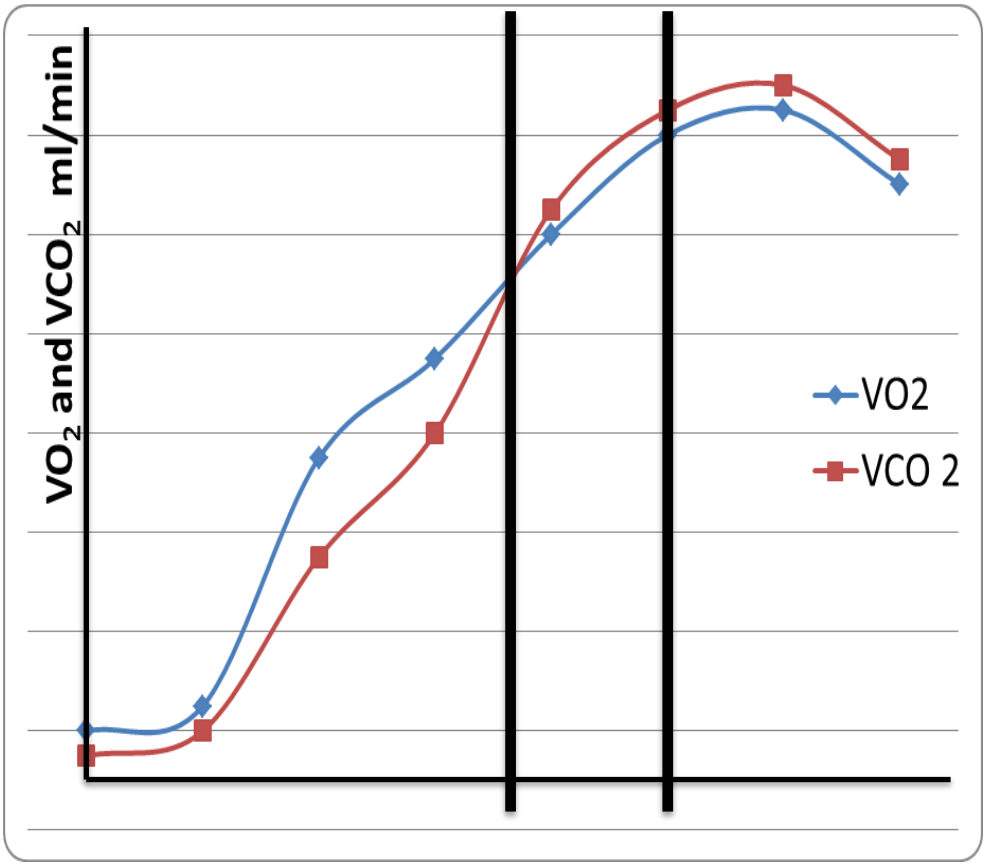


Figure 11 Graph depicting data from the CPET test

The first vertical black line, where the VO_2 and VCO_2 lines cross, indicates the (probable) AT. The second vertical black line marks the end of the exercise phase and the beginning of the recovery phase; the point at which fatigue overcomes the patient or it is deemed appropriate to stop the test. In health this is known as the maximal VO_2 however in disease states, patients frequently only reach a submaximal threshold/peak VO_2.

Assessment of the anaerobic threshold can be highly variable and subjective. Methods have been devised to counter this (Gaskill et al. 2001), they include four separate parameters:

V-slope simplified technique

This technique allows the detection of lactic acidosis during an incremental exercise test. It is visualised by the accelerated uptake of CO_2 output compared to O_2 uptake (Sue et al. 1988; Beaver et al. 1986). The graph plots the VCO_2 against the VO_2. The point at which the gradient alters from 1 is determined as the gas exchange threshold. This method has been found to overestimate the work rate when compared to computerised methods of calculating the V-slope (Schneider et al. 1993).

VCO_2 inflection point

This occurs when there is an inflection point in the work rate/VCO_2 slope. The inflection point represents the respiratory compensation point.

Nadir of VE/VCO_2

VE is the minute ventilation of the lung whilst VE/VCO_2 indicates the efficiency of the ventilation with respect to CO_2 production (Davies et al. 1991). It quantifies the amount of pulmonary ventilation required to clear one unit of carbon dioxide from the lungs. A $VE/VCO_2>34$ is deemed to be a predictor of morbidity and mortality (Brunelli et al. 2012) in colorectal patients and patients with heart failure (Stringer 2010). It indicates that either the stroke volume of a patient is impaired as the lung is poorly perfused or that gas exchange at the lung surface is impaired due to significant lung pathology e.g. COPD.

The nadir of the VE/VCO_2 curve indicates the change from clearing CO_2 efficiently to muscle fatigue and patients tiring. The negative gradient of the curve is attributed

to increase in stroke volume secondary to exercise. At the nadir, the body is at its most efficient at clearing CO_2, after which point the rate of CO_2 production exceeds the rate of clearance, the AT. After the AT, the positive gradient of the slope indicates that the rate of production of CO_2 is greater than the circulating volume that is required to clear it, or the lung volume available for gas exchange.

Respiratory exchange ratio (RER) linear increase

The respiratory exchange ratio (RER) is a reflection of the substrate being metabolised by the body. It is a ratio of the CO_2/O_2 and is a reflection of the respiratory quotient during steady state exercise. The respiratory compensation point is the second non-linear increase in ventilation and decrease in end tidal pCO_2

$RER = VCO_2/VO_2$

At rest, the RER should be approximately 0.7-0.9 (Krogh & Lindhard 1920).

Oxidation of a molecule of ***Fatty Acid***

$23\ O_2 + C_{16}H_{32}O_2 \rightarrow 16\ CO_2 + 16\ H_2O + 129\ ATP$

$RER = VCO_2/VO_2 = 16\ CO_2/23\ O_2 = 0.7$

Once CPET has commenced, a linear increase in RER will occur once AT has been reached. This reflects the body's change in substrate as it converts from aerobic metabolism to anaerobic.

Oxidation of a molecule of ***Carbohydrate***

$6\ O_2 + C_6H_{12}O_6 \rightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP$

$RER = VCO_2\ /VO_2 = 6\ CO_2/6\ O_2 = 1.0$

1.4.2.3. AT and Vascular disease

The AT and percentages of maximal VO_2 have been used in exercise prescriptions. The AT represents the transition from moderate sustainable exercise to heavy intensity and occurs between 40% and 60% of VO_{2max} in untrained individuals, but can increase up to 90% in athletes. In patients with congestive heart failure, concern exists regarding the optimal exercise prescription intensity (Beale et al. 2011). Exercising at the AT has been associated with increased left ventricular wall stress, which may induce LV dysfunction, making a lower intensity more appropriate for this group.

In claudicants, exercise up to their pain threshold has been advocated. Exercising to pain threshold (n=33 claudicants) was found to induce a greater VO_2 and heart rate than exercising to a prescribed intensity of 40% or 70% peak VO_2 (Ritti-Dias et al. 2009). This single study indicated that working to anaerobic threshold requires greater energy expenditure than working to a prescribed intensity level of the peak VO_2.

The broader application of CPET to surgical outcome has identified an AT value ≥11ml/kg/min to be associated with a significantly superior survival (Smith et al. 2009) in non-cardiac surgery.

1.4.2.4. VO2 and Vascular disease

In patients with heart failure, retrospective multivariate data analysis has identified that a VO_2 max <50% of predicted was the most significant predictor of cardiac death (Stelken et al. 1996). VE/VCO_2 slopes were also an independent prognostic marker for patients with heart failure. Recent evidence suggests there is a paucity of data from CPET in vascular surgery to merit it's routine use in the clinical setting, a systematic review identified only six relevant studies which focused on an AT>11ml/kg/min and VE/VCO_2 <43 (Young et al. 2012).

The choice of ergometry method utilised in this thesis required an understanding of the pros and cons of all three commonly used ergometry methods. This has been expanded upon below.

1.4.2.5. Treadmill ergometry

In athletes it is well established that testing is specific to an athlete's event i.e. cycling for cyclists (Sporer et al. 2007), rowing for rowers (Huntsman et al. 2011; Klasnja et al. 2010) etc. In those with claudication, limitation by walking ability may provide a lower reading than an individual's true cardiovascular ability, therefore a modality to mirror this is difficult to identify. Interestingly, the ATS/ACCP erred on the side of cycle ergometry for patients and graded treadmill tests for healthy individuals (Ross 2003). (See Table 8).

Whilst graded treadmill tests are commonly used to provide an assessment of walking time in claudication, questions persist over whether this is the most appropriate testing modality for this disease. Exercise physiologists recommend a test lasting for approximately 8-12 minutes (Albouaini et al, 2007) of continuous walking which excludes all but mild claudicants, leading to potential selection bias.

Meta-regression analysis of graded and constant load treadmill testing identified graded protocols to have the highest reliability for identifying MWD (Nicolaï, Viechtbauer, et al. 2009a). There is no consensus regarding the most appropriate treadmill test protocol for use in claudicants. Whilst many use the Gardner treadmill protocol, this starts with a 0% grade at 2mph which was then increased by 2% every 2 minutes, (Dias et al. 2009; Afaq et al. 2007; Gardner et al. 2008), others have adopted different treadmill speeds and angles of inclination (Bronas et al. 2011; Langbein et al. 2002; Allen et al, 2010). Interestingly, variations in graded treadmill protocols have not increased our insight into which protocol is optimal (Nicolaï, Leffers, et al. 2010a).

Table 8 Summary of the three different CPET assessment modalities used in clinical practice and their suitability

	Cycle ergometry	Graded Treadmill	Arm Crank
Type of person to test	Patient	Healthy person	Patient, preferably not with respiratory compromise*
VO_2 max	Lower than predicted	5-10% Higher than predicted	Is 70% of that achievable in lower extremity exercise
Work rate measurement	Yes Body weight taken out of the equation due to seated position	No Unable to adequately estimate work rate. Holding onto side rails, and own body mass will affect outcome of test.	Yes
Noise/artefact	Less	More	Less
Safety	More	Less	More
Degree of muscle training	Less	More	Lactic acid builds up quicker with this

Other potential problems	Quadriceps fatigue, Seat discomfort		

*Arm crank may interfere with accessory muscles use, and therefore affect those with respiratory disease

1.4.2.6. Cycle ergometry

The majority of early CPET data was treadmill based, however the use of cycle ergometry in CPET in preference to treadmill testing has increased in popularity especially in the clinical environment for patients with a disease state.

Equations to estimate maximal VO_2 by cycle ergometry differ from that derived from that advocated by the ACSM (Lang et al. 1992). Studies identified that the ACSM equation tended to over-estimate maximal VO_2 for men and women (Latin & Berg 1994), as did BMI {Latin:1997fs}. The use of recumbent or upright cycle ergometers (n=31) were also noted to affect the levels derived for VO_2, with a recumbent cycle producing higher VO_2 levels at low watts (15-30), which ceased to be of importance at higher power output levels (greater than or equal to 50W) (Saitoh et al. 2005).

Comparisons of cycling to treadmill testing during CPET (n=10 claudicants) have identified a high correlation between the two for both AT and peak VO_2. Of note, greater values were induced through ramp cycling (10W/ minute) compared to graded treadmill tests (Tuner et al. 2008).

1.4.2.7. Arm ergometry

Whilst largely ignored, arm ergometry has become a new interesting modality for assessing AT and VO_2. Two independent studies have utilised arm ergometry as a training regimen in preference to walking or other lower limb based exercises. Both studies have demonstrated improvements in walking distance and CPET measures, however the CPET was not performed using the arm ergometry (Zwierska et al. 2005; Parr et al. 2009).

1.4.3. CPET and Claudication

Although a summary of the three main ergometry modalities has been included above, a literature review was also performed.

A variety of training programmes and assessment protocols have been utilised in patients with intermittent claudication. Treadmill protocols for assessing walking distance have been highly variable; with the main focus of physiological data on the derived functional indices e.g. claudication and maximal walking distances. In spite of utilising CPET assessments, cardiovascular and respiratory assessment of patients with PAD has been somewhat neglected.

A review of current claudication literature was undertaken to identify

a. which protocols are routinely used in the claudicant population,

b. how comparable these are,

c. what the baseline anaerobic threshold and peak oxygen consumption was in claudicants.

A search was performed of the Embase, Cinahl and Medline databases This identified 175 abstracts, of which only 31 (Afaq et al. 2007; Allen et al, 2010; Barker et al. 2004; Belcaro et al. 1998; Belcaro et al. 2000; Bronas et al. 2011; Cachovan et al. 1999; Crowther et al. 2007; Crowther et al. 2008; Degischer et al. 2002; Dias et al. 2009; Duprez et al. 1992; Gardner et al. 2008; Gardner, Parker, et al. 2010a; Gardner & Poehlman 1995; Gardner, Ritti-Dias, et al. 2010b; Hodges et al. 2007; Khurana et al. 2012; Kirby & Marlow 1987; Kövamees & Brundin 1976; Langbein et al. 2002; Ritti-Dias et al. 2009; Ritti-Dias et al. 2011; Shimizu et al. 1992; Tew, Nawaz, Zwierska, et al. 2009b; Tew, Nawaz, Blagojevic, et al. 2009a; Treat-Jacobson et al. 2009; West, Anderson, Epstein, et al. 2011a; Anon 1997; Wang et al. 2008) were relevant and discussed the use of CPET in claudication. Of these, only 17 provided data on baseline CPET values for patients with intermittent claudication.

Results of the searches and pooling of this data was undertaken using comprehensive meta-analysis V2 (2006 Biostat Inc, Englewood NJ).

1.4.3.1. Protocols routinely used in claudication

The vast majority of studies used a treadmill test during CPET, twelve used the Skinner-Gardner protocol (Dias et al. 2009; Gardner, Parker, et al. 2010a; Hodges et al. 2007; Ritti-Dias et al. 2010; Anon 1997; Afaq et al. 2007; Allen et al. 2010; Crowther et al. 2008; Crowther et al. 2007; Gardner et al. 2008; Gardner, Ritti-Dias, et al. 2010b; West, Anderson, Epstein, et al. 2011a), ten used other differing graded treadmill tests (Bronas et al. 2011; Cachovan et al. 1999; Kirby & Marlow 1987; Treat-Jacobson et al. 2009; Allen et al. 2010; Duprez et al. 1992; Langbein et al. 2002; Shimizu et al. 1992; Tew, Nawaz, Zwierska, et al. 2009b; Tew, Nawaz, Blagojevic, et al. 2009a), five used a fixed constant load treadmill test (Cachovan et al. 1999; Langbein et al. 2002; Belcaro et al. 1998; Degischer et al. 2002; Ritti-Dias et al. 2011), whilst only two used cycle ergometry (Tew, Nawaz, Zwierska, et al. 2009b; Bronas et al. 2011). All studies described the exercise protocol utilised during CPET.

Although the majority of protocols used a treadmill test, a treadmill test was met with reservations on the following basis:

1. The majority of patients were unable to complete a constant load treadmill test at 1.6mph for 5 minutes. Our sports science experts have advocated that a 7-10 minute test is required to assess AR and peak VO_2.
2. Our treadmill testing equipment does not provide sufficient clearance behind it to allow for a crash mat and harness as is recommended by our University sports science department to ensure safe testing.
3. It is advocated by the ACSM that cycle ergometry be used in patients as it is safer than treadmill testing.
4. In claudicants, a graded treadmill test is ideal to assess improvement in symptoms, but this may not identify changes in cardiovascular physiology if the test is terminated prematurely due to lower limb pain. It remains unclear whether walking to maximum pain threshold is equivalent to an individual's cardiac or respiratory maximum capacity. The appropriate CPET testing

modality for claudicants is contentious when assessing peak VO_2 and AT, as leg fatigue secondary to claudication may result in a potential underrepresentation of exercise tolerance.

Therefore a cycle test was opted for in preference to a treadmill test.

Means and standard deviations or standard errors of baseline values of claudicants were entered into the spreadsheet from either the paper directly or from responses received from authors. Where means and standard deviations were used, these were then transformed into standard errors to allow a pooling of the data.

A random effects model was applied to the data as we wished to apply this data to all claudicants and identify a generalisable baseline value for what the peak VO_2 is in this population as a whole.

A random effects model identified the mean peak VO_2 of patients with Fontaine II disease to be 14.75ml/kg/min (standard error 0.56; 95% C.I. 13.64-15.85).

1.4.3.2. Factors affecting peak VO2 and AT

Several studies identified cofounding factors, which influenced baseline peak VO_2 in claudicants. Factors associated with a significant reduction in baseline peak VO_2 included, in order of magnitude pain (peak VO_2 4ml/kg/min lower), smoking (peak VO_2 2.4ml/kg/min lower) or obesity (peak VO_2 1.7ml/kg/min higher). These studies were however non-randomised with only small sample sizes. In healthy, non-age or sex matched, individuals the peak VO_2 varied between 26.5-34.5ml/kg/min, whilst those reported for claudicants were significantly lower at 12.6 to 18.3ml/kg/min. However, it would appear from the inclusion criteria described in these studies, only the fittest of claudicants were selected, suggesting values in the general claudicant population may be much lower.

1.4.3.3. General improvement in peak VO2 following exercise

Studies focused on the effect of exercise in IC, and followed patients up at variable time points. The main follow up point was after a 12 week supervised programme. The results of studies analysing the effect of supervised exercise programmes on CPET outcomes in patients with IC are somewhat contradictory. Several studies have demonstrated an improvement in peak VO_2, generally in the order of 1 - 1.5ml/kg/min from baseline (Bronas et al. 2011; Treat-Jacobson et al. 2009; Allen et al. 2010; Langbein et al. 2002; Tew, Nawaz, Zwierska, et al. 2009b). However, other studies have failed to demonstrate any significant change in peak VO_2 with a supervised exercise programme, in some cases, despite an improvement in MWD (Hodges et al. 2007; Ritti-Dias et al. 2010; Crowther et al. 2008). There was variability in exercise programme duration and timing of follow up assessments between studies.

These results leave several important research questions unanswered. There is a paucity of convincing data to support the hypothesis that improvements in cardiopulmonary parameters contribute to the mechanism by which supervised exercise programmes improve outcomes in claudicants. In addition due to the "selection" of only the fittest claudicants in a majority of theses studies, their generalizability to the whole claudicant population should be questioned. Finally, given the current variability of exercise regimens in the treatment of claudicants, perhaps further research is required to analyse their intensity in order to maximise clinical effectiveness.

1.4.4. Relationship of exercise to other aspects of the athero-inflammatory cascade

Inflammation and atherosclerosis lead to the development of PAD. The use of exercise to ameliorate this cascade, the underlying mechanism and the speed at which this occurs, requires further exploration. Although we have cited reasons for

non-response to exercise; lack of motivation, genetic predisposition, or poor effort within the class; the time required to elicit a response must also be accounted for.

The majority of studies focus on twelve weeks of exercise, however some have focused on shorter durations in non-peripheral vascular disease cohorts. It may be that shorter periods produce some improvements, but not in all. Therefore the correlation between different testing modalities shall be explored as a potential surrogate. If one modality improves, it may indicate that others will follow.

1.5. Quality of Life

Quality of life has become an increasingly important aspect of chronic disease assessments. It allows an assessment of how a disease impacts on the patient's quality of life. Options for assessing patient concerns include qualitative semi-structured interviews (Egberg et al. 2012) and questionnaires (Langley 2004). This thesis focuses on the use of questionnaires for the assessment of quality of life. The use of generic questionnaires has allowed comparison between health and different disease states, these commonly use the EuroQoL 5D (EQ5D) or Mosby Short form 36 health survey (SF36) (Brazier et al. 1993). Use of the EQ5D also allows for the calculation of Quality adjusted life years and subsequent economic analysis (Miller 2005).

More sensitive, disease specific questionnaires are used for focused questions on specific conditions. In claudication these include the walking impairment questionnaire (WIQ) (McDermott, Guralnik, et al. 2008b), the Kings College VascuQoL questionnaire (VascuQoL) (Mazari et al. 2012).

The use of quality of life scales allows the assessment of the impact of a disease on an individual's physical and psychological wellbeing. The changes in quality of life attributed to claudication have in general been negative. A reduction in emotional scores with a higher incidence of depression and isolation mean that these patients

often require treatment for both a physical condition and subsequent psychological complications of isolation and embarrassment (Spronk et al. 2007; Breek et al. 2005).

In health, psychological improvements have been noted secondary to exercise in elderly cohorts (Conn 2010; Matthews et al. 2011; Aichberger et al. 2010) with an increase in positive constructs (happiness, vigor, self-esteem, self-efficacy, optimism and morale) over negative (depression, anxiety). This is mirrored by improvements in PAD quality of life secondary to exercise (Chetter, Spark, et al. 1997b).

Although benefits of exercise are apparent, attendance and compliance with supervised exercise programmes is variable. Cardiac rehabilitation programmes (Wyer et al. 2001) have shown that patients fall into one of three categories;

- Attendees; this group tended to see themselves as active and capable at managing their own condition (psychological model)
- Drop-outs (mixed model)
- Non-attendee who declines straight away; this group had a strong belief that recovery was a medical process that required input from the medical practitioner and medication (medical model). This group felt helpless and that the stress attributed to having the disease further confounded their own health.

1.5.1. Generic groups- control or SEP - Short Form 36 (SF 36)

The short form 36 (SF36) is well established as a valid (Jenkinson et al. 1994; Brazier et al. 1993; McHorney et al. 1993; Garratt et al. 1993; Brazier et al. 1992), reliable (Klevsgard et al. 2002) and responsive (Currie et al. 1995) generic QoL instrument which has been recommended for use in patients with claudication. It is a thirty-six item questionnaire with clusters to form eight health domains and two overall summary scores, one mental and one physical.

The eight domains are physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and finally mental health (MH).

Factor analysis performed in 1994 identified these eight domains to account for 80-85% of the variance within the data and items within each domain correlate ($r>0.4$). The questionnaire was revised in 2000 by Ware *et al* to reduce the ceiling and floor effect. The greater precision was afforded through the use of the improvement in the scoring algorithms (Ware & Sherbourne 1992; Ware 2000).

Test re-test reliability (Sechrest 1984) is a measure of how consistent a measure is between two time points and incorporates the possibility of errors due to fatigue, practice and genuine changes between testing intervals. It can be measured with cronbach's α as a measure of internal consistency. In the eight domains of the SF36, this has been noted to be high, ranging from 0.7 – 0.93. (See Table 9)

Validity is a measure that assesses the content of the questionnaire and its appropriateness, also known as face validity. Content validity is a measure by which each domain of the questionnaire is deemed to have sufficient questions focused on determining that the answer is for that domain (Sechrest 1984). The SF36 covers the main eight domains regarded when assessing quality of life. It does however lack focus and depth on specific conditions but retains its generic nature.

Table 9 Summary of SF36 v2 domains, summary component measures and mean scores

Domain	Correlates highly to:	Items	Mean (SD)	Cronbach's alpha
PF	PCS (r=.85)	10	84.2 (23.3)	.93
RP	PCS (r=.81)	4	80.9 (34.0)	.89
BP	PCS (r=.76)	2	75.2 (23.7)	.90
GH	PCS (r=.69)	5	71.9 (20.3)	.81
VT	MCS (r=.65)	4	60.9 (20.9)	.86
SF	MCS (r=.65)	2	83.3 (22.7)	.68
RE	MCS (r=.78)	3	81.3 (33.0)	.82
MH	MCS (r=.87)	5	74.7 (18.1)	.84
PCS	N/A	35	50.0 (10.0)	.92
MCS	N/A	35	50.0 (10.0)	.88

The use of quality of life of tools for the assessment of disease requires understanding of patient's perceptions. When the SF36 was used in a claudication cohort (n=201), it was demonstrated that all domains were reduced in comparison to the medical clinician's perception and understanding of intermittent claudication (Pell 1995). The improvement in functional walking distance (the point at which patients choose to stop walking) has been noted to be moderately correlated in five of the domains (Kruidenier et al. 2009), physical function ($r = 0.571$), role physical ($r= 0.532$), vitality ($r= 0.416$), pain ($r= 0.416$) and health change ($r= 0.414$, for domains stated, $p<0.05$).

1.5.2. Disease Specific - VascuQoL

The Kings College Vascular Quality of Life Questionnaire (VascuQoL) is a disease specific QoL questionnaire developed at Kings College Hospital, London, UK. The VascuQoL was originally developed to cover the spectrum of Fontaine II-IV disease allowing deterioration of disease specific quality of life to also be detected (de Vries et al. 2005; Mehta et al. 2006). Whilst the ideal claudication questionnaire remains contested (Chong et al. 2006; Golledge et al. 2006) the VascuQoL is a well validated (Golledge et al. 2006) and widely utilised questionnaire specifically for the claudicant population (Mazari et al. 2010b; Mazari et al. 2012; Mehta et al. 2006; Chetter et al. 1997a; Frans et al. 2012; Nordanstig et al, 2012).

The VascuQoL test retest reliability had an intra-class coefficient of 0.94 ($p<0.001$, CI >90%) (Morgan et al. 2001), and improves with walking distance giving it construct validity (the degree to which the questionnaire measured the characteristic being investigated; disease severity). The spearman's correlation coefficient for VascuQol in comparison to ICD and MWD were moderate and significant (Mehta et al. 2006) ($r= 0.554$ and $r= 0.637$; $p<0.001$ respectively). The construct validity for the VascuQoL was greater using the Fontaine classification across all domains (spearman's rank ranged from -0.63 to -0.79, $p<0.001$).

The VascuQoL is comprised of 25 questions, which have seven responses to each question. The resultant domains are activity, symptoms, pain, emotional and social. The scores are graded 1 (worst quality of life) to seven (best quality of life) (Morgan et al. 2001). The cronbach's α for each domain was 0.7-0.8, allowing the final 25 items to remain in the questionnaire. When comparing the VascuQoL to the SF36, the physical domains of pain and activity were highly correlated to PF and RP ($r=0.69$ and 0.56 $p<0.001$), as were the emotional domains with RE ($r=0.66$ $p<0.01$) (Morgan et al. 2001).

The VascuQoL is able to distinguish between differing levels of physical functional ability as determined by the American Medical Centre linear disability scores (ALDS), $r=0.64$, $p<0.03$ with both being able to distinguish between claudication and critical limb ischaemia (Met et al. 2009).

An improvement in VascuQoL was noted to moderately correlate ($r=0.61$, $p<0.001$) (Roberts et al. 2008) with improvements in walking distance secondary to unsupervised regular exercise (one hour of daily walking for 12 weeks).

1.5.3. Cost effectiveness of treatment for IC

The use of generic quality of life questionnaires (SF36 and EQ5D) has been used to identify the change in claudicants' quality of life after treatment (Currie et al. 1995; Bosch & Hunink 2000). The use of QALY's to assess cost effectiveness means that NICE is provided with a standard against which new treatment strategies can be compared to current practice. The threshold set by NICE is approximately £20,000-£30,000 per QALY (Devlin & Parkin 2004).

To determine cost effectiveness, papers have used a Markov decision analytical model of options compared to conservative management (not including exercise). The model focused on treatment strategies for first time presentations of a single >50% stenosis, either infra or supra inguinal distribution, and severe claudication in a

60 year old man. In the model, a Monte Carlo simulation was performed multiple times (n=100000).

1.5.3.1. Exercise versus Invasive Treatment options

Two papers from the Netherlands have produced cost effectiveness data on the treatment of severe new onset claudication. The 2001 paper identified costs to be lower when treatment strategies of exercise alone were used (less than $20,000); however this also had the lowest QALY gain (6.05) which equated to $3,500 per QALY (de Vries et al. 2001). Over the $20,000 threshold, both exercise and angioplasty combination had a QALY gain (6.15) associated with a modest increase in cost (approximately $22,000). The inclusion of surgery however increased the cost to approximately $45,000 for a QALY gain of 6.23. The incremental cost effectiveness ratio therefore rose dramatically to $63,000/QALY for SEP and PTA combined, and higher still for the inclusion of surgery ($230,000/QALY).

The 2003 paper focused on the cost effectiveness of investigations (no investigation vs MRI vs Duplex ultrasound) and treatment strategies (exercise, angioplasty or bypass surgery). They identified that although no test and an exercise prescription was the cheapest option, this was also the least cost-effective (QALY gain of 6.06 at a cost of €6793). The use of a duplex ultrasound followed by either angioplasty or exercise was deemed to be the most cost effective with an incremental cost effectiveness ratio of €20,000/QALY) (Visser et al. 2003).

It is important to remember that in both instances, extrapolation of the cost effectiveness for bilateral claudicants cannot be made and requires a separate model.

A 2008 study from the Netherlands performed a prospective RCT of 151 participants with claudication (Spronk et al. 2008). Lesions at either the SFA or iliac were included, and subjects were randomised to either exercise or angioplasty. Follow up data for one year was obtained and QALY's were calculated. This study demonstrated that by one year, there was no significant difference in either the

quality of life scores, walking distance or QALYs. The costs following revascularisation were higher than that of twice weekly hospital based exercise class undertaken for 24 weeks.

A UK trial, which focused on superficial femoro-popliteal region disease only, performed an economic analysis on the use of angioplasty versus SEP or combination treatment. The use of SEP (€6147.04/ QALY) or SEP and PTA (€10,649.74/ QALY) in combination was deemed most cost effective when compared to PTA alone (€11,777.00/ QALY). This data is unique as it is the largest randomised controlled trial of SEP, PTA and combination treatment in femoro-popliteal disease (Mazari et al. 2013).

1.5.3.2. Different Exercise Treatment options

When focusing on the type of exercise programme used, the costs associated with a home based programmes with hospital appointments to appraise progress, has been explored. However, this Italian retrospective study from 2011 failed to randomise individuals to their home-based programme versus a full hospital based SEP. Therefore the no true cost effectiveness data was present (Malagoni et al. 2011).

Home based exercise programmes with weekly contact by either telephone or lectures from a health professional have recently been compared to fully supervised programmes. A recent systematic review has identified that although home-based programmes improve walking distance over usual care, the improvement is modest in comparison to SEPs (Al-Jundi et al. 2013). The Exercise therapy in PAD (ExitPAD) study randomised 304 participants to receive either walking advice, SEP or SEP with feedback (van Asselt et al. 2011). The cost associated with a SEP was €3407 (mean QALY 0.71), compared to €2304 for walking advice (mean QALY 0.67). Whilst SEP was more costly than WA, it was also deemed to be more effective. Further data is however required to support these findings and provide a

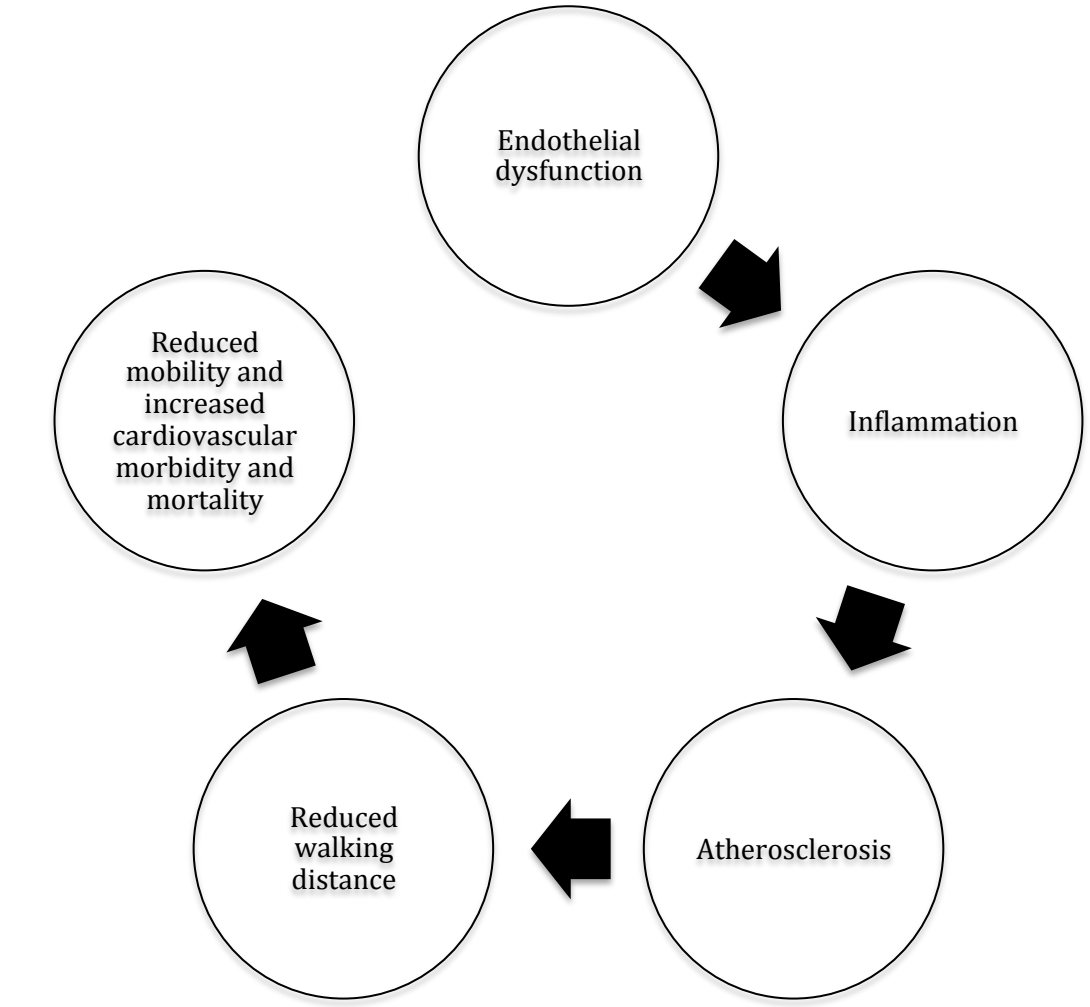
clear understanding of the cost effectiveness of walking advice compared to SEP and supported home based exercises.

1.5.3.3. *Cost effectiveness of Angioplasty and Stents*

Iliac stenting was identified to be less cost effective than exercise after 6 months with a gain in absolute cost effectiveness ratio of $19/m (Treesak et al. 2004). When angioplasty is advocated, the use of whether to routinely stent versus selective placement has also been questioned. Stenting during iliac PTA has been deemed cost-effective over never placing stents (Bosch et al. 2000), with selected stenting also being advocated through the use of modelling. Whilst iliac disease is favoured for selective stent placement, more evidence is required for femoro-popliteal disease (Greenberg et al. 2004; Dosluoglu et al. 2009). A recent non randomised trial of single long stents compared to multiple stents was inconclusive due to methodological flaws (Matsumura et al. 2013).

1.6. Summary

The overall aim of this research is to quantify whether six weeks of exercise will improve cardiopulmonary physiology (see Figure 12).



Can exercise change this cycle?

Figure 12 Summary diagram of research

2. HYPOTHESIS

The overall objective was to determine whether six weeks of supervised exercise was sufficient to improve walking distance, quality of life, cardiac physiology, inflammatory profiles and endothelial function in patients with claudication.

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2.1. Primary Outcomes

Our primary outcome was to identify whether a six week exercise program improved peak VO_2 and anaerobic threshold levels as measured by cardio-respiratory exercise testing.

Although 6 weeks is only half of the recommended program length, previous studies have used this time point (Gremeaux et al. 2010; Sutbeyaz et al. 2010; Kothmann et al. 2009) and found it to provide benefit.

2.2. Secondary Outcomes

We aimed to investigate and document changes following the enrolment in an exercise class for 6 weeks and at 12 weeks in the following secondary outcome measures;

a. Cardiac/Respiratory markers
    i. Resting heart rate
    ii. Maximum Heart rate
    iii. Number of co-morbidities

b. Athero-inflammatory markers
    i. IL-6
    ii. TNF alpha
    iii. High sensitivity C-Reactive Protein

c. Endothelial function
    i. Reactive Hyperaemia Index (RHI) Score
    ii. Augmentation Index

d. Physical functional ability
    i. Timed Up and Go Test
    ii. Chair Stand Time

e. Measures of Lower Limb Ischaemia
    i. Pre & Post exercise Ankle-Brachial Pressure Index (ABPI)
    ii. Treadmill Initial claudication distance
    iii. Treadmill Maximal walking distance

f. Quality of Life
    i. Generic (Short form 36)
    ii. Disease specific (VascuQoL)

Was there a difference over time (baseline, 6 weeks, 12 weeks) when looking at the above outcome measures?

Did walking distance or physical function improve at the same rate as cardiopulmonary exercise physiology parameters? (Namely AT and peak VO2.)

Could these values be utilised to predict who would benefit from exercise? I.e. responders and non-responders to exercise.

3. METHODS

3.1. Ethics

Ethical approval was sought and obtained from the Yorkshire & Humber Research ethics committee; reference number 07/Q1105/12. NHS Research & Design approval was granted from the Hull and East Yorkshire R & D unit.

3.2. Study Design

This was a prospective observational study conducted at Hull Royal Infirmary, Hull HU3 2JZ.

3.2.1. Inclusion Criteria

Community dwelling older adults aged 45 and over

Diagnosis of intermittent claudication – ABPI < 0.9 with symptoms in keeping with intermittent claudication

Willing to partake in 6 weeks of exercise

Ability to walk unaided

3.2.2. Exclusion Criteria

Participants who are unable to provide informed consent

Severe or acute cardiovascular, musculo-skeletal or pulmonary illness

Critical limb ischaemia

Active treatment for cancer

3.3. Recruitment

3.3.1. Patient selection

Consecutive patients with proven arterial lower limb insufficiency referred for SEP were invited to participate in the study. All patients identified through claudication clinics or who were referred from the consultant, were sent a patient information sheet, detailing the aims of the study. After a week, the research fellow Miss Risha Gohil (RG) contacted patients, to see if they were interested in participating. RG then recruited individuals into the study following informed written consent.

3.3.2. Power calculation

There is limited data available on changes in peak VO_2 in claudicants. Based on the paper by Wang et al (Wang et al. 2008):

At 80% power and 5% significance, 32 patients were required to detect a difference of 1.5ml/kg/min peak VO_2 over time. Allowing for a 10% withdrawal and 10% loss to follow up rate, it was estimated that 38 patients would be required to demonstrate significance in the primary outcome measure.

3.3.3. Study duration and expected rate of recruitment

With adjustment for dropouts, an anticipated recruitment rate of 4-5 patients per month was expected. We therefore expected to attain our goal of 38 patients by 10 months.

3.4. Testing schedule

All tests were carried out pre-intervention, 6 and 12 weeks post intervention. Patients underwent two testing sessions at each time point, with each lasting for approximately 2 hours. To ensure that circadian rhythm did not introduce variability,

each participant underwent 6 week and 12 week testing at the same time as their baseline visits were performed. All tests were carried out in the same order for sessions one and two, to reduce variability between patients, and time points.

3.4.1. Session 1

Demographic data was collected and patients underwent a maximal, constant load treadmill test with pre and post ABPI's.

3.4.1.1. *Demographic data*

Initially, a brief interview was performed with each patient to ascertain the following information; patient's age, sex, height (to the nearest 1mm) and weight (to the nearest 0.1 kg) were recorded. Comorbidities, past medical and surgical history, and current medication were also recorded.

3.4.1.2. *Clinical Measures of Physical Function*

Following this, clinical markers of physical function were measured to provide objective evidence of mobility and strength. Markers included:

Timed Up and Go Test (TUG).

The timed "Up & Go" test measured, in seconds, was the time taken by an individual to stand up from a standard arm chair (approximate seat height of 46 cm, arm height 65 cm), walk a distance of 3 meters (approximately 10 feet), turn, walk back to the chair, and sit down again (Podsiadlo & Richardson 1991) (See Figure 13).

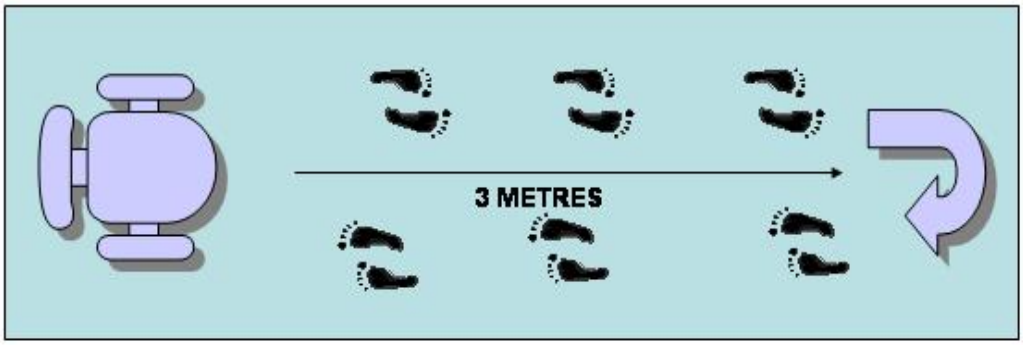

Figure 13 Picture demonstrating the Timed Up and Go (TUG) test

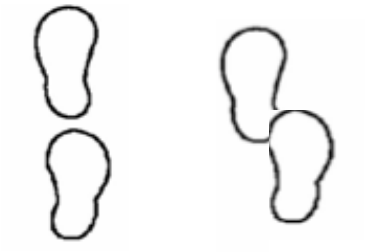

Figure 14 demonstrating the Full Tandem Stance (FTS) on the left and the Semi Tandem Stance (STS) on the right

Repeated Chair Rises; Chair Stance Time (CST)

This test measures leg strength and balance. Participants will sit in a straight-backed chair with their arms folded across their chest and will be asked to stand 5 times consecutively as quickly as possible. The time to complete 5 chair rises will be measured in seconds (Shimada et al. 2011). The participant has a three minute rest period, the test is then repeated. The mean time is used.

Standing Balance

Participants will be asked to hold 3 increasingly difficult standing positions for 10 seconds each: standing with feet together side-by-side and parallel (side-by-side stand), standing with feet parallel with the toes of one foot adjacent to and touching the heel of the opposite foot (semi-tandem stand), and standing with one foot directly in front of the other (tandem stand) (Guralnik et al. 1994). (See Figure 14).

3.4.1.3. Clinical Indicators of Lower Limb Ischaemia

Finally, clinical markers of lower limb ischaemia were measured to provide objective evidence of peripheral arterial disease at baseline and as a screening tool to ensure correct identification of claudicants. Patients were asked to lie down with their shoes and socks removed and their trousers raised up to the knees. A brief neurological assessment was performed to assess strength, tone and power of lower limbs. Following this, resting blood pressures were measured in all four limbs and at both the dorsalis pedis and posterior tibialis.

Claudicants were asked to walk for a maximum of five minutes on a fixed constant load (10% incline) treadmill for 5 minutes at a speed of 2.57km/hr. The resting ankle brachial pressure index (ABPI Re) was measured using a standard sphygmomanometer and a handheld Doppler flow detector with an 8-MHz probe (Huntleigh Technology plc, Cardiff, UK).

Initial claudication distance (ICD) was the distance at which an initial ache or discomfort was felt, this was recorded in seconds on the treadmill and then converted to metres. The time of test termination and reasons for this were also recorded. The length of walking in seconds was then converted to metres and recorded as the maximal treadmill walking distance (MWD), this was recorded up to a maximum of 207m (equivalent of 5 minutes walking). Following treadmill testing, post exercise ABPI (ABPI-Pe) were re-measured in each limb. Pre exercise ABPI levels 0.3>x< 0.9 or evidence of drop in ABPI secondary to exercise resulted in inclusion in the study.

Patient reported walking distance (PRWD) was recorded to a maximum of 1000m.

3.4.2. Session 2

Session 2 lasted two hours, during this time participants underwent a measure of endothelial function, venepuncture and cardiopulmonary exercise testing, always in the aforementioned order.

3.4.2.1. *Endothelial function*

Background

Endothelial function was measured using the 'EndoPAT2000' machine. The EndoPAT2000 measures the peripheral arterial tone (PAT) signal at the fingertips in response to reactive hyperaemia.

The EndoPAT measures endothelium-mediated changes in vascular tone using a unique bio-sensor placed on the fingertips (see Figure 15). It measures the change of digital pulsatile volume at the fingertip with non-invasive single use probes. The probe utilised is a finger plethysmograph that produces uniform pressure to the distal 2/3rds of the finger. This allows for a better attachment to the finger and a continuous recording of the digital arterial pulse wave.

The changes in arterial tone are elicited by creating a down-stream hyperaemic response. This is induced by a standard 5-minute occlusion of the feeding brachial artery (using a standard blood pressure cuff). When the cuff is released, the surge of blood flow causes an endothelium-dependent Flow Mediated Dilatation (FMD). The dilatation in the brachial artery leads to a reactive hyperaemia, which manifests as a bright red arm and change in amplitude on the computer screen for the occluded arm. The increased amplitude is captured as an increase in the peripheral arterial tone (PAT) signal amplitude. The EndoPAT software automatically calculates a post-occlusion to pre-occlusion ratio. From this, the software determines the reactive hyperaemia index (RHI) and the augmentation index (AI). Measurements from the contra-lateral arm are used to control for concurrent non-endothelial dependent changes in vascular tone.

All measurements were recorded anonymously and analysed automatically by an algorithm; thus eliminating intra and inter-observer variability or need for operator interpretation (Selamet Tierney et al. 2009). An index score ≤ 1.67 was taken from the literature to represent endothelial dysfunction (Sobieraj et al. 2012; Peled et al. 2008; Yinon et al. 2006).

Figure 15 EndoPAT finger-tip probe to assess capillary bed dilatation

Testing protocol

Participants were asked to lie supine, and quietly rest for 15 minutes in a thermo-neutral environment prior to testing. Patients were requested to refrain from smoking or heavy exercise in the preceding 24 hours and to have omitted their alpha and calcium channel blockers, but to bring them to the assessment so they may take them after this. They were also asked to refrain from food for the preceding 3 hours. As non-compliance with this was an issue, patients were also asked on arrival when their last cigarette was.

Participants were asked to remove any restrictive jewellery, watches or clothing over the arms, wrists or fingers to prevent venous pooling at these sites, which would create spurious results. Hands were elevated to the level of the heart and supported in this position. The index finger on each hand was the finger of choice, with a blue anchor applied to the middle finger. If there was any injury to that finger, which would prevent its use in the measure of endothelial function, the middle finger of both hands were chosen for the EndoPAT probe and the anchor was applied to the ring finger. They were instructed to try and keep their probes free from contact from any other objects (including the support surface). The blue foam anchor rings was placed proximal to the proximal inter-pharyngeal joint (PIPJ). These anchors act as a support to the tubing from the probes, which lie on the test and control fingers during the procedure. This prevents any artefact from the tubing during the test. The machine works by measuring the change in the capillary beds at the finger tips in patients. A blood pressure cuff is placed on one arm and the other side acts as the control arm (see Figure 16).

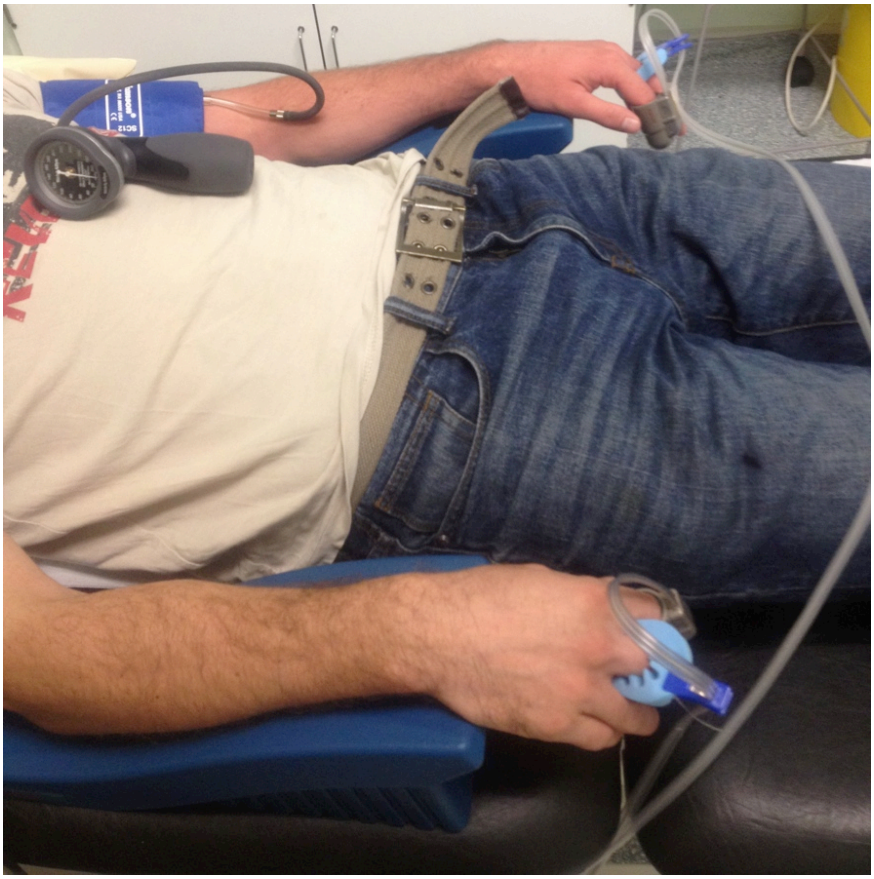

Figure 16 Photo demonstrating the difference between the left test arm, which sustained a period of ischaemia, and the right control arm

The left arm demonstrates reactive hyperaemia with a bright red arm compared to the control (right arm).

After a 5 minute stable baseline recording, the machines settings were altered to amplify the gain up to 20,000. The baseline reading required five minutes, a subsequent 5 minutes with the cuff inflated occluded the blood flow to the arm and allowed for a measure of reactive hyperaemia over the 5 minutes post occlusion to be measured. The test arm was occluded rapidly using a blood pressure cuff inflated between 200-300mmHg for 5 minutes. The initial occlusion was 200mmHg, however any evidence of an incomplete arterial occlusion meant that additional cuff inflation was undertaken to 250mmHg and then up to a maximum of 300mmHg. A ratio of the pre and post occlusion score is standardised to the other arm, which has been acting as the control. The generation of an automatic EndoPAT score removes the question of operator variability and reliability (see Figure 17).

Exclusion criteria for the use of the EndoPAT included patients receiving glycero tri-nitrate (GTN), calcium channel blockers or alpha blockers within the preceding 24 hours. If a test was deemed to have failed due to operator problems, a repeat test was not undertaken for a minimum of an hour. At this point the control arm was used as the new test arm. A repeat test was never required during the duration of this thesis.

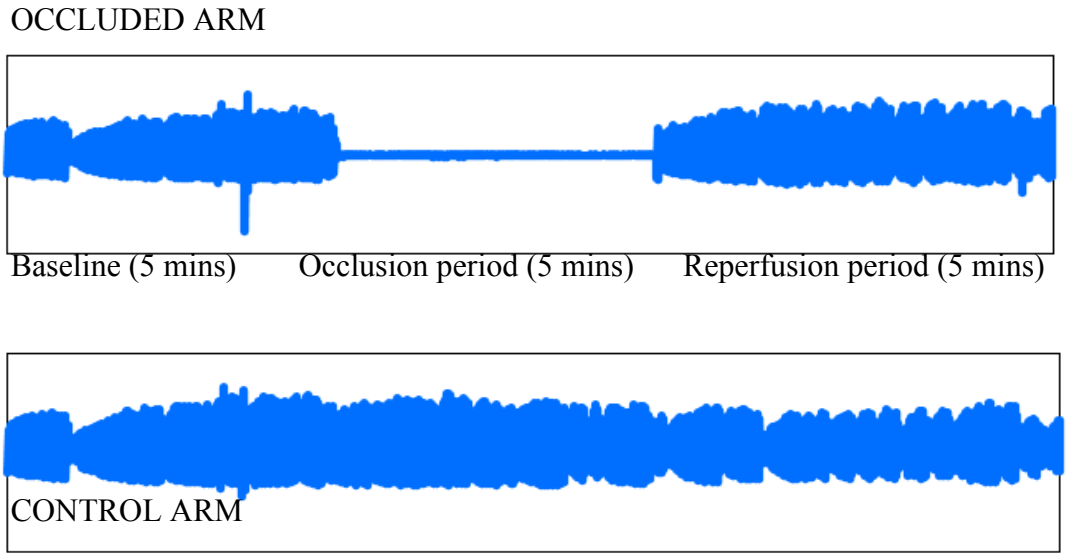

Figure 17 Illustration of the output derived from the EndoPAT machine when calculating the RHI and AI

3.4.2.2. Venepuncture

The non-occluded arm was then occluded using a tourniquet. A sterile alcohol wipe was applied to clean the skin and a 21g green needle was inserted into the antecubital fossa and 10mls of blood taken using a closed vacutainer method. Two yellow serum (gel clot activator) bottles and a purple Ethylene-diamine-tetra-acetic acid (EDTA) bottle were drawn. The two yellow bottles were anonymised and left to stand to clot for 20 minutes. The purple bottle was labelled with patient identifying data and processed within the Hull Royal Infirmary haematology laboratory.

Blood samples were taken at baseline, 6 weeks and 12 weeks to identify if any change occurred with exercise. Tubes were then spun at 45,000rpm for 15 minutes to separate the serum, which was placed in 0.5mls aliquots and stored at -80^{0}C within 24 hours of receiving the sample; the remaining blood was discarded.

The option had originally occurred of taking blood samples prior to undergoing the EndoPAT testing. By taking the blood samples first, there was concern about whether this would affect the integrity of the vessel wall and result in spurious results from the EndoPAT. The alternative was to take samples following the period of reactive hyperaemia. As the non occluded arm was used as the baseline comparator against which the occluded arm was tested, it was felt to be the most steady state option. It is however acknowledged that we do not know how much of a systemic effect occurs secondary to the five minute occlusion period, nor do we know the effect on concentrations of CRP, IL-6 or TNF alpha.

3.4.2.3. Cardio-Pulmonary Exercise Testing (CPET)

CPET cycle ergometry was undertaken post venepuncture. A wireless ECG was connected to the patient, the equipment calibrated and the patient was provided with instructions on how to complete the test.

Calibration of machinery for CPET

Vacuum calibration

The machine requires a two minute period during which time the vacuum pump turns on and self-calibrates. In order to prevent "sensor drift," the vacuum pump turns off if there is longer than forty minutes of inactivity with the CPET software.

Volume calibration

The CPET breath-by-breath analysis is set to detect a maximal volume of 3L. This volume is calibrated against the room temperature and humidity. A 3L syringe is used to pump 3 litres of air past the flow analyser five times (see Figure 18). This is required twice a day to account for any changes in atmospheric pressure.

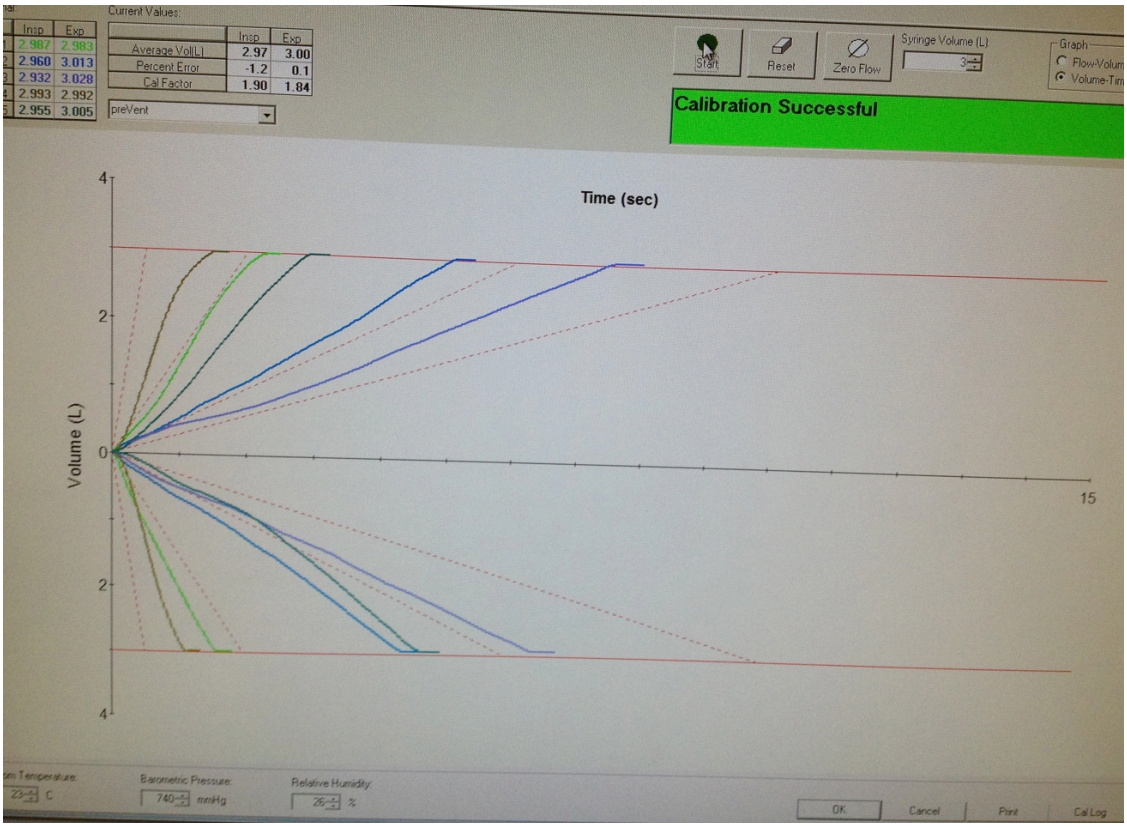

Figure 18 Picture of volume calibration

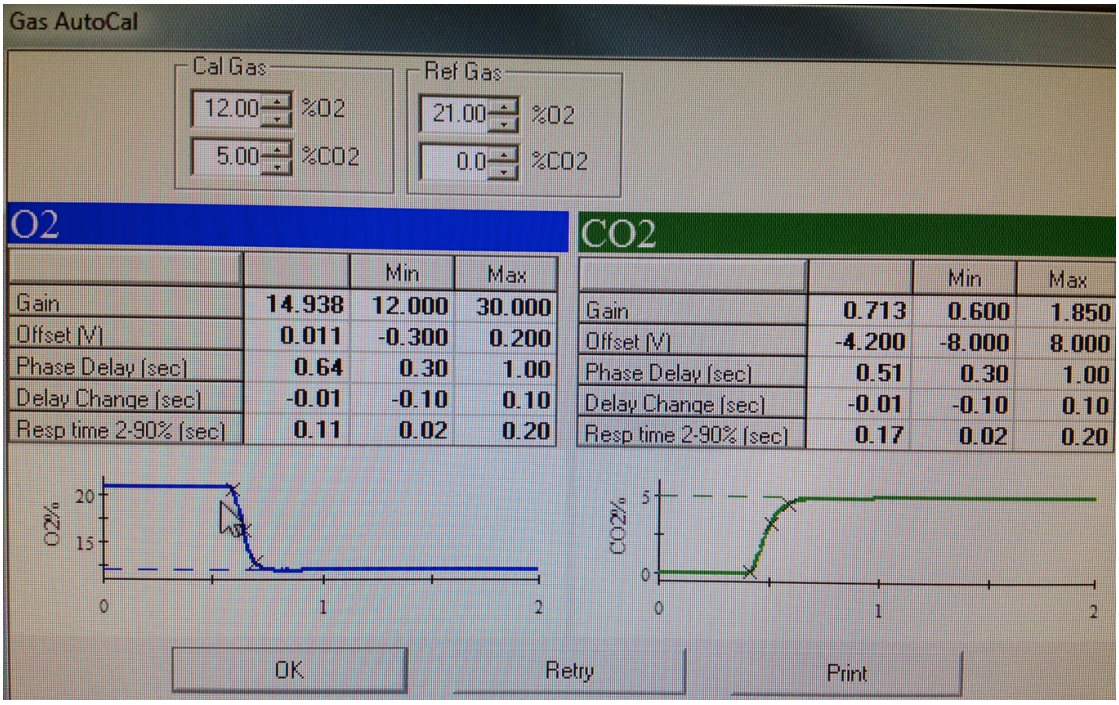

Figure 19 Photo of Gas Calibrations screen

Gas auto-calibration

The breath-by-breath analysis requires the sensor to be calibrated against standard concentrations of carbon dioxide and oxygen. This initial calibration is to calibrate the system to the surrounding air concentrations and against a gas cylinder of each. The CPET gas analyser requires calibration in between each patient test to re-zero the sensor against the environment rather than the previous patient (see Figure 19).

Instructions to patients prior to testing

Anaerobic threshold and peak VO_2 were identified by breath-by-breath analysis of expired air from a tight fitting facemask. To ensure accurate values were detected, a well-fitting mask placed over the mouth and nose was vital. Any air leaks would result in reduced quality data.

In addition, patients were asked to conduct the test in silence. Talking, laughing, coughing were all detected as a higher level of CO_2 expenditure and resulted in an earlier detection of AT and peak VO_2 levels. To prevent this "background noise" from giving patients false low readings, they were advised to communicate via hand signals. Gestures included "a thumbs up" for "I'm fine" (see Figure 20a), flat spread out palm for so-so (see Figure 20b) and tapping on the bike handlebars for "I wish to stop" (see Figure 20c).

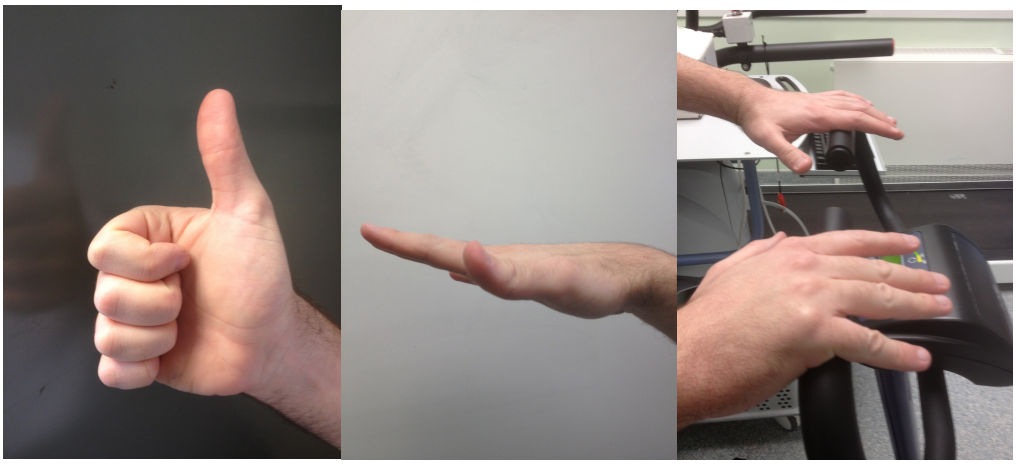

Figure 20 Hand gestures for CPET

a. Thumbs up

b. Hand flat for “so, so”

c. Hands banging on the handlebars for “I wish to stop now”

Safety

During all exercise tests, recruits were monitored by continuous wireless ECG. The test was discontinued if patients developed any ECG evidence of ischaemia (ST elevation > 1mm; or depression >2mm), shortness of breath, dizziness or chest pain.

3.4.2.4. Cycle Ergometry

Bicycle seat height

As the populations height and leg length vary, the height of the bicycle seat requires standardisation. If a seat is too short, the angle at the knee will be more than 5^{o} and result in a less economical movement and a lower AT and peak VO_2 reading. The seat height was adjusted to be in line with an individual's hip height. They were then asked to sit on the bike and fully extend one leg, if the angle at the knee demonstrated any flexion, the seat height was raised until this was eliminated. The seat height was then measured between at two fixed points to ensure subsequent tests were performed at the same height, providing a standardised, repeatable test.

Mask error

The tight fitting facemask or mouthpiece both attach to the sterile pneumotachs. The mouthpieces require a nose clip and for the patient to insert the mouthpiece as they would a snorkel, which can be uncomfortable, difficult for those with a small mouth and unsanitary as the individual is not able to swallow. (All saliva falls forwards through the mouthpiece into a "saliva trap", once this is full, it then falls onto the patient.) In general, this has been poorly tolerated during initial tests in healthy individuals; therefore the facemask has been used preferentially.

The facemask does however have its own drawbacks; primarily that of over breathing as people can feel trapped or that they are suffocating. The subsequent hyperventilation can result in artificially low AT and peak VO_2 readings. To combat

this, individuals had the facemask applied and were then asked to sit quietly for up to two minutes. During this time, the machine was able to continuously perform breath-by-breath analysis to allow adjustment to the facemask and prevent hyperventilation, which in turn allows the RER to lower.

Bike cadence

Recruits underwent a 10-15 minute test on the upright cycle ergometer (see Figure 21). They were asked to pedal at a constant maximum cadence of 60rpm. After two minutes of freewheeling (no resistance), a standardised incremental ramp of 10W/minute was commenced until patients reached their anaerobic threshold (AT), pain threshold (PT) or sub-maximal VO_2. The test was discontinued if their pedal cadence dropped below 40rpm. After completion of the test, the ramp was turned off and patients were advised to continue freewheeling for a further minute. After this time the exercise test was completed and the individual was allowed to rest. The ECG monitoring was stopped once parameters (heart rate and any presence of ST changes) had returned to normal.

Figure 21 Person undergoing a Cardiopulmonary exercise Test on the Upright Exercise Bike

Assessing the data

Gaskill method

The Gaskill method is the standard method of assessment for anaerobic threshold and peak VO_2. It uses 20 second breath by breath averaged data to smooth the data. From this data three graphs are drawn, which are then placed in line to provide the AT.

Winbreak 3.2

Software is available which can smooth the data and exclude outliers. From this data, 20 second averaged data can be calculated. This averaged 20 second data was then used to draw three scatter graphs to determine anaerobic threshold. All scatter graphs had VO_2 along the x-axis. Along the y-axis, the first graph was VCO_2, the second graph was excess CO_2 and the final was VE/VCO_2 (see Figure 22).

Excess CO_2 was calculated as follows: Excess $CO_2 = [(VCO_2)^2/VO_2] - VCO_2$

The change in gradient of each graph was identified and the inflection points were compared to identify the VO_2 closest to all three of these points. This process was undertaken by two independent assessors to determine anaerobic threshold (see Figure 23).

VO_2 measurements were anonymised and then assessed independently by RG and AM (a sports science physiologist). Where the two assessors differed by more than 3%, a third assessor (sports science physiologist- LI) was asked to review the data to give a final opinion. Where all differed, a mean of all three values was given.

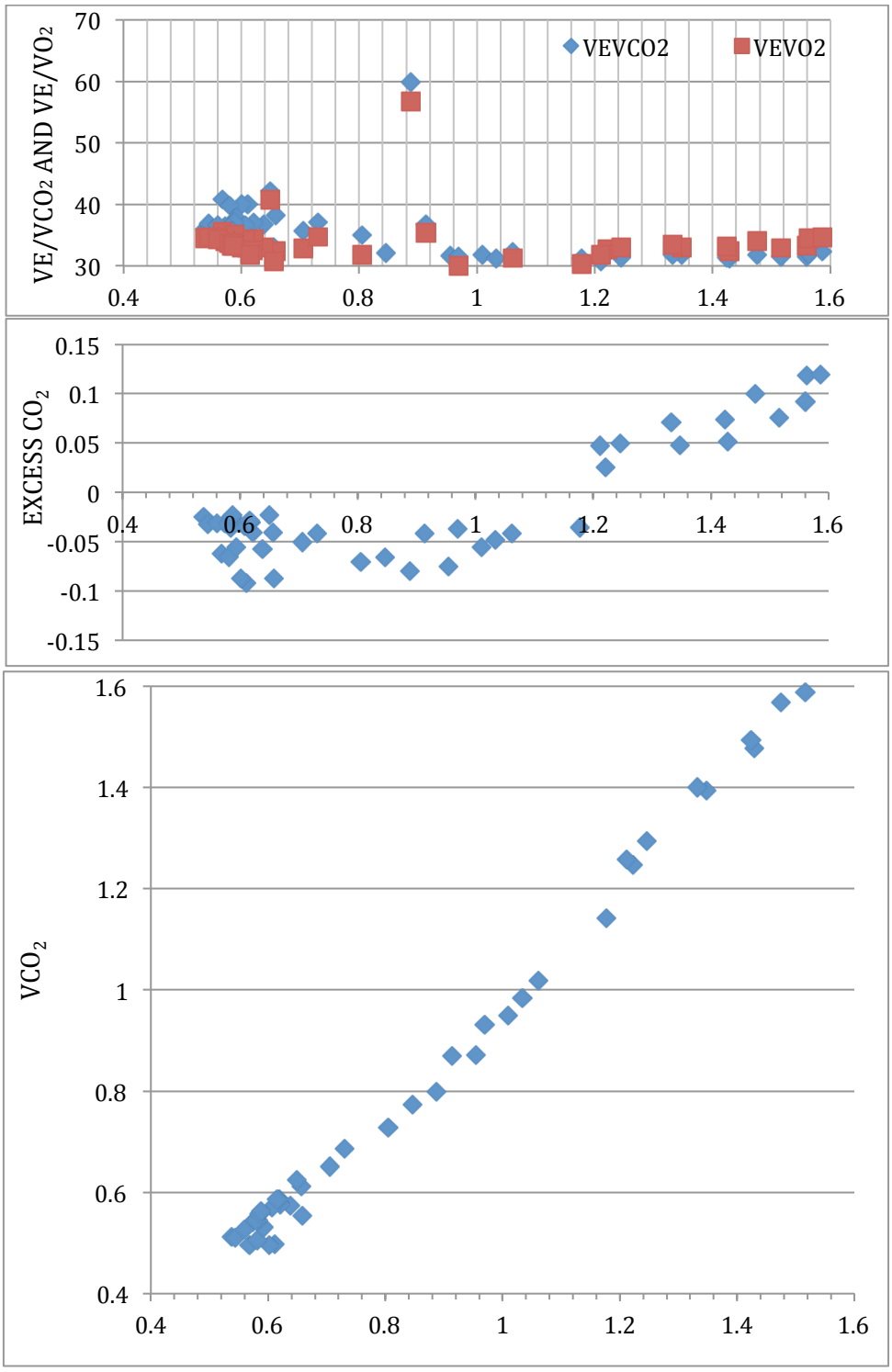

Figure 22 Diagram to show the three graphs required to identify changes in the respiration, which are indicative of the anaerobic threshold

Anaerobic Threshold VO_2 (ml/min)

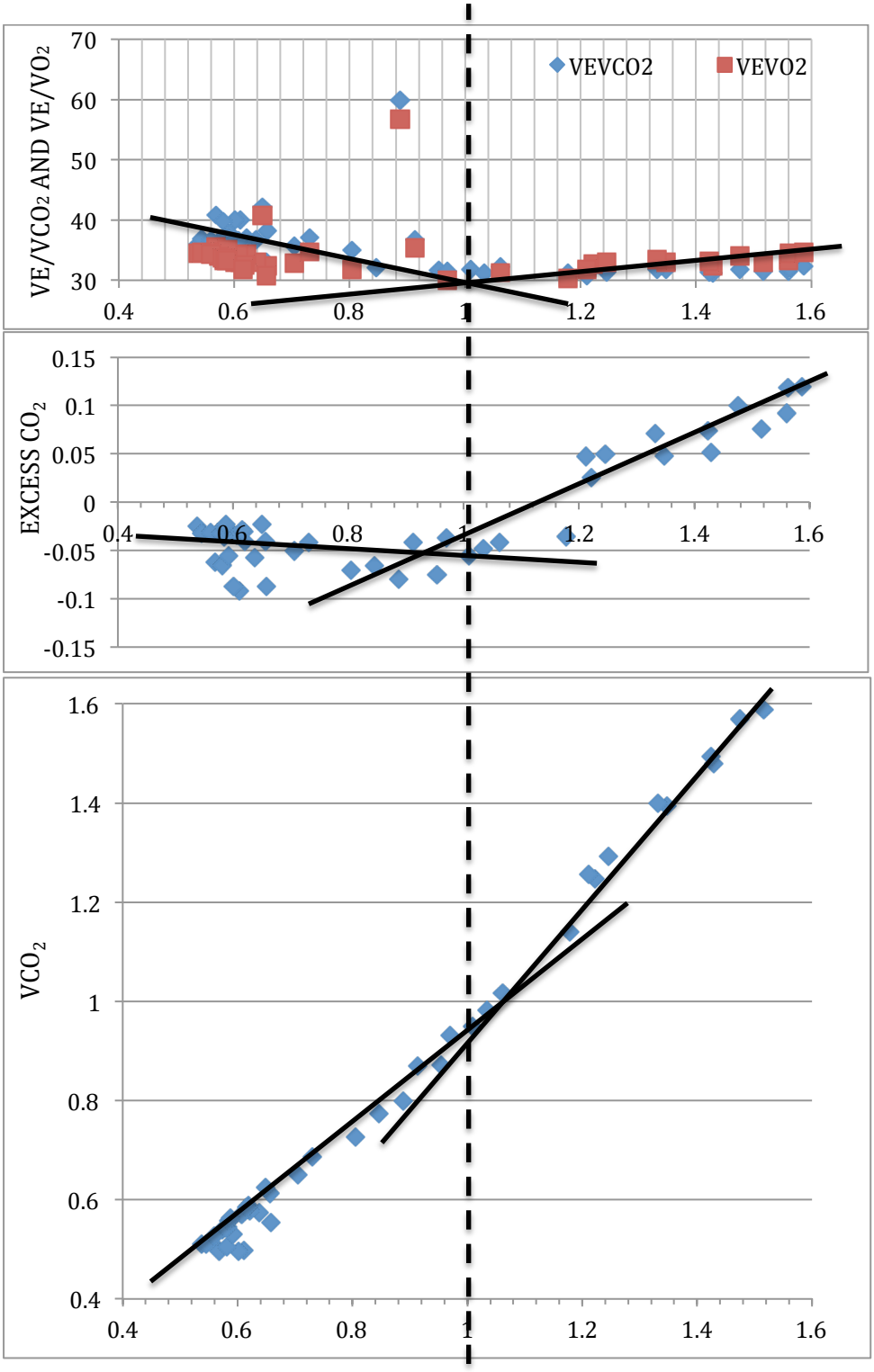

Figure 23 Diagram to show the three graphs required to identify changes in the respiration, which indicates the AT (the solid black lines and single dotted line)

3.4.3. Inflammation

Serum from patients stored at -80°C were defrosted and analysed to assess for the concentration of the following inflammatory markers; high sensitivity CRP, IL-6 and TNF alpha. These cytokines were measured by enzyme linked immuno-sorbant assay (ELISA) techniques (Palmer-Kazen et al. 2009). All ELISA's were performed at the University of Hull, Hardy building under the supervision of L Madden.

3.4.3.1. *Enzyme linked Immuno-sorbant assay (ELISA)*

In brief, the ELISA technique measures the concentration of a protein through the density of colour it produces when bound to chromagens.

The cytokine undergoes numerous incubation steps with antibody substrates to which it binds. These are either bound to a colourless dye or are required to amplify the concentration of the cytokine so that it may be detected. In the final step once all incubations have been performed, the colourless complex is incubated with an enzyme, which converts the solution to a blue colour.

The intensity of the blue staining is determined through the use of an optical density plate reader. Control wells of known concentration are then used to determine the linear relationship between protein concentration and optical density. This allows a quantification of the protein content from each sample.

Method

Stored serum was defrosted in room temperature water baths. Once defrosted, samples were diluted to 1:500 with assay buffer to make a 500ul solution. Each sample was run in duplicate to allow mean absorbance to be calculated.

Pre-coated 96-well plates were washed with 400ul washing buffer to remove any pellet residue and reduce error secondary to well variation. 50ul of distilled water was added to each sample well along with 100ul of the sample. The control wells contained 50ul of distilled water instead of the sample. The plate was covered and incubated with the serum for 2 hours at room temperature with gentle agitation (100rpm).

Wells were emptied and re-washed with 400ul wash buffer per well. This was performed three times followed by inversion onto a drying strip for approximately 5 minutes. Wells were then incubated with 100ul of TMB substrate solution and incubated for a further 10 minutes in the dark, at room temperature with gentle agitation (100rpm).

When the colour started to develop in the plates, this was monitored to ensure that only the highest standard reached a dark blue (a fully saturated colour). At this point, 100ul of stop solution was pipetted into each well (see Figure 24). The colour intensity was measured using a spectro-photometer, which had a primary wavelength of 450nm and reference wavelength of 620nm (610-650nm).

Interpretation

As the samples were diluted to 1:500, the standard curve must be multiplied by the dilution factor to give a true reading. The average absorbance values were calculated for each plate based on the duplicate standards. A standard curve (see Figure 25), of mean absorbance for the standard wells is plotted against the CRP protein concentration. From this a line of best fit was drawn, and subsequently used to calculate sample CRP concentrations according to their mean absorbance value (ebioscience 2013).

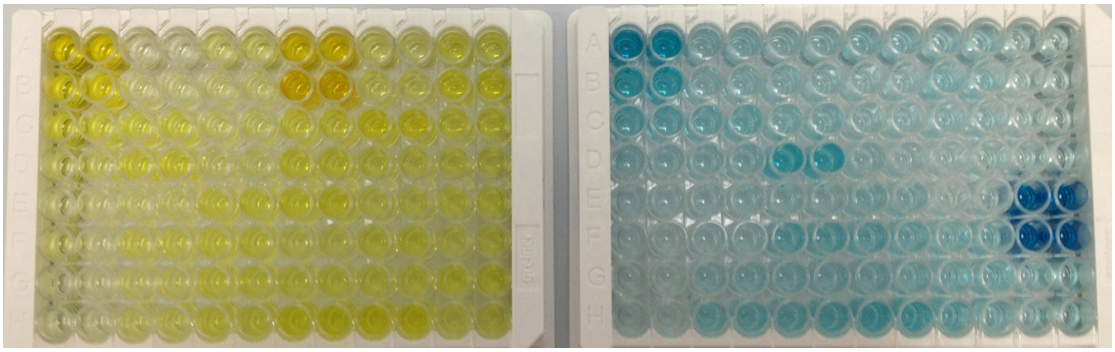

Figure 24 ELISA plates demonstrating the colour change from yellow to blue

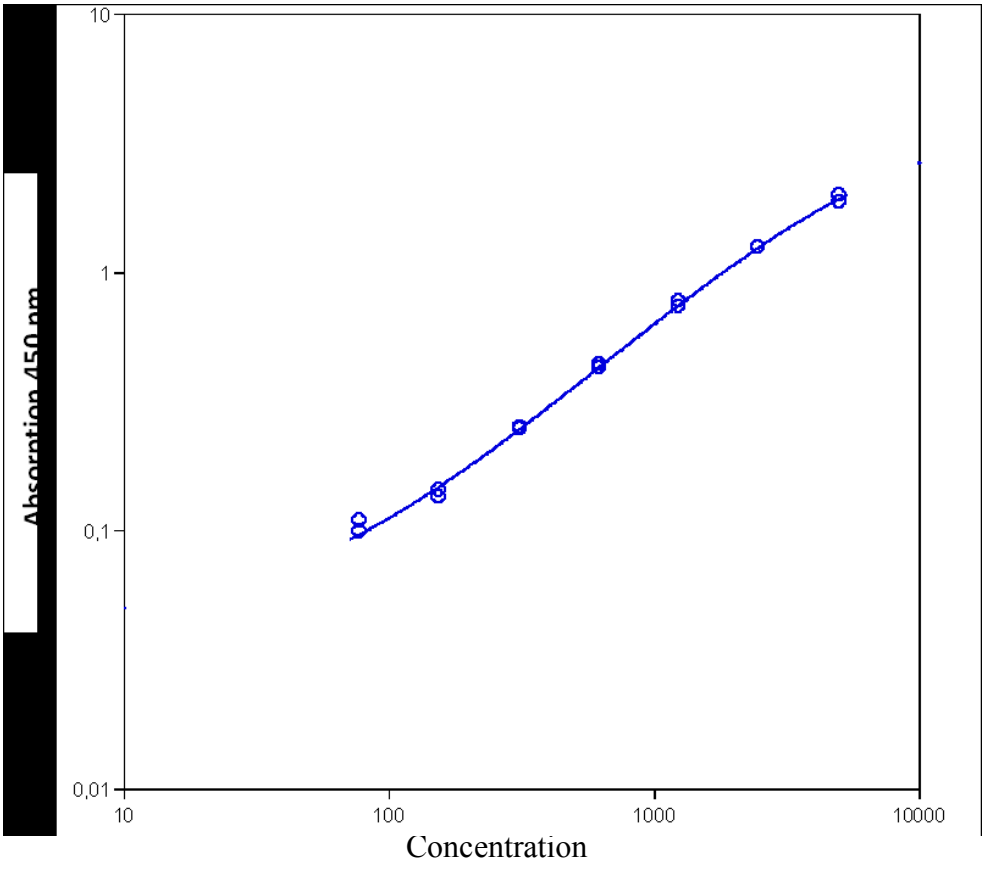

Figure 25 Standard curve of the mean absorbance for the standard wells plotted against the protein concentration being assessed

Samples were analysed for:

3.4.3.2. High sensitivity (hs) C-Reactive Protein (BMS288INSTCE)

CRP is a 110-140kD alpha globulin comprised of five identical subunits. This acute phase protein is synthesised by the liver in response to cytokines. This protein rises up to 48 hours after acute tissue damage occurs, and has been used to assess vascular disease risk (see section on C-Reactive protein (CRP)).

The processing of CRP has shifted to the use of high sensitivity assays, which are now deemed as standard for analysis, and is termed hs-CRP.

The processing of CRP has been associated with numerous fluctuations. E.g. overnight refrigeration and use of NSAIDs or statins. One area of previous concern was the fact that IL-6 has a diurnal variation leading to peaks in the evening. As CRP is produced in response to IL-6, studies have explored whether there is any such variation in CRP levels. In 2001 a study was performed on 13 healthy volunteers to identify any evidence of diurnal variation in hs CRP levels (Meier-Ewert et al. 2001). In health, it was deemed that hsCRP was stable and not subject to change throughout the day. The largest degree of fluctuation was noted to be a median of 0.2mg/L.

To assess the use of commercially available kits, the reliability was compared and found to be high ($r = 0.95$; $P < 0.001$). The test was found to be useful in differentiating those with PAD compared with controls (Rifai et al. 1999).

The manufacturer's calculated intra-assay coefficient of variation for the hs-CRP ELISA was 6.9%, whilst inter-assay variability was 13.1%. The sensitivity of the kit was identified as +/-3pg/ml. The standard curve range for this kit was between 78 - 5,000 pg/mL. (Blood may be sampled from EDTA, citrate or heparinised tubes) (ebioscience 2013) The manufacturers state that the samples have a storage stability of up to 96 hours at -20^{o}C.

3.4.3.3. IL-6 (Human IL-6 high sensitivity ELISA: BMS213HS)

Interleukin-6 is a 21.8kDa single chain protein (see section on Interleukin 6). IL-6 is a cytokine that regulates immune responses. (Blood may be sampled from EDTA or heparinised tubes.) This was a high sensitivity test to quantify IL-6.

Analysis

For the standard curve, the samples and control were diluted to 1:2 and 1:40 respectively. Therefore, when using the standard curve, this must be factored in. The range of the standard curve was from 0.08 - 5.0 pg/mL.

The manufacturer's calculated intra-assay coefficient of variation for the IL-6 ELISA was 4.9%, whilst inter-assay variability was 6.0%. The sensitivity of the kit was identified as +/-0.03pg/ml. The freeze-thaw stability of IL-6 was assessed using five cycles; no loss of immune-reactivity was noted for samples (ebioscience 2012a). The manufacturers have demonstrated stability of samples for up to 24 hours at -20°C.

3.4.3.4. Tumour Necrosis Factor (TNF) alpha (Human TNF alpha ELISA: BMS223INST)

TNF alpha is a polypeptide cytokine produced by macrophages and monocytes. This cytokine alters the vascular endothelium, induces clotting and activates neutrophils. (Blood may be sampled from EDTA, citrate or heparinised tubes.) Unfortunately the kit purchased was not high sensitivity and this will have affected the ability to interpret the results. This mistake was not identified until after the kits had been utilised.

Analysis

For the standard curve, the samples and control were diluted to 1:2. The standard curve range was between 7.8 to 500 pg/mL, outside of these values, the test was deemed inaccurate.

The manufacturer's calculated intra-assay coefficient of variation for the TNF alpha ELISA was 4.9%, whilst inter-assay variability was 9.3%. The sensitivity of the kit was identified as +/-1.65pg/ml. The freeze-thaw stability of TNF alpha was assessed using five cycles; no loss of immune-reactivity was noted for samples (ebioscience 2012b). The manufacturers state the storage stability of the samples to be up to 24 hours at -20^{o}C. ,

3.4.4. Quality of Life

The generic and disease specific quality of life questionnaires were provided to patients in the waiting room prior to session 2's assessment. Individuals were asked to complete these independently and return them to RG by leaving them with the receptionist. All questionnaires were anonymised. Patients had approximately 10-30 minutes to complete the questionnaire, dependent on how long they required to review the questions.

The generic SF-36 questionnaire was analysed using the free online software provided by QualityMetric, Rhode Island, USA (http://www.sf-36.org/demos/SF-36.html). VascuQoL domains were calculated as explained by Morgan et al (Morgan et al. 2001).

3.4.5. The Supervised Exercise Programme (SEP)

Recommendations by the American college of sports medicine (ACSM) have outlined the degree of supervision recommended for patients. They have primarily focused on the resuscitation equipment that should be available and level of staff supervising the class (Balady, et al, 2002b) (see Table 10). They also advocated that resistance training be included for patients undergoing exercise regimens, in addition to 20 minutes of aerobic training three times a week (Phillips & Ziuraitis 2004; Westcott et al. 2009).

Table 10 ACSM 1998 guidelines for the degree of supervision in place of medical patients (Balady, et al. 2002a)

	Level 1	2	3	4	5
Type of facility	Unsupervised exercise room	Single exercise leader	Fitness centre for general membership	Fitness centre offering special programs for clinical populations	SEP: Medically supervised
Personnel	None	Exercise leader Recommended: medical liaison	General manager Health/fitness instructor Exercise leader Recommended: medical liaison	General manager Exercise specialist Health/fitness instructor Exercise leader Medical liaison present	General manager Exercise specialist Health/fitness instructor Exercise leader Medical liaison present
Emergency Plan	Present	Present	Present	Present	Present
Emergency equipment	Telephone in room Signs Encouraged: PAD plan with AED as part of the composite PAD plan in the host facility	Telephone in room Signs Encouraged: sphygmanometer, stethoscope, PAD plan with AED	Telephone in room Signs Encouraged: sphygmanometer, stethoscope, PAD plan with AED (strongly recommended if: membership >2500 or EMS response > 5min from recognition of arrest)	Telephone in room Signs Sphygmanometer, stethoscope, Strongly encouraged: PAD plan with AED	Telephone in room Signs Sphygmanometer, stethoscope, oxygen, crash cart, defibrillator

The SEP lasts for twelve weeks, and each class consists of circuit training. Each circuit lasts two minutes. The six exercises are interspersed with two minutes of walking. Individuals are advised to walk at a speed, which produces claudication pain within the two minutes. The supervised exercise class was cost neutral and has been in place through the vascular department at the physiotherapy suite, H.R.I for the last 8 years. The six exercise stations comprise of both upper and lower limb strengthening exercises in conjunction with aerobic exercises (see Figure 26).

The list below details the six exercises stations performed in the class:

Heel raises:

This requires the individual to stand with their feet half on an elevated platform. With their heels hanging over the platform, an individual is asked to stand up onto their tiptoes and then lower them back down. This movement is repeated for two minutes.

Sit to Stand:

An individual is asked to sit on a chair, place their feet shoulder width apart and their arms across their chest. They are then asked to repeatedly stand up and sit down until two minutes have been completed. They are advised to remember the number of times they have stood up during the two minute period and try and add on a further 2-3 each week as they progress through the course.

Bicycle:

The upright bicycle is used for two minutes. Resistance is set according to the individual's ability and output was defined in watts.

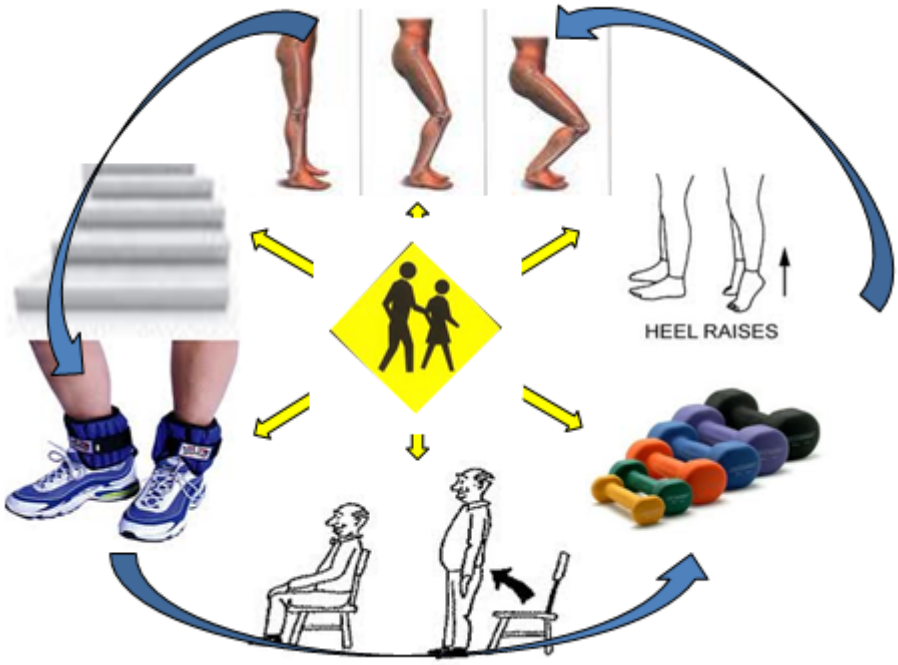

Figure 26 Pictorial representation of the six exercises interspersed with walking in the SEP

Steps:

Patients are asked to step on to the first step of a box alternating legs each time. Depending on the patients balance, physical ability and own confidence, they are either placed on a step with bannister support, without support or a step machine. All individuals are graduated up to the step machine if they are managing well on either of the two static blocks.

Leg Weights:

Varying weights from 2lbs to 10lbs are placed over the lower limb on both legs. Individuals are then asked to sit and slowly extend their knee from a flexed position repeatedly and alternate after every 10 repetitions.

Arm Weights:

Three arm weights, 3.5kg, 4.0kg and 5kg are available. Everyone is started on the lightest weights and graduated up according to their upper arm strength, and dependent upon any pre-existing shoulder or upper arm conditions. Bicep curls are performed in sets with 10 repetitions per set on each arm.

3.5. Statistical Analysis

This thesis was analysed with SPSS version 19 (IBM, Armonk USA). The meta-analysis of baseline CPET data was undertaken using comprehensive meta-analysis version 2 (Biostat, Englewood USA). For analysis of CPET data, Winbreak version 3.7 (Epistemic, Mindworks, Ames USA) was used to produce 20 second averaged data from which AT and peak VO_2 were derived.

The data was analysed non-parametrically. For non-parametrical data, Chi-squared tests where used, when the data distribution was less than 20%, a Likelihood ratio was used. For continuous data, it was analysed by either a Mann-Whitney U test or

Wilcoxon signed rank test (for six week data) or Friedmann's analysis of variance (ANOVA) for twelve week data.

In view of the secondary analysis of data for twelve week outcomes, a Bonferroni correction was applied to this data set (Napieraia 2012). To reduce the risk of a type I error, the level deemed to be statistically significant was raised from 0.05 to 0.05/3 = 0.017. Any values less than 0.017 have been highlighted in yellow, whilst those between 0.05 and 0.018 have been highlighted in blue.

Spearman's rho correlation coefficient was performed as the data was non-parametric and the sample size was small.

4. RESULTS

The results are presented in five sections:

A) The baseline data and the qualitative data obtained with regards to attendance (see section 4.1 and 4.2).

B) A comparison between baseline and 6 weeks for peak VO_2 – the primary outcome measure. In addition, all outcome measures have been explored with regards to changes within six weeks (see section 4.3).

C) Changes over the whole 12 week duration have been explored (see section 4.4) and finally

D) Mortality within twelve weeks has been summarised briefly (see section 4.5). In addition, the correlation between outcomes measured has been investigated (see section 4.6).

E) Stratification of responders and non-responders to exercise (see section 4.7).

F) Audit of uptake to SEP (see section 4.8).

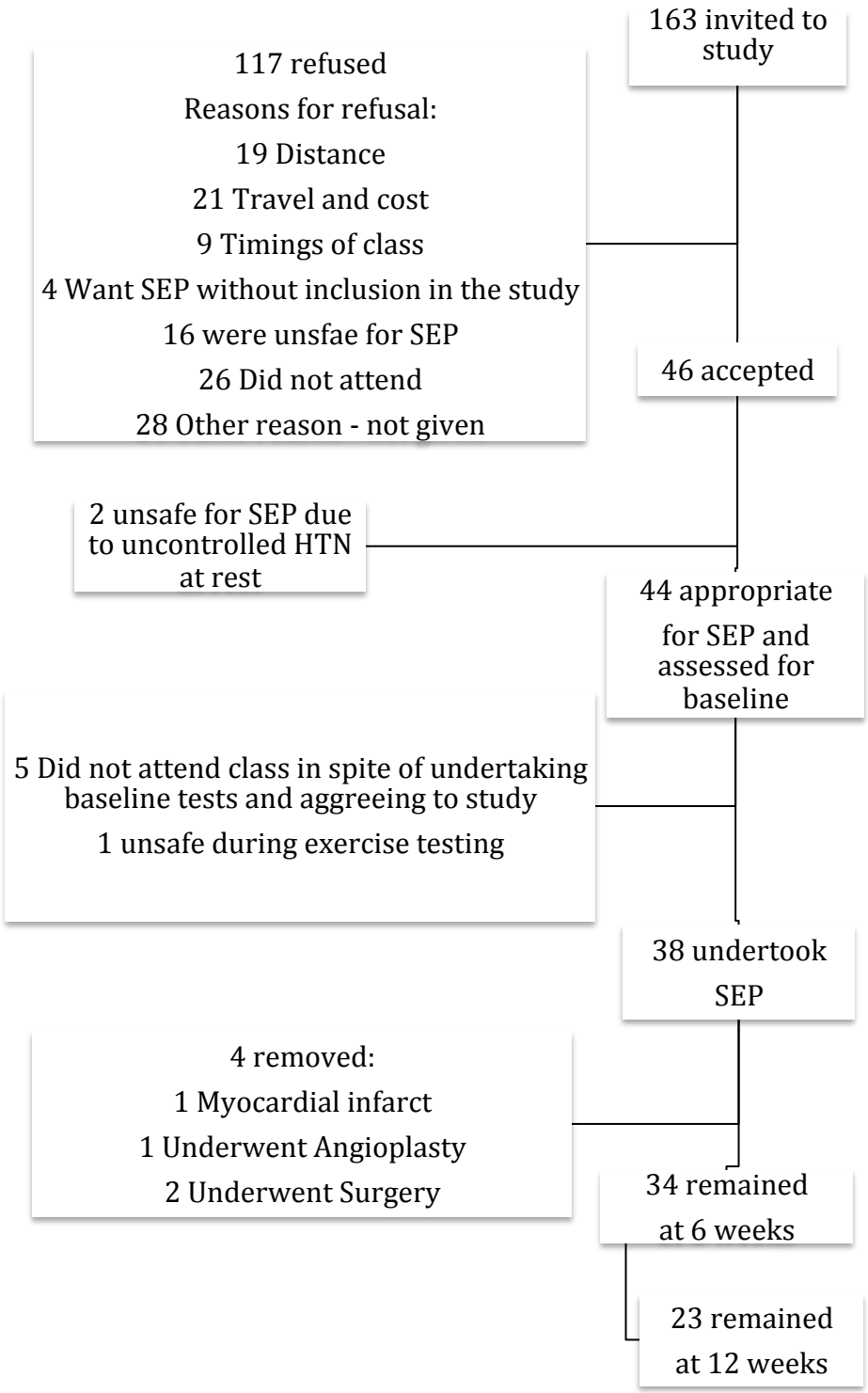

Figure 27 Consort diagram of invited participants

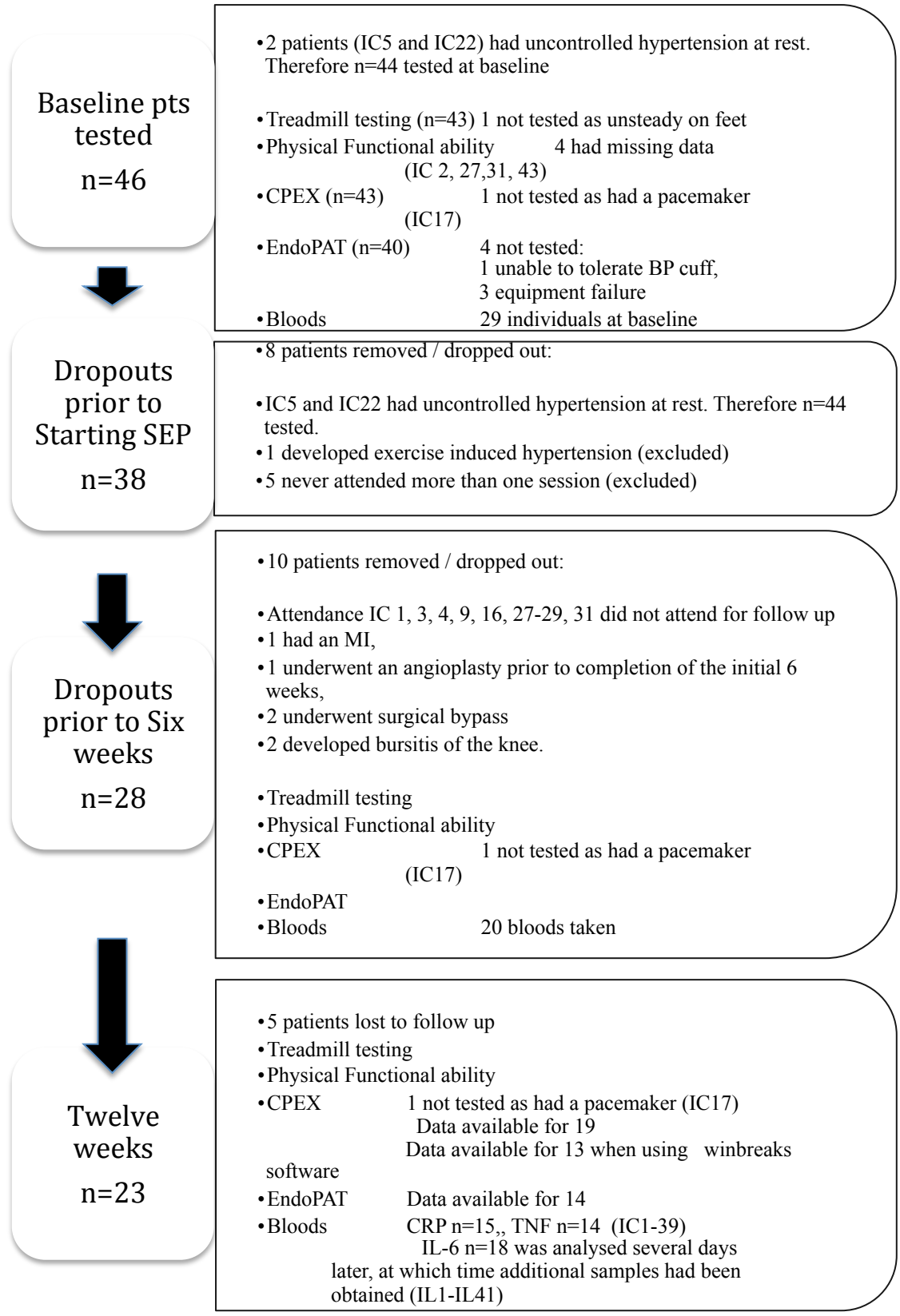

Figure 28 Expanded Consort diagram detailing losses to follow up

4.1. Reasons cited for non attendance

Between 1st August 2011 and 31st October 2012, 163 patients with intermittent claudication were referred to the supervised exercise programme. Only 46 (28.2%) patients were willing to partake in the research. Reasons cited by the 117 patients who declined participation in the research included are listed below and outlined in Figure 27 and 28.

- distance (n=19, 16.2%), simply too far to travel 3 times / week for 12 weeks

- SEP timings (n=9, 7.7%), the SEP classes were scheduled at inconvenient times or clashed with work commitments

- preferred exercise on prescription (n=4, 3.4%), i.e. self directed community exercise programme supported by advice sheet devised by physiotherapy department

- unsafe for assessment (n=16, 13.7%), this group of patients felt they could not tolerate treadmill testing or CPEX testing

- and finally 28 patients (23.9%) gave "other" reasons for declining participation including; "I'd rather not, I just need an angioplasty," "I've done it before and found no benefit," "I exercise plenty already."

In addition 26 patients (22.2%) did not respond to letters inviting them to participate in the SEP and failed to attend an initial assessment for suitability for SEP (n=26, 22.2%).

For those who cited travel and cost (n=21, 17.9%), as preventative problems for attending the class, distance ranged from 8 miles (Beverley) to across the Humber Bridge or further afield, Immingham, Goole, Scunthorpe.

Co morbid conditions prevented 16 patients from participating in the SEP. These included diabetes (clashes with diabetes meal time regimens), uncontrolled epilepsy, emphysema, recent CVA (n=2), stable angina, recurrent falls, partially sighted, musculoskeletal pain (back, hip, knee pain), developed CLI (n=2), hypertension (SBP>220mmHg, n=4).

Only 3 cited the pain in their legs as too disabling to attend the SEP. A further 2 cited their age as the reason (87 and 86 years old) for not attending, 2 were not claudicants (ABPI >0.9 post exercise) and finally 2 felt that the class was pointless.

Seven patients felt that they already exercised sufficiently and that the class would add no additional benefit for them, whilst 2 had previously undergone the SEP and felt fitter but had found no additional benefit in their walking distance.

Commitments such as being a carer (n=2), or work (n=9), were an issue for some. Of those who were interested in the exercise, 4 wished to undergo exercise on prescription, 4 wished to attend but couldn't come regularly, 4 underwent an angioplasty whilst on the SEP waiting list and 10 agreed to SEP but not the research.

Baseline testing was performed on the 46 who provided voluntary informed written consent. From this cohort, a further three were identified as unsafe for the class. Two (IC5, IC22) were identified as having uncontrolled hypertension at rest. Therefore no exercise tests were performed and they were referred back to their GP for an urgent re-assessment. One patient developed exercise induced hypertension with systolic blood pressure up to 220mmHg, which resolved with rest, he was also referred back to his GP.

Of the remaining 43 patients, 5 never attended more than one session of the entire 12 week programme. Therefore only 38 agreed and attended the SEP. Of these, 1 had an MI, 1 underwent an angioplasty prior to completion of the initial 6 weeks, 2 underwent surgical bypass and n=2 developed bursitis of the knee. Of those who attended the class, only 47% were documented as having attended more than 50% of the sessions.

4.2. Baseline variables

In total, 44 underwent a full baseline assessment (see Table 11); 9 women and 35 men, median age was 67.0 years (IQR 61.5 to 73.5 years) and a median BMI of 27.63kg/m^2 (IQR 25.30-30.24kg/m^2).

Table 11 Baseline variables for those enrolled in the SEP

	Count	Percentage %
Gender (Male : Female)	35: 9	79.5: 20.5
Ischaemic Heart Disease	14	31.8
Hypertension	32	72.7
Anti platelet / Anticoagulant	36	81.8
Hypercholesterolaemia or on a Statin	37	84.1
Previous CVA or TIA	5	11.4
Diabetes Mellitus	15	34.1
Osteoarthritis	22	50.0
Current Smoker Ex-smoker Never	17 25 2	38.7 56.8 4.6

Co-morbidities were documented for all patients; with uncontrolled hypertension being a common occurrence. Guidelines suggest that systolic blood pressure should be maintained below 140mmHg or 130 mmHg in diabetics; 72.7% of the participants (n=32) were classed as hypertensive and seven of these had a resting systolic blood pressure > 160mmHg. Of the normo-glycaemic group (n=28), 17 (60.7%) had controlled blood pressure (SBP ≤140mmHg). Of those with diabetes (n=15), 5 (33.3%) had blood pressure lower than 130mmHg.

Diabetes control was not checked as this was not within the remit of this project, however two patients did complain of elevated BMs in spite of being on oral anti-hyperglycaemic agents and letters were again sent to their GP's advising of their concerns. 15 (34.1%) were diabetic and controlled by either insulin or oral hypo-glycaemics, whilst 29 (65.9%) were not diabetic.

The majority of patients were taking antiplatelet therapy (n=36, 81.8%) and statin therapy (n=37, 84.1%). These were usually commenced by their GP immediately after consultation with the vascular consultant or post MI or CABG. Only 4 (9.1%) could not tolerate any form of statin and 3 (6.8%) had never been commenced on one. Of those on an antiplatelet / anticoagulant, 32 (72.7%) were on aspirin, 3 (6.8%) were on clopidogrel, 1 (2.3%) was on warfarin and 8 (18.2%) were not taking either an antiplatelet or an anticoagulant.

4.2.1. Measures of Lower Limb of Ischaemia

4.2.1.1. *ABPI reliability*

To ensure that ABPI measurements were reliable and accurate, the intra-rater reliability of ABPI recordings was performed. Eight consecutive participants were recorded approximately one week apart for their resting ABPI measurements. The reliability of ABPI measurements from16 limbs was assessed. The cronbach's α was calculated to be 0.878 with an inter-item correlation of 0.786.

The inter-rater reliability was not assessed due to a lack of additional personnel to facilitate this. Comparisons were made to the vascular database to ensure that similar values were gained; however this was not always possible and the lag time between a vascular scientist assessment and the research readings varied from one week to four years. In view of the variable time frame, the intra-class coefficient to assess inter-rater reliability was not performed.

4.2.1.2. Baseline characteristics of participants lower limb ischaemia

The majority of patients had demonstrable bilateral disease, with approximately 50% suffering from Rutherford Grade 3 disease, with 70% in an infra-inguinal distribution (see Table 12).

Baseline characteristics of age, gender, co-morbidities were assessed as per Rutherford grade to determine whether there was any difference between the groups (see Table 13).

Overall there were no significant differences between the groups with regards to other co-existing co-morbidities.

Table 12 Proportion of Participants with IC as per; laterality, Rutherford grade, distribution

	Count	Percentage %
Leg Affected (Bilateral)	31	70.5
Rutherford Grade		
Mild (1)	9	20.9
Moderate (2)	12	27.9
Severe (3)	22	51.1
Disease level:		
Supra-Inguinal	5	11.4
Infra-Inguinal	32	72.7
Crural	2	4.5
Mixed	5	11.4

Table 13 Rutherford Grade, demographics and co-morbidities

Demographics and Co-morbidities	Rutherford Grade			P
	I	II	III	
N	9	12	21	
Age; Median (IQR)	65 (60-70)	67 (60-73.5)	67 (66-75)	KW p=0.559
BMI (kg/m^2); Median (IQR)	27.5 (25.2-30.0)	29.8 (25.9-30.8)	27.3 (24.8-29.9)	KW p=0.485
Gender (Male)	6	9	18	LL =1.467, d.f.=2, p=0.480
Ischaemic heart disease	2	4	8	LL =0.779, d.f.=2, p=0.678
Previous CVA/ TIA	0	2	3	LL=2.749, d.f.=2, p=0.253
Hypertension	7	8	15	LL=0.134, d.f.=2, p=0.935
Diabetes Mellitus	2	5	7	LL=1.218, d.f.=2, p=0.544
Hypercholesterolaemia or on a statin	7	8	18	LL=0.822, d.f.=2, p=0.663
Aspirin Usage	6	7	19	LL= 4.069, d.f.=2, p=0.131

LL Likelihood ratio, KW Kruskal-Wallis

4.2.1.3. Constant Load Treadmill Testing

All patients underwent a constant load treadmill test at 2.57km/hr with a 10% incline for a maximum duration of five minutes. A pre and post exercise ankle brachial pressure index was performed in each lower limb to identify both symptomatic and asymptomatic PAD. Initial claudication and maximum walking distances were recorded in seconds and converted to distance, and patients were categorised into Rutherford groups according to their test.

Only one individual did not undergo treadmill walking distances as he was deemed unsafe on the treadmill due to a poor gait and instability. He did however undergo all other tests and had evidence of reduced resting ABPI.

In total, there were 9 (20.9%) patients with Rutherford grade 1 disease, 12 (27.9%) with grade 2 and 21 (51.1%) with grade 3 disease. The median initial claudication distance was 48.92m (IQR 33.08 to 80.59m) and median maximum walking distance was 106.82 (IQR 74.66 to 214.50m). The median pre exercise ABPI was 0.64 (IQR 0.48 to 0.77) and reduced to 0.32 (IQR 0.18 to 0.53m) for post exercise ABPIs (see Table 14).

Reasons cited for termination of the constant load treadmill test included

- test completed in 11 patients (25.6%) i.e. five minutes had elapsed,

- claudication in 24 (55.8%) patients

- shortness of breath in 6 (14.0%)patients.

- in the remaining two cases, no reason was cited for one, and the other developed a headache which terminated the treadmill test.

ABPI of the worst leg pre exercise correlated strongly with post exercise ABPI ($r=0.732$, $p<0.001$). Of note however, ABPI-Pre and ABPI-Post were also negatively correlated with mean Chair Stance Time ($r= -0.585$, $p=0.001$ and $r=-0.680$, $p<0.001$ respectively). MWD (m) was strongly positively correlated with ICD ($r=0.658$, $p<0.001$) and best TUG ($r= -0.325$, $p=0.050$).

Table 14 Baseline measures of Lower Limb Ischaemia

	Median	IQR
Pre exercise ABPI (n=43)	0.64	0.48-0.77
Post exercise ABPI (n=42)	0.32	0.18-0.53
Initial Claudication Distance ICD (n=42; units in m)	48.92	33.08-80.59
Maximum Walking Distance MWD (n=42; units in m)	106.82	74.66-214.50

*One individual did not undergo treadmill walking distances as he was deemed unsafe on the treadmill due to a poor gait and instability. He did however undergo all other tests and had evidence of reduced resting ABPI.

Table 15 Baseline Measures of Physical Functional Ability (n=43)

	Normal values	Median	IQR
Mean TUG (s)	<13.5	7.48	6.13-9.30
Best TUG (s)	<13.5	7.16	5.97-8.90
Mean CST (s)	>16.7	11.60	8.97-12.89
Semi Tandem stance (s)	>10.00	30.00	30.00-30.00
Full Tandem Stance (s)	>10.00	30.00	30.00-30.00

4.2.2. Markers of physical functional ability

Markers of physical functional ability included the timed up and go (TUG) test, chair stand time (CST), tandem stance and semi-tandem stance test (see Table 15). These simple measures of physical function were completed by all patients and were relatively easy to perform.

One person failed the semi-tandem stance, and four had missing values for the test. For the full tandem stance, eight were unable to complete a 30 second stance and there was missing data for the same four missing from the STS (IC2, IC27, IC 31 and IC43).

Mean TUG was strongly positively correlated with best TUG ($r=0.986$, $p<0.001$) and mean CST ($r=0.731$, $p<0.001$).

4.2.3. CPET

Cardiopulmonary exercise testing was performed in all patients except IC17. This gentleman had a pacemaker in situ firing at a constant rate of 60 beats per minute, in view of his inability to alter his cardiovascular physiology with exertion; he did not undergo cycle ergometry to maximal exertion. In total, 43 of the group underwent cycle ergometry (see Table 16).

Table 16 Baseline measurements from Cardiopulmonary Exercise Test using Cycle Ergometry

	Median	IQR
$PeakVO_2$ Unadjusted for weight (ml/min)	.957	.708-1.083
$PeakVO_2$ (ml/kg/min)	12.025	9.688-14.629
Time to reach $PeakVO_2$ (min)	11.00	9.00-13.33
Watts attained at $PeakVO_2$ (W)	67.13	47.89-86.83
VE/VCO_2 at $PeakVO_2$	32.38	28.67-35.50
Heart Rate at $PeakVO_2$ (bpm)	107.50	94.71-121.25

4.2.3.1. AT

Whilst data was provided by the Medgraphics software for an AT for each of the participants, by the Winbreak method, only n=31 (72.1%) were identified to have an anaerobic threshold.

The median AT was identified as 7.40ml/kg/min (IQR 6.52 to 8.58ml/kg/min) and occurred at a median of 46.71 Watts (IQR 38.25-54.00W). The median length of time patients reached their median anaerobic threshold at was 9.3 minutes (IQR 8.0-10.0 minutes).

4.2.3.2. Peak VO_2

Peak VO_2 was not attained in 14% of patients (n=6), as their heart rate exceeded 85% of their maximum [as determined by the Karvenon formula (220 – age = maximum heart rate)]; which led to termination of the test.

Reasons cited for test termination were multiple for each individual with approximately three being cited each time. Whilst the test was explained as an opportunity to establish fitness and would require an individual to exercise to their maximum possible capacity, common reasons for test termination included shortness of breath, pain from the cycle seat, knee pain, feeling that they had done enough, thigh pain, occasionally claudication symptoms and the final problem was an inability to maintain 60rpm.

The investigator terminated cycle ergometer tests early if there were any ECG changes or medical reasons. In only one case was the test terminated for a medical reason, one patient developed a headache whilst exercising and was identified to have developed substantial hypertension with a systolic of 210mmHg. This subsided with rest and the headache also fully resolved. In view of this exercise-induced hypertension, this patient was excluded from the SEP.

In the others, any concerning ECG changes terminated the test early, in each of these cases, all ECG changes reverted back to normal immediately on cessation of exercise. Whilst ECG monitoring was maintained for a further two minutes post exercise to ensure that no ectopics, premature beats or ST changes developed, this remained negative in all cases.

The median peak VO_2 was identified as 12.025mL/kg/min (IQR 9.688 to 14.629mL/kg/min) and occurred at a median of 67.13Watts (IQR 47.89-86.63W). The median length of time patients reached their median peak was 11.0 minutes (IQR 9.0-13.3).

4.2.3.3. Published data on baseline CPET values in patients with claudication

The mean peak VO_2 was 12.535 (S.E. 0.575) for the 43 patients who underwent baseline testing by CPET. When incorporating these values into the pooled analysis with the other 16 studies, the random effects model had a mean peak VO_2 of 14.656ml/kg/min (S.E. 0.548, 95% CI 13.582-15.730ml/kg/min). This was only marginally lower than with the 16 studies only; 14.748ml/kg/min (standard error 0.564; 95% C.I. 13.644-15.853.

When looking at the overall values from each of the 16 studies, only three studies had lower peak VO_2 values than ours; Afaq (Afaq et al. 2007), Bronas (Bronas et al. 2011) and Gardner (Gardner, et al. 2010a).

4.2.4. EndoPAT

The EndoPAT was performed in 40 of the 44 patients at baseline. Of those with no baseline EndoPAT data, one was unable to tolerate the occlusion period, and equipment failure occurred in three patients. Values for the RHI and AI were obtained by automatic analysis by the EndoPAT software (see Table 17).

Table 17 Baseline measurements of Endothelial Function (RHI and I) using the EndoPAT2000

	Median	IQR
Reactive Hyperaemia Index (RHI)	2.22	1.67-2.47
Augmentation Index (AI)	34.59	21.30-50.53
Augmentation Index at 75	28.97	14.91-42.64
Resting Heart rate (bpm)	65.00	58.00-76.00
Systolic Blood Pressure (SBP) (mmHg)	142.00	122.00-152.00
Diastolic Blood Pressure (DBP) (mmHg)	77.00	68.50-81.00

4.2.4.1. *RHI*

The median reactive hyperaemia index was identified to be 2.22 (IQR 1.67 to 2.47). Of those 10 (25.0%) were < 1.67 threshold value and 30 (75.0%) were above, whilst 4 (10.0%) were < 1.35 threshold value. These are the threshold values quoted by the company, below these is associated with a high likelihood of having an angiographically detected coronary lesions.

The groups were compared for co-morbidities, age and gender using non-parametric tests. This was undertaken to assess whether baseline co-morbidities had an influence on whether participants were likely to have a lower RHI. This was assessed for both the 1.67 (see Table 18) and the 1.35 (see Table 19) cut-off threshold values.

RHI correlated moderately with systolic blood pressure (r=0.434, p=0.005). There was no correlation between reactive hyperaemia and the augmentation index.

4.2.4.2. *AI*

The augmentation index had a median value of 34.59 (IQR 21.30-50.53). When comparing between genders, there was no significant difference between the two (MWU p=0.288).

When correlating this with other output measures, this was most closely correlated; ABPI of the worst leg (r=-0.342, p=0.031), heart rate (r=-0.360, p=0.022) and systolic blood pressure (r=0.320, p=0.044).

Table 18 Table comparing co-morbidities between the threshold values for RHI at the 1.67 value

	<1.67	= or >1.67	p
N	10	30	
Age	64.5 (58.0-69.0)	67.5 (62.0-74.0)	0.221^
Gender (male)	8	26	0.629*
IHD	4	10	0.718*
HTN	6	25	0.190*
Hypercholesterolaemia	8	23	1.000*
Diabetes	7	8	0.024*
Bilateral	8	20	0.693*
ABPI (Pre-exercise)	.71 (.44-.77)	.62 (.48-.77)	0.770^
SBP (mmHg)	125.5 (103.0-145.0)	144.5 (129.0-157.0)	0.500^
ICD (m)	54.58 (33.08-82.53)	48.92 (30.70-79.47)	0.674^
MWD (m)	167.13 (90.42-214.50)	100.27 (74.66-202.54)	0.245^
Mean TUG (s)	6.72 (6.31-8.60)	7.62 (6.07-9.52)	0.955^
Mean CST (s)	10.60 (8.97-12.96)	11.41 (8.66-12.35)	0.813^
Anaerobic Threshold (ml/kg/min)	7.40 (6.65-10.90)	7.40 (6.52-8.10)	0.717^
Peak VO_2 (ml/kg/min)	11.11 (9.69-17.21)	12.30 (9.43-14.63)	1.000^

Table 19 Table comparing co-morbidities between the threshold values for RHI at the 1.35 level

	<1.35	= or >1.35	p
N	4	36	
Age	67.0 (61.5-73.5)	66.5 (61.0-72.5)	0.845^
Gender (male)	3	31	0.493*
IHD	1	13	1.000*
HTN	2	29	0.213*
Hypercholesterolaemia	4	27	0.557*
Diabetes	3	12	0.139*
Bilateral	3	25	1.000*
ABPI (Pre-exercise)	.602 (.219-.816)	.636 (.486-.769)	0.648^
SBP (mmHg)	112.5 (101.5-138.5)	143.0 (125.5-152.0)	0.135^
ICD (m)	56.60 (34.52-97.34)	48.92 (30.70-79.47)	0.620^
MWD (m)	214.50 (136.23-214.50)	105.79 (74.66-214.50)	0.212^
Mean TUG (s)	6.44 (5.95-9.02)	7.48 (6.13-9.30)	0.665^
Mean CST (s)	11.75 (9.79 - 18.85)	11.38 (8.66-12.67)	0.566^
Anaerobic threshold (ml/kg/min)	8.00 (4.95-12.00)	7.40 (6.60-8.10)	0.726^
Peak VO_2 (ml/kg/min)	13.48 (8.12-18.28)	12.03 (9.43-14.63)	0.738^

*Fisher's exact test (2 sided), ^Mann Whitney U Test

4.2.5. Quality of Life

4.2.5.1. *Generic Short Form 36*

Baseline data for patients with claudication demonstrated median scores (see Figure 29 and Table 20), which were lower than the UK national averages (see 1.5.1).

The SF36 (v2) was also assessed for differences between grades of ischaemia (Table 21), no significant differences were found.

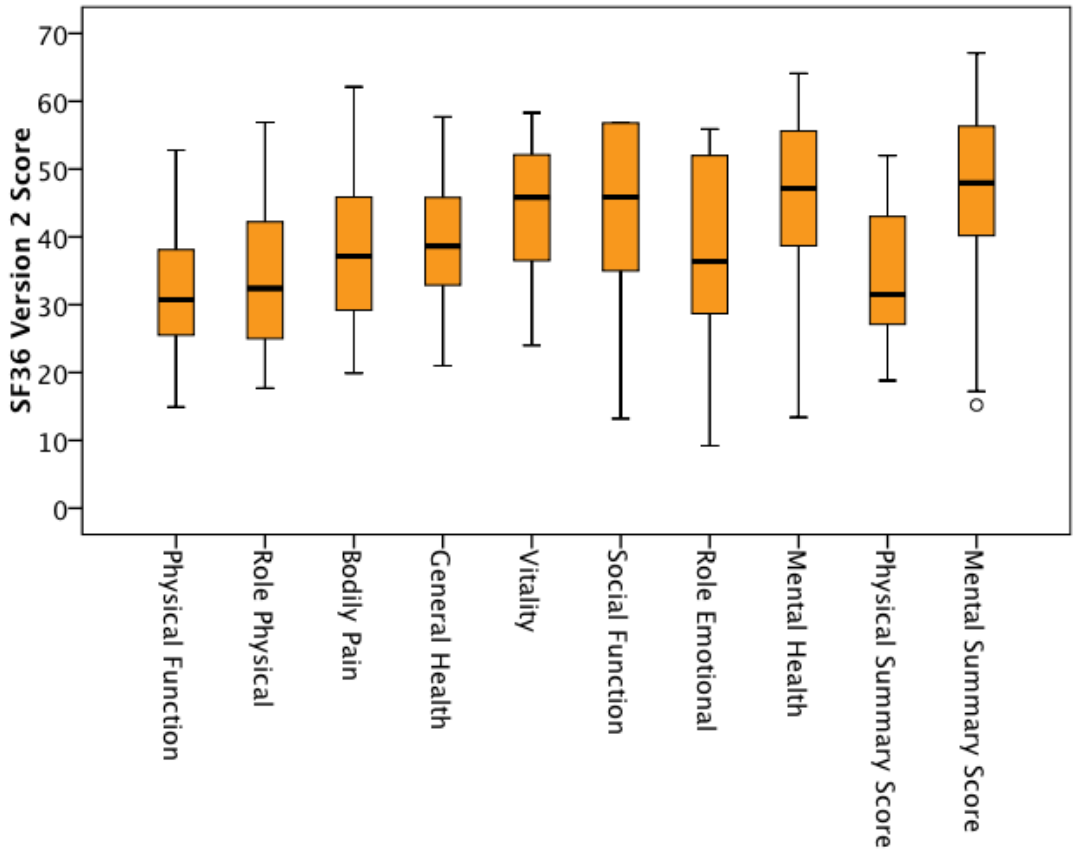

Figure 29 Baseline values of each Generic Quality of Life Domain (SF36 version 2) in patients with IC

Table 20 Baseline Generic Quality of Life measures using the SF-36 compared to the general population

SF 36 Version 2	General population	Patients with IC in study	
	Mean (s.d.)	Median	IQR
Physical Function	84.2 (23.3)	30.75	25.50-38.10
Role Physical	80.9 (34.0)	32.40	25.00-42.20
Bodily Pain	75.2 (23.7)	37.20	29.20-45.85
General Health	71.9 (20.3)	38.60	32.90-45.80
Vitality	60.9 (20.9)	45.80	36.50-52.10
Social Function	83.3 (22.7)	45.90	35.00-56.80
Role Emotional	81.3 (33.0)	36.40	28.70-52.00
Mental Health	74.7 (18.1)	47.20	38.70-55.60
Physical Summary Score	50.0 (10.0)	31.50	27.10-43.00
Mental Summary Score	50.0 (10.0)	48.00	40.20-56.30

Table 21 SF-36 scores and Rutherford Grade

	Rutherford Grade1	Rutherford Grade 2	Rutherford Grade 3	P
Physical Function (PF)	38.1 (27.6-50.7)	29.7 (21.3-33.9)	30.8 (26.6-37.1)	0.133
Role Physical (RP)	39.7 (25.0-56.9)	27.5 (25.0-42.2)	32.4 (22.6-42.2)	0.631
Bodily Pain (BP)	46.1 (33.4-55.4)	33.0 (29.2-45.6)	35.3 (29.2-43.5)	0.181
General Health (GH)	38.6 (35.3-50.6)	41.0 (32.9-44.8)	42.4 (32.9-48.2)	0.859
Vitality (V)	49.0 (45.8-58.3)	36.5 (33.4-45.8)	45.8 (33.4-52.1)	0.133
Social Function (SF)	45.9 (40.5-56.8)	45.9 (35.0-51.4)	40.5 (29.6-56.8)	0.523
Role Emotional (RE)	48.1 (24.8-55.9)	40.3 (32.6-48.1)	32.6 (24.8-52.0)	0.724
Mental Health (MH)	47.2 (35.9-61.3)	44.4 (35.9-52.8)	47.2 (39.4-55.6)	0.836
Physical Summary Score (PSS)	43.7 (30.3-51.1)	28.8 (25.1-41.5)	32.4 (29.2-39.4)	0.156
Mental Summary Score (MSS)	52.2 (37.5-59.8)	48.0 (39.2-50.8)	48.0 (40.6-60.0)	0.836

p value derived by Independent samples Kruskal Wallis Test

4.2.5.2. Disease specific VascuQoL

Disease specific quality of life was determined using the Kings College VascuQoL. The median total VascuQoL score was 4.40 (IQR 3.28-5.48). The five individual domains had median scores of 3.75 (IQR 3.00-4.75) for pain, 5.00 (IQR 3.00-6.50) for social, 3.75 (IQR 2.75-5.13) for activities, 5.25 (IQR 4.50-5.75) for symptoms and 4.86 (IQR 2.86-6.14) for emotional (see Figure 30).

The VascuQoL was also assessed for differences between grades of ischaemia and can be compared to published data (see Table 22 and Table 23). No significant differences were demonstrated.

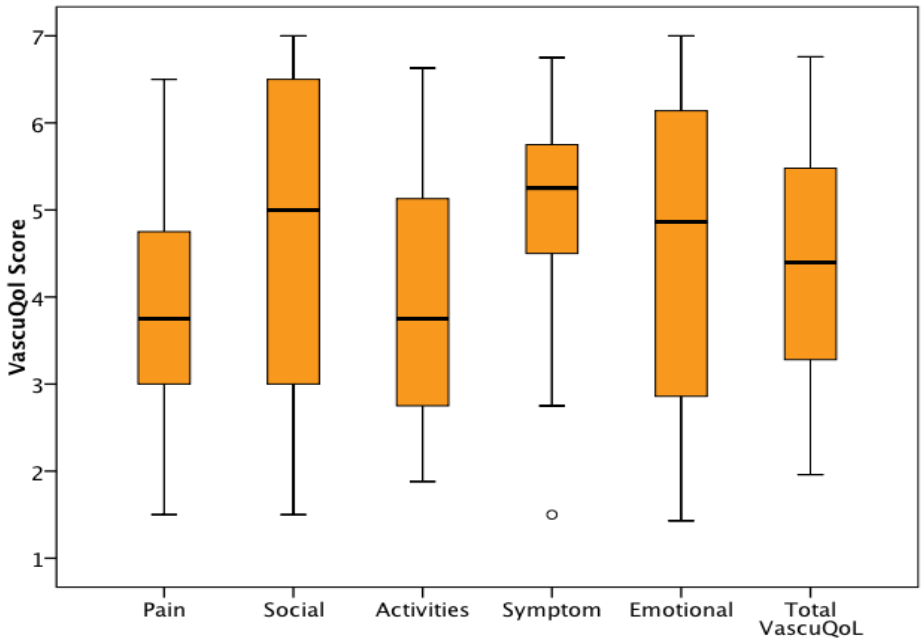

Figure 30 Baseline values for the VascuQoL (disease specific quality of life) in participants with IC

Table 22 Baseline Disease specific Quality of Life measures using the VascuQoL compared to the previously published data in Claudicants

	Patients with IC (n=127) Mean (s.d.) (Nordanstig et al 2012)	Patients with IC in this study	
		Median	IQR
Pain	3.69 (1.18)	3.75	3.00-4.75
Social	4.34 (1.60)	5.00	3.00-6.50
Activities	3.50 (1.12)	3.75	2.75-5.13
Emotional	4.57 (1.29)	4.86	2.86-6.14
Symptoms	4.46 (1.09)	5.25	4.50-5.75
Total	4.05 (1.03)	4.40	3.28-5.48

Table 23 VascuQoL scores and Rutherford Grade

	Rutherford Grade1	Rutherford Grade 2	Rutherford Grade 3	P
Pain	5.00 (3.00-6.25)	4.25 (3.25-5.00)	3.50 (2.75-4.25)	0.098
Social	6.00 (5.00-7.00)	5.50 (3.00-6.00)	4.50 (2.50-5.50)	0.195
Activities	4.38 (2.63-5.88)	3.50 (4.75-5.50)	3.38 (2.25-4.75)	0.415
Emotional	6.14 (4.71-6.71)	4.86 (2.86-6.00)	4.14 (2.86-5.57)	0.175
Symptoms	5.50 (4.75-6.50)	5.00 (4.75-5.50)	5.25 (4.00-5.75)	0.448
Total	5.00 (4.32-6.36)	4.00 (3.36-5.12)	4.28 (3.08-5.08)	0.213

p value derived by Independent samples Kruskal Wallis Test

4.3. Six Week data

At six weeks, attendance was reduced for SEP, of those who started the programme; only 28 (63.6%) attended for the 6 week follow up. One patient did not respond, two dropped out of the SEP, one was called for an angioplasty prior to completion of the initial six weeks (IC 1, 3, 4, 9, 16, 27-29, 31).

Whilst overall data for the 12 weeks has also been analysed, the primary outcome measure for this thesis was whether a 6 week exercise programme could lead to 1.5ml/kg/min change in AT or peak VO_2. In view of this, both time points have therefore been analysed.

Baseline characteristics such as age, height and smoking status were unlikely to change during the twelve-week programme and were therefore not retested. Only BMI and calf circumference were re-assessed at 6 and 12 weeks as training was postulated to have an improvement on these (see Table 24).

Table 24 The difference between Baseline and six weeks of exercise for measures of weight loss and muscle bulk

Characteristics	Baseline Median (IQR)	Six Weeks Median (IQR)	Wilcoxon Signed Rank Test (p)
BMI (kg/m^2)	27.63 (25.27-30.30)	27.51 (24.12-29.63)	0.414
Affected Calf Circumference (cms)	30.00 (28.00-32.40)	27.51 (24.12-29.63)	0.624

4.3.1. Measures of Lower Limb of Ischaemia

At six weeks repeated measures of markers of lower limb ischaemia were performed and compared to baseline measures (see Table 25).

When looking at ABPI measures pre and post exercise between baseline and six weeks, there was no significant difference between the measures (Wilcoxon Signed Rank p=0.098, p=0.130 respectively).

Constant load treadmill testing between baseline and six weeks demonstrated a significant difference for initial claudication distance but not maximum walking distance.

Table 25 The difference between Baseline and six weeks of exercise for measures of Lower Limb Ischaemia

Measures of Lower Limb Ischaemia	Baseline Median (IQR)	Six Weeks Median (IQR)	Wilcoxon Signed Rank Test (p)
Pre exercise ABPI	.64 (.48-.77)	.60 (.51-.67)	0.098
Post exercise ABPI	.32 (.18-.53)	.35 (.29-.56)	0.130
Initial Claudication Distance (m)	48.92 (32.68-81.56)	78.18 (42.03-115.57)	<0.001
Maximum Walking Distance (m)	106.82 (74.66-214.50)	155.35 (89.83-214.50)	0.062

4.3.2. Measures of Physical Functional Ability

Markers of Physical Functional Ability were also re-measured at six weeks to assess for any improvement in balance turning ability and speed (see Table 26). Both the mean TUG and CST demonstrated improvements in speed.

Table 26 The difference between Baseline and six weeks of exercise for measures of Physical Functional Ability

Measures of Physical Functional Ability	Baseline Median (IQR)	Six Weeks Median (IQR)	Wilcoxon Signed Rank Test (p)
Mean TUG (s)	7.48 (6.13-9.30)	6.71 (5.82-8.70)	0.034
Mean CST (s)	11.60 (8.90-12.91)	9.80 (7.86-11.44)	0.003
Semi-Tandem Stance (s)	30.00 (30.00-30.00)	30.00 (30.00-30.00)	1.000
Full Tandem Stance (s)	30.00 (30.00-30.00)	30.00 (30.00-30.00)	0.893

4.3.3. CPET

A difference of 1.5ml/kg/min after 6 weeks was the primary outcome measure of this study.

In total, it was expected that 32 patients would attend for 6 weeks; however the target recruitment was 38 patients. In spite of over recruiting by 6 (15.8%), this was still insufficient for the target sample size of 32. Our study population consisted of 28 participants at six weeks.

The mean difference in peak VO_2 detected between these two time points (baseline and 6 weeks) was -0.724 (S.E.M. 0.505) using a paired t test (p=0.163). The changes in AT (see Table 27) and peak VO_2 (see Table 28) demonstrate no significant improvement in either.

Table 27 The difference between Baseline and six weeks of exercise for AT

Measures of CPET at AT	Baseline Median (IQR)	Six Weeks Median (IQR)	Wilcoxon Signed Rank Test (p)
AT	.740 (.652-.858)	.760 (.595-1.008)	0.306
Excess CO_2 (L/min)	.720 (.555-.875)	.720 (.508-.875)	0.313
Simplified method VCO_2/VO_2 (L/min)	.635 (.555-.838)	.655 (.525-.985)	0.937
VCO_2/VO_2 (L/min)	.655 (.555-.820)	.655 (.528-.805)	0.839
VE/VCO_2 (L/min)	31.40 (28.75-34.57)	30.68 (29.08 - 35.84)	0.647
Watts at AT	46.71 (38.25-54.00)	50.89 (36.48-69.18)	0.338
Heart rate at AT	90.29 (81.00-100.25)	90.13 (83.41-100.21)	0.777

Table 28 The difference between Baseline and six weeks of exercise for Peak VO_2

Measures of CPET at Peak VO_2	Baseline Median (IQR)	Six Weeks Median (IQR)	Wilcoxon Signed Rank Test (p)
Peak VO_2 (ml/min)	.957 (.708-1.083)	1.017 (.738-1.369)	0.179
Peak VO_2 (ml/kg/min)	12.025 (9.688-14.629)	12.794 (11.200-17.095)	0.172
Time at Maximum (mins)	11.00 (9.00-13.33)	12.17 (9.75-13.58)	0.564
Watts at Peak VO_2 (W)	67.13 (47.89-86.63)	77.21 (59.42-95.30)	0.086
VE/VCO_2	32.38 (28.66-35.50)	31.83 (29.70-37.19)	0.302
Heart Rate at Peak VO_2 (bpm)	107.50 (94.71-121.25)	110.99 (98.83-122.46)	0.657

4.3.4. EndoPAT

An assessment of reactive hyperaemia, augmentation index, resting heart rate and blood pressure was undertaken when using the EndoPAT (see Table 29). No significant difference was noted in any of the parameters.

Table 29 The difference between Baseline and six weeks of exercise for measures of Endothelial Function (EndoPAT2000)

Measures of EndoPAT	Baseline Median (IQR)	Six Weeks Median (IQR)	Wilcoxon Signed Rank Test (p)
RHI	2.22 (1.65-2.47)	2.12 (1.84-2.58)	0.513
AI	34.59 (20.76-52.43)	27.48 (19.45-40.12)	0.879
AI at 75%	28.97 (14.76-44.04)	24.76 (13.05-31.01)	0.831
Heart Rate (bpm)	65.0 (57.5-76.5)	65.0 (60.8-74.5)	0.917
Systolic BP (mmHg)	142.0 (122.0-152.0)	144.5 (128.0-151.0)	0.751
Diastolic BP (mmHg)	77.0 (68.3-81.0)	77.0 (68.8-84.0)	0.518

4.3.5. Quality of Life

The generic SF36 version 2 scores displayed are the non-normalised scores (see Table 30). A significant improvement in domain scores for role physical and the mental summary score were seen. The change in VascuQoL over the six weeks (see Table 31), show an improvement in the activity, emotional and total domain scores.

Table 30 The difference between Baseline and six weeks of exercise for measures of Generic Quality of Life as measured by the SF36

	Baseline	6 week	Wilcoxon Signed Rank Test
Physical Function	30.75 (25.50-38.10)	38.10 (29.70-43.35)	0.065
Role physical	32.40 (25.00-43.40)	42.20 (34.80-52.00)	0.002
Bodily Pain	37.0 (29.20-45.98)	45.20 (34.80-51.10)	0.107
General Health	38.60 (32.90-45.80)	41.00 (32.90-50.60)	0.430
Vitality	45.80 (36.50-52.10)	49.00 (39.60-56.75)	0.109
Social Function	45.90 (35.00-56.80)	56.80 (40.50-56.80)	0.101
Role Emotional	36.40 (26.75-52.00)	52.00 (40.30- 55.90)	0.054
Mental Health	47.20 (38.70-55.60)	52.80 (43.00-58.45)	0.307
Physical Summary Score	31.50 (26.40-43.15)	37.40 (32.40-45.00)	0.170
Mental Summary Score	48.00 (39.70-56.60)	54.00 (48.30-60.50)	0.037

Table 31 The difference between Baseline and six weeks of exercise for measures of Disease Specific Quality of Life as measured by the VascuQoL

	Baseline	6 week	Wilcoxon signed rank (p)
Pain	3.8 (3.0-4.9)	4.5 (3.6-5.5)	0.194
Social	5.0 (3.0-6.5)	6.0 (4.5-7.0)	0.437
Activities	3.8 (2.7-5.3)	5.0 (3.9-5.6)	0.005
Symptoms	5.3 (4.5-5.9)	5.5 (4.9-6.0)	0.236
Emotional	4.9 (2.9-6.1)	6.0 (4.9-6.4)	0.032
Total	4.4 (3.3-5.6)	5.4 (4.5-5.8)	0.013

4.4. Twelve Week data

Data was analysed for both the primary outcome measure of changes after 6 weeks alongside changes after the standard duration of 12 weeks of exercise. This allowed an assessment of the impact of a full standard length SEP on recognised measures of lower limb ischaemia and other claudication parameters; i.e. quality of life and physical functional ability. In addition with the inclusion of CPET, endothelial function and inflammatory markers as outcome measures, we initiated investigations to assess the mechanism by which SEP improves clinical outcomes in claudicants.

Data is displayed as box and whisker plots with an accompanying summary table of the actual values. Significant changes over time were measured using a Friedman's ANOVA and are denoted by an asterisk at the end of the title.

4.4.1. Measures of Lower Limb of Ischaemia

Table 32 The difference between baseline, six and twelve weeks of exercise for measures of Lower Limb Ischaemia

Measures of Lower Limb Ischaemia	Baseline	Six Weeks	Twelve weeks	Friedman's ANOVA
Pre exercise ABPI	.64 (.49-.82)	.60 (.54-.74)	.68 (.58-.79)	0.260 n=23
Post Exercise ABPI	.33 (.20-.61)	.36 (.29-.58)	.45 (.37-.65)	0.029 n=22
Treadmill ICD (m)	60.78 (32.68-85.30)	83.38 (41.48-135.36)	126.97 (64.81-214.50)	<0.001 n=21
Treadmill MWD (m)	133.65 (83.95-214.50)	179.39 (105.33-214.50)	214.50 (210.17-214.50)	0.036 n=22

4.4.2. Measures of Physical Functional Ability

Table 33 The difference between baseline, six and twelve weeks of exercise for measures of Physical Functional Ability

Physical Functional Ability	Baseline Median (IQR)	Six Weeks Median (IQR)	Twelve weeks Median (IQR)	Friedman's ANOVA
Mean TUG (s)	7.43 (6.06-9.18)	6.49 (5.43-7.49)	6.67 (5.80-7.28)	n=20, p=0.005
Mean CST (s)	11.20 (8.68-12.34)	10.05 (7.52-11.44)	9.04 (7.13-10.77)	n=22, p<0.001
Semi-Tandem Stance (s)	30.00 (30.00-30.00)	30.00 (30.00-30.00)	30.00 (30.00-30.00)	n=23
Full Tandem Stance (s)	30.00 (30.00-30.00)	30.00 (30.00-30.00)	30.00 (30.00-30.00)	n=23, p=0.913

4.4.3. CPET

Table 34 The difference between baseline, six and twelve weeks of exercise for measures of AT as measured by Cardio-Pulmonary Exercise (CPET)

Measures of CPET at AT	Baseline	Six Weeks	Twelve weeks	Friedman' s ANOVA
AT (L/min)	.795 (.675-.870)	.770 (616-1.052)	.810 (.670-1.133)	n=13, p=0.116
Excess CO_2 (L/min)	.760 (.627-.870)	.745 (.543-.908)	.815 (.613-.980)	n=20 p=0.118
Simplified method VCO_2/VO_2 (L/min)	.700 (.545-1.190)	.820 (.620-.1.130)	.740 (.545-1.025)	n=9, p=0.819
VCO_2/VO_2 (L/min)	.770 (.610-.830)	.670 (.550-.840)	.710 (.580-.890)	n=19, p=0.321
VE/VCO_2 (L/min)	30.50 (28.46-33.82)	30.17 (27.40-33.27)	30.14 (28.43-34.06)	n=13, p=0.926
Watts at AT (W)	49.60 (40.69-56.86)	53.50 (40.75-69.92)	49.44 (37.99-85.46)	n=13, p=0.092
Heart rate at AT (bpm)	91.75 (95.52-83.04)	89.50 (79.83-100.10)	94.33 (87.10-108.00)	n=13, p=0.116

Table 35 The difference between baseline, six and twelve weeks of exercise for measures of Peak VO_2 as measured by Cardio-Pulmonary Exercise (CPET)

Measures of CPET at peak VO_2	Baseline	Six Weeks	Twelve weeks	Friedman's ANOVA
Peak VO_2 (L/min)	.975 (.807-1.361)	1.017 (.818-1.501)	1.049 (.891-1.415)	n=20, p=0.086
Peak VO_2 (ml/kg/min)	13.803 (11.174-16.415)	14.128 (11.704-18.560)	13.607 (12.233-17.162)	n=20, p=0.157
Time at Peak VO_2 (min)	11.833 (10.083-15.333)	12.500 (9.750-15.250)	12.000 (10.333-13.750)	n=20, p=0.962
Watts at Peak VO_2 (W)	71.778 (56.569 -108.978)	83.214 (59.425-111.159)	78.396 (56.929-112.792)	n=20, p=0.086
VE/VCO_2 at Peak VO_2	30.639 (28.617-35.403)	31.333 (29.696- 33.750)	31.444 (29.948-35.111)	n=20, p=0.915
Heart Rate at Peak VO_2 (bpm)	116.336 (97.125- 127.323)	114.625 (98.864-126.103)	113.603 (103.445-125.317)	n=20, p=0.705

Table 36 The difference between baseline, six and twelve weeks of exercise for measures of Endothelial Function as measured by the EndoPAT

Measures of EndoPAT	Baseline	Six Weeks	Twelve Weeks	Friedman's ANOVA (n= , p=)
RHI	2.24 (1.69 -2.39)	2.13 (1.99-2.50)	2.33 (1.86-2.88)	n=14, p=0.807
AI	28.00 (15.79-42.63)	31.51 (17.21-42.42)	26.57 (19.27-38.01)	n=14, p=0.076
AI at 75%	19.77 (13.36-33.57)	24.69 (18.64-33.60)	22.15 (16.68-26.20)	n=14, p=0.135
Heart Rate (bpm)	63.0 (60.0-75.0)	65.0 (60.0-69.0)	68.0 (58.0-76.0)	n=15, p=0.981
Systolic BP (mmHg)	141.0 (125.5-148.0)	143.0 (128.5-150.0)	140.0 (122.5-149.0)	n=17, p=0.901
Diastolic BP (mmHg)	79.0 (72.5-81.0)	77.0 (71.5-84.0)	76.0 (68.0-82.5)	n=17, p=0.898

4.4.4. Inflammatory markers

Inflammatory markers were measured in patients (IC1 to IC39 for TNF and CRP, and IC1-IC41 for IL-6) that attended and consented for blood tests. ELISAs for the three inflammatory markers were measured in 29 individuals at baseline, 20 at six weeks and 15 at twelve weeks, in view of the small numbers analysed, the median and IQR are displayed.

4.4.4.1. *High sensitivity C-Reactive Protein*

Results from the baseline, six week and twelve week levels of those tested have been illustrated (see Figure 31) with actual values included in the table (see Table 37).

The intra-assay mean coefficient of variance was calculated as 5.49% (s.d. 5.97%). Two plates were required to run all of the samples; the individual plate CV for plate 1 was 4.53% (s.d. 4.96%) and the mean CV% for plate 2 was 6.86% (s.d. 6.94%).

Table 37 Summary measurements of CRP at baseline, six and twelve weeks

Mean CRP (mg/L)	Median	IQR	Friedman's Two-way ANOVA
Baseline	0.429	0.268-1.056	n=15
Six Weeks	0.490	0.191-0.713	p=0.936
Twelve Weeks	0.507	0.281-0.813	

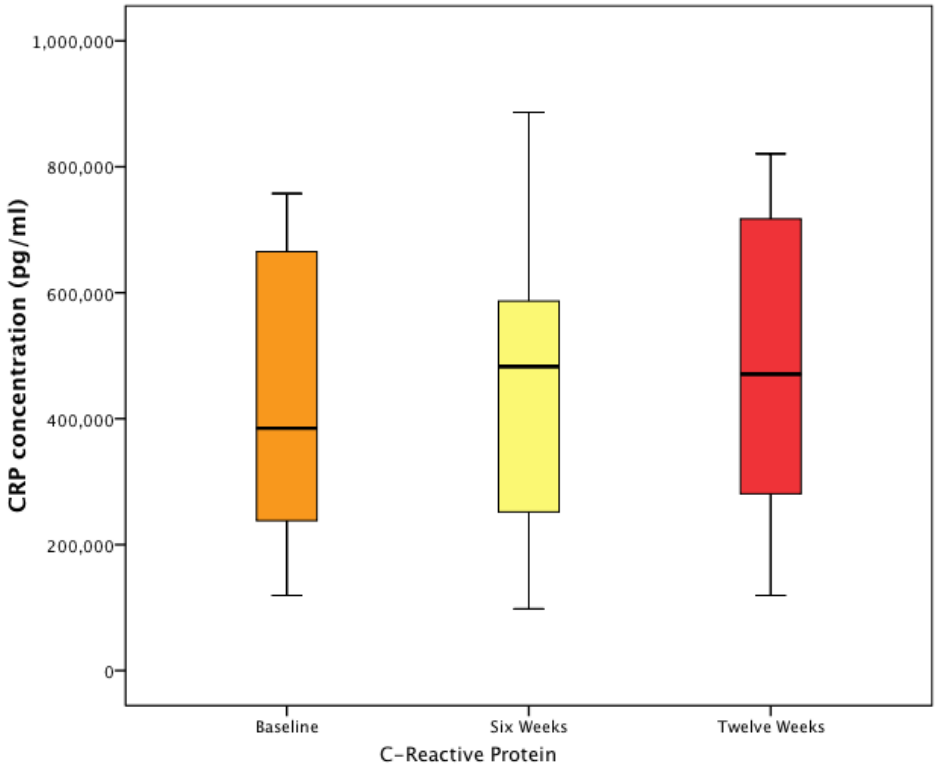

Figure 31 Box and Whisker Plot of change in CRP over time

4.4.4.2. Interleukin-6

Results from the baseline, six week and twelve week levels of those tested have been illustrated (see Figure 32) with actual values included in the table (see Table 38).

The intra-assay mean coefficient of variance was calculated as 5.10% (s.d. 4.54%). Two plates were required to run all of the samples. Unfortunately the original data, which determined which samples were run on each plate, is not available due to electronic corruption of the data file. Therefore inter-assay coefficients were not possible to calculate.

Table 38 Summary measurements of IL-6 at baseline, six and twelve weeks

Mean IL-6 (pg/ml)	Median	IQR	Friedman's Two-way ANOVA
Baseline	1.483	1.038-2.222	n=18 p=0.494
Six Weeks	1.760	1.187-2.213	
Twelve Weeks	1.770	1.178-3.166	

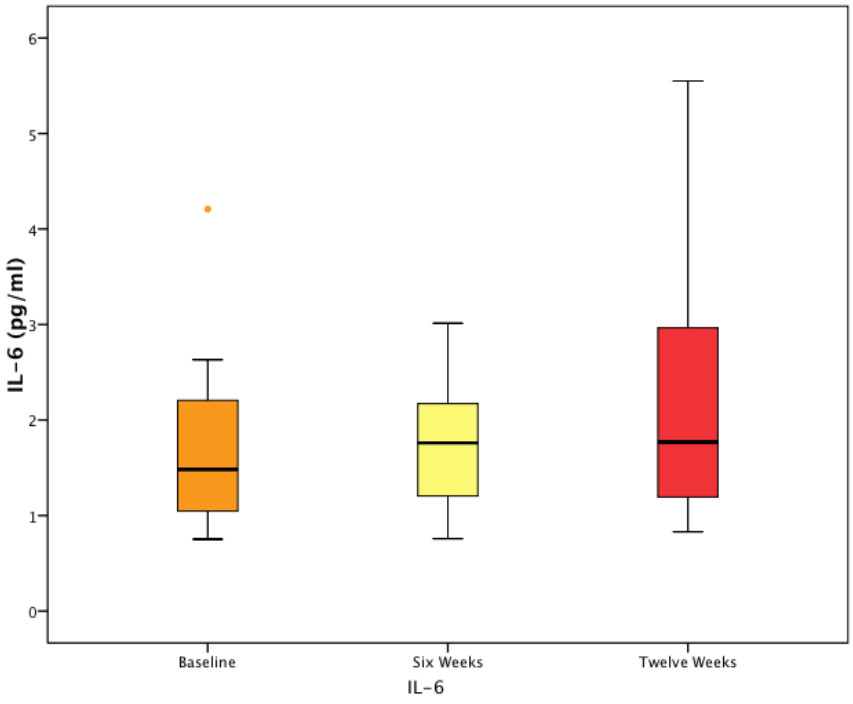

Figure 32 Box and Whisker Plot of change in Interleukin-6 over time

4.4.4.3. Tumour Necrosis Factor alpha

Results from the baseline, six week and twelve week levels of those tested have been illustrated (see Figure 33) with actual values included in the table (see Table 39).

The intra-assay mean coefficient of variance was calculated as 33.23% (s.d. 21.35%). Two plates were required to run all of the samples; the individual plate CV for plate 1 was 25.69% (s.d. 20.52%) and the mean CV% for plate 2 was 48.31% (s.d. 13.57%).

Table 39 Summary measurements of TNF alpha at baseline, six and twelve weeks

Mean TNF alpha (pg/ml)	Median	IQR	Friedman's Two-way ANOVA
Baseline	0.000	0.000-0.334	n=14
Six Weeks	0.000	0.000-2.453	p=0.163
Twelve Weeks	0.000	0.000-0.000	

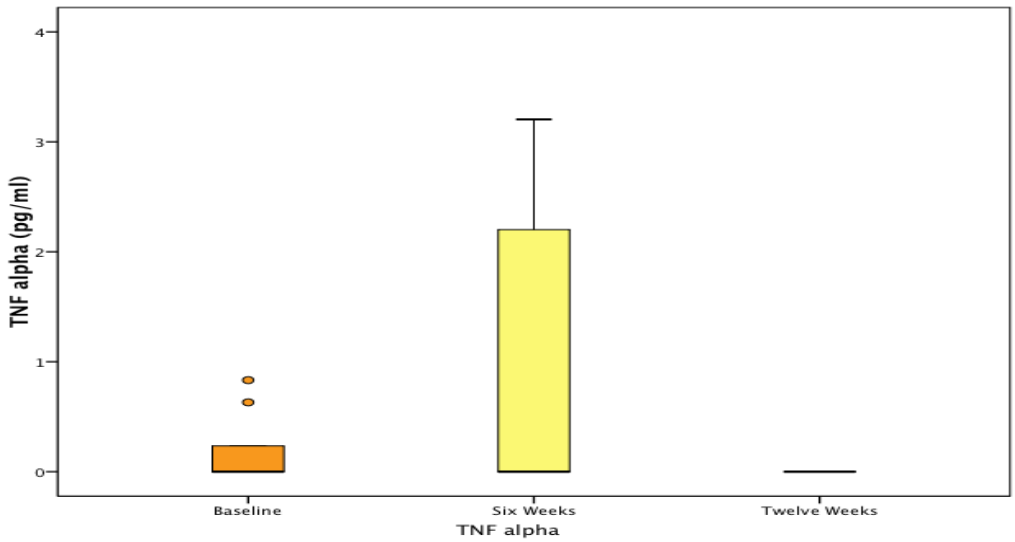

Figure 33 Box and Whisker Plot of change in TNF alpha over time

4.4.5. Quality of life

4.4.5.1. *Generic*

The following ten graphs depict the changes in each domain of the generic quality of life questionnaire (SF36) over the 12 week period. In each graph, the y axis is the numerical score of the SF36, and across the x axis are the three time points.

4.4.5.2. *Disease Specific*

The following six graphs depict the changes in each domain of the disease specific quality of life questionnaire (VascuQoL) over the 12 week period. In each graph, the y axis is the numerical score of the VascuQoL, and across the x axis are the three time points.

Table 40 The difference between baseline, six and twelve weeks of exercise for measures of Generic Quality of Life as measured by the SF36

	Baseline	6 week	12 Week	Friedman ANOVA
Physical Function	38.10 (27.60-44.40)	39.15 (31.28-47.55)	39.15 (30.23-45.98)	n=20, p=0.368
Role physical	38.50 (25.63-48.90)	45.85 (35.43-52.00)	43.40 (32.40-54.40)	n=20, p=0.007
Bodily Pain	45.60 (33.40-55.40)	48.20 (41.40-55.40)	48.20 (38.55-54.33)	n=20, p=0.430
General Health	42.40 (37.70-47.00)	41.00 (36.20-52.95)	41.00 (36.95-50.55)	n=20, p=0.824
Vitality	47.40 (38.03-54.43)	53.65 (42.70-58.30)	50.55 (42.70-52.10)	n=20, p=0.006
Social Function	51.40 (40.50-56.80)	56.80 (45.90-56.80)	56.80 (41.85-56.80)	n=20, p=0.167
Role Emotional	48.10 (33.55-55.90)	53.95 (41.28-55.90)	50.05 (32.60-55.90)	n=20, p=0.010
Mental Health	48.60 (41.60-55.60)	54.20 (45.10-58.48)	52.80 (44.40-58.50)	n=20, p=0.385
Physical Summary Score	41.00 (30.03 (48.13)	43.60 (36.45-45.60)	42.65 (35.25-45.73)	n=18, p=0.154
Mental Summary Score	51.00 (41.58-59.85)	57.40 (50.90-60.53)	55.45 (42.03-58.93)	n=18, p=0.014

Table 41 The difference between baseline, six and twelve weeks of exercise for measures of Disease Specific Quality of Life as measured by the VascuQoL

	Baseline	6 week	12 Week	Friedman ANOVA
Pain	4.3 (3.3-5.9)	5.0 (3.9-5.6)	5.0 (3.9-6.0)	n=21, p=0.051
Social	6.0 (5.0-7.0)	6.0 (5.0-7.0)	7.0 (5.0-7.0)	n=21, p=.150
Activities	4.8 (2.9-5.7)	5.3 (4.3-5.9)	5.6 (4.1-6.1)	n=21, p=0.031
Symptoms	5.5 (4.9-6.3)	5.8 (5.0-6.3)	6.0 (5.3-6.5)	n=21, p=.075
Emotional	5.9 (4.1-6.5)	6.0 (5.0-6.4)	6.3 (5.4-6.5)	n=21, p=0.024
Total	5.0 (4.1-5.9)	5.7 (4.7-6.0)	5.8 (5.1-6.4)	n=21, p=.072

4.5. Mortality

During the study, three deaths occurred, one secondary to a lung cancer recurrence, one secondary to a myocardial infarction and the third as a complication of femoro-popliteal bypass surgery in a patient who developed critical limb ischaemia.

4.6. Correlation

Secondary outcome measures were focused on whether a change in peak VO_2 would correlate with a change in any of the other measures:

- Measures of Lower Limb Ischaemia: ABPI Re, ABPI Pe, Treadmill ICD and MWD
- Physical functional ability: TUG, CST

4.6.1. Correlation between measures of lower limb ischaemia and peak VO2

Table 42 Correlation matrix of measures of lower limb ischaemia against peak VO_2

		Peak VO_2 (ml/min)	Peak VO_2 (ml/kg/min)	AT (ml/min)
ABPI Worst Leg	Correlation Coefficient	0.162	0.177	-0.267
	Sig. (2-tailed)	0.300	0.256	0.147
	N	43	43	31
Post Ex ABPI Worst	Correlation Coefficient	0.093	0.138	-0.176
	Sig. (2-tailed)	0.570	0.398	0.344
	N	40	40	31
ICD (m)	Correlation Coefficient	0.417**	0.554**	0.177
	Sig. (2-tailed)	0.008	<0.001	0.349
	N	39	39	30
MWD (m)	Correlation Coefficient	0.495**	0.688**	0.183
	Sig. (2-tailed)	0.001	<0.001	0.325
	N	41	41	31

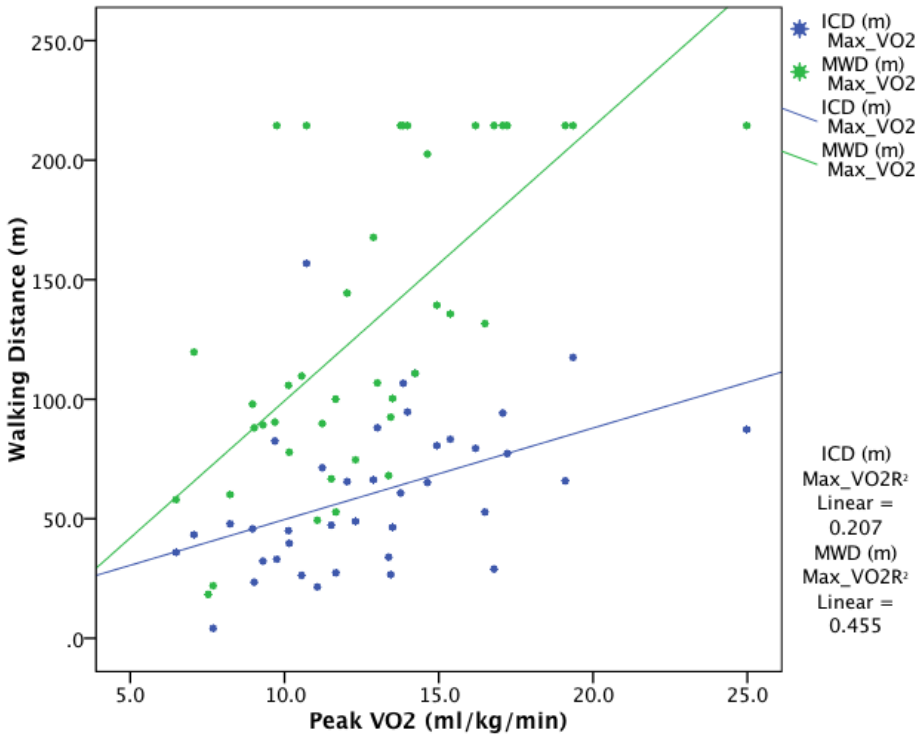

Figure 34 Scatter plot of Peak VO_2 against ICD and MWD

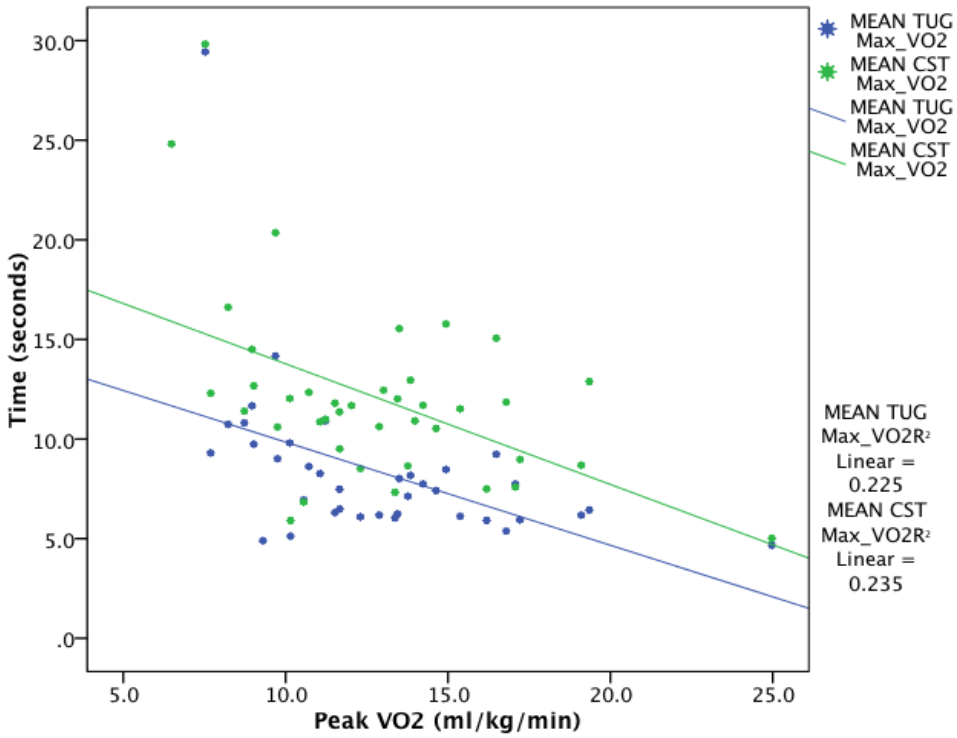

Figure 35 Scatter plot of Peak VO_2 against measures of physical function (TUG and CST)

4.6.2. Correlation between measures of Physical Function and peak VO_2

Table 43 Correlation matrix of measures of physical function against peak VO_2

		Peak VO_2 (ml/min)	Peak VO_2 (ml/kg/min)	AT (ml/min)
Mean TUG (m)	Correlation Coefficient	-0.514**	-.579**	-0.620**
	Sig. (2-tailed)	0.001	<0.001	<0.001
	N	37	37	28
Mean CST (s)	Correlation Coefficient	-0.341*	-0.351*	-0.230
	Sig. (2-tailed)	0.032	0.027	0.221
	N	40	40	30
Semi Tandem stance (s)	Correlation Coefficient	-0.014	0.014	-0.097
	Sig. (2-tailed)	0.931	0.931	0.612
	N	39	39	30
Full Tandem Stance (s)	Correlation Coefficient	0.235	0.300*	0.052
	Sig. (2-tailed)	0.149	0.064	0.784
	N	39	39	30

4.7. Exercise Responders

4.7.1. Peak VO_2

To assess whether the heterogeneity of the sample was influencing the results, the peak VO_2 values were examined. Individuals were stratified by whether or not they had improved their peak VO_2 over the course of the twelve weeks. Those who peaked at 6 weeks and then subsequently fell below their original peak VO_2 were grouped within the non-responders group for failure to maintain an improvement.

Of the 43 participants, 30 were included in the analysis. Of these, 18 were classed as having improved, and 12 had not.

In comparing the two groups there was no significant difference between them for their baseline peak VO_2 values, or their subsequent values achieved with training. Independent Mann Whitney U tests; baseline p=0.415, six weeks p=0.090, twelve weeks p=0.127 (see Figure 36).

In view of the lack of significance between the two groups when trying to identify those who respond and those who do not respond to exercise, a second restriction was placed on the groups. The group was divided into those who had improved their peak VO_2 by ≥1.5ml/kg/min. This resulted in 12 now being placed in the exercise responder category and 18 in the non-responder group. Independent Mann Whitney U tests; baseline p=0.200, six weeks p=0.611, twelve weeks p=0.235 (see Figure 37).

An independent samples Mann Whitney U test was performed to assess whether any differences were present between the two groups for continuous data (see Table 44).

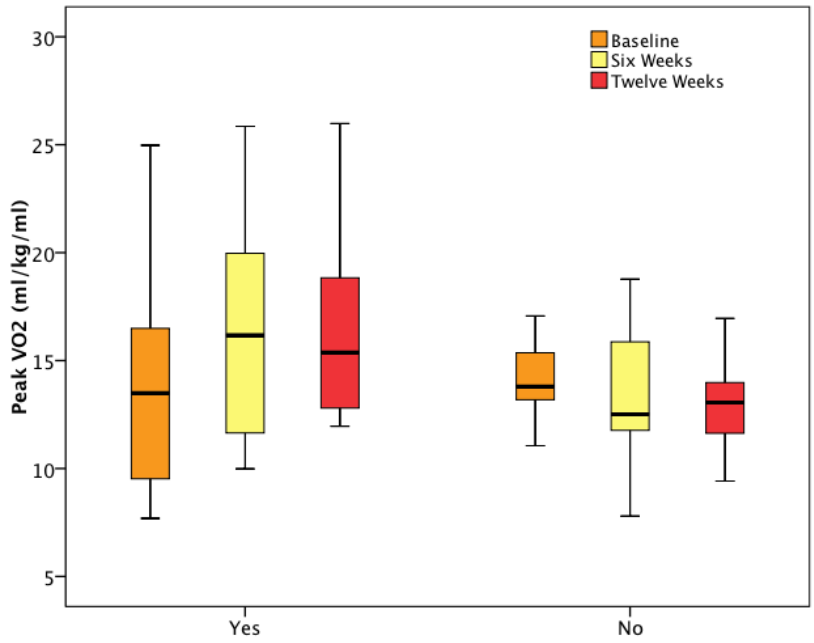

Figure 36 Increase in peak VO_2 over twelve week period

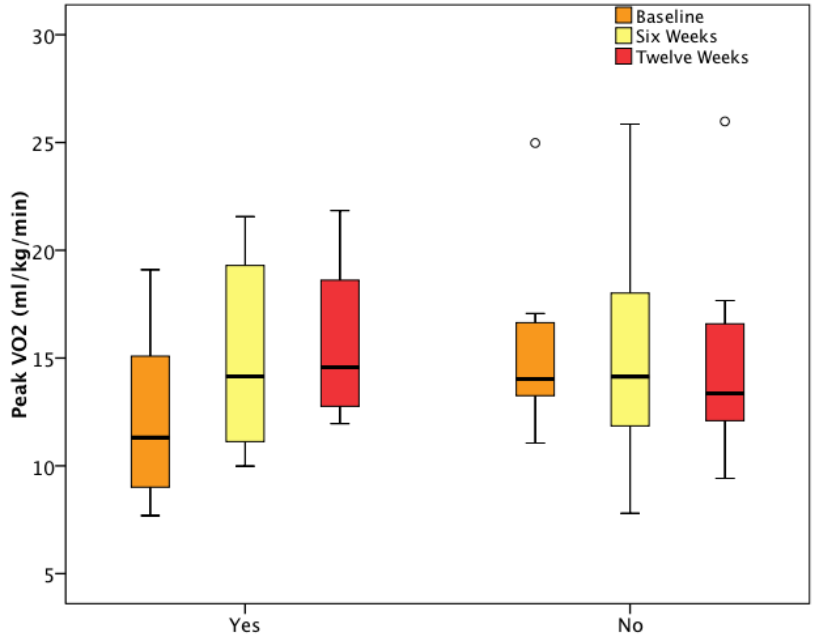

Figure 37 Box and Whisker plot to demonstrate the difference between the exercise responders (Yes group: improved peak VO_2 by 1.5ml/kg/min over twelve weeks) and non-responders (No group)

Table 44 Differences between responders and non-responders by Peak VO_2

	Peak VO_2 Improved by 1.5ml/kg/min				Independent Mann Whitney U Test (p)
	Yes		No		
	Median	IQR	Median	IQR	
Age	69.0	66.5-71.0	65.5	61.0-75.0	0.692
BMI (kg/m2)	27.9	25.2-30.7	27.3	23.4-28.0	0.325
Resting Heart rate (bpm)	57.5	54.5-65.0	71.0	62.5-80.0	0.007
ABPI Worst Leg	0.71	0.56-0.81	0.59	0.52-0.70	0.232
Post Ex ABPI Worst	0.46	0.15-0.65	0.29	0.18-0.43	0.458
ICD (m)	33.9	26.6-66.3	60.8	45.0-87.3	0.134
MWD (m)	144.4	88.0-214.5	110.8	89.8-214.5	1.000
Mean TUG (s)	6.2	6.0-9.3	7.8	5.9-8.3	1.000
Mean CST (s)	10.9	8.7-12.0	11.7	9.0-12.5	0.404
PeakVO_2 (ml/kg/min)	12.5	9.2-13.7	13.6	11.1-16.5	0.200
Baseline AT (L/min)	0.77	0.66-0.81	0.70	.67-.86	1.000
RHI	2.3	1.7-2.6	2.3	1.7-2.5	0.829
AI	35.2	28.0-56.2	34.4	12.1-44.1	0.373
Mean CRP ($x10^3$)	532.2	280.5-929.0	392.9	127.0-757.5	0.291
Mean TNF alpha	0.00	0.00-0.99	0.00	0.00-0.63	0.767
Mean IL6	1.49	1.05-2.27	1.58	1.13-2.21	0.666

4.7.2. Walking Distance

As the use of peak VO_2 failed to demonstrate any insight into who improves and who does not, the same stratification was applied to participants walking distance. An arbitrary threshold of a 50% increase in walking prior to ICD was classed as an exercise responder.

The use of the 50% gain in ICD meant that 17 of the 30 participants demonstrated an improvement over time (see Table 45).

Table 45 Differences between responders and non-responders by 50% improvement in ICD

	50% Improvement in Walking Distance				Independent Mann Whitney U Test (p)
	Yes		No		
	Median	IQR	Median	IQR	
Age	69.0	65.0-72.0	66.0	62.0-75.0	1.000
BMI (kg/m2)	27.	25.2-30.1	27.3	23.4-29.5	0.563
Resting Heart rate (bpm)	61.5	57.0-7.5	67.0	60.0-71.0	0.351
ABPI Worst Leg	0.64	0.54-0.84	0.54	0.46-0.69	0.079
Post Ex ABPI Worst	0.40	0.18-0.66	0.29	0.18-0.40	0.199
ICD (m)	54.1	32.7-83.4	52.8	36.0-77.2	0.948
MWD (m)	191.1	90.9-214.5	105.8	77.8-144.4	0.232
Mean TUG (s)	6.3	5.9-8.0	8.2	6.1-9.8	0.097
Mean CST (s)	10.8	8.1-11.9	12.0	11.0-13.0	0.050
PeakVO_2 (ml/kg/min)	13.4	11.5-16.2	11.6	10.1-15.9	0.616
Baseline AT (L/min)	0.75	0.66-0.86	0.70	0.68-0.81	0.948
RHI	2.3	1.7-2.8	2.0	1.4-2.3	0.118
AI	37.6	29.2-56.8	34.4	22.4-42.1	0.387
Mean CRP ($x10^3$)	362.9	231.7-757.5	728.4	392.9-1359.0	0.176
Mean TNF alpha	0.12	0.00-0.99	0.00	0.00-0.00	0.118
Mean IL6	1.26	1.01-2.27	1.86	1.54-2.21	0.029

When comparing the two different exercise stratifications, there was no significant difference between the two groups when using a two-tailed Fisher's exact test (p=0.054, d.f. =1), but was significant with a one tailed test (p=0.040, d.f. =1) (see Table 46).

Table 46 Overlap between two exercise responder groups

		Improved Walking Distance		Total
		Yes	No	
Improved peak VO_2 by 1.5ml/kg/min	Yes	10	2	12
	No	7	9	16
Total		17	11	28

4.8. AUDIT

4.8.1. Introduction

In order to ensure that the target of 38 patients entering into the SEP was reached, the referral rate to the SEP by the clinical vascular team was audited. The audit was focused primarily on the numbers of patients being referred for enrolment in the supervised exercise programme, but the number of patients referred for exercise advice and the number of patients receiving no advice was also of interest.

4.8.2. Methods

A retrospective audit was undertaken to assess whether the management of patients with intermittent claudication seen in the vascular outpatient clinic at Hull Royal Infirmary from 1st June – 31st August 2010 inclusive was in line with the ACC/AHA guidelines 2005 for the management of patients with Peripheral Arterial Disease (Hirsch 2006). Audit office approval was sought and gained in advance.

The audit department provided a list of new patients, attending outpatient clinics over the study period. The hospital Patient Centre network was searched for response letters from the vascular consultants to the GP's initial referral letter. In order to be deemed as complying with guidelines, the response letter was required to contain a statement recommending exercise or a referral to the supervised exercise programme. If no mention of walking or exercise was present in the letter, this was marked as a failure. Due to a delay in letters being transferred onto the system or diagnoses being made after additional exercise treadmill tests or MRAs, the audit did not commence until January 2011.

Audit criteria were; a minimum of 80% referred for exercise (by either walking advice or SEP) was deemed acceptable.

Using a specifically designed proforma, data was collected on an excel spreadsheet. Data was then analysed in Excel and disseminated to the vascular team.

4.8.3. Result

4.8.3.1. *Results of Initial Audit*

The Hull Royal Infirmary SEP audit was performed between 1st June & 31st August 2010. Over the three month period, the consultant vascular surgeons saw 673 new patient referrals. The proportion of patients who were referred for varicose veins, claudication, and aneurysms are demonstrated, see Figure 38.

Of the 172 patients referred with a differential diagnosis of intermittent claudication, 65 had normal treadmill exercise tests and were discharged from clinic. The remaining 107 patients had the diagnosis of intermittent claudication confirmed, however only 18 patients were given exercise advice and only 11 were referred for SEP. Therefore only 29 / 107 (27.1%) patients received management which complied with the guidelines, less than the "acceptable" level set at 80%. The department therefore failed the audit.

Of those who were referred for the SEP, only 5 of the 11 undertook the program. The remaining six included one above knee amputee whose suction prosthesis loosened when exercising. Three patients declined participation and 1 patient failed to attend the SEP. The final patient was deemed unsuitable for the SEP due to poor mobility (he required a mobility scooter!)

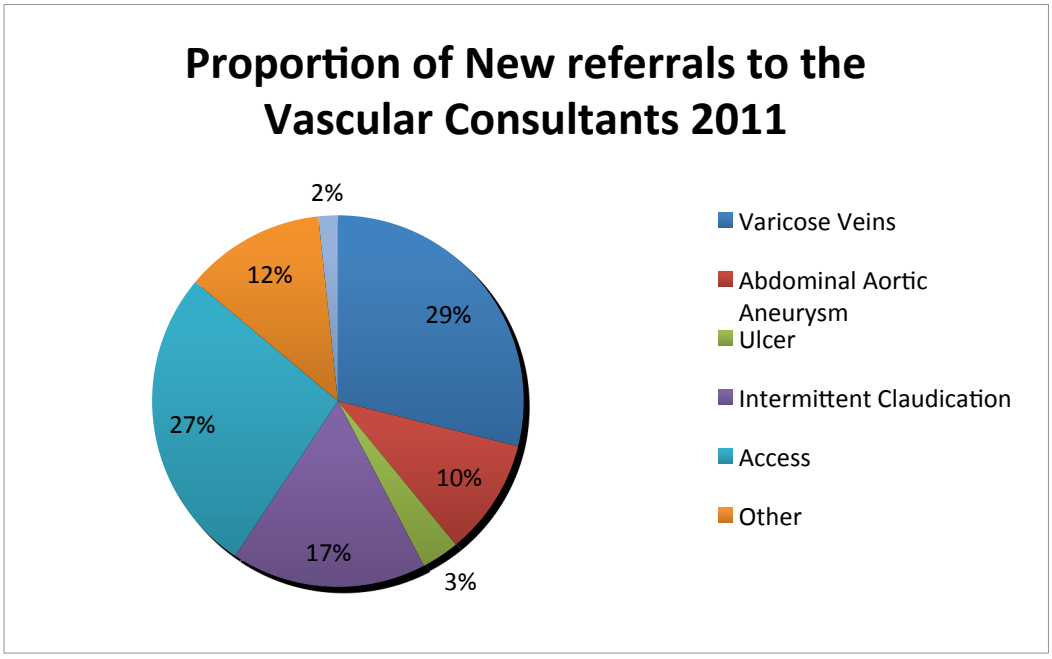

Figure 38 Proportion of new referrals to the Vascular consultants 2011

4.8.3.2. Dissemination to the Vascular Team and agreed outcomes

The audit was disseminated to the vascular consultants and resulted in a new approach to the vascular consultant's letter. The response letters to the General Practitioners were stipulated to include a referral or recommendation with regards to commencing patients on a form of exercise. The re-audit was agreed to start four months following the presentation of the results.

The consultants agreed to guidelines being implemented for the SEP and the subsequent re-audit, these were;

1. Exclude any patient who declined SEP in clinic, but to ensure that this is documented in the GP letter in order to highlight exercise as a primary treatment option.
2. Exclude those with the following medical conditions:
    a. Unstable angina
    b. Exercise limiting shortness of breath
    c. Amputees

4.8.3.3. Results of Re-Audit

The re-audit was re-approved by the audit department and undertaken during February to March 2013. This focused on the time period from 1st July to 31st August 2012. Over the three month period, 693 new patients were reviewed in the vascular clinic by six consultants, and the junior members of their team. The full breakdown of the referrals is illustrated (see Figure 39).

Of the 162 possible claudicant referrals, 58 (8.3%) had normal treadmill exercise tests and were discharged from clinic. The remaining 104 (15.0%) were treated as having intermittent claudication. Of those, 25 (25.2%) were referred for supervised exercise and 31 (29.8%) were given walking advice. An additional 8 patients declined or were deemed unsuitable for SEP. The remaining 40 patients (38.4%) had no documentation in the response letter to the GP regarding exercise advice. The re-

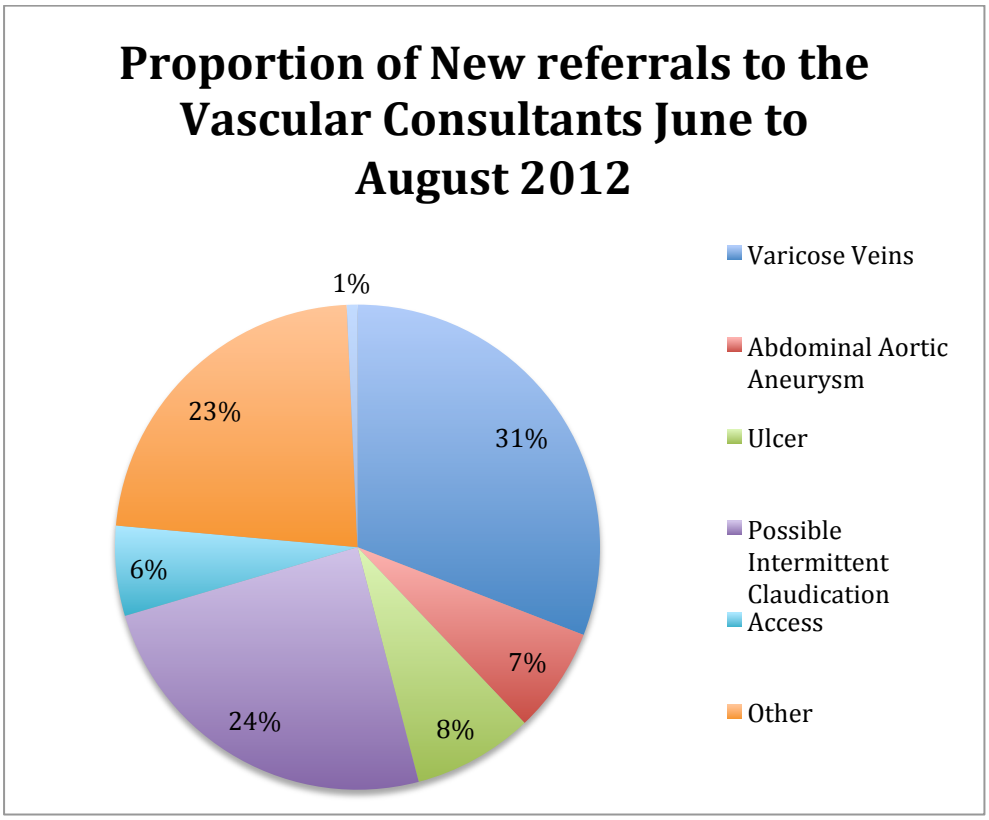

Figure 39 Proportion of new referrals to the Vascular consultants 2012

Table 47 Breakdown of Claudicants exercise advice

2012	Walking advice	SEP	Unsuitable / declined	Nil
June	10 (30.3%)	5 (15.2%)	0 (0%)	18 (54.5%)
July	10 (24.4%)	16 (39.0%)	5 (12.3%)	10 (24.4%)
August	11 (36.7%)	4 (13.3%)	3 (10.0%)	12 (40.0%)

audit demonstrated an improved awareness of the guidelines with walking advice or SEP referral increasing from 10% to 61.5% (see Table 47).

Risk factor modification over the three months, a secondary outcome of the audit, was adequately addressed with regards to aspirin (73.2%) and statin (69.0%) uptake as well as smoking cessation advice (63.3%). Hypertensive status was poorly recorded with two people requiring further blood pressure assessment by their GP.

4.8.3.4. Discussion and Agreed actions from re-audit

At the re-presentation of this second audit, the following were decided upon:

1. Whilst an improvement in SEP referrals and exercise advice for those with IC had occurred, this did not translate into an increased uptake to the SEP. To improve uptake, it was decided that all patients should be explicitly told that they require a period of exercise prior to being considered for any further intervention.
2. An attendance rate greater than 75% at the SEP was required. Without this, patients would be deemed to have failed a proper trial of SEP and would therefore not be deemed acceptable for further intervention.
3. Due to a high proportion of patients failing to attend for SEP, underlying reasons for failure to attend need to be explored at the time of referral. I.e. any mitigating circumstances need to be explored in clinic prior to referral. A high proportion of patients have been noted to have dependants, or work commitments that preclude attendance.
4. Given the clinical need as determined by NICE (NICE 2012), funding and additional help to run satellite sites for SEP in Hull as well as the surrounding community would need to be explored.
5. In working with the local councils on increasing the availability of exercise on prescription for patients with claudication; a move from standard referral forms to those for cardiac rehabilitation referral forms were recommended.

4.8.4. Discussion

The improvement in the referral rate for SEP also resulted in an increased number of patients actually undertaking SEP. These findings should be augmented by NICE's PAD recommendations (September 2012 (NICE 2012)) which reinforce the value of exercise programmes for claudicants.

5. DISCUSSION

5.1. Summary of results

Overall the results from this prospective observational study are somewhat limited by the high attrition rate and under-recruitment. Two key things were however highlighted from our initial recruitment, firstly claudicants were failing to engage with the SEP service and secondly active management of risk factors is still a key target area.

Primary care risk factor modification remains poor with 40% still as current smokers and 50% with persistent hypertension despite best medical therapy. Our cohort of patients had Rutherford grade III claudication, which was reflected by a low median AT (7.40ml/kg/min) and peak VO_2 (12.0ml/kg/min).

Improvements were demonstrated after both six and twelve weeks of exercise in initial claudication distance ($p<0.001$) and in quality of life. Surprisingly, the VascuQol lost significance by twelve weeks, whilst the generic SF-36 continued to show positive improvements (vitality ($p=0.006$), and role emotional ($p=0.010$), and the mental summary score ($p=0.014$)). No significant changes were noted in EndoPAT, CPET or inflammatory markers. These findings have been expanded upon further in sections below.

5.2. Hull Royal Infirmary Supervised Exercise Programme (HRI SEP)

Of those who undertook the HRI SEP, uptake for the first six weeks reduced from a 100% to 47.5%. The literature generally focuses on those who have undergone a minimum of 50% of the SEP. If we were to limit our data to that select group, this would exclude 53% of our cohort. As uptake was poor from our original 163 patients, with only 44 consenting to participate, and only half completing the full 12-week programme (n=20) we decided not to limit our findings to those who had undertaken over 50% of the programme. Acceptance of SEP as an effective treatment for claudication and compliance with the programme are serious problems. In this study, excluding those with poor attendance would have introduced potential bias and weaken any findings. The poor recruitment and compliance rate found in this study is common in many IC based studies and has previously led to the early

abandonment of multicentre trials (Greenhalgh et al. 2008). It would appear a brief psychological intervention aimed at modifying illness perception and walking belief has lasting effects on walking ability (Cunningham et al. 2013; Cunningham et al. 2011). Perhaps the clinical and cost effectiveness of combining cognitive behavioural therapy with a SEP merits further investigation in terms of walking based outcomes, recruitment to SEP and compliance with it. Even with the relatively poor compliance, this study demonstrated similar findings to those reported, namely an improvement in walking distances, physical function and quality of life.

These benefits are well documented and are evidenced in the guidelines recommending the routine use of SEP as the primary intervention for claudication (NICE 2012). Our departmental audit clearly demonstrated our unit to be requiring additional support to adhere to these guidelines, once implemented this improved our referral rate when we closed the audit loop. Of note however, whilst referral rates improved, patient uptake to the programme did not. This indicates the barriers to SEP participation are complex and numerous, certainly in excess of that which could be resolved in a brief clinic consultation, and may merit further investigation.

The patients who enrolled and complied with the SEP demonstrated significant motivation and enthusiasm as evidenced in improvements in their quality of life and walking distances. However, no significant improvements were recorded in other outcome measures including, cardiopulmonary exercise measures, endothelial function or levels of inflammatory markers. This may primarily be due to a type 2 statistical error i.e. the study was underpowered in regard to these specific outcome measures. Alternatively, the psychological effect of SEP may be greater than was previously acknowledged (Cunningham et al. 2011; Cunningham et al. 2013; Galea et al. 2013). An alternative hypothesis is that the duration of the SEP was of inadequate duration to produce a significant improvement in walking outcomes. In addition, it has been demonstrated that a programme $\geq$ 6 months is required for the psychological understanding to sustain behavioural changes (Anshel 2007) in exercise, eating or smoking (Crouch et al. 2011; Read et al. 2001). SEP of shorter

duration are associated with reversal of these improvements and ultimately loss of value of the intervention (Anshel 2007).

We originally planned to use the SEP as a stepping-stone to a community based exercise programme through exercise on prescription and then later enrolment into the gyms for membership. However variability in availability of exercise on prescription according to location made this prohibitive. Therefore the hypothesis that these patients will sustain their walking and lifestyle improvements after only a three-month intervention is postulated rather than supported by evidence (Pavey et al. 2011; Jones et al. 2005). New evidence suggests that two months of exercise increases walking distances, but slows after this. Therefore a minimum of two months may be of benefit, but sustained benefit beyond this is of question (Gardner et al. 2012).

Within our group, the lack of improvement in the anaerobic threshold or peak ventilatory threshold may be in part attributed to the lack of monitoring during the class. In order to ensure maximum benefit from the SEP it is recommended that patients exercise at a pre specified intensity. This intensity should induce a heart rate 40-60% of maximal heart rate. We attempted to validate that patients were exercising at the correct intensity using heart rate using monitors. This proved impossible due to poor patient adherence, the inability of many patients to accurately record the heart rate using the monitor's pre and post each exercise and the loss of signal that frequently occurred between the chest straps and the heart rate monitor watches. We were therefore, unfortunately unable to validate the exercise intensity achieved in this patient cohort. In view of this, the SEP was administered as it has been for the previous ten years. Patients were asked to exercise to an intensity that initiated their claudication pain, and then encouraged to try and exercise through this if possible, but rest as and when they feel is required. This subjective aspect of the SEP has been previously quantified using the BORG RPE scale, which demonstrated it to have limited value in patients with leg pain as it does not correlate with their heart rate (Scherr et al. 2013) as it was originally designed to do (McDermott et al. 2012).

5.3. Baseline claudicant Characteristics

The management of cardiovascular risk factors in claudicants remains poor in primary care (Cassar, et al. 2003b). It should be standard care for claudicants to be commenced on an antiplatelet, a statin and to be provided with smoking cessation advice (Hughson et al. 1978). The compliance with smoking cessation advice is poor, as demonstrated by the 40% who remained current smokers during the SEP. Hypertension was present in 73% of the population (n=32) and was poorly managed in 10 i.e. not in accordance with hypertension management guidelines. Median baseline systolic blood pressure was 142mmHg, indicating that > 50% of participants had a resting blood pressure greater than recommended.

Risk factor management is a crucial given the 5% annual cardiovascular mortality in patients with claudication. Ideally this should be managed in primary care but is the responsibility of all health care professionals involved in the management of these patients (Cassar, et al. 2003a). Efficient communication is required to ensure this is not neglected. The problem of suboptimal risk management in this cohort of patients has been recognised since the 1970's, and in spite of forty years of awareness, continues to be an issue (Brotons et al. 2011; O'Donnell et al. 2011).

5.3.1. Measures of Lower Limb Ischaemia and Physical Functional Ability

The reliability for ABPI measurements was high ($\alpha = 0.878$). This suggests that changes in ABPI over time were not secondary to error but were true changes in the peripheral arterial supply of participants.

Baseline assessment demonstrated that 50% of participants were Rutherford III and approximately 70% had infra-inguinal disease. The relatively high incidence of bilateral disease and comorbidities suggests significant systemic atherosclerosis in this patient cohort, but is a true reflection of the general health of our local population. This may also explain the lower than expected generic quality of life and peak VO_2 values at baseline in our cohort.

The measures for speed and turning ability, the CST and TUG test, were strongly positively correlated. This is also supported by the literature (Schaubert & Bohannon 2005; Khalil et al. 2010; Wright et al. 2010), and one could therefore be used as a surrogate marker for the other. Since ABPI-Pre and ABPI-Post exercise were moderately negatively correlated with mean CST ($r= -0.585$, $p=0.001$ and $r=-0.680$, $p<0.001$ respectively) and MWD (m) had only a weak positive correlation with best TUG ($r= -0.325$, $p=0.050$), the CST would appear to be the most valid measure of physical functional ability in this patient cohort. In addition, the CST is easier to perform, as it required a chair and stopwatch rather than additional space to walk.

5.3.2. Measures of CPET

The exercise ability of those who underwent testing was poor, with a low median anaerobic threshold (7.40ml/kg/min) and median peak VO_2 (12.0ml/kg/min). Given that the threshold for consideration of surgery is an AT of 11ml/kg/min, our cohort's deconditioned baseline state is highly important (Young et al. 2012). As this group would be considered for more invasive treatment should conservative treatment with exercise and risk factor modification fail, their low baseline leaves them at an increased risk of cardiovascular morbidity and mortality if surgery were to be considered.

The AT was derived by three different methods. The Gaskill method averaged data every 20 seconds, this was then graphically plotted and assessed by two independent assessors. This is the gold standard and was utilised in this study. The other two methods of calculating AT, the excess CO_2 and the simplified VO_2 method, underestimated the AT by 0.4 and 1.0 respectively. This demonstrates the variability associated with calculating the AT. The use of lactate thresholds and regular lactate samples to improve accuracy in AT calculation were not feasible in this study (see section 9.2). A further consideration is that the AT will reflect the onset of claudication and may again be an inaccurate marker in patients with claudication.

Peak VO_2 is often easier to identify, although this is also subject to variation. Reasons for terminating an exercise test are not simply exhaustion. Reasons cited by our cohort, included discomfort from the bicycle seat, leg fatigue or boredom. All participants were encouraged to do the best they could so that this would provide them with a personal goal. Interestingly this type of motivation resulted in responses spanning from interest, to fear of failure and embarrassment. The need for exploration of the underlying psychological beliefs attached to exercise (Anshel 2007), their own walking ability and reasons for stopping tests early is of great importance given the governments encouragement of exercise strategies (NICE 2012).

The mean peak VO_2 was 12.313 (S.E. 0.637) for the 43 patients who underwent baseline CPET. This was significantly lower than pooled data from the 16 previously published studies of CPET in claudicants – mean peak VO_2 was 14.748ml/kg/min (standard error 0.564; 95% C.I. 13.644-15.853). It is unclear why the participants from Hull and East Riding have a mean peak VO_2 that is 2ml/kg/min lower than other claudicant populations. This may be due to a higher level of socioeconomic deprivation, higher proportion of smokers or lower daily exercise in the local population. Alternatively, stringent inclusion and exclusion criteria may have resulted in a positive selection bias in the other populations, and account for the higher peak VO_2.

5.3.3. Measures of Endothelial Function

The median RHI-PAT was 2.22 (IQR 1.67 to 2.47), with significant endothelial dysfunction present in 10 (25%) and 4 (10%) of participants at baseline according to the RHI-PAT thresholds of 1.67 and 1.35 respectively. The low frequency of endothelial dysfunction in our cohort seems surprising given their low peak VO_2 and anaerobic thresholds. As other studies have used these thresholds to highlight high-risk populations, the low rate of endothelial dysfunction in our cohort raises

questions. Either the test is unable to accurately quantify the presence of endothelial dysfunction in our cohort, or there is a lower level than previously anticipated.

Recent data suggests that the former hypothesis is true, that the EndoPAT may lack sensitivity to detect endothelial dysfunction in the peripheral vascular disease cohort (Kiani et al. 2013; Allan et al. 2013). Other recent studies have supported this notion in other cohorts; renal failure and type II diabetics (Moerland et al. 2012), hypertension (Takase & Higashimura 2013). Further assessment of the EndoPAT is needed against brachial artery ultrasound in specific patient cohorts.

The only comorbidity associated with a significantly higher risk of endothelial dysfunction (RHI threshold 1.67) was diabetes. This finding has been supported by another study (Hamburg et al. 2008), however the lack of change secondary to other risk factors such as smoking, hypertension or hypercholesterolemia is of surprise. This lack of results may be due to the lack of a healthy age-matched control population against which the values could be compared or low numbers.

An improvement in endothelial function secondary to exercise was hypothesised from the start of this thesis. Previous data has suggested that exercise increases endothelial nitric oxide synthase, therefore improving endothelial function (Ghisi & Pinho 2010; Kojda & Hambrecht 2005). It was postulated that in view of the association between activity, nitric oxide and endothelial function, a positive association might be present. No correlation was identified between the RHIPAT and clinical indicators of lower limb ischaemia or measures of cardiopulmonary exercise tolerance. The literature suggests that endothelial dysfunction plays a major role within the cascade leading to increasingly severe peripheral arterial disease (Vita & Hamburg 2010), however the exact mechanism remains only partially explored. This studies negative result was initially unexpected, however as evidence grows with respect to the sensitivity of the EndoPAT in peripheral vascular disease, findings are actually in keeping with current literature.

The AI correlated with resting ABPI ($r=-0.342$, $p=0.031$), heart rate ($r=-0.360$, $p=0.022$) and systolic blood pressure ($r=0.320$, $p=0.044$). Given the relationship of

AI to arterial pulse wave pressures, this is unsurprising. Of note, the degree of reactive hyperaemia as measured by the RHI-PAT, and potential arterial stiffness (AI) were not related.

5.3.4. Measures of Quality of Life

Our data is supported by the literature, and demonstrates that individuals with IC have a reduced quality of life in comparison to published values in healthy population. This was also reflected in the disease specific quality of life questionnaire, which was reduced across all domains with a median ranging from 4-5. As with the SF-36, this was not affected by worsening Rutherford grade.

We were unable to demonstrate an association with increasing lower limb ischaemia (deteriorating Rutherford grade) and deteriorating generic or disease specific quality of life. A study by Wann-Hansson (Wann-Hansson et al. 2004) demonstrated that the Nottingham health profile and SF-36 were both sensitive at identifying changes in claudication over a years duration.

The lack of association or difference present within our Rutherford groups against the VascuQol is of surprise (Fainita et al. 2005). This may be attributed to low numbers, leading to a type II error or too narrow a cohort of PAD patients, i.e. claudicants only, and no healthy controls or patients with critical limb ischaemia for comparison. Our cohort have a higher quality of life as per the VascuQol when compared to other claudicants, but a low generic quality of life when compared to the general population. This is an area that needs further exploration to identify whether our cohort were relatively well claudicants who therefore lacked the motivation to undergo SEP, or whether they simply lacked insight and felt that there health was equivalent to those surrounding them. If surrounded by other family members with equivalent poor health, this may have affected their perception of their own problems.

Due to low numbers, we have not explored the effect of co-morbidity or gender (Oka et al. 2003) on quality of life. Given the low proportion of females within the study, this aspect may have failed to highlight any significant findings. Had there been further time and resources, socioeconomic status, gender, age would have been used to allow for comparison against normalised values.

5.4. Post SEP

5.4.1. Measures of Lower Limb Ischaemia and Physical Function

At both six and twelve weeks of exercise an improvement in initial claudication distance was noted ($p<0.001$), and the speed of completing the chair stance time decreased by 1 and ½ seconds ($p<0.001$). At twelve weeks, speed of completion of the mean TUG also significantly reduced. The benefit of a 1-2 second improvement in speed is of uncertain clinical relevance. An improvement in functional measures has been identified in other disease states when undertaking a six week exercise programme (de Oca et al. 2005). The TUG and CST are associated with lower limb strength and turning ability, therefore an improvement in these measures may act as a surrogate for these. The CST also correlated significantly to walking distance, which may allow a quick assessment of fitness within vascular clinics.

Other improvements seen at 12 weeks; post exercise ABPI ($p=0.029$), and MWD ($p=0.036$), lost significance when using the Bonferroni correction. The change in MWD is an expected change that has been well-documented in the literature (Watson et al. 2008). Whilst the changes in walking distance are well recognised, the improvement in ABPI is uncommon (Mazari et al. 2012), and it is unclear whether this was a type I error or true finding.

5.4.2. Measures of CPET

As previously stated, the study was underpowered by 4 participants despite over-recruitment. The mean difference calculated peak VO_2 was 0.63ml/kg/min after 6 weeks of exercise; this was less than half the improvement deemed to be clinically significant. Both the peak VO_2 and the AT did not significantly improve over the six weeks, or at three months.

These findings are in direct contrast to other publications focused on the effect of SEP in other chronic atherosclerotic diseases (metabolic syndrome associated with polycystic ovarian syndrome (PCOS) (Orio et al. 2008), and cardiac disease; acute MI (Che et al. 2008), heart failure (Kubinyi et al. 2003). Patients with heart failure were randomised to three groups; progressive increased workload, interval training or a control group. Whilst both training groups increased the duration they were able to exercise for, METS, peak VO_2, the group who undertook an increased workload showed a better outcome without any cardiac detriment. The same principle of continued incremental physiotherapy was applied to the Gardner study (Gardner et al. 2011) of home based physiotherapy with outpatient monitoring. Without regular monitoring to adjust and increment exercise appropriately, there remains a risk that patients will remain static and only push themselves a minimal amount. This was demonstrated in the peak VO_2 improvement, which demonstrated no change in the control group, but improvements in the SEP and home exercise groups.

Due to difficulties implementing the heart rate monitors within our group, this may have a negative confounding on outcomes from the class and explain the lack of improvement seen within this study.

5.4.3. Measures of Endothelial Function

In this study, there was no improvement in measures of endothelial function; RHI or AI at either six or 12 weeks. This is in direct contrast to other studies of claudicants using BAUS to measure endothelial function (Andreozzi, Leone, et al. 2007a)) or cardiopulmonary exercise tolerance.

Current literature associates regular exercise with an improvement in endothelial function (Lippincott, Desai, et al. 2008b; Lippincott, Carlow, et al. 2008a), and sedentary behaviour resulting in the opposite. After four weeks of exercise, an improvement in endothelial function was identified in patients with coronary artery disease (Hambrecht, Wolf, et al. 2000b). A period of 30 days of enhanced external counter-pulsation (EECP) in patients with symptomatic coronary artery disease has also been demonstrated to improve endothelial function as measured by EndoPAT (Bonetti et al. 2003). Whilst EECP is not a form of exercise, it has been used to aid cardiac patients (Yang & Wu 2013; Manchanda & Soran 2007)with heart failure (Bonetti et al. 2003), or angina (Kitsou et al. 2010).

It may be that the measures of endothelial function used in this study were not sufficiently sensitive to detect subtle changes (Allan et al. 2013) or the negative result in this study is a real finding given the lack of detectable improvement in CPET and inflammatory markers. The disparity noted by Allan et al has also been corroborated in patients with hypertension (Takase & Higashimura 2013), leaving some questions about the validity of the EndoPAT in non-healthy cohorts. An alternative hypothesis is that the exercise programme was not sufficient stimulus to produce a change. Perhaps a more targeted exercise programme may be required to ensure that claudicants are exercising at a sufficient intensity to gain the cardiovascular and endothelial benefits? Or a parallel use of CBT with the SEP may lead to greater benefits and a demonstrable improvement in endothelial function.

5.4.4. Measures of Quality of Life

Quality of life measures did improve when measured by the generic SF36 form, but only the role physical ($p=0.002$). All of the other domains showed a non-significant improvement. It remains a possibility that the cohort at 6 weeks was too small to demonstrate an improvement in the other domains, or that the re-appraisal was too early. This is supported by the improvements seen at twelve weeks. By twelve weeks, an additional three domains gained significance, vitality ($p=0.006$, role

emotional (p=0.010), and the mental summary score (p=0.014). The lack of improvement in all domains is not represented in the literature (Malagoni et al. 2011).

When looking at the disease specific VascuQoL, only the total (p=0.013) and activities (p=0.005) domains were of significance at six weeks. In addition, pain, emotional and social domains demonstrated a non-significant improvement in quality of life. There are no studies that provide insight into how quality of life improves, or whether an improvement in one domain leads to improvement in others. This makes it difficult to ascertain whether there is a recognisable pattern of quality life improvement associated with SEP for claudicants.

Of note, after twelve weeks of exercise, there was no demonstrable improvement in quality of life measures as measured by the VascuQoL. It remains unclear why domains lost significance and the overall improvement was seen purely in the psychological domains; this is in contrast to other studies (Mazari, Gulati, et al. 2010b).

5.4.5. Measures of Athero-inflammatory markers

Inflammatory markers, (hsCRP, IL-6 and tNF alpha) were measured in the participants at baseline, six and twelve weeks. Over the whole twelve week period, no significant alteration in values occurred. This suggests that either the intensity of exercise was insufficient to result in a change, or the exercise in isolation has no significant effect on improving inflammatory markers. A recent study by Mika *et al* of two different exercise training modalities (pain free walking compared to exercising whilst having moderate pain) over a twelve week period also demonstrated no decrease in CRP values for either group (Mika et al. 2012). This finding of no change in CRP was also supported by Schalger's study of best medical therapy compared to SEP which followed patients up at 3, 6 and 12 months (Schlager et al. 2012). Findings are contradictory however (Tisi et al. 1997), with a 24 week

follow up of patients undergoing arm or leg ergometry demonstrating a reduction in CRP when compared to controls (Saxton et al. 2008).

The failure to identify any change may be due to the change in circulating cytokines being seen as an acute phenomenon (within an hour post exercise) rather than more long-term effect (Palmer-Kazen et al. 2009). This has been seen with IL-6 (Andreozzi, Martini, et al. 2007b), TNF alpha (Signorelli et al. 2003) and CRP, but is not always supported (Collins et al. 2006).

5.5. Correlation with peak VO_2

The expense associated with CPET makes it a costly and time consuming piece of equipment required for gauging fitness secondary to a SEP. Identifying other easy, reliable measures that will provide a measure which correlates highly with peak VO_2 may allow for the use of surrogate markers.

There was a strong correlation between walking distances and weight-adjusted peak VO_2 (MWD: $r=0.688$, $p<0.001$), but not AT (MWD: $r=0.325$, $p=0.325$). This was also supported by current data from Allen *et al* (Allen et al. 2010). (ICD correlated with peak VO_2 $r^2=0.43$, $p<0.01$), and Treat Jacobson *et al* (Treat-Jacobson et al. 2009) (MWD and change in peak VO_2, $r=0.45$; $p= 0.005$). Mean TUG was associated with a moderate, significant inverse correlation with peak VO_2 ($r=-0.579$, $p<0.001$). Given that an improvement occurs in both this and walking ability, the three may be closely associated with changes in physical function and walking being demonstrated quicker than any present in peak VO_2. The use of a TUG test in clinic would be a quick and easy way of establishing fitness, and could potentially be used as a surrogate for places who are yet to gain access to a CPET session within their pre-assessment clinics.

CPET was highly correlated with other exercise markers; resting SBP, time to peak, watts to peak and maximum HR. This is unsurprising as the other markers are all derived at the same time as a participant achieves their peak VO_2. Unfortunately,

there was no association with endothelial function, markers of inflammation or quality of life.

5.6. Exercise Responders

This thesis has failed to demonstrate convincing evidence that the mechanism by which SEP improves walking distances, physical function and QoL is related to changes in cardiopulmonary fitness, endothelial function or the inflammatory atherosclerotic process.

In view of this, we also interrogated the data to identify which of the cohort responded to exercise. Responders were grouped as either those who had improved their walking distance by 50%, or those whose peak VO_2 had improved by 1.5ml/kg/min. Using both of these strategies failed to identify any significant differences between the groups.

Peak VO_2 has demonstrated that "responders" had a lower resting heart rate, but this result was not reproduced within the walking distance group. Therefore this result may be due to chance and error rather than a true novel finding. Further work with a larger cohort and a focus on medications such as beta-blockers would be of interest.

As mentioned earlier, the influence of genotype on response to exercise has been explored within some large genomic studies. To be able to predict who will benefit from exercise, and who would fail to respond would allow streamlining of the current NHS practice. I.e. All claudicants are referred for exercise, and those who fail fall into one of two categories; those who are offered invasive treatment, and those who aren't. As claudicants can find SEP painful, for those who are likely to fail, re-routing them back to the clinic early for a re-discussion of options would seem appropriate. For those who fail exercise and are not a candidate for invasive treatment, further work is required to explore what treatment options can be provided.

As the importance of SEP continues to increase, and funding streams are reviewed to allow this resource to be implemented throughout the UK, the ability to predict responders and non-responders would be of benefit. Further work into this area continues to be required.

5.7. Adherence to SEP

NICE advocates that all patients with IC undertake an exercise programme, however the uptake to our programme was low at 30%. The use of exercise programmes in other specialities such as cardiac rehabilitation reflects this finding (Jolly et al. 2006). One issue with claudication rehabilitation, which is not seen with cardiac rehabilitation, is the presence of centralisation of services. Centralisation has been advocated as a life saving measure to improve surgical and anaesthetic care, morbidity and mortality (Holt & Matt Thompson 2010; Jensen 2006). The underlying reason for the improvement in outcomes remains contentious, it is advocated that an increase in endovascular treatments at larger centres may account for part of this improvement (Marlow et al. 2010; Matthew Thompson et al. 2011b; Loftus, 2014). The change in location of provision of vascular services has affected routine outpatient services. The availability of SEP for claudicants varies considerably throughout the country, and with the centralisation of vascular services, the likelihood of SEPs being available to claudicants in smaller hospitals is likely to fall. We certainly did not have the option to run SEP in "spoke" centres e.g. Grimsby or Scunthorpe. This centralisation of services to the large "hub" seemed to have a negative impact on recruitment rates into this study, with a significant number of patients finding the travel and associated costs unacceptable / unfeasible. A potential solution to this problem is the advent of exercise on prescription (Simmons et al. 2013). These community exercise programmes are available to patients with claudication in the East Riding of Yorkshire, who are willing to spend £33 on 20 sessions. This would seem a potentially viable option to SEP in the tertiary referral centre, however its clinical and cost effectiveness would need to be clearly

demonstrated (Pavey et al. 2011; Jones et al. 2005) – perhaps a fruitful topic for future research.

The belief that access to exercise programmes should be free, funded by the NHS, was common in our patient cohort, Whilst the price remains low at £33, and perhaps less expensive than travelling and parking at Hull Royal Infirmary 3 times a week, the cost saving was recognised by a minority. For the majority the cost or a lack of governmental help through benefits was prohibitive. The median age of the cohort was 67 years, suggesting that a significant proportion were retired, possible carer for partners or grandchildren, living on a pension or benefits. This may have been one of the major impacts on uptake as 3 of the 163 wished to undergo the exercise on prescription programme.

In addition to patient related barriers to the uptake of exercise on prescription in the community, another potential barrier was noted when discussing this service with the lead physiotherapists and managers for the East Riding area. They demonstrated a relative lack of awareness about claudication, its association with an increased cardiovascular risk, and impaired quality of life. Clearly a programme of staff education would be required before the widespread adoption of claudication into the community exercise on prescription programme.

Local inconsistencies in funding also became apparent during the study. Patients who lived within the Hull area had no access to an exercise on prescription programme, as the Primary Care Trusts in Hull did not fund these. Therefore claudicants with work commitments from Hull, who were unable to attend the SEP, had no provision for additional support for structured exercise in a supportive environment. Instead, for those groups the only option was exercise advice and an explanatory sheet outlining the class programme should they wish to recreate a similar programme for themselves at home. Adherence to this exercise advice was not analysed as it fell beyond the remit of this study.

6. LIMITATIONS

Whilst this thesis has maintained rigour in the methodology for testing patients, ethical constraints and the need to maintain high patient compliance resulted in some inherent bias. The bias that has limited the applicability of these results has been discussed further below.

6.1. Inter-test reliability

Whilst intra and inter test reliability was recorded when possible, this was not done for the EndoPAT. The inter-test reliability for this equipment has not been stated in the literature, and therefore the natural variation between tests is unknown.

Due to the cost implications, (£40/pair of probes), the time involved and the requirement for individuals to re-attend at regular intervals, this was not explored. Ideally repetitive tests after 24 hours, one week and then possible weekly after this would allow recognition of whether variation occurred. The number of patients required to test this is also unknown. Whilst three additional separate research groups were using the EndoPAT within the University of Hull, a co-ordinated effort to gain this data was never successful.

6.2. Selection bias

Consecutive participants identified from the consultant vascular clinics were referred to the supervised exercise programme. Only one third of those referred actually agreed to undertake the SEP as recommended by their consultant as first line treatment.

Those who agreed to SEP participation were motivated and either felt compelled to as this had been recommended, or felt that if they didn't help themselves, then they could not expect any change to occur in their health.

The class was held at the therapies centre, Hull Royal Infirmary between 16:00 to 17:00 every Monday, Wednesday and Friday. The timings of the SEP predispose the class to uptake by those who are retired and able to be free during the daytime.

Various reasons for non-participation were provided by the two thirds who declined the SEP. Reasons included the distance required to travel to the class; which could be from as little as 1 mile up to 30 miles from the class. Unfortunately, no expenses to support the cost of travel to and from the class were possible. Whilst many asked for transport, none were willing to be collected at around 14:00 and picked up at 17:30 spending an afternoon three times a week travelling to and from the hospital. As this was the only alternative available for many patients, this deterred many from attending.

For the younger cohort, work prevented many from attending the class. As the class is research led but supported by the NHS facilities, flexibility on the timing of the class or additional staffing members to allow further classes was not possible.

At present, no community SEP specifically for claudication exists in the Hull and East Riding area. Efforts are currently being made to liaise with the local council and exercise on prescription teams to implement such a programme. Further research into the efficacy and uptake of this programme would be required to determine its cost effectiveness in our local area. Patients with claudication coming towards the end of the SEP frequently expressed interest regarding local community SEP as completion of the hospital based SEP frequently leaves them feeling isolated and unsupported again. The financial implication of supporting a continuous SEP remains an issue. Whilst these patients feel that they should be allowed access to SEP as their treatment programme, the question remains, at which point patients should pay for their own health? Especially if they persist in smoking, poor diets or return to more sedentary behaviours post SEP.

Whilst there is inherent selection bias by the patients with predominantly the most motivated attending, the question remains of what should happen to the non-

motivated dis-interested cohort who would rather undergo a medical treatment and will not consider anything other than an intervention (PTA or surgery).

6.3. Attrition Bias

One of the key factors affecting all research studies pertaining to claudicants is the slow recruitment rate and subsequent attrition rate. Whilst a stipulation of some studies is that those who undertake them must be willing to attend all sessions for exercise and complete the 12 week course, our patients varied in whether they felt that they could commit to this. In addition, reasons for non-attendance were secondary to other medical appointments for themselves or their partners, commitments for grandchildren and finally holidays. In view of this, no single participant managed a full 100% attendance to the SEP. Regular training is required for improvements to be seen, making the lack of regular commitment and attrition for follow-up a key area of focus in future work. Whilst the data here suggests that no improvement has been demonstrated, a better attendance and lower attrition rate alongside more individualised exercise programmes may be the key to improving cardiovascular fitness.

6.4. SEP Bias: Motivation

Different individuals ran the SEP; RG, HB, RS, TW, LG, EC and AF. Whilst RG and HB oversaw the majority of the classes (approximately 80%), a small minority were taken by several different nursing (EC or AF) or medically trained staff (RS, TW, and LG).

The use of additional staff introduced supervision bias, as the effort and enthusiasm provided by SEP supervisors would differ on an individual basis. The two regular SEP supervisors were RG and HB, when they were unavailable, additional help was sought. The alternative was to cancel classes and prevent progression of the class due to the lack of people to facilitate it. In view of this, whilst the use of five additional

members to supervise the class was not ideal, the practicality of cancelling 20% of classes was also unfair for those who had committed to a 12 week programme. The effect of the supervisor on the level of improvement has not been commented on or discussed in claudication literature. Published data suggests that individual feedback and tailored instructions has been shown to benefit patients (Gardner et al. 2011). The use of a constant supervisor would possibly allow recognition of when to encourage people to do more. Without a stable input into the class, there is the potential to lose rapport, the focus for improvement; motivation and morale can also be lost. This group morale may also contribute to improvements in quality of life and walking distances, as the need to attend a 'social' class is potentially a highly significant factor for improved attendance as the class progresses beyond six weeks (Jones et al. 2009).

6.5. Blinding

This prospective observational study was un-blinded. Participants underwent the same procedures in the same order at each assessment, with feedback provided at the end of each coupled session. The research tests for CPET, treadmill walking distance and bloods were all taken by the same person to remove any inter-observer variability. All data collected was processed anonymously. The CPET data was assessed by two different assessors and a third if a high degree of variability was present. All EndoPAT data was derived from a computer algorithm preventing any observer bias. Bloods were anonymised at the time of receiving the sample, ELISAs were then performed on the anonymised tubes over three separate days.

6.6. Time constraints

Numbers were limited and recruitment was halted due to time constraints. Had more time been available, additional recruitment would have occurred to achieve the sample size. There is however no current consensus on how much of an

improvement would be expected from a 6 or 12 week exercise class with regards to peak VO_2 or anaerobic threshold.

To compensate for the lack of data available from individual small studies, including this one, data from the studies with pre and post exercise values were initially collected with a view to pooling the data. A lack of randomised controlled trials and no consistent reporting within the studies meant that this was not explored further and was consequently abandoned. The only pooled data analysis of prospective studies and observational series was undertaken to gauge baseline values for the claudicant's peak VO_2.

7. CONCLUSIONS

This prospective observational study of SEP in claudicants confirms previous work with respect to improvements in walking distances, quality of life and physical functional ability. These improvements are seen as early as 6 weeks in our programme; longer may be required to confer a benefit on endothelial and cardiovascular function. These findings concur with the current evidence available in PAD.

Data from other studies has shown that simple cognitive behavioural therapy (CBT) has improved walking distances in claudicants. It remains unclear whether the benefit in walking occurs secondary to a psychological intervention, or training. As heart rates were unable to be monitored, and cardiopulmonary physiology did not improve, improvements within the muscle is a final hypothesis for why exercise improves claudication waking distance. Evidence on the improvement of the muscle at the cellular level exists with revascularisation, as well as data on the effect of ischaemia (Clyne et al. 1985). The effect of exercise on lower limb calf muscle structure and metabolism in claudicants remains unclear.

8. FUTURE DIRECTIONS

The results from this study appear to have produced more questions than definitive answers, but have corroborated some findings available already in the literature. As such, further work is required to explore major themes raised in this thesis; the uptake to SEP, the role of CPET in claudication, and the underlying mechanism by which patients benefit.

8.1. The future of SEP

The future of SEP for claudication is secure as it is supported by NICE and will therefore require funding to be the first line treatment of all claudicants. The transition seems to be relatively clear for clinicians, but the same ethos is not apparent in patients. Many patients seem to view SEP as a stalling tactic that will delay them from having their angioplasty or surgery. In addition, uptake of SEP remains disappointingly low. Lessons from cardiac rehabilitation are required to allow this area to progress and develop as required.

Cardiac rehabilitation remains at the forefront of exercise research in patients with cardiovascular disease and has evolved to include a "heart manual". A health technology assessment (HTA) programme compared four hospital based exercise programmes to the "heart manual" supplemented with cardiac nurse input (Jolly et al. 2009). Outcome measures included smoking cessation, reduction in blood pressure, cholesterol, hospital anxiety depression (HAD) scores, improved exercise capacity and class adherence. Overall, there were no significant differences in the clinical outcomes between either trial arms. A cost analysis identified the hospital programme to be £157/patient compared to £198/patient with the "heart manual". *If* patients travel expenditure was taken into consideration, cost for the hospital programme increased to £182/patient. A systematic review of exercise programs in sedentary over 65 year olds, identified the level of supervision be the main cost; gym based, €35,665/Quality adjusted life year (QALY); instructor led walking,

€86,877/QALY and community based programs, €19,425/QALY (Garrett et al. 2011).

The HTA assessment felt that no supervision *may* be safe in those of **low to moderate risk,** without excessive cost implications. A claudication manual is currently not in existence, and with fiscal constraints the feasibility of implementing a new nurse led exercise service with telephone/home visits is questionable.

With the backing of NICE, this may be a sustainable future alternative to SEP, with regular physiotherapy input to ensure that the manual is updated and followed appropriately; as an exercise workbook. To date the closest that studies have come to such a manual is an RCT by Gardner *et al* (Gardner 2011)which advocates the use of unsupervised exercise with regular assessments and meetings to gauge progress.

8.2. The role of CPET

CPET continues to use peak VO_2 measures in addition to AT to assess for cardiovascular fitness. In patients with IC, the optimal CPET remains contentious. The use of a graded treadmill test to exhaustion can be performed, but this may prevent true assessments of fitness as it will be limited by lower limb PAD. Alternatives, such as arm or leg cycle ergometers are of value; the applicability of these to fitness however requires further investigation.

The cycle ergometer was used in this study to allow participants to exercise for approximately 8-10 minutes allowing the gas exchange ratios to be calculated.

8.2.1. Lactate threshold (LT)

The anaerobic threshold is a point at which the metabolism changes from a balance of aerobic and anaerobic metabolism, to predominantly anaerobic. This shift is due to the cells working at maximal capacity to utilise oxygen, and occurs when the

metabolic requirements of the cells exceed this. The by-product of this is lactate. The change in lactate concentration can be defined in numerous ways:

1. Lactate threshold
    a. *Breakpoint:* This is the highest VO_2 attained during incremental exercise prior to a rise in blood lactate. Alternative names for the breakpoint are the *onset of plasma lactate accumulation*, *anaerobic threshold* and *aerobic threshold*.
    b. *Delta 1mM:* this is the VO_2 associated with a 1mM increase above the baseline blood lactate concentration.
    c. *2.5mM blood lactate concentration:* this is the VO_2 observed during incremental exercise associated with a blood lactate concentration of 2.5mM.

2. Onset of blood lactate accumulation (OBLA): this is the VO_2 associated with a blood lactate concentration of 4mM. This is also named the *anaerobic threshold*. Biochemically it is arbitrarily measured as a blood lactate level exceeding 4mM (resting lactate blood levels are 1mM). More accurate assessment of the lactate threshold can occur by serial blood lactate tests during graded exercise testing.

3. Individual anaerobic threshold (IAT): this was the highest VO_2 that can be maintained prior to a continuous increase in blood lactate accumulation. IAT is also called the *maximal steady state.*

The increase in lactic acid results in a change in respiration to allow excess CO_2 to be expired and help revert the acidosis. Therefore the lactate threshold occurs at a similar time point to the anaerobic threshold. This allows non-invasive testing of inspired and expired air ratios (the respiratory quotient) to determine when the AT and consequently the LT have occurred.

The simultaneous use of LT with CPET testing may increase the accuracy of AT identification. As with AT assessment, controversy exists regarding which lactate measurement should be used. In addition, it is important to note that the blood lactate doesn't reflect muscle lactate production. It reflects the difference between muscle lactate efflux and inherent muscle metabolism clearance of lactate. To accurately assess lactate concentrations, the use of a three minute steady state period is required at each time of sampling, making the use of a treadmill Gardner protocol of relevance, and the bike redundant. In addition, the site of testing (arterial venous or capillary) must remain constant as the concentrations will vary and require a correction factor to equate one to the other.

8.2.2. Why do claudicants benefit from exercise

Our current data still provides no insight into why claudicants walking distances improve with training. Other units have noted the psychological impact of CBT may have a significant impact on walking distances and quality of life.

It is unclear whether PAD patients have an inherent fear that exercise advice, SEP and CBT help them overcome, thereby allowing them to improve daily function. Whilst other groups (Cunningham et al. 2011; Cunningham et al. 2013) continue to explore these avenues, we have an alternative hypothesis, which remains poorly explored.

Muscle histology changes secondary to exercise facilitate the re-conditioning and re-training of the ischaemic muscle. Whilst we are aware of the changes secondary to ischaemia, there is limited evidence of the changes that occur in ischaemic muscle secondary to exercise. The majority of these studies have been small and focused on murine models whilst the changes in adults remain unanswered.

8.2.3. Muscle in Claudication

Exercise improves exercise capacity and lower limb blood flow in rats with lower limb ischaemia, however a 40% deficit remains when compared to age matched normal control rats (Mathien & Terjung 1990). This implies that the improvements secondary to exercise are not solely due to blood flow but an improvement in skeletal muscle metabolism (Parmenter, Raymond & Singh 2011a; Terjung et al. 1988), just as in health.

Mitochondria are organelles within cells that convert glucose (in aerobic metabolism) or other substrates into ATP (energy for the cells). Any alteration in mitochondrial function will result in an alteration in the energy supply to the cell (Kemp et al. 1995). In health improvements are noted in the metabolic oxidative capacity of muscle namely citrate synthase (Erney et al. 1991) and succinic dehydrogenase activity (Holm et al. 1975).

Morphological changes in skeletal muscle mitochondria are present in patients with PAD, these include hyperplasia, proliferation, ballooning of the cristae and paracrystalline inclusions (Sjöström et al. 1982). The alterations are most likely secondary to ischaemia. In addition, mitochondrial DNA damage has been noted (Bhat et al. 1999). Structural and genetic defects in the mitochondria are deemed to be the cause of the energy dysfunction and utilisation in peripheral arterial disease (Wang et al. 1999). Only 25% of claudicants have been identified to have normal functioning mitochondria. A plethora of insults must therefore account for the subsequent change in claudicating muscle (Brass et al. 2000). So far data has shown a correlation between walking distance and nuclear magnetic resonance spectroscopy but has not produced correlation between disease severity and walking capability. This dichotomy has been supported by the work done by Zatina *et al* (Zatina et al. 1986), who demonstrated that re-vascularisation did not reverse muscle mitochondrial dysfunction.

8.2.4. RCT in muscle (Ethics No 11/YH/0210)

PAD is associated with an alteration at the vascular endothelium, increased levels of inflammatory markers, an alteration in muscle histology and change in physical activity of individuals. The increased sedentary nature of patients with PAD may in turn exacerbate the disease process, something that may be partially reversible by exercise. As exercise remains a mainstay for patients with claudication, encouragement of patients to attend these classes and improved accessibility remains important.

The underlying ischaemia reperfusion cycle associated with exercise has been a concern, as acute inflammatory changes may hasten atherosclerotic progression. However, data from long term training programmes does not support this. The mechanism and associated changes therefore requires further clarification.

We aim to tie the initial baseline levels of endothelial dysfunction in with inflammatory markers and identify whether these levels correlate with muscle fibre types, walking distances and overall cardiovascular status as measured by peak VO_2. We know that exercise leads to an increase in capillarisation around type II fibres and quantities of VEGF mRNA increases. However, the increase between these two has not been correlated to one another or functional physiological measurements of aerobic capacity (VO_2 max). We hope to focus on these two outcomes initially and then go on to establish whether any link exists between this and mitochondrial function and enzyme activity.

8.2.4.1. *Aims*

We aim to investigate and document the changes in muscle architecture, physiology and biochemical changes at a cellular level following the enrolment in an exercise class for 6 weeks. We aim to identify whether a change in muscle architecture correlates with a change in VO_2 max, anaerobic threshold or walking distances.

8.2.4.2. *Study design*

This will be a randomised controlled trial conducted at Hull Royal Infirmary, Hull HU3 2JZ. Randomisation will occur by using a sealed envelope method. Simple randomisation of equal numbers of suitable participants to each of two groups;

a) 6 week supervised exercise program

b) Normal care (no exercise)

A control group will also be recruited consisting of healthy aged- and sexed-matched participants for use in the muscle architecture aspect of the study. 20 patients, who are undergoing lower limb varicose vein surgery, will be recruited as healthy controls.

Muscle biopsies will be undertaken as an intraoperative procedure, a tissue sample from the medial head of gastrocnemius will be taken from all patients enrolled in the trial. Samples will be processed for the percentage of fibre types I, IIa and IIx and this will be correlated with levels of peak VO_2.

Inclusion Criteria

Community dwelling older adults aged 45 and over

Diagnosis of intermittent claudication – ABPI < 0.9 with symptoms in keeping with intermittent claudication

Undergoing surgery for claudication; Infrainguinal bypass

Ability to walk without assistance.

Exclusion Criteria

Participants who are unable to provide informed consent

Severe or acute cardiovascular, musculo-skeletal or pulmonary illness

Critical limb ischaemia

Active treatment for cancer

Rheumatoid arthritis or anyone receiving disease modifying anti-rheumatic drugs (DMARDs)

8.2.4.3. *Study duration and expected rate of recruitment*

The study is envisaged to be carried out over a 2 year period and began recruitment in September 2013. This unit currently undertakes 40 infra-inguinal bypasses for claudication per year. Assuming the number stays constant, this will allow recruitment of between 2-3 patients per month.

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10. APPENDICES

10.1. Questionnaires used in the study

10.1.1. SF36 version 2

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Thank you for completing this survey!

For each of the following questions, please tick the one box that best describes your answer

1. In general, would you say your health is?

○Excellent ○Very Good ○Good ○Fair ○Poor

2. Compared to one year ago, how would you rate your health in general now?

○Much better now than one year ago

○Somewhat better now than one year ago

○About the same

○Somewhat worse now than one year ago

○Much worse than one year ago

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, Limited a lot	Yes Limited a little	No, Not Limited at all
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.			
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf			
Lifting or carrying groceries			
Climbing several flights of stairs			
Climbing one flight of stairs			
Bending, kneeling or stooping			
Walking more than one mile			
Walking several hundred yards			
Bathing or dressing			

yourself			

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
Cut down on the amount of time you spent on work or other activities					
Accomplished less than you would like					
Were limited in the kind of work or other activities					
Had difficulty performing the work or other activities (e.g. it took extra effort)					

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
Cut down on the amount of time you spent on work or other activities					
Accomplished less than you would like					
Did work or other activities less carefully than usual					

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

○Not at all ○Slightly ○Moderately ○Quite a bit ○Extremely

7. How much bodily pain have you had in the past 4 weeks?

○None ○Very Mild ○Mild ○Moderate ○Severe ○Very Severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all A little bit Moderately Quite a bit Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks …

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
Did you feel full of life?					
Have you been very nervous?					
Have you felt so down in the dumps that nothing could cheer you up?					
Have you felt calm and peaceful?					
Did you have a lot of energy?					
Have you felt disheartened and low?					
Did you feel worn out?					

Have you been happy?					
Did you feel tired?					

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

○All of the time ○Most of the time ○Some of the time ○A little of the time ○None of the time

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
I seem to get ill more easily than other people					
I am as healthy as anybody I know					
I expect my health to get worse					
My health is excellent					

10.1.2. VascuQoL Questionnaire

Instructions: These questions ask you how you have been affected by poor circulation to your legs over the last two weeks.

You will be asked about the symptoms you have had, the way that your activities have been affected and how you have been feeling.

Please read each bit of the answer and then tick the one that applies best to you.

If you are unsure about how to answer a question, please give the best answer you can.

There is no right or wrong answer.

Please answer every question. Thank you.

1. In the last two weeks I have had pain in the leg (or foot) when walking ……

(Tick one)

1. All of the time	☐ 1
2. Most of the time	☐ 2
3. A good bit of the time	☐ 3
4. Some of the time	☐ 4
5. A little of the time	☐ 5
6. Hardly any of the time	☐ 6
7. None of the time	☐ 7

2. In the last two weeks I have been worried that I might injure my leg ……

(Tick one)

1. All of the time	☐ 1
2. Most of the time	☐ 2

3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

3. In the last two weeks **cold feet have given me** ……. *(Tick one)*

1. A very great deal of discomfort or distress ☐ 1
2. A great deal of discomfort or distress ☐ 2
3. A good deal of discomfort or distress ☐ 3
4. A moderate amount of discomfort or distress ☐ 4
5. Some discomfort or distress ☐ 5
6. Very little discomfort or distress ☐ 6
7. No discomfort or distress ☐ 7

4. In the last two weeks, because of the poor circulation to my legs, **my ability to take exercise or to play any sports has been** ….. *(Tick one)*

1. Totally limited, couldn't exercise at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

5. In the last two weeks **my legs have felt tired or weak** …. *(Tick one)*

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4

5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

6. In the last two weeks, because of the poor circulation to my legs, **I have been restricted in spending time with my friends or relatives** ….. *(Tick one)*

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

7. In the last two weeks I have had pain in the foot (or leg) after going to bed at night ….. (Tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

8. In the last two weeks **pins and needles or numbness in my leg (or foot)** have caused me …. *(Tick one)*

1. A very great deal of discomfort or distress ☐ 1
2. A great deal of discomfort or distress ☐ 2
3. A good deal of discomfort or distress ☐ 3
4. A moderate amount of discomfort or distress ☐ 4

5. Some discomfort or distress ☐ 5
6. Very little discomfort or distress ☐ 6
7. No discomfort or distress ☐ 7

9. In the last two weeks **the distance I can <u>walk has improved</u>** …. *(Tick one)*

1. Not at all (tick this if distance is unchanged or has decreased) ☐ 1
2. A little ☐ 2
3. Somewhat ☐ 3
4. Moderately ☐ 4
5. A good deal ☐ 5
6. A great deal ☐ 6
7. A very great deal ☐ 7

10. In the last two weeks, because of the poor circulation to my legs, **my ability to walk has been** *(tick one)*

1. Totally limited, couldn't walk at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

11. In the last two weeks being (or becoming) housebound has been a concern of mine …. *(Tick one)*

1. A very great deal ☐ 1
2. A great deal ☐ 2
3. A good deal ☐ 3
4. Moderately ☐ 4
5. Somewhat ☐ 5
6. A little ☐ 6
7. Not at all ☐ 7

12. In the last two weeks I have been concerned about having poor circulation to my legs …. *(Tick one)*

1. All of the time	☐ 1
2. Most of the time	☐ 2
3. A good bit of the time	☐ 3
4. Some of the time	☐ 4
5. A little of the time	☐ 5
6. Hardly any of the time	☐ 6
7. None of the time	☐ 7

13. In the last two weeks I have had pain in the foot (or leg) when I am at rest …..

(Tick one)

1. All of the time	☐ 1
2. Most of the time	☐ 2
3. A good bit of the time	☐ 3
4. Some of the time	☐ 4
5. A little of the time	☐ 5
6. Hardly any of the time	☐ 6
7. None of the time	☐ 7

14. In the last two weeks, because of the poor circulation to my legs**, my ability to climb stairs has been** ….. (Tick one)

1. Totally limited, couldn't climb stairs at all	☐ 1
2. Extremely limited	☐ 2
3. Very limited	☐ 3
4. Moderately limited	☐ 4
5. A little limited	☐ 5
6. Only very slightly limited	☐ 6
7. Not at all limited	☐ 7

15. In the last two weeks, because of the poor circulation to my legs**, my ability to take part in social activities has been** …. *(Tick one)*

1. Totally limited, couldn't socialise at all	☐ 1
2. Extremely limited	☐ 2
3. Very limited	☐ 3
4. Moderately limited	☐ 4
5. A little limited	☐ 5
6. Only very slightly limited	☐ 6
7. Not at all limited	☐ 7

16. In the last two weeks, because of the poor circulation to my legs**, my ability to perform routine household work has been** ….. *(Tick one)*

1. Totally limited, couldn't perform housework at all	☐ 1
2. Extremely limited	☐ 2
3. Very limited	☐ 3
4. Moderately limited	☐ 4
5. A little limited	☐ 5
6. Only very slightly limited	☐ 6
7. Not at all limited	☐ 7

17. In the last two weeks ulcers in the leg (or foot) have given me pain or distress *(Tick one)*

1. All of the time	☐ 1
2. Most of the time	☐ 2
3. A good bit of the time	☐ 3
4. Some of the time	☐ 4
5. A little of the time	☐ 5
6. Hardly any of the time	☐ 6
7. None of the time (tick this if you do not have leg ulcers)	☐ 7

18. Because of poor circulation to my legs, the overall range of activities that I would have liked to do in the last two weeks has been ….. *(Tick one)*

1. Severely limited – most activities not done ☐ 1
2. Very limited ☐ 2
3. Moderately limited – several activities not done ☐ 3
4. Slightly limited ☐ 4
5. Very slightly limited – very few activities not done ☐ 5
6. Hardly limited at all ☐ 6
7. Not limited at all – have done all the activities that I wanted to ☐ 7

19. In the last two weeks the poor circulation to the legs have made me feel frustrated ….. (Tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

20. In the last two weeks when I do get pain in my leg (or foot) it has given me ….

(Tick one)

1. A very great deal of discomfort or distress ☐ 1
2. A great deal of discomfort or distress ☐ 2
3. A good deal of discomfort or distress ☐ 3
4. A moderate amount of discomfort or distress ☐ 4
5. Some discomfort or distress ☐ 5
6. Very little discomfort or distress ☐ 6
7. No discomfort or distress ☐ 7

21. In the last two weeks I have felt guilty about relying on friends or relatives …..

(Tick one)

1. All of the time	☐ 1
2. Most of the time	☐ 2
3. A good bit of the time	☐ 3
4. Some of the time	☐ 4
5. A little of the time	☐ 5
6. Hardly any of the time	☐ 6
7. None of the time	☐ 7

22. In the last two weeks, because of the poor circulation to my legs**, my ability to go shopping or carry bags has been** ….. *(Tick one)*

1. Totally limited, couldn't go shopping at all	☐ 1
2. Extremely limited	☐ 2
3. Very limited	☐ 3
4. Moderately limited	☐ 4
5. A little limited	☐ 5
6. Only very slightly limited	☐ 6
7. Not at all limited	☐ 7

23. In the last two weeks I have worried I might be in danger of losing a part of my leg or foot *(tick one)*

1. All of the time	☐ 1
2. Most of the time	☐ 2
3. A good bit of the time	☐ 3
4. Some of the time	☐ 4
5. A little of the time	☐ 5
6. Hardly any of the time	☐ 6
7. None of the time	☐ 7

24. In the last two weeks the distance I can walk has become less …..

1.	A very great deal	☐ 1
2.	A great deal	☐ 2
3.	A good deal	☐ 3
4.	Moderately	☐ 4
5.	Somewhat	☐ 5
6.	A little	☐ 6
7.	Not at all – tick if distance is unchanged or has increased	☐ 7

25. In the last two weeks I have been depressed about the poor circulation to my legs ….. (Tick one)

1. All of the time	☐ 1
2. Most of the time	☐ 2
3. A good bit of the time	☐ 3
4. Some of the time	☐ 4
5. A little of the time	☐ 5
6. Hardly any of the time	☐ 6
7. None of the time	☐ 7

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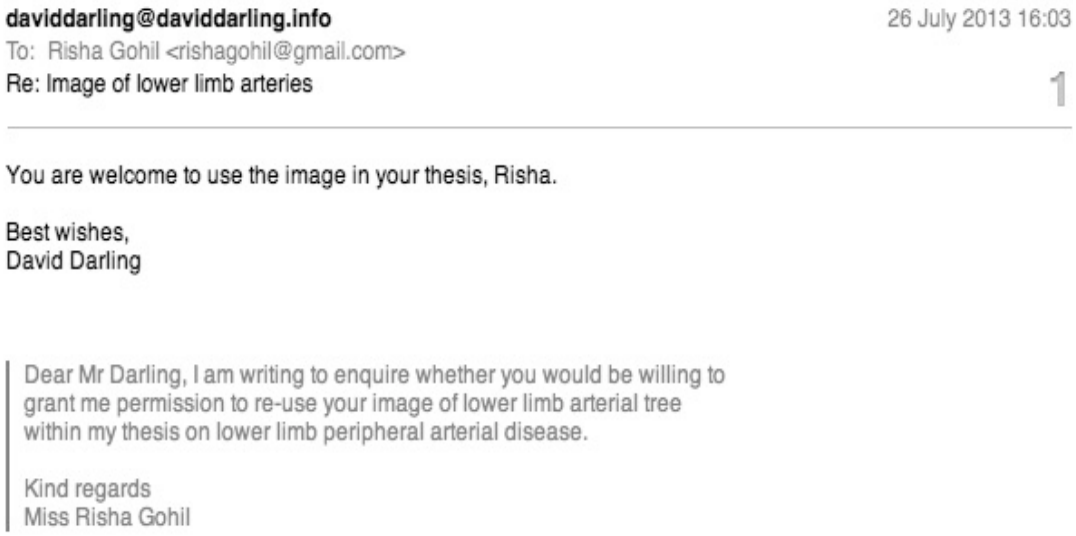

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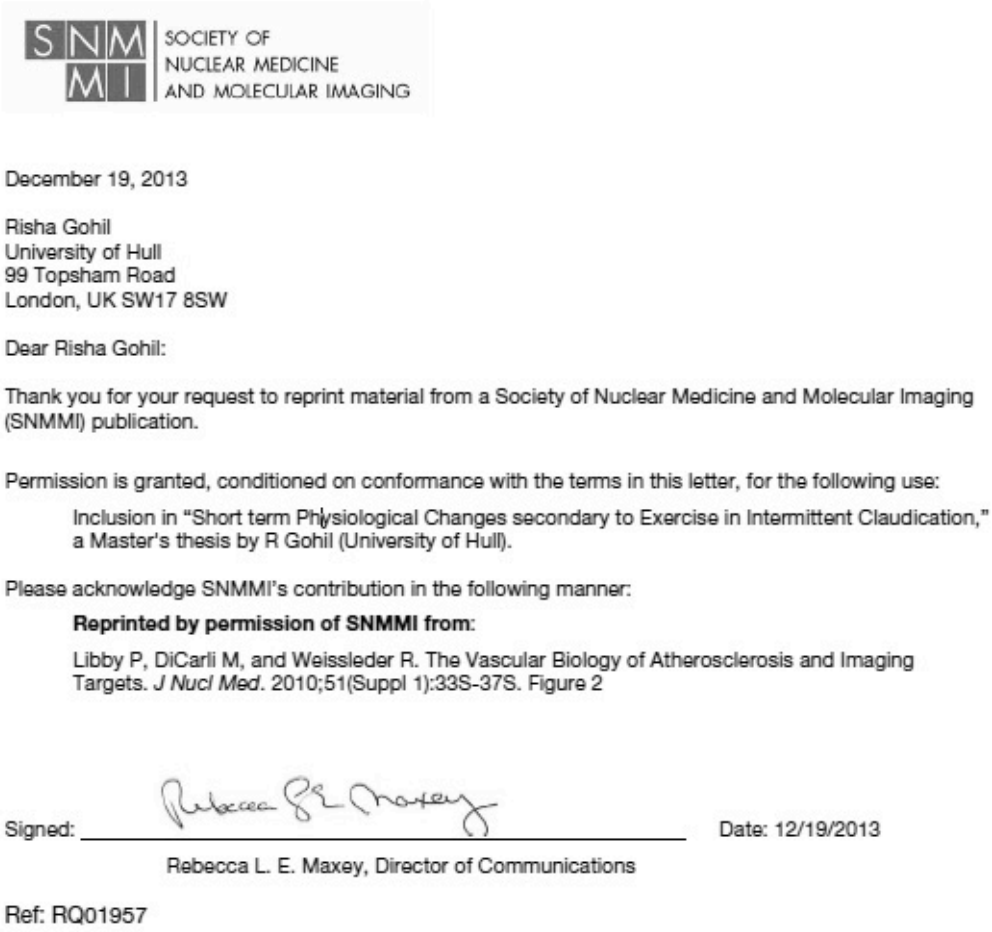

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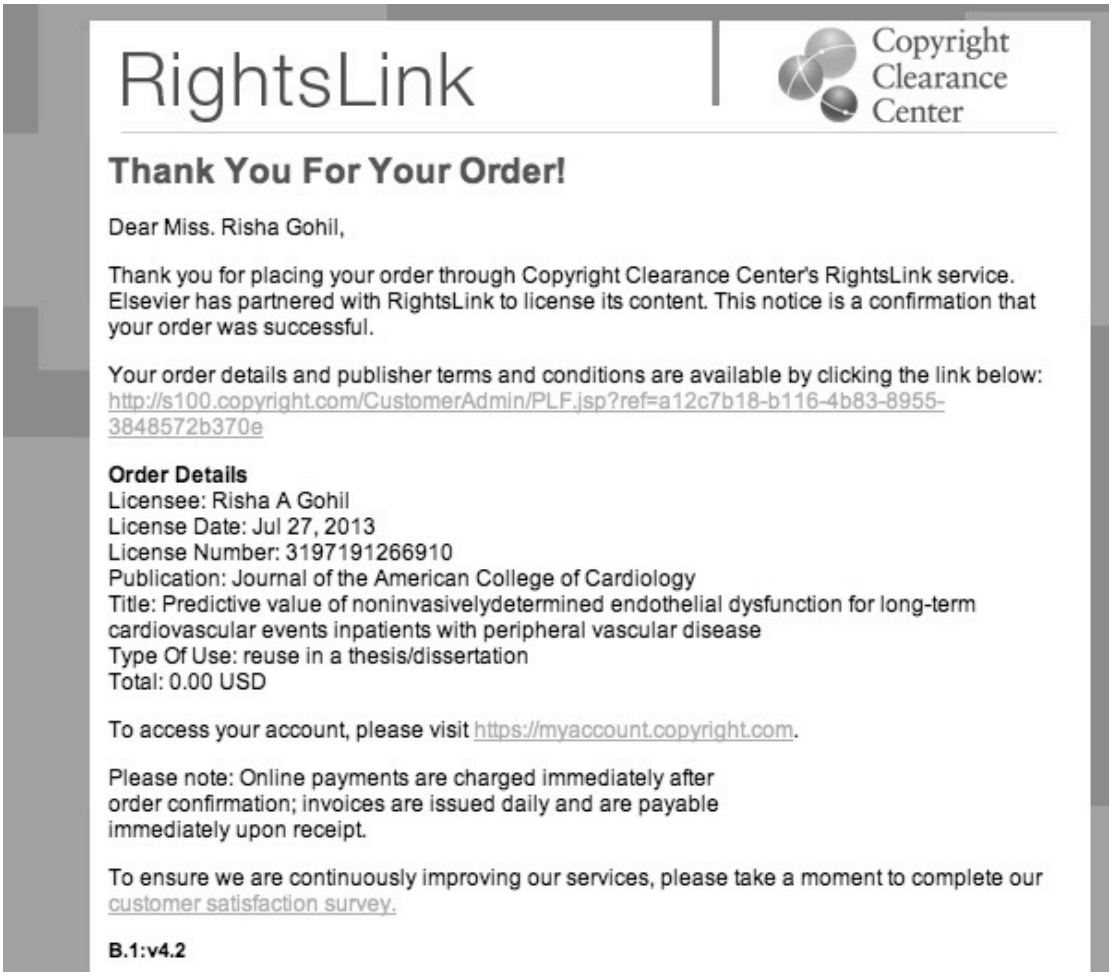

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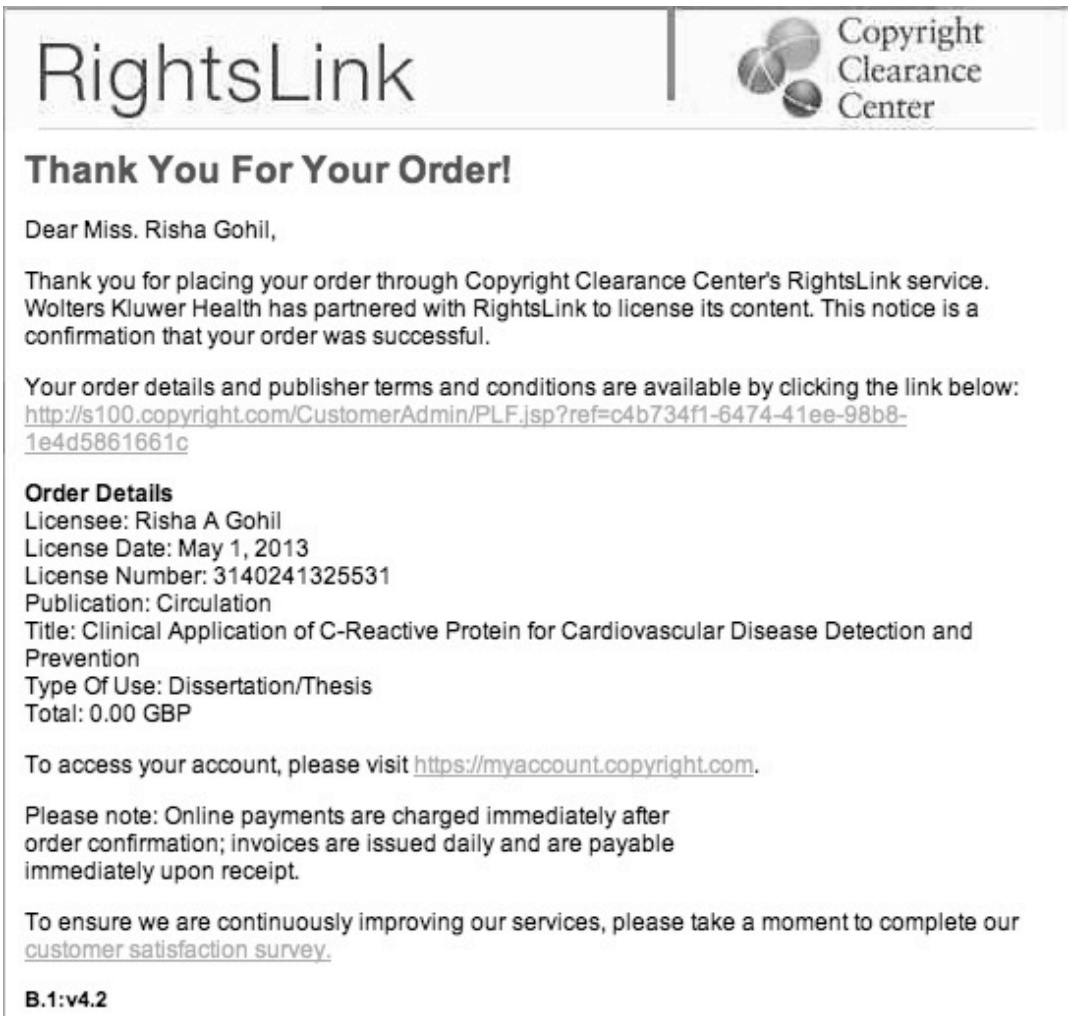

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Gohil R, Mockford KA, Mazari FA, Khan JA, Vanicek N, Chetter IC, Coughlin PA. Balance Impairment, Physical Ability, and Its Link with Disease Severity in Patients with Intermittent Claudication. Ann Vasc Surg. 2012:27(1) 68-74

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Lane R, Ellis B, Watson L, Leng GC. Exercise for Intermittent Claudication. Cochrane Database Syst Rev 2014:CD00990

❖ Glossary

Anaerobic Threshold The exercise VO_2 above which anaerobic high energy phosphate production supplements aerobic high energy phosphate production with a consequent increase in lactate production and lowering of cellular pH.

Exercise above the AT results in a metabolic acidosis and alteration in gas exchange to increase CO_2 output and slowing of VO_2 kinetics.

Gardner treadmill test Treadmill test which is set at 2 miles per hour with a 0% incline for the first 2 minutes. After which, the incline increases by 2% every 2 minutes

Intermittent claudication Muscular leg pain, which occurs on exercise that is relieved by a short rest

Incidence The ratio of people affected compared with the population at risk measured in a specific time period. It is the number of newly diagnosed cases in a specific time period.

Max VO_2 The highest possible oxygen uptake that a given subject can achieve for a given form of ergometry. Maximum uptake may be determined by repeated studies at higher and higher work rates to determine the highest possible value during a single maximum work rate test.

Odds ratio Calculated as the ratio of patients exposed to patients not exposed to a risk factor

Poiseuille's Law Flow (Q) is related to the lumen's radius (r), length (L) of the vessel and viscosity (ή) of the fluid and pressure gradient along the vessel (P)

Prevalence The ratio of people affected compared with the population at risk of a specific disease. It reflects the frequency of the disease. It is the number of cases alive on a certain date.

Relative risk The ratio of the incidence of a specific disease in one group compared to the incidence of that disease in another group.

Storey Protocol Increase in workload 1MET/2 min stage

Submaximal / Peak VO_2 The highest oxygen uptake achieved during a maximum work rate test.

Storey Protocol

VCO_2 Carbon dioxide output. The amount of CO_2 exhaled from the body into the atmosphere per unit time, expressed in ml/min or L/min.

VE Minute Ventilation. The volume of air exhaled from the body in one minute.

VO_2 Oxygen uptake. The amount of oxygen extracted from the inspired gas in a given period of time, expressed in ml/min or L/min.

V-slope method A technique that allows detection of the onset of lactic acidosis during an incremental exercise test when one notes an accelerated rate of CO_2 output (VCO_2) compared to oxygen uptake (VO_2).

❖ **Abbreviations**

AA	Abdominal aorta
Ab	Antibody
ABPI	Ankle Brachial Pressure Index
ABPI Re	Resting Ankle Brachial Pressure Index
ABPI Pe	Post Exercise Ankle Brachial Pressure Index
ACC	American College of Cardiology
ACSM	American College of Sports Medicine
ADP	Adenosine di-phosphate
AGE	advanced glycation end-products
AHA	American Heart Association
AI	Augmentation Index
AT	Anaerobic threshold
A/MWD	Actual/Maximal Walking Distance
BAUS	Brachial Artery Ultrasound
BMI	Body Mass Index
BP	Bodily Pain
CABG	Coronary Artery Bypass Graft
CARE Trial	Cholesterol and Recurrent Events Trial
CDC	Centre of Disease Control
CI	Confidence Interval

CIA	Common iliac artery
CFA	Common femoral artery
CLI	Critical Limb Ischaemia
CPET/X	Cardiopulmonary Exercise Test
Cr	Creatinine
COPD/COAD	Chronic Obstructive Pulmonary/Airways Disease
CST	Chair Stance Time
CVA	Cerebrovascular event
DFA	Deep femoral artery or profunda femoris
DM	Diabetes mellitus
DMARDs	Disease modifying anti-rheumatic drugs
ECG	Electrocardiograph
EDTA	Ethylene-diamine-tetra-acetic acid
EECP	Enhanced external counter-pulsation
EIA	External iliac artery
ELISA	Enzyme Linked Immuno-sorbant Assay
ExACT	The Exercise versus Angioplasty in Claudication Trial
FBC	Full Blood Count
FEV_1	Forced Expiratory Volume
FMD	Flow mediated dilatation
FTS	Full Tandem Stance

FVC	Forced Vital Capacity
GH	General Health
GP	General Practioner
GTN	Glycero tri-nitrate
Hb	Haemoglobin
Hct	Haematocrit
HDL	High density lipoprotein
HR	Heart Rate
HRI	Hull Royal Infirmary
HRP	Horseradish peroxidase
Hs CRP	High sensitivity C-reactive Protein
HTN	Hypertension
IC	Intermittent claudication
ICD	Initial Claudication Distance
IL-6	Interleukin 6
IMT	Intima media thickness
ISCVS	International Society for Cardiovascular Surgery
IQR	Interquartile range
kg	Kilogramme
l	Length of the stenotic area
LDL	Low density lipoprotein

LT	Lactate Threshold
MCV	Mean Cell Volume
MH	Mental Health
MHR	Maximum Heart Rate
MI	Myocardial Infarction
MIMIC	Mild and Moderate Intermittent Claudication Trial
MRA	Magnetic Resonance Angiogram
MSS	Mental Summary Score
MVV	Maximum Voluntary Ventilation
MWD	Maximum Walking Distance
Na	Sodium
NHANES	National Health and Nutritional Examination Survey
NICE	National Institute of Clinical Excellence
OBACT Treatment Study	Olso Balloon Angioplasty versus Conservative
OBLA	Onset blood Lactate Accumulation
PA	Popliteal artery
PAD	Peripheral arterial disease
PAT	Peripheral Arterial Tone
PARTNERS for Survival	PAD Awareness, Risk, and Treatment: New Resources
PCOS	Polycystic Ovarian Syndrome

Peak/Submaximal VO_2	Highest achieved effort Ventilatory oxygen threshold
PF	Physical Function
PGI-1_2	Prostacyclin
PIPJ	Proximal inter-phalangeal joint
PRINCE	PRavastatin INflammation /CRP Evaluation
PRWD	Patient reported Walking Distance
PSS	Physical Summary Score
PT	Pain Threshold
PTA	Percutaneous Transluminal Angioplasty
RE	Role Emotional
RP	Role Physical
Q	Flow through the vessel
QoL	Quality of Life
QALY	Quality of Life Adjusted Life Year
r	Radius of the vessel
RAGE	Receptors for advanced glycosylation end-products
RCT	Randomised controlled trial
RER	Respiratory Exchange Ratio
RHI	Reactive Hyperaemia Index
RHR	Resting Heart Rate
RR	Respiratory Rate

rpm	Revolutions per minute
SBP	Systolic Blood Pressure
SEP/T	Supervised Exercise Programme/Therapy
SF	Social Function
SF36	Short Form 36
SFA	Superficial femoral artery
SPPB	Short Physical Performance Battery Score
STS	Semi Tandem Stance
SVC	Slow Vital Capacity
TASC	Trans-Atlantic Inter-Society Consensus Document

On Management of Peripheral Arterial Disease

TIA	Transient Ischaemic Event
TMB	3,3',5,5'-Tetramethylbenzidine
TNF	Tumour Necrosis Factor
TPT	Tibio-peroneal trunk
TUG	Timed Up and Go Test
UEs	Urea and Electrolytes
η	Viscosity of fluid (in this instance it is blood viscosity)
V	Vitality
VascuQoL	Kings College Vascular Quality of Life questionnaire
VE	Ventilatory Equivalent

VO_2	Ventilatory Oxygen
VCO_2	Ventilatory Carbon dioxide
WOSCOPS	West of Scotland Coronary Prevention Study