Physiological effects of exercise training in patients with peripheral vascular disease

Garry Alan Tew

A thesis submitted in partial fulfilment of the requirements of
Sheffield Hallam University
for the degree of Doctor of Philosophy

September 2009

Collaborating Organisations: Sheffield Teaching Hospitals NHS Foundation Trust
(Northern General Hospital)

PLEASE COVER AND DO NOT DIGITISE AT THE UNIVERSITIES REQUEST

FIG 2.1 P6
TABLE 2.1 P8
TABLE 2.2 P9
FIG 2.2 P11
FIG 2.3 P14
FIG 2.4 P22
TABLE 2.3 P26
TABLE 2.4 P29
FIG 3.1 P47
FIG 3.4 P53
FIG 3.5 P55
FIG 3.6 P57
FIG 8.1 P114
FIG 8.2 P120
DO NO INCLUDE THE APPENDICES

Contents

Acknowledgements

I wish to acknowledge and thank the Sheffield Vascular Institute at the Northern General Hospital for supporting these collaborative studies and Sheffield Hallam University for hosting and funding them.

My thanks are extended to the people who helped make these studies possible, namely my supervisors Dr John Saxton and Mr Shah Nawaz. Dr Saxton, my Director of Studies, supervised my studies on a day-to-day basis, and his relentless work and support is formally acknowledged. Mr Nawaz, my co-supervisor, clinically assessed all of the claudicants who participated in Section 1 of this thesis, and I am grateful for his support and commitment in the recruitment process.

I wish to acknowledge the support of the staff at the Sheffield Vascular Institute for allowing access to recruit patients onto these studies. Special thanks are also extended to Dr Irena Zwierska and Dr Markos Klonizakis, for helping me develop the necessary skills for effectively assessing and training the patients, and for undertaking the preliminary research upon-which my studies are based.

Thanks are extended to Miss Helen Lloyd, Mrs Alayne Flowers, Mr Neil Donovan, Mr Martin Stout, Mr Mark Black, and Mr Alan Ruddock, for providing technical assistance during these studies, and to Professor Edward Winter for his informative discussions with me about various aspects of the physiology of exercise.

These studies, and the findings thereof, would not have been possible without the participation, commitment, dedication, enthusiasm, and co-operation of all of the patients recruited. I remain eternally grateful to you all.

This thesis is dedicated primarily to my family. I shall forever be indebted for your support throughout my education. In particular, I would like to thank my wife, Natalie, for standing by my side all of the way. Your love was felt throughout the years and this gave me strength to persevere. My deepest love is extended to you all now and forever.

Statement of Originality

Section 1: The strategy of using arm-crank exercise training to improve walking performance in patients with intermittent claudication has previously been described in studies conducted by members of our investigatory team (Walker *et al.*, 2000; Zwierska *et al.*, 2005). After these preliminary studies, further research was needed to help understand physiological adaptations that underpinning improvements in walking performance after arm-crank exercise training. For the intermittent claudication studies, all of the recruitment, initial consultations and about 99% of all assessment and training sessions were performed by myself. Miss Helen Lloyd provided technical assistance in the majority of assessment sessions. Statistical analyses were performed by myself, with guidance and verification of findings confirmed by Dr John Saxton.

Section 2: Skin microvascular function after varicose-vein surgery was poorly characterised and there were no published data about the effects of acute or chronic exercise on microvascular function. Therefore, the aims of this research were to compare cutaneous microvascular function between patients who have recently had varicose-vein surgery and age-matched healthy controls, and to investigate whether or not any impairment of function could be alleviated by acute or chronic lower-limb exercise. For this research, Dr Markos Klonizakis completed the recruitment and initial consultations. We both participated in all of the assessment sessions and supervised the training sessions between us. Statistical analyses, data interpretation and writing of this report were performed by myself.

With the exception of any statements to the contrary, all the data presented in this report are the result of my own efforts. In addition, no parts of this report have been copied from other sources. I understand that any evidence of plagiarism and/or the use of unacknowledged third party data will be dealt with as a serious matter.

Signed.....

Date.....

Abbreviations

ABPI	ankle-brachial pressure index
ACE	angiotensin-converting enzyme
ACh	acetylcholine
ATP	adenosine triphosphate
CD	claudication distance
CEAP	Clinical-Etiology-Anatomy-Pathophysiology
cGMP	cyclic guanosine monophosphate
EDHF	endothelium-derived hyperpolarising factor
LDL	low-density lipoprotein
MET	metabolic equivalent
MRT	mean response time
MWD	maximum walking distance
NIRS	near-infrared spectroscopy
NO	nitric oxide
OR	odds ratio
PAD	peripheral arterial disease
PTA	percutaneous transluminal angioplasty
PU	perfusion units
RPE	rating of perceived exertion
SD	standard deviation
SEM	standard error of the mean
SEPS	superficial endoscopic perforator surgery
SNP	sodium nitroprusside
SNS	sympathetic nervous system
StO_2	calf muscle oxygen saturation
τ	time constant
TD	time delay
TEM	technical error of measurement
tHb	total haemoglobin/myoglobin
$\dot{V}O_2$	rate of oxygen consumption

List of Figures

List of Tables

Publications and conference presentations arising from this research

Published articles

Tew GA, Nawaz S, Zwierska I, Blagojevic M, Saxton JM (2009). Physiological predictors of maximum treadmill walking performance in patients with intermittent claudication. *International Journal of Sports Medicine*. **30**, 467-472.

Tew GA, Nawaz S, Zwierska I, Saxton JM (2009). Limb-specific and cross-transfer effects of arm-crank exercise training in patients with symptomatic peripheral arterial disease. *Clinical Science*, **117**, 405-413.

Klonizakis M, Tew GA, Michaels J, Saxton JM (2009). Impaired microvascular endothelial function is restored by acute lower-limb exercise in post-surgical varicose-vein patients. *Microvascular Research*, **77**, 158-162.

Klonizakis M, Tew GA, Michaels J, Saxton JM (2009). Exercise training improves cutaneous microvascular endothelial function in post-surgical varicose-vein patients. *Microvascular Research*, **78**, 67-70.

Published abstracts

Oral communication at the Yorkshire Vascular Forum, Garforth, Leeds, Oct 2008

Tew GA, Nawaz S, Zwierska I, Blagojevic M, Saxton JM (2009). Physiological predictors of maximum treadmill walking performance in patients with intermittent claudication. *Annals of The Royal Society of Surgeons of England.**

*Awarded 'Registrar Research Prize'.

Oral communication at the BASES Annual Conference, Brunel University, Sept 2008.

Tew GA, Nawaz S, Zwierska I, Saxton JM (2008). Effects of arm-crank training on walking performance and lower-limb circulation in claudicants. *Journal of Sports Sciences*, **26**, Suppl. 2, S33.

Oral presentation at the European Society for Clinical Hemorheology and Microcirculation Annual Meeting, Switzerland, June 2009.

Klonizakis M, Tew GA, Michaels J, Saxton JM. (2009). Effects of exercise training on small-blood-vessel function in post-surgical varicose-vein patients. *Clinical Hemorheology and Microcirculation*, **42**, 203.

Other presentations

Oral communication at the BASES Student Conference, University of Bedfordshire, March 2008.

Tew GA, Nawaz S, Zwierska I, Saxton JM. Effects of arm-crank training on walking performance and lower-limb circulation in claudicants.*

*Awarded 'Best Post-Graduate Oral Presentation Prize'.

Oral communication at the Yorkshire Vascular Forum, Garforth, Leeds, Oct 2008

Tew GA, Saxton JM, Nawaz S, Zwierska I. Arm-crank training improves walking performance and lower-limb oxygen delivery in patients with symptomatic peripheral arterial disease.

Oral communication at the East Midlands Surgical Society annual meeting, King's Mill Hospital, Mansfield, Oct 2008

Tew GA, Saxton JM, Nawaz S, Zwierska I. Arm-crank training improves walking performance and lower-limb oxygen delivery in patients with symptomatic peripheral arterial disease.*

*Awarded 'Registrar Research Prize'.

Poster presentation at the BASES Student Conference, University of Hull, March 2009.

Tew GA, Klonizakis M, Michaels J, Saxton JM. Effects of exercise training on small-blood-vessel function in post-surgical varicose-vein patients.*

*Runner-up prize in the postgraduate poster presentation session.

Accepted for poster presentation at the BASES Annual Conference, Leeds Metropolitan University, September 2009.

Tew GA, Klonizakis M, Michaels J, Saxton JM. Effects of exercise training on small-blood-vessel function in post-surgical varicose-vein patients.

Abstract

This work sought to identify physiological effects of aerobic exercise training in patients with lower-limb vascular disease.

The main aim of the studies in Section 1 of this thesis was to investigate potential mechanisms by which arm-crank exercise training evokes improved walking performance in patients with intermittent claudication. In Study 1, multiple regression analysis was used to identify key physiological predictors of walking performance in this patient group. The three variables included in the final regression model were peak oxygen uptake, calf muscle oxygenation at 1 min and time-to-minimum calf muscle oxygenation. The results suggest that cardiopulmonary fitness and the ability to match oxygen delivery to metabolic demand are important determinants of walking performance in claudicants. In Study 2, a randomised, controlled trial investigated limb-specific and cross-transfer effects of arm-crank exercise training in claudicants. After 12 weeks of training, patients showed improvements in walking performance and specific cardiopulmonary fitness and calf muscle oxygenation variables. The results suggest that the improvement in walking performance is attributable, at least in part, to improved lower-limb oxygen delivery.

The main aims of the studies in Section 2 of this thesis were to compare cutaneous microvascular function between post-surgical varicose-vein patients and age-matched healthy controls and to investigate whether or not any impairment of function is alleviated by acute and chronic lower-limb exercise. The results suggest that post-surgical varicose-vein patients have microvascular endothelial dysfunction that can be corrected both by acute and chronic moderate-intensity lower-limb exercise. Attenuation of microvascular abnormalities might be important for reducing the risk of venous ulceration in this patient group.

Collectively, this thesis provides evidence that aerobic exercise training is an effective stimulus for evoking favourable physiological adaptations in patients with lower-limb vascular disease. Therefore, aerobic exercise training can generally be considered a useful adjunct therapy for these patients.

Section 1: Exercise training for patients with intermittent claudication

Chapter 1: General introduction

The physiology of exercise is the study of how the body responds and adapts to exercise. The beneficial effect of regular exercise on health and fitness is not a new concept and has been appreciated since at least the time of Hippocrates (around 400 BC; Porter, 1997). However, only in recent decades, with the increasing focus on health-based research, have physiological consequences of habitual inactivity and regular exercise become more fully understood.

A one-size-fits-all approach should not be used with exercise training prescription; what might be suitable for one group of people could be inappropriate or even dangerous for another. Accordingly, it is important to identify suitable exercise strategies for different groups and in particular for clinical populations. It is in such populations that the greatest risks of adverse effects of exercise training can be found.

Exercise training is often recommended as an adjunct therapy for patients with peripheral arterial disease (PAD) (Hirsch *et al.*, 2006), which is a common form of peripheral vascular disease caused primarily by atherosclerosis. PAD is characterised by chronic luminal narrowing of the arterial beds that supply blood to non-coronary arteries. A common symptom of PAD is intermittent claudication, a cramp-like muscle pain that occurs during exercise because of limited limb blood flow and skeletal muscle metabolic abnormalities. It usually occurs in the calf muscles of one or both legs during walking, and is generally associated with arterial luminal obstruction >50% within the aorto-iliac, femoral, and/or popliteal arterial segments (Garcia, 2006).

Intermittent claudication reduces walking performance to about 50% of that seen in healthy individuals of a similar age (Regensteiner *et al.*, 1993). Impaired walking ability can precipitate a chain of events resulting in further deconditioning and functional decline, eventual physical disability, loss of independence and impaired quality of life. Additionally, since atherosclerosis is a systemic disease process, individuals with PAD are at a greatly increased risk of major cardiovascular events. For example, Criqui *et al.* (1992) showed that, after multivariate adjustment for age, sex and other risk factors for cardiovascular disease, patients with PAD had a 6-fold higher risk of cardiovascular-related death than patients without PAD. When the disease advances further, patients

can also experience pain at rest, leg ulceration and/or tissue necrosis. Here, limb amputation might ensue in the absence of surgical revascularisation. Therefore, the goals of treatment for patients with intermittent claudication are to relieve exertional symptoms, improve walking capacity, improve quality of life, and to prevent and retard the progression of systemic atherosclerosis and adverse cardiovascular outcomes (Hirsch *et al.*, 2006; Norgren *et al.*, 2007).

Supervised exercise training is an important treatment strategy for patients with intermittent claudication because it can induce concomitant improvements in functional capacity (Gardner and Poehlman, 1995; Leng *et al.*, 2000; Watson *et al.*, 2008), quality of life (Gartenmann *et al.*, 2002; Keo *et al.*, 2008) and cardiovascular risk factors such as hypercholesterolaemia and hypertension (Izquierdo-Porrera *et al.*, 2000). Walking is the most commonly advocated exercise training modality for these patients (Hirsch *et al.*, 2006; Norgren *et al.*, 2007). However, since walking can be painful, the desire and ability of these patients to perform such activity might be limited. Indeed, in clinical practice, there is evidence that nearly half of patients refrain from regular walking exercise (Bartelink *et al.*, 2004). As upper-limb arterial disease is over 20 times less frequent than lower-limb arterial disease (Welling *et al.*, 1981), patients are less likely to experience ischaemic muscular pain during arm exercise. There is evidence that arm-crank exercise training is well tolerated by claudicants and can improve walking performance to a similar extent as lower-limb cycle-ergometry training (Zwierska *et al.*, 2005). However, the extent to which physiological mechanisms account for this improvement is unclear. Section 1 of this thesis comprises a literature review about management of PAD and two studies. The aim of Study 1 was to identify key physiological predictors of walking performance in claudicants using multiple regression analysis. The aim of Study 2 was to investigate potential mechanisms by which arm-crank exercise training evokes improved walking performance in this patient group.

Chapter 2: Literature review - Management of peripheral arterial disease

2.1 Disease epidemiology

Reported prevalence rates of PAD vary widely depending on the population and the study methodology. However, total disease prevalence based on objective testing, evaluated in several epidemiologic studies, is in the range of 3 to 10%, increasing to 15 to 20% in persons over 70 years (Criqui *et al.*, 1985; Hiatt *et al.*, 1995a; Selvin and Erlinger, 2004). Although it is commonly believed that PAD is more prevalent in men than women, recent research now indicates that both genders are equally affected (Meijer *et al.*, 1998; Selvin and Erlinger, 2004). Around 10 to 35% of patients with PAD have the symptom of intermittent claudication (Hirsch *et al.*, 2006; Chi and Jaff, 2008). As with PAD, the prevalence of intermittent claudication increases with age, with 3 to 7% of people over the age of 60 years being affected (Dormandy and Rutherford, 2000; Cimminiello, 2002). In the Framingham Heart Study, the annual incidence if intermittent claudication in people older than 65 years was 61 cases per 10,000 person years in males and 54 cases per 10,000 person years in females (Kannel and McGee, 1985).

2.2 Risk factors, aetiology and pathogenesis of atherosclerotic disease

In the third National Health and Nutrition Examination Survey (Selvin and Erlinger, 2004), the adjusted odds ratio (OR) for PAD prevalence was significantly greater with tobacco use (OR, 4.2), African-American ethnicity (OR, 2.4), glomerular filtration rate <60 $mL \cdot min^{-1}$ (OR, 2.2), diabetes mellitus (OR, 2.1), and hypercholesterolaemia (OR, 1.7). The risk of PAD progressing to critical limb ischemia is increased with diabetes mellitus (OR, 4.0), tobacco use (OR, 3.0), an ankle-brachial pressure index (ABPI) <0.7 (OR, 2.0), ABPI <0.5 (OR, 2.5), age greater than 65 years (OR, 2.0), and hypercholesterolaemia (OR, 2.0).

Several novel biomarkers, including C-reactive protein, lipoprotein(a), homocysteine, and D-dimer, have been associated with the development of systemic atherosclerosis (Caslake *et al.*, 2000; Ridker *et al.*, 2001; Libby, 2002). Elevated circulating concentrations of C-reactive protein are associated with higher all-cause mortality and adverse cardiovascular outcomes in patients with (Vidula *et al.*, 2008) or without

(Vainas *et al.*, 2005) known atherosclerotic vascular disease. High concentrations of some inflammatory biomarkers are also predictive of high short-term mortality (Libby, 2001; Libby *et al.*, 2002).

The term atherosclerosis is derived from the Latin words *athero* meaning gruel or porridge, and *sclerosis* meaning hardening. It is the process in which deposits of fatty substances, cholesterol, cellular waste products, calcium, and other substances build up in the inner lining of arteries. This build-up is called plaque. Plaques can be dispersed throughout the medium- and large-sized arteries, but usually they form at arterial branch-points (bifurcations) where high shear stress causes endothelial damage, for example in the legs where the popliteal artery branches off from the femoral artery (Ouriel, 2001).

Atherosclerosis is a complex, multi-factorial, multi-step disease that involves chronic inflammation at every step, from initiation to progression (Mallika *et al.*, 2007). The PAD risk factors described above, all contribute to the pathogenesis by aggravating the underlying inflammatory process. Figure 2.1 depicts the progression of atherosclerosis in cardiovascular diseases. Atherosclerosis is thought to be initiated by one or more factors that cause dysfunction of, or damage to, the endothelial lining of the arterial wall. Such factors might include high shear stress, high circulating low-density lipoprotein levels, prolonged high blood pressure, carbon monoxide from cigarette smoke and high blood glucose concentrations in diabetes mellitus.

Figure 2.1 Progression of atherosclerosis in cardiovascular diseases (Higashi and Yoshizumi, 2003)

At the injury site, fats, cholesterol, platelets, cellular waste products, calcium and other substances collect in the inner layer of the arterial wall. Contact with platelets, lipids and other components of blood stimulate smooth muscle cells in the arterial wall to proliferate abnormally. As an atherosclerotic plaque develops and enlarges, it progressively obstructs blood flow. What begins as a relatively harmless fatty streak can develop into a complicated lesion with associated cardiovascular complications. An additional danger is that a plaque might provide a roughened surface that attracts platelets, initiating clot formation and further obstructing blood flow. Moreover, a thrombus or piece of thrombus might dislodge to become an embolus and obstruct blood flow distally.

In PAD, flow-limiting atherosclerotic plaques are major contributors towards the development of ischaemic symptoms. Whether a lesion is haemodynamically significant depends on both the degree of the stenosis and flow velocities. Flow velocity at rest has been measured as low as 20 $cm \cdot s^{-1}$ in the femoral artery. At this rate, a diameter reduction of >90% would be required for a lesion to become haemodynamically significant (Meru *et al.*, 2006). Metabolic requirements in the distal tissues of an exercising, active individual are higher, and femoral velocities might need to increase up to 150 $cm \cdot s^{-1}$. At this rate, a stenosis of even 50% can cause a significant pressure and flow gradient leading to inadequate oxygen delivery (Meru *et al.*, 2006).

2.3 Prognosis and natural history of intermittent claudication

Despite the functional impairment caused by intermittent claudication, its natural history, in terms of the risk of disease progression with respect to disability and eventual limb loss, is relatively benign. Patients with PAD can often remain at the same level of walking impairment for years if not offered specific treatments (Regensteiner and Hiatt, 1995). Intermittent claudication can, however, progress in one of five ways: (i) improvement; (ii) stabilisation; (iii) worsening of the disease, but with no revascularisation needed; (iv) worsening of the disease with revascularisation required; and (v) a requirement for amputation, usually after disease progression (Aquino *et al.*, 2001). Most claudicants (about 75%) improve spontaneously or remain stable (Fowkes, 2001). Symptoms worsen in 25% of patients, with about 5% requiring amputation within 5 years and about 5 to 10% developing critical limb ischaemia (Table 2.1).

Although relatively few deaths are directly attributed to PAD, its presence has potent morbidity and mortality implications. Since the risk factors for atherosclerotic disease of the legs are no different from those in other areas, such as in the heart or brain, it is no surprise that PAD is strongly associated with other cardiovascular diseases such as coronary artery and cerebrovascular disease. In a review by Weitz *et al.* (1996), at the time of diagnosis of intermittent claudication, at least 28% of patients had concomitant coronary artery disease and 10% concomitant cerebrovascular disease. Patients with PAD have a 5-year mortality of 15 to 30%, with 75% of these deaths attributed to cardiovascular causes (Weitz *et al.*, 1996). A further 20% of PAD patients will have a non-fatal cardiovascular event within 5 years (Weitz *et al.*, 1996). Overall, claudicants have a mortality rate that is about 2.5 times greater than that of age-matched heathy controls (O'Hare *et al.*, 2004).

2.4 Diagnosis of intermittent claudication

The diagnosis of intermittent claudication usually begins with a medical history and physical examination. The site of the arterial stenosis is often associated with specific leg symptoms. Aorto-iliac disease might produce hip, buttock and thigh pain, as well as calf pain. Femoro-popliteal disease is usually associated with calf pain only. Tibial artery disease might also produce calf pain or, more rarely, foot pain and numbness. Other diagnostic approaches to locate lesions include duplex scanning, magnetic resonance angiography, catheter angiography, and computed tomographic angiography;

however, these techniques are usually reserved for revascularisation candidates. The severity of the ischaemia can be classified according to either the Fontaine or Rutherford categories (Table 2.2).

Intermittent claudication must be distinguished from other causes of leg pain such as neurological disorders, venous obstructive disease, chronic compartment syndrome, lumbar disease/spinal stenosis, osteoarthritis and inflammatory muscle diseases. The medical history should also document risk factors for atherosclerotic disease, such as smoking, diabetes, hypertension, hyperlipidaemia and a family history of atherosclerotic disease. Physical examination should document diminished or absent pulses in the femoral, popliteal, posterior tibial and dorsalis pedis arteries. Femoral artery bruits, caused by turbulence from focal stenoses, should also be documented.

Clinical diagnosis is usually confirmed by assessing the ankle-brachial pressure index (ABPI). The ABPI is the ratio of systolic blood pressure at the ankle (usually higher of posterior tibialis and dorsalis pedis pressures) relative to that at the arm (usually higher of the two brachial artery pressures), measured with the patient in a resting supine position (Figure 2.2). A normal ABPI value is 1.00 to 1.29. Indeed, the ankle pressure

will generally exceed the brachial pressure by 10 to 15 mmHg in healthy individuals as a result of higher peripheral resistance at the ankles. A resting ABPI of ≤0.90 has a sensitivity of 95% and a specificity of 100%, relative to contrast angiography, for detecting a stenotic lesion in the limb of ≥50% (Fowkes, 1988). Resting ABPI values of 0.41 to 0.90 represent mild-to-moderate PAD and are often associated with intermittent claudication, whereas values of ≤0.40 represent severe PAD (Hirsch *et al.*, 2006). An abnormal ABPI (≤0.90) is a potent predictor of cardiovascular events and premature mortality (Newman *et al.*, 1999).

Figure 2.2 Measurement of the ABPI (Norgren *et al.*, 2007)

Resting ABPI values >1.29 are considered falsely elevated, most likely due to vessel wall rigidity caused by medial calcinosis commonly associated with diabetes, end-stage renal disease and advanced age (Stein *et al.*, 2006). This rigidity might become so severe that pressures cannot be obtained in which case the result is recorded as "non-compressible". In this situation, the toe-brachial index might be a better test for assessing lower-limb perfusion, because small arteries (as in the toes) are less susceptible to calcification (Brooks *et al.*, 2001). As normal toe pressures are lower than brachial and ankle pressures, a toe-brachial index <0.70 is considered diagnostic of PAD.

If ABPIs are normal at rest but symptoms strongly suggest intermittent claudication (as often occurs in the presence of extensive collateralisation), ABPIs should be obtained before and after a treadmill exercise test to confirm diagnosis of PAD. The post-exercise ABPI measurement relies on the principle that walking induces profound peripheral vasodilatation and decreased leg peripheral resistance. In normal individuals, the brachial and ankle blood pressures rise together and maintain their normal relationship with exertion. In contrast, in individuals with lower-limb PAD, despite the increased central blood pressure, maximal exercise-induced ischaemic vasodilation in the claudicating limb is associated with development of a significant blood pressure gradient across lower-limb arterial stenoses. Thus the post-exercise ABPI will fall from its baseline value. A drop in ABPI of >0.15 after maximum walking exercise is considered representative of PAD (Baker and Dix, 1981). Treadmill walking tests are also useful for quantifying patients' functional capacity.

2.5 Current management of peripheral arterial disease

2.5.1 Overall strategy

The current treatment strategy for PAD is shown in Figure 2.3. For patients with intermittent claudication, the goals of treatment are to relieve exertional symptoms, improve walking capacity, improve quality of life, and to prevent and retard the progression of systemic atherosclerosis and adverse cardiovascular outcomes (Chi and Jaff, 2008). The treatment options for intermittent claudication vary according to the degree of disease and co-morbidity of the patient. Classic teaching has stressed conservative, non-operative therapy for intermittent claudication, including exercise training and risk factor modification. This approach is based on the frequently cited

benign natural history of lower-limb PAD and the marginal long-term benefits of surgery as it pertains to risk of limb loss (Taylor *et al.*, 2008). Revascularisation procedures are thought to be necessary in <10% of patients with intermittent claudication (Rowlands and Donnelly, 2007), and is generally reserved for those with proximal (aorto-iliac) disease, lifestyle/occupation limiting symptoms and/or for those where conservative management has failed (Norgren *et al.*, 2007).

Figure 2.3 Overall treatment strategy for peripheral arterial disease (Norgren *et al.*, 2007). BP, blood pressure; HbA1c, glycosylated haemoglobin; LDL, low density lipoprotein; MRA, magnetic resonance angiography; CTA, computed tomographic angiography.

2.5.2 Revascularisation procedures

Because of the variability of individual limb ischaemic symptoms and variable impact of these symptoms on quality of life, patients should be selected for revascularisation on the basis of: the severity of their symptoms; a significant disability as assessed by the patient; failure of conservative therapies; lack of significant co-morbid conditions; vascular anatomy suitable for the planned revascularisation; and a favourable risk/benefit ratio. Patients selected for possible revascularisation usually undergo additional imaging studies as required, such as duplex ultrasound, magnetic resonance angiography and/or catheter angiography, to determine whether their arterial anatomy is suitable for endovascular or surgical revascularisation. The determination of the best method of revascularisation (endovascular vs. open surgery) for treatment of intermittent claudication is based upon the balance between risk of a specific intervention and the degree and durability of the improvement that can be expected from this intervention. In general, endovascular procedures are best suited to focal stenoses in the larger, proximal vessels, whereas surgery is best applied to multi-level occlusions involving smaller and more distant vessels (Almahameed and Bhatt, 2006).

2.5.2.1 Endovascular revascularisation

An endovascular procedure is one that is performed inside the blood vessels with a catheter. This relatively-less-invasive revascularisation approach results in reduced mortality and morbidity compared with open surgery, although long-term patency rates appear higher for the latter (Norgren *et al.*, 2007). Percutaneous transluminal angioplasty (PTA), with or without stent placement, is the most common endovascular intervention in patients with lower-limb PAD (Almahameed and Bhatt, 2006). This technique involves threading a small catheter across the stenosis and inflating a balloon attached to the catheter tip. This flattens/compresses the plaque against the arterial wall, thus increasing the vessel lumen and facilitating blood flow. A small metal coil (stent) is sometimes inserted to help preserve vessel patency. Other endovascular procedures include atherectomy and laser therapy.

The goals of PTA include improved ABPI, walking performance, quality of life and vessel patency. The technical and initial clinical success of PTA generally exceeds 90%, with a 30-day mortality of 0 to 3% (van der Zaag *et al.*, 1996; Adam *et al.*, 2005; Vogel *et al.*, 2007). Durability of patency after PTA appears greatest for focal lesions in

proximal vessels, in non-diabetic, non-smoking patients (Hirsch *et al.*, 2006). In claudicants treated for significant arterial stenoses, 3-year patency rates for iliac and femoro-popliteal PTA procedures have been reported as 68 and 74%, respectively (Kandarpa *et al.*, 2001). Although PTA appears to improve ABPI, walking performance and quality of life (Cassar *et al.*, 2003; Spronk *et al.*, 2005; Breek *et al.*, 2007), its efficacy compared to conservative management is poorly established. In a review article comparing PTA and exercise training studies involving claudicants (Spronk *et al.*, 2005), PTA appeared more effective at improving resting ABPI (0.71 to 0.91 vs. 0.64 to 0.71, respectively), whereas exercise training resulted in greater improvements in maximum treadmill walking distance (155 to 222 vs. 289 to 713 m, respectively). The latter might be explained, at least in part, by improved lower-limb muscle metabolism after exercise training (Hiatt *et al.*, 1996), which is not apparent after PTA.

2.5.2.2 Surgical revascularisation

Surgical treatment is usually reserved for individuals who do not derive adequate functional benefit from non-surgical therapies, who have limb arterial anatomy that is amenable to obtaining a durable clinical result, and in whom the cardiovascular risk of surgical revascularization is low. Surgical options include autogenous or synthetic bypass, endarterectomy or an intra-operative hybrid procedure. Bypass surgery involves grafting a piece of surgically-excised vein or a synthetic replacement so that arterial stenoses/occlusions are circumnavigated. A bypass surgery is named for the artery that will be bypassed and the arteries that will receive the re-routed blood. The three most common peripheral vascular bypass surgeries are: (i) Aorto-bifemoral bypass surgery, which re-routes blood from the abdominal aorta to the two femoral arteries in the groin; (ii) femoro-popliteal bypass surgery, which re-routes blood from the femoral artery to the popliteal arteries above or below the knee; and, (iii) femoro-tibial bypass surgery, which re-routes blood between the femoral artery and the tibial artery. Endarterectomy involves surgical removal of plaque from an artery. This is usually reserved for stenosis of the aorta, common iliac or common femoral arteries that are unsuitable for PTA.

The technical and initial clinical success of bypass surgery generally exceeds 90% (Jamsen *et al.*, 2001; Kashyap *et al.*, 2008), with a 30-day mortality of around 0 to 6% (van der Zaag *et al.*, 1996; Adam *et al.*, 2005; Hirsch *et al.*, 2006). Five- and 10-year patency rates after aorto-bifemoral bypass surgery in claudicants have been reported as

91 and 86%, respectively (de Vries and Hunink, 1997). Successful lower-limb arterial surgical revascularisation can effectively reduce or eliminate symptoms of claudication, normalise ABPI, and improve quality of life (Holtzman *et al.*, 1999; Gardner and Killewich, 2001; Shechter *et al.*, 2003). In contrast, other findings suggest that bypass surgery is ineffective for improving 6-min walking distance (85 ± 9 to 101 ± 11 m; P = 0.739) or habitual physical activity levels (Gardner and Killewich, 2001), and bypass graft failure has the potential to convert a claudicant into a patient with more severe leg ischaemia, requiring further surgery to avoid limb loss.

2.5.3 Pharmacotherapy for intermittent claudication

Patients with intermittent claudication should receive pharmacological treatment for their cardiovascular risk factors and co-existing diseases to help prevent cardiovascular events (myocardial infarction, stroke, death) associated with atherosclerosis. However, this approach will typically not provide a significant reduction or elimination of symptoms of claudication (Norgren *et al.*, 2007). Thus, claudication drug therapy for relief of symptoms typically involves different drugs than those that would be used for risk reduction (an exception might be lipid-lowering therapy). A number of types of drugs have been promoted for symptom relief, with varying levels of evidence to support their use.

Cilostazol (Pletal) is a phosphodiesterase III inhibitor that is licensed in the USA, Japan, and parts of Europe (UK, Ireland, Germany) for the improvement of maximal and pain-free walking distances in patients with intermittent claudication. This drug has the best overall evidence for treatment benefit in this patient group. The underlying mechanism of action is not entirely clear, but weak vasodilatory activity, anti-platelet effects (improving collateral flow) and possibly a direct biochemical action on muscle oxygen consumption and pain threshold (via increases in cyclic AMP) might all contribute to the improved walking distances (Rowlands and Donnelly, 2007). Current guidelines suggest that cilostazol treatment should be stopped after 6 months if it is not clinically benefiting an individual patient and/or if their claudication symptoms have subsided (Rowlands and Donnelly, 2007).

A meta-analysis of placebo-controlled trials involving patients with moderate-to-severe intermittent claudication has shown a 40% improvement in maximum walking distance,

relative to placebo, after 6 months treatment with 100 mg cilostazol twice daily (Thompson *et al.*, 2002). Cilostazol treatment can also result in improved quality of life (Regensteiner *et al.*, 2002). Cilostazol is generally well tolerated (Rowlands and Donnelly, 2007), and is not associated with excess cardiovascular deaths (Pratt, 2001). However, because of safety concerns with milrinone, cilostazol is contra-indicated in patients with heart failure and/or serious ventricular arrhythmias (Rowlands and Donnelly, 2007). The most common side effect is headache, occurring in around 20% of patients (Thompson *et al.*, 2002). Transient diarrhoea, palpitation and dizziness (possibly because of cilostazol's vasodilatory effects) have also been recorded in around 2% of patients (Thompson *et al.*, 2002).

Naftidrofuryl has been available for treating intermittent claudication for over 20 years in several European countries. It is a 5-hydroxytryptamine type 2 antagonist, which ameliorates symptoms of PAD through vasodilation and improved muscle metabolism (Lehert *et al.*, 1990). A meta-analysis of placebo-controlled trials involving patients with intermittent claudication has shown a 37% improvement in pain-free walking distance, relative to placebo, after 6 months treatment with 200 mg naftidrofuryl thrice daily (De Backer *et al.*, 2008). Naftidrofuryl treatment can also result in improved quality of life (D'Hooge *et al.*, 2001). Naftidrofuryl seems to be well-tolerated with side effects limited to minor gastric symptoms (De Backer *et al.*, 2008).

L-Carnitine and propionyl-L-carnitine appear to improve muscle metabolism and exercise performance in patients with PAD (Hiatt, 2004). Propionyl-L-carnitine has been shown in patients with PAD to improve phosphocreatine synthesis after exercise (Taylor *et al.*, 1996), endothelial function (Cipolla *et al.*, 1999), and muscle strength (Barker *et al.*, 2001). These metabolic and endothelial effects might explain, at least in part, their clinical benefit.

Propionyl-L-carnitine (an acyl form of carnitine) appears more effective than L-carnitine for improving walking distances in claudicants (Brevetti *et al.*, 1992). In two multicenter trials of a total of 730 patients, treadmill walking distances improved significantly more with propionyl-L-carnitine (54 to 98%) than placebo (25 to 54%) (Brevetti *et al.*, 1999; Hiatt *et al.*, 2001). The drug also improved quality of life and had minimal side effects as compared with placebo. A dose-titration study suggested an

optimal dose of propionyl-L-carnitine of 2 grams/day (Brevetti *et al.*, 1995). Additional trials in a broad population of patients with claudication are necessary to establish the overall efficacy and clinical benefit of these drugs.

Statins are drugs used to lower cholesterol concentrations in people with or at risk from cardiovascular disease. They are most effective for lowering low-density lipoprotein (LDL) cholesterol, with expected reductions in the range of 20 to 63% for patients with PAD (Samson, 2008). Currently available statins include atorvastatin (Lipitor), pravastatin (Pravachol), and simvastatin (Zocor). Statins lower cholesterol by inhibiting the enzyme hydroxymethylglutaryl coenzyme A reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis. Inhibition of this enzyme in the liver stimulates LDL receptors, resulting in an increased clearance of LDL from the bloodstream and a decrease in blood cholesterol levels. Statin treatment is indicated for all patients with PAD to achieve a target LDL cholesterol value of <2.59 mM (<100 $mg \cdot dL^{-1}$) (Hirsch *et al.*, 2006).

Statin therapy reduces the risk of cardiovascular events in patients with PAD (Collins and Brittenden, 2004). It might also improve symptoms of intermittent claudication (Buchwald *et al.*, 1996; Pedersen *et al.*, 1998). Indeed, a retrospective analysis found that simvastatin therapy reduced the risk of new or worsening claudication (Pedersen *et al.*, 1998). Furthermore, a prospective trial demonstrated that treatment with 80 mg atorvastatin daily for 12 months increased pain-free walking distance (63%), but not maximum walking distance or quality of life, in patients with intermittent claudication (Mohler *et al.*, 2003). Two additional single-centre studies have also suggested a similar benefit (42 to 119%) regarding pain-free walking distance with simvastatin treatment (Aronow *et al.*, 2003; Mondillo *et al.*, 2003). Statins probably improve pain-free walking distance via anti-inflammatory and plaque-stabilising effects, rather than cholesterol-lowering effects (McDermott *et al.*, 2003; Mondillo *et al.*, 2003; Collins and Brittenden, 2004). Statins are generally well-tolerated and have only two major side effects that occur rarely, namely raised liver enzymes and skeletal muscle damage (Samson, 2008). The relative benefit of the statin therapies for claudication symptoms remains unclear.

2.5.4 Management of cardiovascular risk factors and co-existing disease

Patients with PAD have multiple atherosclerosis risk factors and extensive atherosclerotic disease, which puts them at markedly increased risk for cardiovascular events, similar to patients with established coronary artery disease (Selvin and Hirsch, 2008). Thus, patients with PAD are deemed high risk and require intensive cardiovascular risk factor modification to retard the atherosclerotic disease process and help prevent clinical events. Unfortunately, the PAD population appears largely under-treated. For example, in a cross-sectional survey of the US population (n = 7571), less than 50% of people with PAD who had hypertension were adequately controlled (Selvin and Hirsch, 2008). The reasons for such under-treatment are poorly understood, but might include under-diagnosis and/or poor awareness of the strong association between PAD and cardiovascular morbidity and mortality. Cardiovascular risk reduction strategies include exercise training, smoking cessation, anti-platelet drug therapy, lipid-lowering therapy, and anti-hypertensive and glycaemic control treatments.

2.5.4.1 Smoking cessation

Tobacco smoking, the most important modifiable risk factor in PAD (Chi and Jaff, 2008), has haematological effects that play a pivotal role in PAD, such as increased blood viscosity, and promotion of endothelial dysfunction and platelet aggregation (Ambrose and Barua, 2004). Smoking cessation has been shown to be beneficial in reducing not only the risk for lower-limb PAD progression in patients with claudication (Jonason and Bergstrom, 1987), but also the risk for cardiovascular events such as myocardial infarction and stroke (Mohler, 2004). Individuals who smoke more than 10 cigarettes daily are more likely to have restenosis after endovascular procedures (Schillinger *et al.*, 2004) and, similarly, those who have had lower-limb bypass surgery and stopped smoking have less graft failure compared with those who continue to smoke (Willigendael *et al.*, 2005). However, a meta-analysis by Girolami *et al.* (1999) showed that smoking cessation alone does not significantly improve maximum walking distance in claudicants (46.7 m, 95% confidence interval, -19.3 to 112.7 m). Thus, smoking cessation in combination with a treatment strategy that is designed to improve functional capacity (e.g. exercise training) seems the ideal strategy. Physician advice, combined with behaviour modification, nicotine replacement therapy and/or other smoking-cessation medication (e.g. Bupropion Hydrochloride [Zyban], Varenicline

[Champix]), has been recommended as the best strategy for smoking cessation (Norgren *et al.*, 2007).

2.5.4.2 Anti-platelet drug therapy

Platelet activation is increased in patients with PAD suggesting an underlying pro-thrombotic state (Cassar *et al.*, 2003). Life-long anti-platelet therapy is recommended for all patients with PAD to reduce the risk of cardiovascular events via decreased platelet aggregation and inhibited thrombus formation (Hirsch *et al.*, 2006; Norgren *et al.*, 2007). The Antithrombotic Trialist Collaboration (Antithrombotic Trialists' Collaboration, 2002), a meta-analysis of 287 studies involving 135,000 patients randomised to receive placebo or anti-platelet therapy, found that in the subset of patients with PAD treated with anti-platelet therapy that there was a 23% reduction in cardiovascular events, with similar benefits among patients with intermittent claudication and patients undergoing lower-limb revascularisation. Aspirin (75 to 160 mg daily) is the preferred first-line anti-platelet therapy, with clopidogrel (75 mg daily) being a useful alternative (Norgren *et al.*, 2007). Higher doses and/or combination therapy are generally avoided due to fear of increased risk of major bleeding (Norgren *et al.*, 2007).

2.5.4.3 Anti-hypertensive drug therapy

Treatment of high blood pressure is indicated in patients with PAD to reduce the risk of cardiovascular events and blood pressure-attributable progression of peripheral vascular problems (Hirsch *et al.*, 2006; Singer and Kite, 2008). Optimal treatment targets are <140/85 mmHg, with a lower target of <130/80 mmHg in the presence of diabetes mellitus or chronic renal disease (Singer and Kite, 2008). Regarding drug choice, all drugs that lower blood pressure are effective at reducing the risk of cardiovascular events. Hypertension treatment guidelines from the National Institute for Health and Clinical Excellence and the British Hypertension Society are summarised in Figure 2.4. Calcium-channel blockers or thiazide diuretics are recommended as first-line agents for people aged >55 years or for black patients of any age. Angiotensin-converting enzyme (ACE) inhibitors are first-line agents for people aged <55 years. Most patients will require multiple agents to achieve desired blood pressure goals (Norgren *et al.*, 2007).

Figure 2.4 Hypertension treatment guidelines from the National Institute for Health and Clinical Excellence and the British Hypertension Society (Sever, 2006)

2.5.4.4 Glycaemic control

Diabetes is present in around 12 to 20% of patients with PAD (Hiatt *et al.*, 1995a; Meijer *et al.*, 1998) and concomitant diabetes amplifies the already high risk of cardiovascular events (Mohler, 2007). Indeed, atherosclerosis tends to follow a more aggressive course in diabetics, leading to both macro- and micro-vascular complications (Aquino *et al.*, 2001). Aggressive blood glucose lowering can prevent microvascular complications of diabetes (particularly retinopathy and nephropathy), but its effect on macrovascular complications is less definitive (Chi and Jaff, 2008). Therefore, further research is necessary to define the role of glycaemic control in the management of cardiovascular complication of diabetes in patients with PAD. Nevertheless, to reduce the risk of microvascular events, patients with diabetes and PAD should be treated aggressively to reduce their glycosylated haemoglobin to <7% (Hirsch *et al.*, 2006).

2.5.5 Other treatments for intermittent claudication

2.5.5.1 Intermittent pneumatic compression

Intermittent pneumatic compression is a mechanical technique normally used to prevent deep vein thrombosis and treat leg ulcers and oedema. The device usually involves enclosing the lower leg and foot in an inflatable, fabric appliance such as a cuff-like legging or boot that intermittently exerts pressure on the limb. This action activates the calf muscle pump, causing an acute, two- to four-fold increase in arterial leg inflow (Delis, 2005). This flow enhancement is attributable to an increased arteriovenous pressure gradient, transient abolishment of peripheral sympathetic autoregulation, and a shear-stress mediated increase in nitric oxide bioavailability (Delis, 2005). It has been suggested that the enhanced leg inflow during intermittent pneumatic compression application might promote arterial collateralisation, attenuating the increased fixed component of peripheral resistance due to atherosclerotic arterial stenoses/occlusions (Delis and Nicolaides, 2005). Contraindications to this technique include a recent venous thrombosis and congestive heart failure (Delis, 2005).

Delis and Nicolaides (2005) investigated the effects of 5-months intermittent pneumatic compression therapy on walking performance, haemodynamics and quality of life in patients with intermittent claudication. Pain-free walking was increased by 197%, maximum walking distance by 212%, and ABPI values were significantly improved (resting 0.59 to 0.69, post-exercise 0.22 to 0.36) suggesting improved collateral

circulation (Delis, 2005). Most aspects of quality of life (assessed using the SF-36 questionnaire) were also improved. These findings are supported by other reports (Delis *et al.*, 2000; Kakkos *et al.*, 2005). Potential stimuli for the development of collateral circulation after intermittent pneumatic compression therapy include an increase in pressure gradient around the arterial block and increased flow volume and velocity around the block (Delis, 2005). Thus, intermittent pneumatic compression appears a useful alternative therapy for patients with intermittent claudication. However, larger studies with long-term follow-up are needed to establish the role for this treatment modality in the future.

2.5.5.2 Therapeutic angiogenesis

Angiogenesis is the growth and proliferation of new blood vessels from existing vascular structures. Therapeutic angiogenesis aims to improve tissue perfusion by the delivery of angiogenic cytokines to stimulate this process. Delivery of angiogenic growth factors can be carried out in the form of the protein itself, or through gene transfer methods using viral or plasmid vectors. The advantages of local gene transfer are prolonged expression of the protein, theoretically leading to sustained angiogenesis, and reduced systemic exposure to angiogenic cytokines, the long-term effects of which remains unknown (Ghosh *et al.*, 2008).

Pre-clinical experiments involving animal models of hind-limb and coronary ischaemia showed promising results of angiogenesis and improved perfusion after transfer of certain genes (Mack *et al.*, 1998; Gowdak *et al.*, 2000). However, studies involving claudicants have shown mixed findings. In a small phase 1, double-blinded, placebo-controlled study, administration of 30 $\mu g \cdot kg^{-1}$ basic fibroblast growth factor via the femoral artery on 1 or 2 consecutive days increased calf blood flow (strain gauge plethysmography) 1 month (66%) and 6 months (153%) later (Lazarous *et al.*, 2000). Intra-arterial basic fibroblast growth factor appeared safe and well-tolerated. In a randomised, placebo-controlled study, intra-arterial administration of recombinant fibroblast growth factor-2 (30 $\mu g \cdot kg^{-1}$) increased maximum walking time by 19% and resting ABPI by 10%, measured 90 days later (Lederman *et al.*, 2002). There was also no difference in adverse events between control and intervention groups. However, there were no changes in pain-free walking time, quality of life or self-perceived walking ability. A recent meta-analysis of five randomised clinical trials (total $n = 508$)

also reported that there were no differences between control and gene therapy groups for any outcomes, irrespective of whether low- or high-dose gene therapy was used (Ghosh *et al.*, 2008). Thus, given the available evidence, it appears premature to make any recommendations regarding the relative efficacy and safety of angiogenic growth factors for the treatment of intermittent claudication. Furthers trials are needed to clarify the role of this novel treatment strategy.

2.5.6 Exercise training

A substantial number of patients with intermittent claudication are unsuitable for revascularisation procedures, for example, those with diffuse PAD, those in whom revascularisation procedures have failed, and those whose symptoms are not sufficiently disabling to justify the risks of invasive procedures. For these patients, conservative therapy programmes, such as exercise rehabilitation in conjunction with risk factor management are a possibility. Exercise training, in particular, has been recommended for over 50 years as an effective and low-risk means to help patients with symptomatic PAD improve their walking ability (Regensteiner and Hiatt, 1995).

Exercise as a treatment for claudication is not new, with improvements in walking ability having been described from as early as 1898 (cited in Stewart and Lamont, 2001). The data supporting the efficacy of supervised exercise programmes to alleviate claudication symptoms are impressive (Gardner and Poehlman, 1995; Robeer *et al.*, 1998; Girolami *et al.*, 1999; Leng *et al.*, 2000; Watson *et al.*, 2008). Exercise-induced improvements in walking ability enhance routine daily activities, quality of life and community-based functional capacity (Regensteiner *et al.*, 1996). A meta-analysis of 21 randomised and non-randomised trials of exercise training studies for claudicants, revealed mean improvements in pain-free and maximum walking times of 180% and 120%, respectively (Gardner and Poehlman, 1995). A separate meta-analysis evaluating only randomised, controlled trials concluded that exercise training improved maximal walking ability by a mean value of 150% (Leng *et al.*, 2000). The greatest improvements in walking ability were attained when each exercise session lasted ≥30 min, sessions took place ≥3 times per week, when the exercise modality was walking to near-maximal claudication pain and when the programme lasted ≥6 months. As a result of these data, guidelines for management of patients with PAD advocate supervised exercise rehabilitation for the initial treatment of patients with intermittent claudication

(Hirsch *et al.*, 2006). The key elements of an exercise programme for patients with intermittent claudication are summarised in Table 2.3.

Table 2.3 Key elements of a therapeutic claudication exercise training programme (Hirsch *et al.*, 2006)

* These general guidelines should be individualised and based on the results of treadmill stress testing and the clinical status of the patient. PAD, peripheral arterial disease; ABPI, ankle-brachial pressure index.

In addition to the benefits of regular exercise on limb ischaemic symptoms, exercise training is associated with an improved cardiovascular risk profile. Indeed, physical activity both prevents and treats many established risk factors including elevated blood pressure, insulin resistance and glucose intolerance, elevated triglyceride concentrations, low concentrations of high-density lipoprotein cholesterol and obesity (Thompson *et al.*, 2003). Moreover, other less established risk factors that can impact patients with PAD can also be improved by supervised exercise training, including high sensitivity C-reactive protein (Saxton *et al.*, 2008), homocysteine (Ali *et al.*, 1998), blood rheology (Ernst and Matrai, 1987) and autonomic function (Lucini *et al.*, 2002). Thus, there is good reason to suggest that exercise training for intermittent claudication might reduce the incidence of cardiovascular events; however, this remains to be established in clinical trials.

Few comparative prospective trials have been conducted comparing the use of revascularisation procedures and exercise training for intermittent claudication. The results of the available studies also appear dependent on the population studied, length of treatment, choice of outcome measures, and method of application of each intervention. Perkins *et al.* (1996) observed that exercise training conferred a greater improvement in walking distances than angioplasty. In the exercise-trained patients, mean maximum walking distance increased from 100 to about 450 m, with a much smaller improvement displayed in the angioplasty group (90 to 150 m). Exercise training also appears more cost-effective than angioplasty (Treesak *et al.*, 2004; Spronk *et al.*, 2008). However, such findings are not universal. Indeed, a comparative meta-analysis reported mean symptomatic success rates at 6 months of 38.4 and 76.6% after exercise training and angioplasty, respectively (Chong *et al.*, 2000). In another trial, arterial bypass surgery improved walking performance to a similar extent as exercise training (173 vs. 150%). However, complication rates were higher for surgery compared exercise training (18 vs. 8%). Thus, at present there are inadequate data to effectively compare the efficacy exercise training to that of revascularisation procedures. However, exercise training is generally adopted as a first-line treatment, since it has lower associated risks and it does not interfere with the option for revascularisation should this become necessary (Hirsch *et al.*, 2006).

Exercise training can be promoted in two formats: unsupervised home-based exercise and supervised hospital or community-based exercise training (Milani and Lavie, 2007). Although home-based programmes can generate several health benefits (Milani and Lavie, 2007), the usefulness of unsupervised exercise programmes is not well established as an effective treatment of claudication, and significant improvements in walking ability have not been consistently demonstrated with this format of exercise prescription (Savage *et al.*, 2001). A recent meta-analysis of 8 randomised, controlled trials compared supervised exercise programmes with non-supervised exercise programmes for people with intermittent claudication (Bendermacher *et al.*, 2006). Supervised exercise therapy showed statistically significant differences in improvements of pain-free and maximal treadmill walking distances compared with non-supervised exercise regimens, with moderate effects sizes at three months of 0.58 and 0.61, respectively. These translated to mean improvements in favour of the supervised group of about 125 and 150 m, respectively. Plausible explanations for this greater improvement include a higher intensity of exercise and better adherence of those completing supervised exercise training (Bendermacher *et al.*, 2006).

Given the substantial improvements in walking distances produced by supervised exercise training and the relatively poor results of simply giving advice, it remains surprising that supervised programmes are largely under-utilised in clinical practice. The major limitation of exercise rehabilitation has been described as being the lack of availability of a supervised setting to refer patients (Norgren *et al.*, 2007). Indeed, a survey of consultant surgeons with an interest in vascular disease in the UK and Ireland showed that supervised exercise programmes were only available to 27% of consultants (Stewart and Lamont, 2001). Other barriers to participation in a supervised exercise programme include lack of motivation and transportation and time issues for patients. Such factors need consideration during the development of exercise programmes for this patient group.

To date, the most utilised and effective mode of exercise therapy for patients with intermittent claudication appears to be treadmill-walking exercise in a hospital-based setting (Regensteiner and Hiatt, 1995). Programmes of supervised walking exercise have consistently been shown to improve functional capacity, with increases ranging 44 to 300% and 25 to 442% for pain-free and maximum walking distances, respectively

(Regensteiner, 1997). As a result, current PAD management guidelines recommend walking as the preferred exercise modality (Hirsch *et al.*, 2006; Norgren *et al.*, 2007).

However, there are various potential problems associated with walking exercise for patients with intermittent claudication. Firstly, claudicants are limited in their ability to walk and the physical discomfort encountered during walking is probably a major reason why this strategy has failed to achieve widespread popularity (Walker *et al.*, 2000). Walking exercise also requires patients to be well motivated and not to have severe co-morbid conditions such as disabling angina, dyspnoea or arthritis, which might render exercise to be impossible (Whyman and Ruckley, 1998). Weight-bearing exercise such as walking is also inappropriate for diabetic patients with peripheral neuropathy, as these patients are at increased risk of foot ulceration in the absence of appropriate footwear. Further disadvantages with outdoor-based walking programmes include the deterrents of heavily polluted air, poor weather conditions and difficult walking terrains (e.g. steep hills and uneven surfaces). Common patient anxieties associated with walking exercise are listed in Table 2.4.

Therefore, adherence to walking can be difficult for many patients with claudication, and this is reflected in the relatively high drop-out rates of about 30% from walking programmes (Womack *et al.*, 1997). Alternative modes of exercise might thus be useful for this patient group. However, the effectiveness of other modes of exercise such as

cycling, resistance training, pole-striding and arm-cranking has been studied to a very limited extent.

Cycling is an attractive alternative exercise modality to walking because it is a relatively easy, inexpensive and safe exercise to perform and is also a popular mode of transport in some countries (Sanderson *et al.*, 2006). Stationary cycling might also be associated with a reduced risk of falling compared to walking exercise in patients who are frail and/or very deconditioned (Askew *et al.*, 2002). While acute physiologic responses to stationary cycling and treadmill walking are similar in patients with intermittent claudication (Askew *et al.*, 2002), the effects of chronic cycle training on walking performance remain unclear. Claudicants completing a 24-week cycle exercise programme experienced improvements of 57 and 31% for pain-free and maximum walking distances, respectively (Zwierska *et al.*, 2005). However, in the only comparative prospective trial to date, Sanderson *et al.* (2006) observed improvements in walking distances in those who performed walking exercise training, but not in those who performed cycle training. However, these findings might be explained, at least in part, by the short training period (6 weeks) and small sample size ($n = 40$).

Strength training of the leg muscles has also been evaluated because of the observation that patients with PAD have leg muscle weakness (Regensteiner *et al.*, 1993). In a 12-week comparative study, Regensteiner *et al.* (1996) observed improvements in walking performance and peak oxygen uptake after walking exercise training, but not after strength training, in patients with intermittent claudication. Despite these findings, resistance training is recommended as a useful adjunct to aerobic exercise training in claudicants (Hirsch *et al.*, 2006), as it can induce favourable improvements in muscular strength and endurance.

Pole-striding exercise (walking using modified ski poles), which uses muscles of the upper and lower body in a continuous movement similar to cross-country skiing, might lead to improved exercise tolerance in patients with PAD. Oakley *et al.* (2008) reported that claudicants could walk 38% further on a standardised treadmill test (3.2 $km \cdot h^{-1}$ at 4% gradient) compared to regular walking. This improvement was accompanied by a reduction in peak pain (4.3 ± 0.5 vs. 5.6 ± 0.5 on the Borg CR-10 scale), and a 16.5% increase in oxygen consumption. The authors concluded that pole-striding might be a

useful strategy for improving cardiovascular fitness and walking capacity in patients with intermittent claudication. This conclusion is supported by a previous study that demonstrated improvements in maximum treadmill walking time (10.3 ± 4.1 to 15.1 ± 4.5 min), peak oxygen uptake (16.7 ± 4.0 to 19.5 ± 4.6 $mL \cdot kg^{-1} \cdot min^{-1}$) and the physical component of quality of life (38.3 ± 9.4 to 43.4 ± 9.3) (Collins *et al.*, 2005). However, no study has directly compared the effectiveness of pole-striding with walking exercise.

A further alternative training modality is arm-crank exercise. Such exercise is usually performed on a mechanically-braked ergometer, which permits accurate measurement of power output. Arm-cranking is commonly used for fitness testing and exercise conditioning in individuals with lower-limb problems or spinal cord injury (Balady 1993; Wang *et al.*, 2000). Potential advantages of arm-cranking for patients with intermittent claudication are two-fold. Firstly, patients do not experience the ischaemic muscular pain that is associated with lower-limb exercise. Secondly, upper-limb exercise might not induce the systemic inflammatory response associated with the relative ischaemia-reperfusion and neutrophil activation that is associated with lower-limb exercise (Nawaz *et al.*, 2001). No study has directly compared arm-cranking with walking exercise. However, arm-cranking appears to induce improvements in walking distances that are comparable to those achieved using cycle exercise (Zwierska *et al.*, 2005) and it can also improve markers of systemic inflammation (Saxton *et al.*, 2008). Thus, arm-crank exercise appears a useful alternative training modality for claudicants, and especially for those who are unable or unwilling to perform lower-limb exercise training.

Exercise training is of generally low risk for patients with cardiovascular disease (Leng *et al.*, 2000; Casillas *et al.*, 2007). Serious adverse events are rare, but can occur, particularly in response to intense and inappropriate training (Siscovick *et al.*, 1984). Potential hazards include musculo-skeletal injury, and cardiovascular complications such as myocardial infarction (Casillas *et al.*, 2007). Furthermore, diabetic patients with severe distal neuropathy might also develop foot lesions in the absence of appropriate footwear (Norgren *et al.*, 2007).

To limit the risk of these hazards occurring, contra-indications to exercise training must be identified and appropriate exercise recommendations made. Contra-indications

include recent electrocardiographic changes or acute cardiac event, unstable angina, uncontrolled cardiac arrhythmias, symptomatic aortic stenosis or heart failure, and acute infections, amongst many others (American College of Sports Medicine, 2000). The prevalence of contra-indications in claudicants ranges from 9 to 34% depending on the population studied (Norgren *et al.*, 2007). It is recommended that patients undergo a medical examination and a treadmill exercise test with concomitant 12-lead electrocardiographic monitoring before a therapeutic exercise programme is initiated, so that any potential contra-indications can be identified (Hirsch *et al.*, 2006).

Although the effectiveness of supervised exercise training is established, the mechanisms of enhanced walking distances are not clearly understood. The improvements in walking distances after supervised exercise rehabilitation might be explained by several mechanisms, including changes in central and peripheral circulatory function, localised skeletal muscle adaptations, and/or psycho-physiological adaptations such as increased pain tolerance.

There is evidence that aerobic exercise training can improve central cardiac pumping capacity in patients with intermittent claudication. Aerobic exercise training is known to cause left ventricular hypertrophy (Fagard, 1997), which, in turn, contributes to increased resting and exercising stroke volume (Wilmore and Costill, 1999). With increased stroke volume, a lower heart rate is needed to maintain a given cardiac output. Arm-crank exercise training has been shown to reduce sub-maximal lower-limb exercise heart rate in patients with intermittent claudication (Walker *et al.*, 2000), thus providing indirect evidence of enhanced cardiac stroke volume. A post-training increase in peak oxygen uptake, as observed previously (Gardner *et al.*, 2005; Zwierska *et al.*, 2005), is also indicative of enhanced stroke volume, given that an increase in stoke volume is known to account for most of the rapid improvement in cardio-respiratory fitness observed after short-term aerobic exercise training in previously sedentary individuals (Saltin *et al.*, 1968). An improvement in central cardiac pumping capacity could potentially have a significant impact on exercise tolerance (Tan *et al.*, 2000).

Exercise training might also improve walking distances in claudicants via peripheral circulatory adaptations that increase, or more effectively distribute, blood flow to the legs. An increase in absolute limb blood flow could result from the development of a

collateral blood supply that bypasses areas of arterial stenosis/occlusion. Initial studies on rats (Nicholson *et al.*, 1992) and dogs (Weiss *et al.*, 1992) with ligated femoral arteries demonstrated that, after exercise training, limb blood flow returned to pre-ligation values. However, there is little evidence of an increase in absolute limb blood flow after exercise training in patients with intermittent claudication (Gardner and Poehlman, 1995; Tan *et al.*, 2000; Watson *et al.*, 2008). Work using venous occlusion plethysmography has demonstrated no change in peak post-occlusive blood flow after an otherwise successful exercise programme (Zetterquist, 1970; Mannarino *et al.*, 1989; Hiatt *et al.*, 1990). Johnson *et al.* (1989) used duplex ultrasonography to demonstrate that in claudicants peak common femoral blood flow was unchanged after exercise training despite an increase in maximum walking distance of over 40%. Similar results were reported by Sorlie and Myhre (1978) who used a thermodilution technique to measure blood flow.

Alpert *et al.* (1969) found that the clearance rate of xenon-133 injected into the calf muscles of claudicants increased after exercise training. Since most studies do not show any increase in blood flow, these authors proposed the concept of enhanced blood redistribution after training. They hypothesised that there was a diversion of blood from minimally active muscle (low oxygen extraction rate) to exercising muscles (high oxygen extraction rate). This idea was supported by studies showing reduced femoral venous oxygen saturation during exercise in claudicants after exercise training (Zetterquist, 1970; Sorlie and Myhre, 1978). However, the increased lower-limb oxygen uptake might not be due simply to a redistribution of blood flow to more active muscles as the metabolic capabilities of the muscle fibres also change after exercise training (see below).

An enhancement of blood distribution after exercise training in claudicants could possibly occur via improved endothelial vasodilator function. Patients with PAD have decreased endothelial function compared to age-matched healthy controls (Yataco *et al.*, 1999), and this appears to be due, at least in part, to increased oxidative stress (Loffredo *et al.*, 2007). While acute high-intensity exercise worsens endothelial function (Rossi *et al.*, 2002; Andreozzi *et al.*, 2007), chronic exercise training improves it (Brendle *et al.*, 2001; Andreozzi *et al.*, 2007). The mechanisms underpinning this observation are not fully understood, although it is suggested that they are, at least in part, nitric oxide-

dependent. Increased nitric oxide production might occur through upregulated endothelial cell gene expression in response to repeated exposure of shear and/or circumferential wall stress with each exercise training session (Green *et al.*, 2004). Alternatively, exercise training might increase nitric oxide bioavailability via reduced nitric oxide quenching by oxygen free radicals (Adamopoulos *et al.*, 2001). Indeed, exercise training is known to reduce the production of radical species (Leeuwenburgh and Heinecke, 2001) and enhance anti-oxidant defences (Sen, 1995). Further research is needed to clarify the exact role of each of these potential mechanisms.

Other potential circulatory adaptations to exercise training that could improve muscle blood flow and walking performance in claudicants include capillarisation, improved blood rheology, and increased blood volume. Wang *et al.* (2008) reported that improvements in calf muscle capillary-to-fibre ratio after a 12-week walking exercise programme were significantly and positively associated with improvements in pain-free walking time ($r = 0.62$ to 0.69). Training-induced improvements in blood rheology have also been reported in some studies (Ernst and Matrai, 1987), but not others (Tan *et al.*, 2000). An increase in plasma volume, as occurs with endurance training (Fellmann, 1992), leads to a decrease in haematocrit, which might be a factor in the reduction of blood viscosity (Chien *et al.*, 1966).

Although the adaptive response of skeletal muscle in PAD patients is variable (Tan *et al.*, 2000), common changes include a selective loss of muscle fibres (type I or II), generalised muscle fibre atrophy and impaired skeletal muscle mitochondria function (Tan *et al.*, 2000; Brass *et al.*, 2004). Such changes have been suggested to be important contributors to exercise intolerance in PAD (Green 2002; Brass *et al.*, 2004).

Exercise training in healthy adults leads to numerous changes in muscle structure and function, which are dependent upon the type, intensity, and duration of the exercise performed. Endurance training has been shown to increase the proportion of oxidative muscle fibres (Saltin, 1977; Hoppeler, 1986), increase the number of mitochondria per muscle fibre (Holloszy and Coyle, 1984), increase oxidative enzyme activity (Hoppeler, 1986), slow utilisation of muscle glycogen and blood glucose, and elicit a greater reliance on fatty acid oxidation (Holloszy and Coyle, 1984).

In PAD patients, exercise training might have a favourable impact on the citric acid cycle and activation of the respiratory chains (Sorlie and Myhre, 1978; Lundgren *et al.*, 1989) by increasing the size and number of mitochondria, and enhancing mitochondria oxidative enzyme activity, which allows available oxygen to be utilised more efficiently (Holm *et al.*, 1973). Regular exercise might also upregulate fatty acid oxidation in intermittent claudication patients (evident from a reduction in respiratory exchange ratio, 0.89 ± 0.07 to 0.83 ± 0.05; (Hiatt *et al.*, 1990), which might allow exercise to continue for a longer period of time. Exercise training also has a favourable effect on carnitine metabolism. Acylcarnitine is a marker of metabolic dysfunction and is inversely correlated with walking performance in patients with PAD (Hiatt *et al.*, 1987). Exercise training has been shown to reduce plasma concentrations of acylcarnitines (12.5 ± 8.4 to 9.6 ± 8.8 μM), with greatest reductions found in those patients with the most improved walking performance (Hiatt *et al.*, 1990). Therefore, localised skeletal muscle adaptations probably contribute to the improvement in walking distances that is observed with regular lower-limb exercise training.

Patients with intermittent claudication have an altered walking gait that favours stability over speed (Gardner *et al.*, 2001; McDermott *et al.*, 2001). This causes an increase in the oxygen cost of walking (decreased economy). Claudicants might respond to an exercise rehabilitation programme by showing a decrease in steady-state oxygen consumption during treadmill testing (Tan *et al.*, 2000). For example, Gardner *et al.* (2001) observed a significant improvement in walking economy (12.7 ± 0.5 to 11.2 ± 0.4 $mL \cdot kg^{-1} \cdot min^{-1}$) after a 6-month walking exercise programme in patients with intermittent claudication. Furthermore, changes in maximum walking distance were negatively associated with changes in walking economy (r = -0.50, P = 0.013). While increased walking economy is probably due to improved walking biomechanics, the available evidence suggests that walking gait is unchanged after long-term exercise training (Crowther *et al.*, 2008). Calf muscle strength and endurance has been shown to be improved after treadmill-walking training, with changes in the latter being significantly associated with changes in walking capacity (Wang *et al.*, 2006).

The contribution of psychological mechanisms to improved walking performance after exercise training in claudicants cannot be dismissed. For example, Zwierska *et al.* (2005) observed that improvements in walking distance after programmes of upper- or lower-

limb exercise training were accompanied by an increase in exercise pain tolerance. It is also possible that the improvements in walking performance can be explained partially by the Hawthorne effect (Wind and Koelemay, 2007). The Hawthorne effect describes the effect that the awareness of being under observation can alter the way a patient behaves or positively influence the outcome of a study as the control patients in some of the studies showed an increase in walking distance at the end of the study period. Finally, the belief that exercise will lead to an improvement in walking status also appears a good predictor of outcome (Rosfors *et al.*, 1990).

To summarise, there is inadequate evidence to attribute the functional benefit of exercise training, as is often believed, to collateralisation; in contrast, clinical improvement appears more likely to be due to a combination of mechanisms, including improved cardiac function, skeletal muscle metabolism, lower-limb muscular strength and endurance, endothelial function, walking economy, and/or pain tolerance.

2.6 Aims of the studies

The primary determinants of walking impairment in claudicants are poorly understood. Therefore, the aim of Study 1 was to identify key physiological predictors of maximum treadmill walking performance in claudicants using multiple regression analysis, with a view to gaining further insight into the underlying mechanisms of functional impairment that could be the target of future treatment interventions. Additionally, improved knowledge of the underlying mechanisms of functional impairment could aid our understanding of how exercise interventions promote enhanced functional capacity. A mixture of haemodynamic, cardiopulmonary fitness and calf muscle oxygenation variables were included in the statistical analyses. Haemodynamic measures included resting ABPI and peak calf blood flow. Cardiopulmonary fitness measures included peak oxygen uptake (peak $\dot{V}O_2$), ventilatory threshold and pulmonary $\dot{V}O_2$ kinetics. Calf muscle oxygenation variables included calf muscle oxygen saturation (StO_2) at 1 min and time-to-minimum StO_2. We hypothesised that all of the physiological variables listed above would be significantly associated with maximum treadmill walking distance, but that the strongest associations would be for the calf muscle oxygenation and cardiopulmonary fitness measures.

Arm-crank exercise training can improve walking performance in patients with intermittent claudication (Zwierska *et al.*, 2005); however, the mechanisms underpinning this change remain unclear. Therefore, the main aim of Study 2 was to investigate potential physiological mechanisms by which arm-crank exercise training evokes improved walking performance in this patient group. The principal research question was does arm-crank exercise training could evoke significant changes in lower-limb O_2 delivery? We hypothesised that the arm-crank exercise training would evoke significant improvement in walking performance and that this improvement would be attributable, at least in part, to enhanced lower-limb O_2 delivery.

Chapter 3: Methods

3.1 Overview of Studies 1 and 2

Study 1 sought to identify key physiological predictors of maximum treadmill walking performance in claudicants using multiple regression analysis, with a view to gaining further insight into the underlying mechanisms of functional impairment that could be the target of future treatment interventions. Forty-five patients performed an incremental treadmill-walking test to determine maximum walking distance. Peak $\dot{V}O_2$ and ventilatory threshold were recorded during this test using breath-by-breath gas analysis. Calf muscle StO_2 at 1 min and time-to-minimum StO_2 were also measured using near-infrared spectroscopy. On other occasions, resting ABPI, peak calf blood flow (strain-gauge plethysmography), and pulmonary $\dot{V}O_2$ kinetics (breath-by-breath gas analysis) during moderate-intensity walking were assessed. A forward stepwise multiple regression analysis was performed to determine predictors of maximum treadmill walking distance.

Study 2 investigated the mechanisms of improved walking performance after arm-crank exercise training in patients with intermittent claudication. Fifty-seven claudicants were randomly allocated to an arm-crank exercise group or a non-exercise control group. The exercise group trained twice weekly for 12 weeks. At baseline and 12 weeks, patients completed incremental tests to maximum exercise tolerance on both an arm-crank ergometer and on a treadmill. Respiratory variables were measured breath-by-breath to determine peak $\dot{V}O_2$ and ventilatory threshold. Near-infrared spectroscopy was used in the treadmill test to determine changes in calf muscle StO_2. Patients also completed a square-wave moderate-intensity treadmill-walking protocol to determine pulmonary $\dot{V}O_2$ kinetics. Mixed-model (group-by-time) analyses of covariance were used to detect changes in outcome measures between groups.

3.2 Patient recruitment for Studies 1 and 2

3.2.1 Selection and recruitment

Clinically-diagnosed patients with stable intermittent claudication were selected and recruited from clinical notes, from the Sheffield Vascular Institute, at the Northern General Hospital, Sheffield, UK. Selection was based on patient's medical history, previous physical examination and on the inclusion and exclusion criteria described

below. In accordance with previous suggestions, nursing home residents, wheelchair-bound patients, and patients with lower-extremity amputation(s) were excluded because their function was uniquely impaired (McDermott *et al.*, 2002a). Vascular consultants also referred suitable patients onto this study.

Patients satisfying the study criteria were sent a letter (Appendix 1), with an attached patient information sheet (Appendix 2) and a leaflet describing the facilities and directions to The Centre for Sport and Exercise Science at Sheffield Hallam University. The letter clearly stated that there was no obligation or pressure to participate in this study. If patients did not wish to participate, their future medical care would not be jeopardised. Patients declining to be contacted were requested to leave an answer-phone message by a specific date.

Patients who had not left an answer-phone message declining to take part were presumed to have a possible interest in study participation. These patients were contacted via telephone, and vetted to ascertain that they still fulfilled the study criteria. All patient questions were answered. Patients satisfying the study criteria were invited to The Centre for Sport and Exercise Science for an initial consultation session to view the facilities and to discuss all aspects of the study.

The composition of the two study groups, the randomisation procedure, potential benefits of the exercise programme, the required commitment to the training sessions, and the measurements that were to be taken during the assessment sessions were all discussed, as were the requirements from patients if randomised to the control group. Suitability was re-assessed on the basis of previous history, in accordance with the study inclusion and exclusion criteria.

3.2.2 Inclusion criteria

Patients were included in this study on the basis of:

- Clinical symptoms and signs of lower-limb PAD supported by the presence of usual risk factors
- Symptoms of stable intermittent claudication of $\geq$12 months
- A resting ABPI $\leq$0.9

- A decrease in ABPI of ≥0.15 after maximum walking exercise if resting ABPI was >0.9
- No revascularisation procedures within the last 12 months
- Ability to undertake exercise testing and training
- No exercise-limiting angina
- No shortness of breath
- No severe arthritis

3.2.3 Exclusion criteria

Patients were excluded on the basis of:

- Symptoms of intermittent claudication for <12 months
- Inability to obtain an ABPI measurement due to non-compressible vessels
- Significant change in walking ability within the last 12 months (denoting unstable claudication)
- Exhibiting features of critical limb ischaemia
- Abnormal resting electrocardiogram readings
- A revascularisation procedure or other major surgery within the previous 12 months
- Patients taking medication specifically for claudication (e.g. cilostazol)
- If the initial assessment established that the patient suffered from severe arthritis or unstable cardiopulmonary symptoms such as shortness of breath or exercise-limiting angina

This research was carried out in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the North Sheffield Research Ethics Committee (Appendix 3). Written informed consent was obtained from each patient prior to investigation.

3.2.4 Power calculations for determination of sample size

The sample size determination table of Milton (1986) was used to estimate the number of participants needed for Study 1. This study included seven predictor variables in a model anticipated to explain 60% of the variance in maximum walking distance. Based on this, a sample size of 40 should have been large enough to assure that any predictor

variable contributing an additional 5% of explained variance to the model (if entered last) be significant at the 0.05 level.

The primary outcome variable for the calculation of sample size for Study 2 was maximum walking distance due to intolerable claudication pain, since pain-free walking distance is recognised as a less reproducible walking performance measure in incremental walking assessments (Labs *et al.*, 1999). In a preliminary study, maximum walking distance increased from a baseline (mean ± SD) of 289 ± 127 to 427 ± 219 m after a 6-week arm-crank exercise training programme (Walker *et al.*, 2000). An improvement of this magnitude is considered to be clinically important in patients with PAD. On the basis of these data, and taking into account a possible patient drop-out of 30% over the 12-week study period, recruitment of 30 patients for each group yielded an 80% power to detect an increase in maximum walking distance of this magnitude at the alpha value of 0.05.

3.2.5 Medical examination

Prior to entering the study, all patients underwent a medical examination performed by Mr Shah Nawaz (Consultant Vascular Surgeon, Northern General Hospital, Sheffield, UK). During this examination, the diagnosis of PAD was further confirmed by the Doppler assessment of ABPI (described below). Some patients had previously undergone duplex ultrasonography and/or intra-vascular peripheral angiography assessments, and these results were made available during the examination. About two-thirds of the patients had claudication due to superficial femoral artery disease, as determined by angiogram, duplex scanning or examination of pulses. The remainder of patients had distal (below-knee) arterial disease.

During the medical examination, details of surgical history, co-morbid conditions, and risk factors such as smoking status were assessed. The physical examination also included vital signs and cardiopulmonary and musculoskeletal evaluation. Current medication was also confirmed. Patients on long-term medication continued on their treatment. Blood pressure was taken (manual sphygmomanometer) and a resting 12-lead electrocardiogram (Cardioperfect, Welch Allyn, USA) was performed with the patient in the seated position, to identify evidence of arrhythmias, myocardial ischaemia or previous myocardial infarction.

Absolute contraindications to formal exercise testing included recent electrocardiographic changes or acute cardiac event, unstable angina, uncontrolled cardiac arrhythmias, symptomatic aortic stenosis or heart failure, and acute infections (American College of Sports Medicine, 2000). Patients with abnormal resting electrocardiogram readings were excluded from participation in the study. Relative contraindications to exercise testing included elevated blood pressure (systolic blood pressure >200 mmHg and/or diastolic blood pressure >110 mmHg), musculoskeletal disorders that are exacerbated by exercise, valvular heart disease, complex ventricular ectopy, and uncontrolled metabolic diseases (American College of Sports Medicine, 2000). Each patient's initial arm-crank exercise test to maximum exercise tolerance (described below) was completed in the presence of Mr Nawaz. All other sessions were undertaken in the presence of personnel who had received training in advanced-life-support procedures. All patients' general practitioners were notified in writing of their study participation.

3.3 Outcome measures

Patients were fully accustomed to the assessment protocols prior to baseline data collection. Outcome measures were assessed over three separate days, within a 5- to 10-day period, at baseline and 12 weeks. Patients were instructed not to perform any vigorous exercise in the 24 hours before an assessment and to abstain from caffeine and nicotine intake for at least 2 hours before an assessment. On day 1, patients performed an incremental arm-crank exercise test. On day 2, skin and calf blood flow measurements were obtained before completion of an incremental treadmill-walking test. On day 3, patients completed a physical activity questionnaire as well as a square-wave, moderate-intensity, treadmill-walking protocol to determine pulmonary $\dot{V}O_2$ kinetics.

3.3.1 Functional capacity

Functional capacity was assessed using an incremental treadmill-walking test. Resting ABPI is frequently a poor predictor of walking performance in patients with symptomatic PAD (Szuba *et al.*, 2006), which means that monitoring ABPI alone is inadequate for assessing the impact of the disease on functional capacity. For this reason, walking performance is usually assessed using a standardised treadmill test. Treadmill

testing is also used in the clinical setting to exceed the capacity of the lower-limb collateral circulation in 5% of patients with PAD who have a normal resting ABPI, thereby helping to establish the diagnosis of exercise-induced leg pain (McDermott *et al.*, 2002b). After treadmill exercise, ABPI characteristically decreases in patients with PAD due to a decrease in systolic pressure at the ankle, relative to an increase in pressure proximal to the site of stenosis.

Following a Transatlantic conference on clinical trials guidelines in PAD (Labs *et al.*, 1999), two internationally-accepted treadmill protocols were recommended: (i) Constant-pace treadmill protocol - constant walking speed of 3.2 $km \cdot h^{-1}$ at 12% gradient; and, (ii) graded (incremental) treadmill protocol - starting horizontally at a constant speed of 3.2 $km \cdot h^{-1}$, but with the gradient increasing in pre-defined steps (e.g. 2%) at pre-defined time intervals (e.g. every 2 min). The main variables measured in such tests of walking performance are (i) distance or time to the onset of claudication pain (CD), and (ii) maximum walking distance or time (MWD), at which point patients can no longer tolerate the claudication pain. Patients must report the onset of claudication pain verbally and CD is considered a less reliable walking performance measure than MWD, particularly in incremental tests (Hiatt *et al.*, 1995b; Labs *et al.*, 1999). To reduce measurement error, it is good practice to ensure that patients are fully accustomed to the testing procedures before assessment, as many elderly people are not familiar with treadmill walking. In addition, it is important to confirm that patients terminated the test due to intolerable claudication pain and not due to some other reason, for example, breathlessness or unrelated exercise pain due to co-morbidities that are common in this patient group.

Constant-pace tests are generally easier to administer and do not require a programmable treadmill. In addition, there is a larger historical database derived from constant-pace tests, as many of the earlier published studies used such protocols. However, incremental protocols have the advantage that they can be used to assess walking performance in more heterogenous patient populations with wide-ranging walking abilities (Hiatt *et al.*, 1995b; Regensteiner and Hiatt, 1995). Furthermore, incremental protocols are likely to be more useful for re-assessing patients after a treatment intervention (in which an improvement is expected), as they do not exhibit the 'ceiling' effects which are more characteristic of constant-pace protocols. Incremental

treadmill protocols are also considered to have higher test-retest reproducibility in comparison to constant-pace protocols (Hiatt *et al.*, 1995b; Regensteiner and Hiatt, 1995; Labs *et al.*, 1999). Coefficients of variation in the range of 30 to 45% for CD and MWD have been reported for constant-pace tests, in comparison to 15 to 25% for CD and 12 to 13% for MWD on incremental tests (Hiatt *et al.*, 1995b).

Each patient completed an incremental treadmill-walking test, during which the speed was kept constant (3.2 $km \cdot h^{-1}$) but the grade was varied, starting horizontally, and then increasing by $1\% \cdot min^{-1}$. Grade increments of $1\% \cdot min^{-1}$ were used instead of the typical 2% every 2 min to make the protocol more 'ramp-like', and thus facilitating the determination of ventilatory threshold.

All patients confirmed to be pain-free prior to commencing this test. Resting heart rate (12-lead electrocardiogram; Cardioperfect, Welch Allyn, USA), blood pressure (manual sphygmomanometer) and capillary blood lactate concentration (section 3.3.1.3) were recorded. Near-infrared spectroscopy (section 3.3.1.4) and breath-by-breath gas analysis (section 3.3.1.2) data were also collected at rest during a two-minute period with the patient stood still astride the moving treadmill belt.

After the baseline period, the patient was instructed to start walking on the treadmill. Patients verbally reported the onset of claudication pain, at which point CD was recorded. Patients were encouraged to continue walking until they could no longer tolerate the claudication pain, at which point MWD was noted and the test was terminated. Test-retest reproducibility work with eight patients indicated that the technical errors of measurement (TEMs; Gore, 2000) for CD and MWD were 22% and 6%, respectively.

Heart rate, gas exchange and near-infrared spectroscopy data were recorded throughout the test. Perceived exertion and leg pain responses (Borg's 6-20 RPE and CR-10 scales, respectively; Borg, 1998) were recorded at one minute intervals. Blood pressure and blood lactate were assessed immediately post-exercise. Patients were monitored for $\geq$15 minutes after the walking test to ensure that heart rate and blood pressure had returned to resting values and were stable.

3.3.1.1 Heart rate, perceived exertion and perceived leg pain

The heart rate, perceived exertion and pain responses at MWD were used to assess the consistency of effort and degree of leg pain experienced during each walking assessment at the different time points of the study.

3.3.1.2 Expired gas variables

Oxygen consumption, carbon dioxide production, minute ventilation, and other respiratory variables were recorded breath-by-breath (MedGraphics Ultima CardiO2, Minnesota, USA). The system oxygen and carbon dioxide analysers were calibrated before each test using gases of known concentrations. Inspired and expired volumes were also calibrated using a 3-L syringe. All breath-by-breath data collected were stored to computer disk for analysis. Ventilatory threshold was identified using the *V*-slope method (Beaver *et al.*, 1986) and peak $\dot{V}O_2$ was recorded as the highest value over any 20-s averaged period. At maximum exercise tolerance, the peak values for all other respiratory variables were recorded.

3.3.1.3 Capillary blood lactate

The pain experienced during claudication is associated with elevated blood lactate concentration resulting from anaerobic metabolism. 25-µl finger prick blood samples were taken to identify capillary blood lactate concentration before and immediately after exercise using a portable lactate analyser (YSI 1500 Sport, Ohio, USA). Increased lactate tolerance, which would be indicated by increased end-exercise blood lactate values, is a potential mechanism by which arm-crank exercise training could improve walking performance in claudicants.

3.3.1.4 Calf muscle oxygenation

Calf muscle oxygenation (StO_2) data were recorded during the incremental treadmill-walking test using near-infrared spectroscopy (NIRS). NIRS is an optical technique that can be used for the non-invasive measurement of tissue oxygenation and haemodynamics. It is based on the relative tissue transparency for light in the near-infrared region and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. Haemoglobin, myoglobin, and to a lesser extent cytochrome oxidase, are the most important chromophores absorbing near-infrared light in muscle tissue.

Haemoglobin is the main component of erythrocytes and the oxygen carrier of the blood. Myoglobin is present within the muscle cell and facilitates intracellular oxygen transport. Due to identical spectral characteristics, it is not possible with NIRS to distinguish between haemoglobin and myoglobin. However, the contribution of myoglobin to the overall signal has previously been described as minimal (~10%; Mancini *et al.*, 1994). Therefore, the degree of light absorption is thought to be primarily dependent on the amount of oxygen attached to haemoglobin in the small arterioles, venules and capillaries (Boushel *et al.*, 2001). Cytochrome oxidase is the terminal enzyme of the mitochondrial respiratory chain reaction transferring the electrons to molecular oxygen. Because the amount of cytochrome oxidase in muscle is relatively low as compared with haemoglobin and myoglobin, changes in cytochrome oxidase are lost within the noise of the signal. Therefore, the contribution of cytochrome oxidase was neglected in the studies of this thesis.

The continuous-wave near-infrared spectrophotometer used in this thesis (NIRO-300, Hamamatsu Photonics, Japan) generates light at four wavelengths (775, 810, 850 and 910 nm), which is transported to the tissue by means of an optical fibre bundle called an optode. A second optode transports the light to the detector and is placed parallel to the light source, directly on the skin over the muscle or other tissue of interest. The light penetrates the skin, sub-cutaneous fat layer and muscle, and is either scattered or absorbed within the tissue. The light scattering originating from the source occurs in any direction, but the light detected by the second optode is thought to describe a banana shape (Boushel *et al.*, 2001). Back-scattered light is returned as an optical signal and analysed using a procedure called spatially resolved spectroscopy to produce a ratio of oxygenated haemoglobin/myoglobin to total haemoglobin/myoglobin, expressed as a StO_2 percentage (Figure 3.1). Calf muscle StO_2 changes during ambulation can be used to reflect the balance between oxygen delivery and tissue oxygen utilisation; a mismatch being reflected by a drop in StO_2 relative to baseline.

Figure 3.1 Schematic of spatially resolved spectroscopy. Light attenuation in tissue is measured at several focal points from the light source and the slope of light attenuation versus distance is used to estimate the proportion of the oxyhaemoglobin to total haemoglobin, from which a percentage oxygen saturation value is derived (Boushel *et al.*, 2001).

The NIRS optodes were placed on the lateral head of the gastrocnemius muscle of the most diseased leg (lowest ABPI; Figure 3.2). Source-detector seperation was 5 cm. The optodes were housed in an optically dense rubber holder, which ensured that their position, relative to each other, was fixed and invariant. The optode assembly was secured on the skin surface with double-sided tape and then covered with Coban band (3M Health Care Inc., USA) to minimise the intrusion of extraneous light and loss of near-infrared transmitted light from the field of interrogation (Figure 3.2).

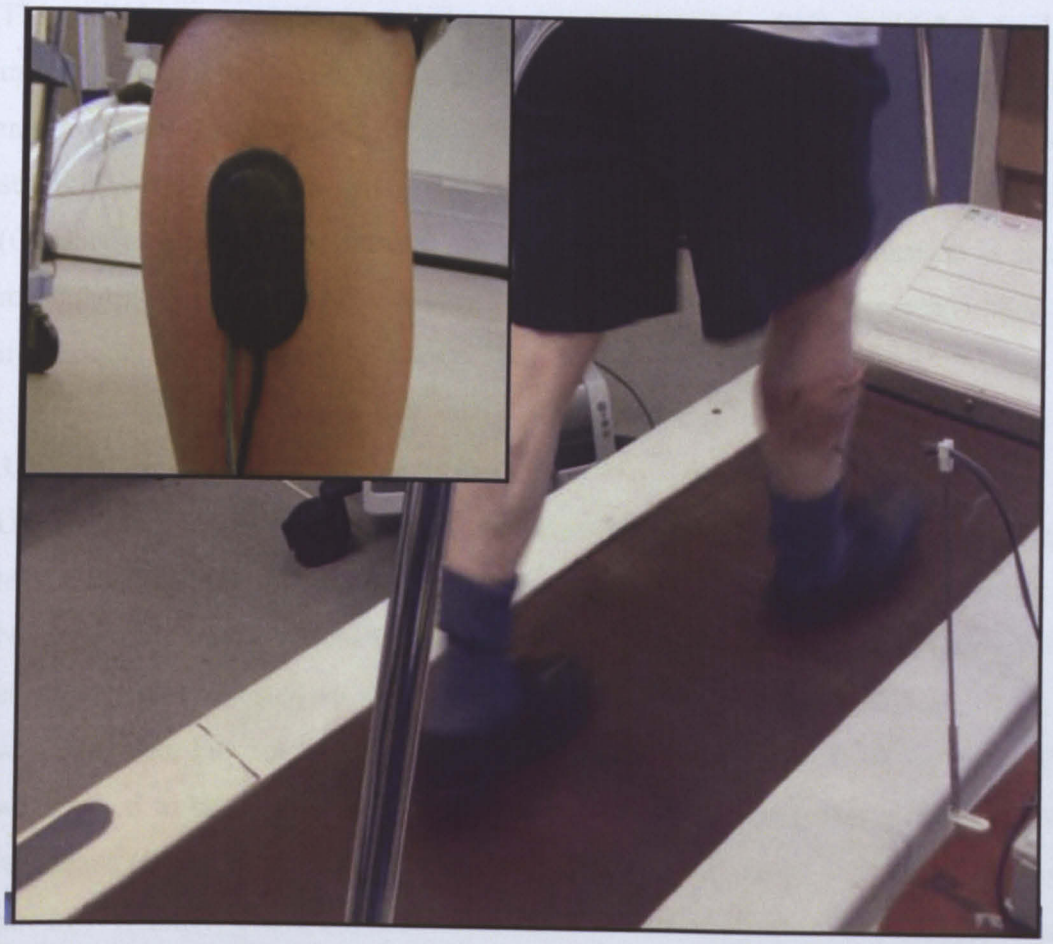

Figure 3.2 Positioning of the near-infrared probes on the lower-limb for measurement of calf muscle oxygen saturation.

The variables assessed by near-infrared spectroscopy were StO_2 at absolute (e.g. rest, 1 min, 2 min) and relative (e.g. MWD) time-points, and time-to-minimum StO_2. Time-to-minimum StO_2, is thought of as a key NIRS measure in claudicants because it is strongly correlated with maximum treadmill walking performance in these patients (Gardner *et al.*, 2008), suggesting that patients with faster deoxygenation of the active musculature (presumably because of inadequate blood [= oxygen] supply) have greater impairment in exercise performance. This variable had a TEM of 8%.

3.3.2 Upper-limb exercise performance

Upper-limb exercise performance was assessed using incremental arm-crank exercise test. For this test, an electronically-braked cycle ergometer (Lode Excalibur Sport, Netherlands) was positioned specifically for arm-crank exercise (Figure 3.3). The ergometer was adjusted so that the crank axle was at shoulder level and so the elbow was extended, but not locked when the handgrip was farthest from the body. Patients were asked to maintain a cadence of 50 $rev \cdot min^{-1}$. After a 2-min warm-up against no resistance (0 W), the work rate was increased in a continuous and ramped fashion at a rate of 7 $W \cdot min^{-1}$. The assessment terminated at maximum exercise tolerance. Heart rate, exertion, pain, blood lactate and gas exchange variables were recorded as in the incremental treadmill-walking test above. Peak work rate was also recorded and was subsequently used to prescribe the training intensity for those taking part in the arm-crank exercise training programme. Ergometer calibration procedures indicated that the accuracy of the ergometer was 1 to 6% in the range of 50 to 400 W at 50 $rev \cdot min^{-1}$.

Figure 3.3 Electronically-braked cycle ergometer positioned for arm-crank exercise

3.3.3 Pulmonary oxygen uptake kinetics

Pulmonary $\dot{V}O_2$ kinetics were assessed using breath-by-breath gas analysis measurements made during a square-wave, moderate-intensity treadmill-walking protocol. At the onset of exercise, an immediate increase in adenosine triphosphate (ATP) production in the active muscle cells is required to meet the increased metabolic demand. Measurements of muscle $\dot{V}O_2$, however, reveal that the ATP supplied by oxidative phosphorylation as a proportion of the total ATP requirement rises comparatively slowly, such that a steady-state matching between ATP utilisation and ATP supply through oxidative metabolism might not be achieved until at least 2 min after the onset of exercise (Figure 3.4). The initial rise in muscle $\dot{V}O_2$ can be well described by an exponential function, and can thus be described with an equation of the form:

$$Y(t) = Y(b) + A \cdot [1 - e^{-(t-TD)/\tau}] \qquad [1]$$

where Y represents pulmonary $\dot{V}O_2$ at any time (t), b represents baseline, A is the amplitude of the increase in Y above the baseline value, τ is the time constant defined as the time required for $\dot{V}O_2$ to reach 63% of its final amplitude (Jones and Poole, 2005), and TD is the time delay.

Figure 3.4 Schematic to demonstrate the typical response of muscle oxygen consumption after the onset of constant, moderate-intensity exercise. ATP turnover at the myofibril cross bridges increases instantaneously, but muscle $\dot{V}\ O_2$ increases relatively slowly, not attaining a steady state until approximately 2 min in this example (Jones and Poole, 2005).

The time constant, τ, is a measure of the time required for $\dot{V}O_2$ to reach 63% of its final amplitude in response to moderate-intensity (sub-gas exchange threshold) exercise: when two time constants have elapsed, $\dot{V}O_2$ will have attained about 86% of its final amplitude (i.e. 0.63 + 0.63 × (1.0 – 0.63) = 0.86); when three time constants have elapsed, $\dot{V}O_2$ will have attained approximately 95% of its final amplitude; and when four time constants have elapsed, $\dot{V}O_2$ will have attained more than 98% of its final amplitude and the response will be essentially complete. Low values of τ therefore represent 'fast' $\dot{V}O_2$ response kinetics (e.g. a τ of 20 s means that a steady-state $\dot{V}O_2$ is attained in approximately 80 s after the onset of muscle contractions; i.e. 4 × 20 s), whereas high values of τ represent 'slow' $\dot{V}O_2$ kinetics (a τ of 50 s means that a steady-state $\dot{V}O_2$ is attained in approximately 200 s; 4 × 50 s).

The τ of the $\dot{V}O_2$ response after an increase in metabolic rate is thought to be an important determinant exercise tolerance because, for the same increase in metabolic rate above baseline, an individual with fast $\dot{V}O_2$ kinetics will incur a smaller 'oxygen deficit' (Figure 3.5) and thus presumably experience a smaller fall in muscle phosphocreatine concentration, a smaller increase in lactate and hydrogen ion production, and a reduced degradation of muscle glycogen, compared with an individual with slow $\dot{V}O_2$ kinetics (Demarle *et al.*, 2001). Indeed, depletion of muscle high-energy phosphates and a reduction in muscle pH have both been implicated in the fatigue process (Fitts, 1994).

Figure 3.5 Examples of the oxygen deficit that would be incurred by individuals with different values for the phase II $\dot{V}O_2$ time constant (τ) after the onset of exercise. For the same increase in metabolic rate, represented by the $\dot{V}O_2$ attained in the steady state, an individual with fast $\dot{V}O_2$ kinetics would incur a much smaller oxygen deficit than would individuals with slower $\dot{V}O_2$ kinetics (Jones and Poole, 2005)

The measurement of muscle $\dot{V}O_2$ kinetics is invasive and technically challenging, and not suitable for routine laboratory use. Fortunately, the available evidence indicates that measurement of $\dot{V}O_2$ kinetics at the lung provides an accurate and convenient method of estimating the kinetics of O_2 consumption at the level of the working muscles (Barstow *et al.*, 1990; Grassi *et al.*, 1996; Rossiter *et al.*, 1999). One obvious difference between the dynamic profiles of muscle and pulmonary $\dot{V}O_2$ kinetics after the onset of exercise is the existence of an additional component in the pulmonary $\dot{V}O_2$ response in the first 15 to 20 s of exercise (Figure 3.6). This 'phase I' response represents an increase in $\dot{V}O_2$, resulting from a rapid increase in blood flow through the lung consequent to the immediate increase in cardiac output at the onset of exercise and, importantly, does not reflect an increased muscle O_2 consumption. The arrival of deoxygenated blood at the lung, resulting from an increased muscle O_2 consumption after the onset of exercise, is marked by a fall in the end-tidal pressure of O_2, and it is this that signifies the end of phase I and the beginning of phase II, where phase II represents the predominant, or fundamental, exponential rise in $\dot{V}O_2$ towards the expected steady state.

Figure 3.6 Schematic to demonstrate the typical response of pulmonary O_2 uptake after the onset of constant, moderate-intensity exercise. Note the similarity to the muscle $\dot{V}\ O_2$ response schematized in Figure 3.4. The time constant describing the predominant, near-exponential, rise in pulmonary $\dot{V}\ O_2$ in phase II has been shown to provide a close representation of the time constant for the increase in muscle $\dot{V}\ O_2$ (Jones and Poole, 2005).

The absolute $\dot{V}O_2$ measured at the lung will always be higher than the absolute $\dot{V}O_2$ measured across the working muscles due to the O_2 cost associated with cardiac and ventilatory work, and the maintenance of posture, etc. This will vary according to the exercise mode and intensity, but the difference is typically about 10 to 15% during cycle exercise (Jones and Poole, 2005). Although the responses are 'offset' by some 10 to 15 s, there is evidence that the τ describing the rate with which pulmonary $\dot{V}O_2$ rises in phase II reflects the τ for O_2 consumption in the exercising muscles, to within ±10% (Barstow *et al.*, 1990; Grassi *et al.*, 1996; Rossiter *et al.*, 1999).

The limitations to the rate at which $\dot{V}O_2$ rises after the onset of exercise continue to be debated (see Grassi [2006] for review). However, it is generally accepted that $\dot{V}O_2$ kinetics are slowed in PAD patients (Bauer *et al.*, 1999; Bauer *et al.*, 2004a), and that endurance exercise training results in a speeding of the phase II $\dot{V}O_2$ response (Demarle *et al.*, 2001; Koppo *et al.*, 2004; Berger *et al.*, 2006). Broadly speaking, a speeding of $\dot{V}O_2$ kinetics can occur in response to enhanced O_2 delivery, enhanced O_2 utilisation through localised metabolic adaptations, or a combination of the two. In Study 2, we investigated if arm-crank exercise training could speed pulmonary $\dot{V}O_2$ kinetics assessed during steady-state walking exercise. A speeding of $\dot{V}O_2$ kinetics in this situation could be interpreted as resulting from enhanced lower-limb O_2 delivery given that changes in O_2 utilisation are generally confined to exercise-trained skeletal muscles (Tordi *et al.*, 2001).

For each patient, breath-by-breath $\dot{V}O_2$ was measured at rest (2 min standing with feet astride the treadmill belt) and during a 6-min constant, moderate-intensity walk. The treadmill speed and gradient were individually set to elicit either 90% ventilatory threshold recorded in the incremental walking test or a perceived exertion of 'light' to 'somewhat-hard' if ventilatory threshold could not be detected (n = 22). The majority of patients completed the exercise transitions at a speed of 3.2 $km \cdot h^{-1}$ and gradient of 0 to 2%. The transition from standing to walking was performed three times with a 20-min seated-rest period between each exercise transition (Bauer *et al.*, 1999). $\dot{V}O_2$ data were processed for each exercise transition using a custom-made software programme, described previously (Bauer *et al.*, 2004a). Data points were removed if >3 standard deviations from the local, 5-point mean (Lamarra *et al.*, 1987), interpolated to 1-s

intervals, and then ensemble-averaged to yield a single response for each patient. The first 30 s of data after the onset of exercise (i.e. phase 1) were deleted. Phase 2 kinetics were then assessed using equation [1]. The τ had a TEM of 18%. The mean response time (MRT) was also calculated (MRT = TD + τ) to provide an indication of the overall response dynamics (Jones and Poole, 2005).

3.3.4 Lower-limb haemodynamic impairment

3.3.4.1 Ankle-brachial pressure index

Whilst in a recumbent position, and with the lower-limb area exposed, the systolic pressures in the posterior tibial and dorsalis pedis arteries were determined in both feet using an 8-MHz Doppler probe (MD2, Huntleigh Healthcare, UK) and manual sphygmomanometer. The systolic pressure in the brachial artery of each arm was also recorded, and is equal to that pressure when the Doppler signal resumes after gradual cuff pressure release. The ABPI for each leg was calculated by dividing the mean values from the dorsalis pedis and posterior tibial arteries by the mean values from the brachial artery in each arm (McDermott *et al.*, 2000). The higher of the two brachial pressures was used if the two brachial pressures differed by more than 10 mmHg, in which case subclavian stenosis was suspected (McDermott *et al.*, 1998; McDermott *et al.*, 2002a). The lowest leg ABPI value was used in all analyses, since any training-induced effects were envisaged to be more perceptible in this limb. Post-exercise ABPI measurements were not performed because capillary lactate measurements were being taken at that time.

3.3.4.2 Resting and peak calf blood flow

Calf blood flow was measured under resting and post-occlusive conditions in the leg with the lowest ABPI using venous-occlusion mercury-strain-gauge plethysmography. Venous occlusion plethysmography is a simple, non-invasive tool to study limb blood flow in humans. The general idea behind this technique is that a 'collecting' cuff is inflated around the upper arm or thigh to a pressure less than diastolic so that arterial inflow to a limb continues whereas venous outflow is obstructed. Under these circumstances, the limb 'swells' and the volume of the limb increases. If the veins of the limb under study are relatively empty by positioning them above heart level, the rate of increase in limb volume is thought to be proportional to the rate of arterial inflow (Joyner *et al.*, 2001).

The volume increase of the limb during venous occlusion is often measured using a mercury-in-Silastic strain gauge. Here, a thin Silastic tube is filled with mercury, and a small electrical current is passed through the mercury. When the veins are occluded and the limb expands, the Silastic is stretched, which reduces the diameter of the tubing and increases the electrical resistance. Properly calibrated, the change in electrical resistance has a linear relationship with the change in limb circumference and hence provides an estimate of volume or flow.

Venous-occlusion plethysmography can be used to measure post-occlusive reactive hyperaemia, which is the phenomenon of increased blood flow that follows relief of ischaemia and is a result of dilation of resistance vessels. This vasodilation has been attributed to myogenic relaxation of the vessels (Joyner *et al.*, 2001) and local release of mediators and metabolites such as adenosine (Shinoda *et al.*, 1997), prostaglandins and nitric oxide (Engelke *et al.*, 1996; Dakak *et al.*, 1998). Post-occlusive reactive hyperaemia data are commonly used in the evaluation of structural changes in the circulation (Joyner *et al.*, 2001), and leg blood flow during reactive hyperaemia has been shown to be depressed in PAD patients compared to age-matched heathy controls (Sanada *et al.*, 2005).

For this thesis, a 12-cm venous occlusion cuff (Hokanson, Bellevue, USA) connected to an adjustable air pressure source (Hokanson, Bellevue, USA) was attached to the distal thigh, and a mercury-strain gauge attached around the thickest part of the calf (Figure 3.7). An arterial occlusion cuff was also attached around the ankle to exclude the circulation of foot. The measured calf was supported slightly above heart level to facilitate venous outflow between repeat venous occlusions. Resting calf blood flow was assessed using repeated 10-s venous occlusions at 50 mmHg. Each venous occlusion was followed by a 5-s period of no occlusion. Before these measurements were taken, an arterial occlusion of 200 mmHg was applied at the ankle for 1 min. Arterial inflow was calculated from the slope of the volume change over a 4-s interval immediately after cuff inflation and mean-averaged for 5 consecutive venous occlusions (Thijssen *et al.*, 2005). Peak post-occlusive calf blood flow was recorded as the highest flow observed over any 2-s period following a 3-min arterial occlusion of 200 mmHg at the distal thigh (Thijssen *et al.*, 2005). The TEMs for resting and peak calf blood flow were 6 and 8%, respectively.

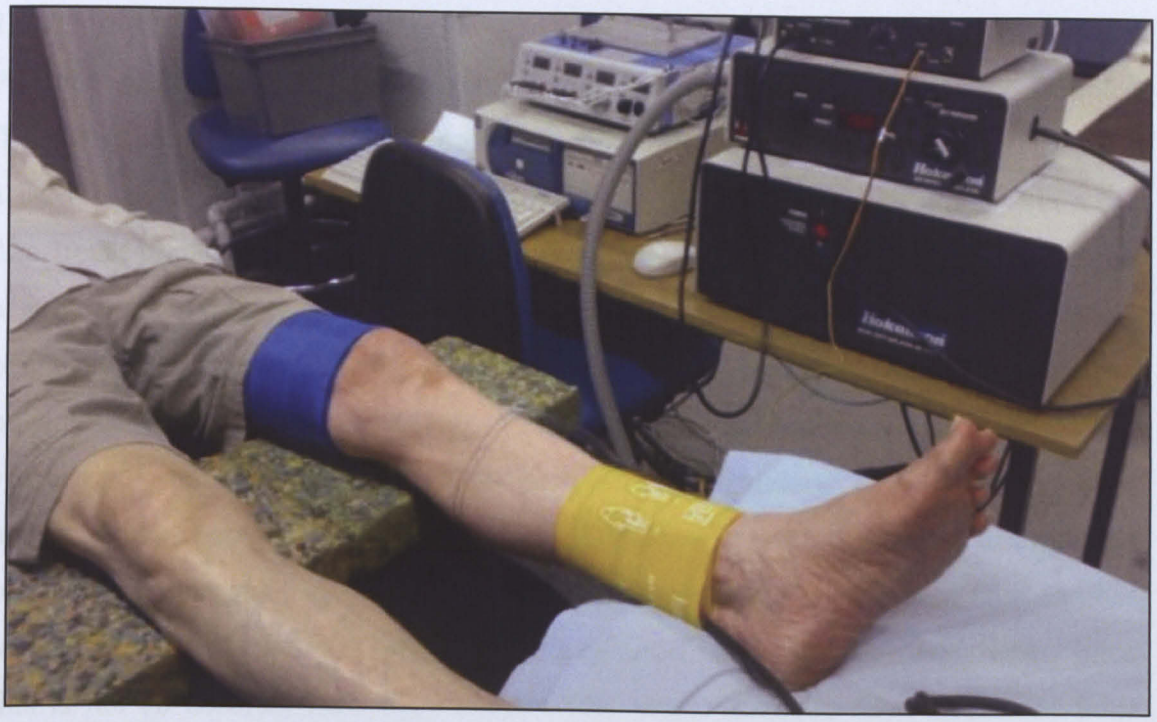

Figure 3.7 Resting and peak calf blood flow experimental set-up

3.3.4.3 Cutaneous microvascular function

Patients' cutaneous (skin) microvascular function was assessed using laser Doppler fluximetry combined with arterial occlusion. Laser Doppler fluximetry involves passing a laser light down a fibre optic cable which is attached to the skin surface via a bi-directional probe. The probe allows back-scattered light to be detected, some of which is subject to a Doppler wavelength shift by moving erythrocytes, and the other portion being reflected straight back by static components of a limited volume of tissue. The magnitude and frequency of the Doppler shift is directly related to the concentration and velocity of moving erythrocytes in both thermoregulatory and nutritional skin microvascular networks. Therefore, laser Doppler fluximetry cannot discriminate between thermoregulatory or nutritional skin perfusion, unless combined with another technique. Changes in skin blood flow are expressed in arbitrary perfusion units, since this is not an absolute flow measurement (the vessel diameter from which the laser Doppler signal originates is unknown), but a relative change in perfusion. Post-occlusive reactive hyperaemia is often used in combination with laser Doppler fluximetry to assess microvascular function (Yvonne-Tee *et al.*, 2006), and the reactive hyperaemia response is blunted and delayed in patients with intermittent claudication compared to age-matched healthy controls (Rossi and Carpi, 2004).

During the skin microvascular measurements, patients laid supine with their legs supported so that the dorsum of the foot was 10 cm above heart level (Morales *et al.*, 2005). The dorsal area of the foot to be studied (the leg with the lowest ABPI) was cleaned with an alcohol wipe and allowed to dry. Cutaneous red cell flux, an index of skin blood flow, was measured using a laser-Doppler probe (PF457; Perimed AB), connected to a laser Doppler fluxmeter (PF5001; Perimed AB). The probe was positioned between the second and third metacarpal radius and the signal was continuously monitored via an online software chart recorder (PSW; Perimed AB). After a two-minute basal flux recording, a 12-cm cuff (Hokanson, Bellevue, USA) placed proximal to the knee joint was inflated to 30 mmHg above the systolic arm pressure, or 200 mmHg (whichever the higher value), for 3-min (Morales *et al.*, 2005), using a rapid cuff inflation/deflation device (Hokanson, Bellevue, USA). After sudden release of the occlusion, the laser Doppler signal was recorded for 10 minutes to obtain the reactive hyperaemia response. Resting and peak skin flux responses were recorded and the TEM for peak skin flux was 35%. Skin temperature was also measured at an

adjacent skin site using a skin thermister (Squirrel model 1010, Cambridge, UK). Figure 3.8 shows the skin microvascular function experimental set-up.

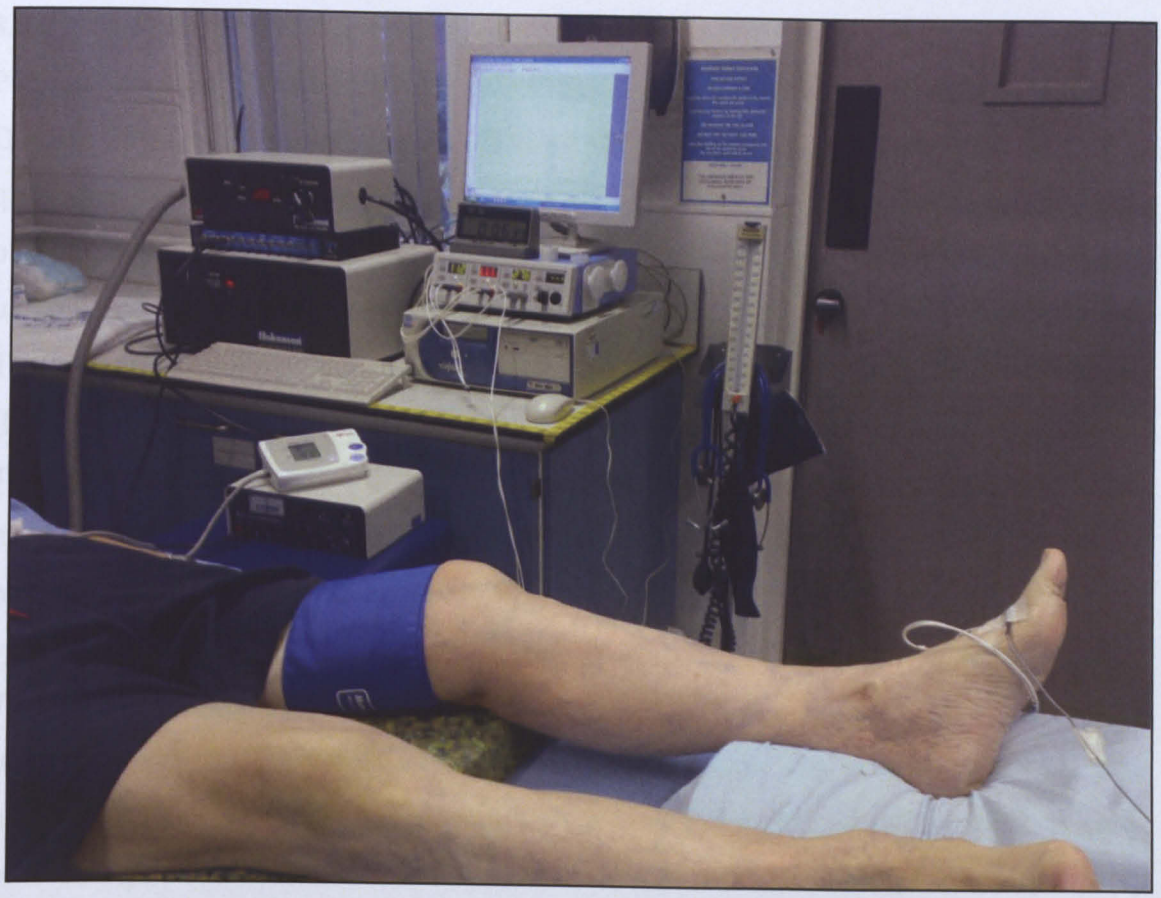

Figure 3.8 Skin microvascular function experimental set-up

3.3.5 Habitual physical activity levels

Patients' habitual physical activity levels were assessed using the Peripheral Arterial Disease-Physical Activity Recall (PAD-PAR) questionnaire (Appendix 4). This questionnaire is specific for PAD patients, and provides a global measure of habitual physical activity levels by estimating the total energy expenditure of the patient at work, in the home, and during leisure/recreational time (Sallis *et al.*, 1985; Hiatt *et al.*, 1995b). The amount of energy expenditure for each activity is expressed as metabolic equivalent (MET) hours per week. One MET equals 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ of oxygen consumption. The PAD-PAR has been modified from the original version (Sallis *et al.*, 1985) to be more appropriate for patients with claudication who can perform only low levels of physical activity (Hiatt *et al.*, 1995b).

The PAD-PAR was administered at baseline and 12 weeks. For each category (work, household and leisure/recreational activities), the patient was asked to estimate the number of hours per week spent within the category during the preceding week. A card with a range of physical activities (Appendix 5) was used as a prompt for the patients, who were asked about specific activities within each intensity domain (ranging very light to heavy). For each activity, the number of hours spent in that activity was calculated (hours per day × days per week). Activities are classed according to the following scale: very light (0.9 to 2.0 METs), light (2.1-3.0 METs), moderate (3.1-5.0 METs) and heavy (5.1 to 7.0 METs). The hours per week for each category were summed to determine the total hours per week. Data are reported in MET hours per week (hours per week × the MET value of the activity) (Hiatt *et al.*, 1995b).

3.4 Randomisation process for Study 2

Upon completing all baseline assessments, patients were randomised (random selection without replacement) using a computer programme (nQuery Advisor 6.0, Statistical Solutions, Ireland) to either an arm-crank exercise training group or a non-exercise control group. The randomisation procedure was conducted by a member of the research team not involved in the recruitment, accustomisation or assessment processes. Patients were randomised into the groups regardless of sex, age, severity of symptoms, current medication or smoking status.

3.5 Supervised exercise sessions in Study 2

Patients allocated to the arm-crank exercise training programme were invited to complete twice weekly supervised arm-crank exercise training sessions for 12 weeks. Training was usually undertaken on a group basis (maximum of 4 patients), with the sessions equally balanced during the week; sessions were normally organised for Mondays and Thursdays. This provided a structure and allowed patients time to recover between sessions. Alternative training sessions were offered, if needed, to maintain twice-weekly attendance.

The exercise training sessions were performed using friction-braked arm ergometers (Monark 881E, Varberg, Sweden; Figure 3.9). Each session began with a 2-min warm-up period consisting of low-intensity (15 W) arm-cranking. Patients then trained in cycles of 2 min exercise at a crank rate of 50 rev·min^{-1}, followed by 2 min rest, for a total exercise time of 20 min in a 40-minute session (Walker *et al.*, 2000; Zwierska *et al.*, 2005). This strategy enables a greater volume of higher intensity exercise to be performed in a given amount of time than can be achieved using continuous exercise of a similar nature, and therefore optimises the stimulus for cardiovascular adaptations. The intensity of exercise was set initially at 60 to 70% of the peak work rate achieved in the incremental arm-crank exercise test. Each patient's intensity of exercise was individually progressed over the 12-week training period to ensure they exercised at a perceived exertion of around 13 ('somewhat-hard').

Figure 3.9 Patients undertaking arm-crank exercise training

Each patient wore a short-range radio-telemetry monitor (Polar A1, Kempele, Finland) during each session to allow continuous heart rate monitoring. Heart rate, exertion, and pain were recorded at the end of the first and last 2-min exercise intervals. Each session finished with a 2-min cool-down period consisting of low-intensity (10 to 15 W) arm-cranking.

Patients allocated to the control group were informed of the benefits of an active lifestyle but did not undertake any supervised exercise. This was reinforced by telephone every fortnight during the study period. Control patients undertook assessments at identical time points as those in the exercise group (i.e. at baseline and 12 weeks).

3.6 Statistical analyses

3.6.1 Study 1

Potential physiological predictors of MWD, including resting ABPI, peak calf blood flow, peak $\dot{V}O_2$, ventilatory threshold, τ, StO_2 at 1 min and time-to-minimum StO_2, were evaluated for their association with MWD by first univariate analysis using the Pearson's correlation test and then adjusted to multivariate analysis using forward stepwise multiple linear regression. The most significant factor (i.e. the one that would result in the largest likelihood ratio statistic) was added to the model at each step and the process continued until no further significant contributing factor could be added. Variance inflation factors were calculated and a correlation matrix constructed to help identify the presence of multicollinearity in the final regression model. A variance inflation factor >10 would have suggested that multicollinearity might be biasing the regression model (Myers, 1990). Analyses were performed using SPSS for Windows statistical software (version 16.0; SPSS, Inc., Chicago, IL) and statistical significance was set at $P \leq 0.05$. Data are presented as mean ± SD.

3.6.2 Study 2

Outcome measures were first tested for normal distribution using the Kolmogorov-Smirnov goodness-of-fit test. Histograms of all outcome measures at all time-points were obtained to confirm normality of distribution. Homogeneity-of-variance checks were performed using Levene's test. The following outcome measures were normalised

using logarithmic transformation before analysis: arm-crank ventilatory threshold, arm-crank peak power output, arm-crank peak lactate, arm-crank peak pain, resting and peak calf blood flow, resting and peak skin blood flux, treadmill-walking peak exertion, treadmill-walking peak pain, time-to-minimum calf StO_2, and CD.

Differences in group characteristics were assessed using independent *t*-tests and Chi-squared tests. Mixed-model (group-by-time) analyses of covariance were used to detect changes in outcome measures between groups, with baseline data used as the covariate to improve the sensitivity of the analysis (Vickers and Altman, 2001). Paired-samples *t*-tests were used to interpret significant interaction effects. Bivariate relationships were assessed using the Pearson product-moment correlation coeffecient (*r*). Only data for patients who completed the study were included in the analyses and no adjustments were made for multiple comparisons. All statistical analyses were performed using SPSS for Windows version 16 (SPSS Ltd, Woking, UK), with significance set at $P \leq 0.05$. Data are expressed as mean ± SD unless otherwise stated.

Chapter 4: Study 1 - Physiological predictors of maximum treadmill walking distance in patients with intermittent claudication

4.1 INTRODUCTION

The main symptom of lower-limb peripheral arterial disease (PAD), intermittent claudication, is prevalent in around 5% of the UK population aged 55 to 74 years (Fowkes *et al.*, 1991; Bainton *et al.*, 1994). This symptom is experienced as a cramp-like leg pain during walking, which is relieved by rest. Patients with intermittent claudication have a marked impairment in walking performance (Green, 2002). Several factors contribute to this limitation, including reductions in lower-limb blood flow and skeletal muscle metabolic abnormalities (Brass and Hiatt, 2000; Green, 2002). However, the relative contribution of each of these factors is poorly understood.

To develop effective strategies that prevent physical disability and improve quality of life in patients with intermittent claudication, a clearer understanding of key factors which influence walking performance is needed. Measures of haemodynamic impairment, such as the ankle-brachial pressure index (ABPI), appear to explain only a small proportion of the variation in walking performance in this population (Gardner *et al.*, 1992; Szuba *et al.*, 2006). Such evidence is often used to argue that other factors, such as muscle metabolism, are important contributors to exercise intolerance in PAD (Brass and Hiatt 2000; Green 2002; Brass *et al.*, 2004). However, measures of haemodynamic impairment collected at rest (including ABPI and peak post-occlusive calf blood flow), are limited in that they do not provide any information on the extent of muscle ischaemia experienced during exercise.

In contrast, near-infrared spectroscopy (NIRS), is a non-invasive technique that can be used to measure haemoglobin/myoglobin oxygen saturation (StO_2) of the painful calf muscles during walking (Comerota *et al.*, 2003; Bauer *et al.*, 2004b; Afaq *et al.*, 2007; Bauer *et al.*, 2007; Gardner *et al.*, 2008), hence providing insight into the balance between oxygen delivery and oxygen utilisation. Recent evidence suggests that certain characteristics of the calf muscle StO_2 response during walking are correlated with walking performance parameters in claudicants (Afaq *et al.*, 2007; Gardner *et al.*, 2008), and could therefore be useful in studies that are aimed at evaluating the mechanisms of clinical improvement with exercise training, pharmacological therapy, or limb revascularisation.

Other factors that could explain the wide variability in walking performance in patients with intermittent claudication include peak oxygen uptake (peak $\dot{V}O_2$), ventilatory threshold, and $\dot{V}O_2$ kinetics, as such measures of cardiopulmonary fitness are correlated with exercise performance in other populations, including trained runners (Kilding *et al.*, 2006), overweight adolescents (Drinkard *et al.*, 2001), and patients with advanced heart failure (Cahalin *et al.*, 1996).

Previous researchers have described univariate relationships between walking performance and various haemodynamic, NIRS and cardiopulmonary fitness measurements in claudicants. However, no studies have used multiple regression analysis to investigate which combination of these variables explains most of the variation in walking performance in the same cohort of patients. Hence, the purpose of this study was to identify key physiological predictors of walking performance in claudicants using multiple regression analysis, with a view to gaining further insight into the underlying mechanisms of functional impairment that could be the target of future treatment interventions.

4.2 METHODS

A detailed description of the methods used in this study is provided in Chapter 3.

Participants

Forty-five male patients with stable intermittent claudication were recruited from the Sheffield Vascular Institute at the Northern General Hospital, Sheffield, UK. Demographic data for the patient cohort are summarised in Table 4.1.

Table 4.1 Characteristics of the 45 male patients with intermittent claudication

Variable	Values, mean ± SD and %
Age (years)	69 ± 9
Body mass (kg)	81.3 ± 13.7
Stature (cm)	174.8 ± 5.4
Duration of claudication (months)	76 ± 89
Previous myocardial infarction (% yes)	18
Previous stroke (% yes)	16
Diabetes (% yes)	22
Hypertension (% yes)	69
Smoking status (%)	
Current	36
Previous	53
Never	11

Outcome measures

Each patient performed an incremental treadmill-walking test to determine maximum walking distance (MWD), peak $\dot{V}O_2$ and ventilatory threshold. Calf muscle oxygenation (StO_2) at 1 min and time-to-minimum StO_2 were also measured using near-infrared spectroscopy. On other occasions, peak calf blood flow, resting ABPI and pulmonary $\dot{V}O_2$ kinetics were assessed.

Statistical analyses

A forward stepwise multiple regression analysis was performed to determine predictors of MWD. Variance inflation factors were calculated for the predictor variables included in the final regression model. Statistical significance was set at $P \leq 0.05$.

4.3 RESULTS

All patients had mild-to-moderate PAD with exercise-limiting intermittent claudication. Measures of walking performance, haemodynamic impairment, cardiopulmonary fitness, and calf muscle StO_2 are presented in Table 4.2. Typical NIRS profiles are depicted in

Figures 4.1 and 4.2. Figure 4.3 illustrates a typical (processed) pulmonary $\dot{V}O_2$ response during the square-wave, moderate-intensity treadmill walking protocol.

Table 4.2 Measures of walking performance, haemodynamic impairment, cardiopulmonary fitness and calf muscle StO_2

Variable	**Values, mean ± SD**
MWD (m)	523 ± 262[a]
Resting ankle-brachial pressure index	0.71 ± 0.17[a]
Resting calf blood flow (%·min^{-1})	2.70 ± 0.97[a]
Peak calf blood flow (%·min^{-1})	10.05 ± 4.97[a]
Peak $\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	17.8 ± 3.9[a]
Ventilatory threshold (ml·kg^{-1}·min^{-1})	12.0 ± 2.7[b]
τ (s)	45.9 ± 12.2[c]
StO_2 at rest (%)	47 ± 8[c]
StO_2 at 1 min (%)	41 ± 12[c]
StO_2 at MWD (%)	38 ± 13[c]
Time-to-minimum StO_2 (s)	345 ± 330[c]

MWD, maximum walking distance; $\dot{V}O_2$, pulmonary oxygen uptake; τ, $\dot{V}O_2$ time constant; StO_2, calf muscle oxygen saturation. [a] $n = 45$; [b] $n = 25$, [c] $n = 43$.

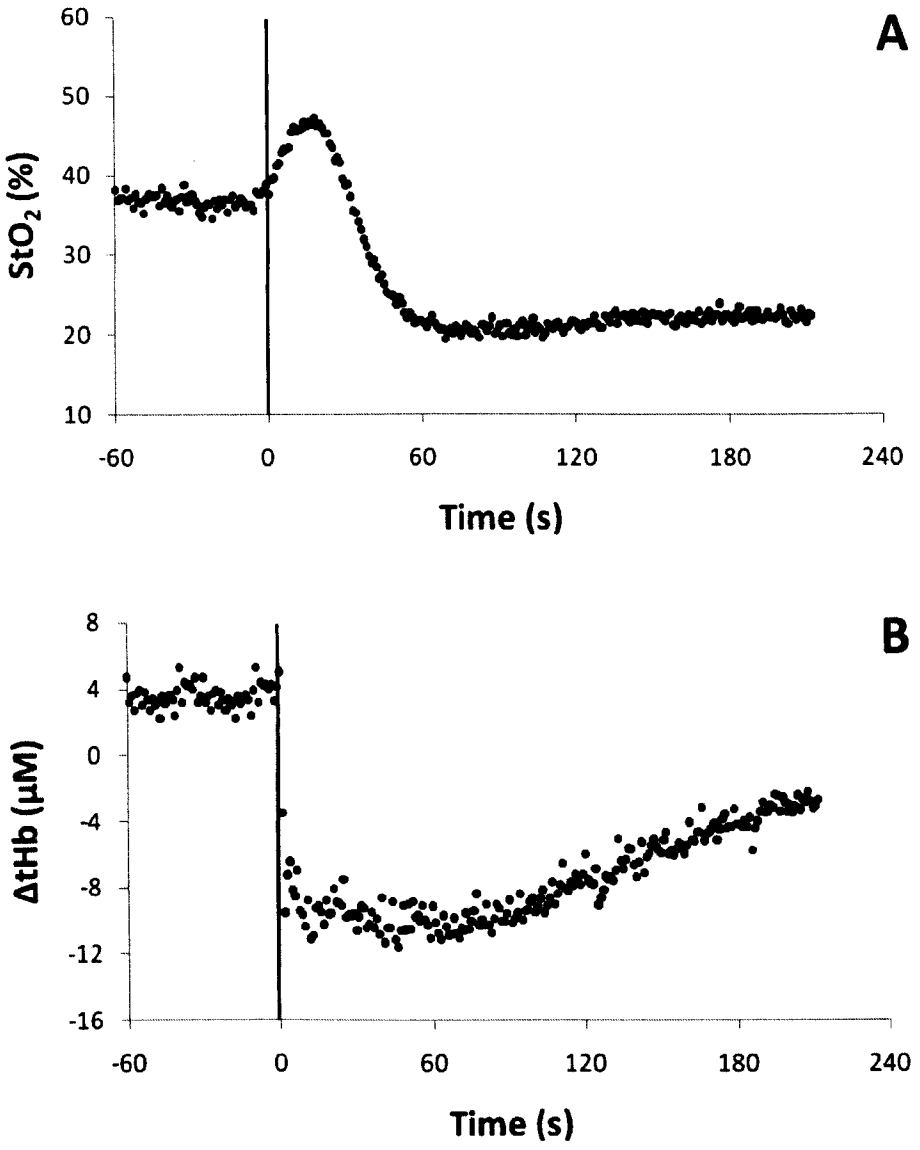

Figure 4.1 Representative near-infrared spectroscopy profile from a claudicant during graded treadmill exercise. A, calf muscle oxygenation (StO_2; represents the balance between oxygen delivery and tissue oxygen utilisation; B, total haemoglobin/myoglobin (tHb; represents microvascular blood volume changes). Vertical line represents exercise onset.

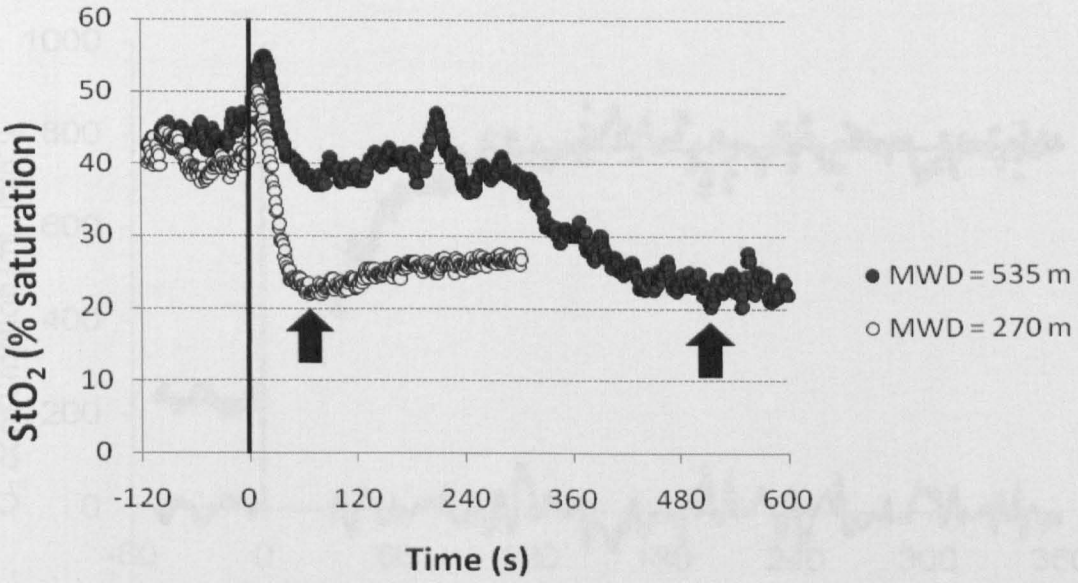

Figure 4.2 Calf muscle oxygen saturation (StO_2) data before and during the incremental treadmill walking test for patients with different walking abilities. The vertical line represents exercise onset and the arrows indicate the time at which StO_2 reaches its minimum value. Note that, for the poorer performer, there is a very early mismatch between oxygen delivery and tissue oxygen utilisation, reflected by a sharp drop in StO_2 relative to baseline, and a low time-to-minimum StO_2 value. MWD indicates maximum walking distance.

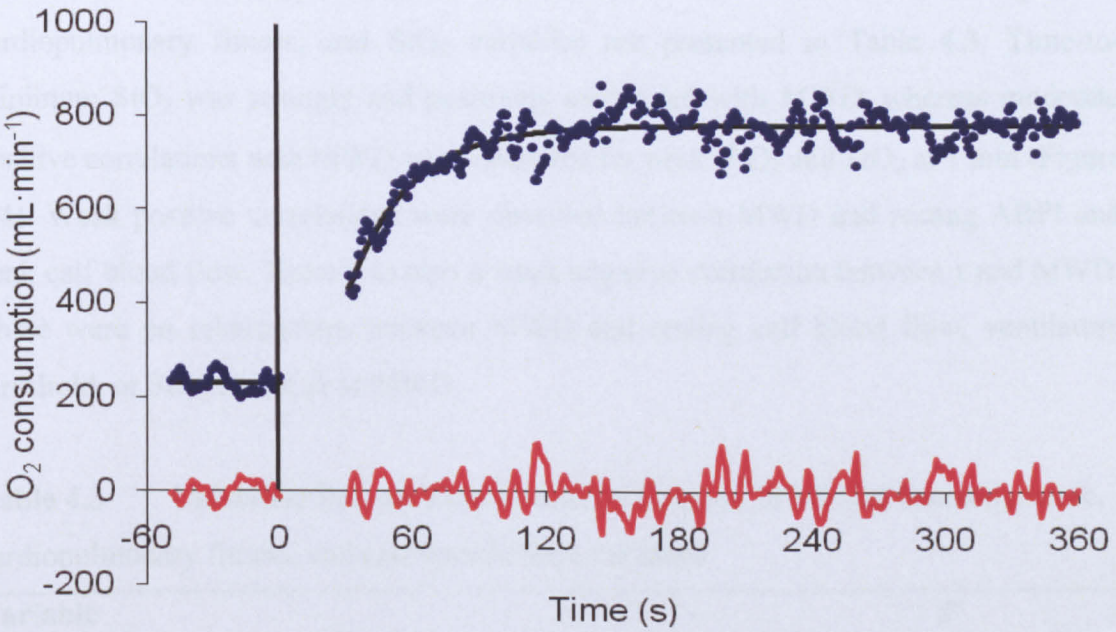

Figure 4.3 Adaptation of pulmonary $\dot{V}O_2$ during a step change from rest to moderate-intensity walking. Mono-exponential fit of data is also illustrated (τ = 44 s). Vertical line represents exercise onset. The values on the horizontal axis are the residuals.

Univariate relationships between MWD and the various haemodynamic, cardiopulmonary fitness, and StO_2 variables are presented in Table 4.3. Time-to-minimum StO_2 was strongly and positively associated with MWD, whereas moderate positive correlations with MWD were observed for peak $\dot{V}O_2$ and StO_2 at 1 min (Figure 4.4). Weak positive correlations were observed between MWD and resting ABPI and peak calf blood flow. There was also a weak negative correlation between τ and MWD. There were no relationships between MWD and resting calf blood flow, ventilatory threshold, or StO_2 at rest or at MWD.

Table 4.3 Univariate Pearson's correlation (*r*) between MWD and haemodynamic, cardiopulmonary fitness, and calf muscle StO_2 variables

Variable	***r***	***P***
Resting ABPI	0.379	0.008
Resting calf blood flow	0.132	0.388
Peak calf blood flow	0.328	0.019
Peak $\dot{V}O_2$	0.553	<0.001
Ventilatory threshold	0.141	0.192
τ	-0.358	0.012
StO_2 at rest	0.075	0.622
StO_2 at 1 min	0.519	<0.001
StO_2 at MWD	0.178	0.241
Time-to-minimum StO_2	0.752	<0.001

MWD, maximum walking distance; ABPI, ankle-brachial pressure index; $\dot{V}O_2$, pulmonary oxygen uptake; τ, $\dot{V}O_2$ time constant; StO_2, calf muscle oxygen saturation

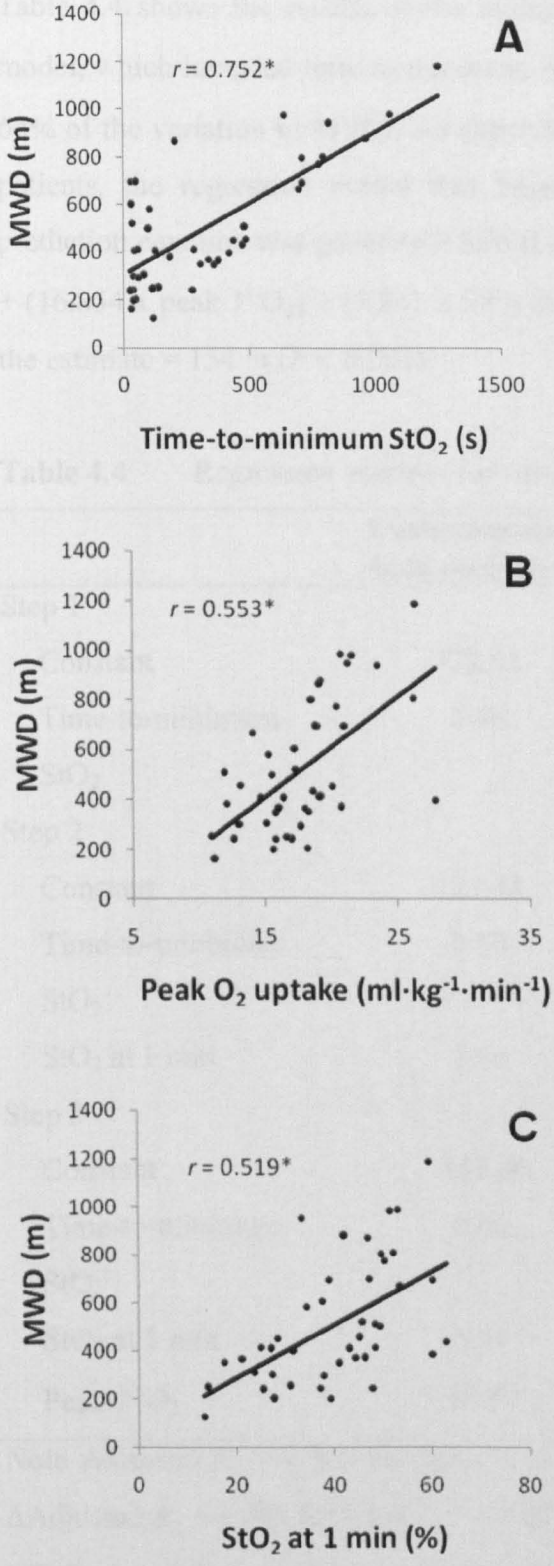

Figure 4.4 Scatter-plots: A, MWD and time-to-minimum StO_2; B, MWD and peak $\dot{V}\ O_2$; and, C, MWD and StO_2 at 1 min. Linear regression lines are illustrated to highlight relationships. $*P < 0.001$.

MWD, maximum walking distance; StO_2, calf muscle oxygen saturation

Table 4.4 shows the results of the multiple regression analysis. The final regression model, which included time-to-minimum StO_2, peak $\dot{V}O_2$ and StO_2 at 1 min, explained 64% of the variation in MWD. As either StO_2 or peak $\dot{V}O_2$ data were missing for four patients, the regression model was based on a sample size of 41. The following prediction equation was generated: MWD (m) = -153 + (0.395 x time-to-minimum StO_2) + (16.854 x peak $\dot{V}O_2$) + (5.841 x StO_2 at 1 min); adjusted $R^2 = 0.64$, standard error of the estimate = 154 m ($P < 0.001$).

Table 4.4 Regression analysis for variables predicting MWD

	Unstandardised beta coefficient	**Standard error**	**Standardised beta coefficient**
Step 1			
Constant	322.52	39.54	
Time-to-minimum StO_2	0.58	0.08	0.75*
Step 2			
Constant	127.48	91.56	
Time-to-minimum StO_2	0.50	0.09	0.65*
StO_2 at 1 min	5.44	2.33	0.26*
Step 3			
Constant	-153.00	152.71	
Time-to-minimum StO_2	0.40	0.09	0.51*
StO_2 at 1 min	5.84	2.22	0.28*
Peak $\dot{V}O_2$	16.85	7.54	0.25*

Note Adjusted $R_2 = 0.566$ for Step 1; ΔAdjusted $R_2 = 0.056$ for Step 2 ($P = 0.025$); ΔAdjusted $R_2 = 0.046$ for Step 3 ($P = 0.032$). *$P \leq 0.032$.

MWD, maximum walking distance; StO_2, calf muscle oxygen saturation; $\dot{V}O_2$, pulmonary oxygen uptake

Table 4.5 provides a matrix of the correlation coefficients for the three variables that were included in the final regression model. Time-to-minimum StO_2 was moderately

and positively associated with StO_2 at 1 min and peak $\dot{V}O_2$, whereas StO_2 at 1 min was not correlated with peak $\dot{V}O_2$. The variance inflation factors were all <1.6, suggesting that multicollinearity was not biasing the regression model.

Table 4.5 Correlation matrix for the three variables included in the final regression model

	Time-to-minimum StO_2	StO_2 at 1 min	Peak $\dot{V}O_2$
Time-to-minimum StO_2	1.000*	0.388*	0.502*
StO_2 at 1 min	0.388*	1.000*	0.081
Peak $\dot{V}O_2$	0.502*	0.081	1.000*

*$P < 0.05$.

StO_2, calf muscle oxygen saturation; $\dot{V}O_2$, pulmonary oxygen uptake

4.4 DISCUSSION

As the primary determinants of impaired walking ability in intermittent claudication are poorly understood, the purpose of this study was to identify physiological predictors of maximum treadmill walking performance in claudicants using multiple regression analysis.

The observed haemodynamic, walking performance and cardiopulmonary fitness values are typical for those with Fontaine stage II PAD. Additionally, the most common pattern of calf muscle StO_2 change during the incremental treadmill-walking test was similar to that in previous reports (Bauer *et al.*, 2004b; Afaq *et al.*, 2007; Gardner *et al.*, 2008). At the onset of exercise, there was an initial transient increase in calf muscle StO_2, likely due to the activation of the muscle pump (Bauer *et al.*, 2004b). This was followed by a sharp decline in calf muscle StO_2 towards the end of the first minute of exercise, and then a relatively stable value throughout the remainder of the test (Figure 4.1A). Afaq *et al.* (2007) suggested that during incremental treadmill-walking exercise there might be a progressive increase in calf blood flow that contributes to the observed

plateau in calf muscle StO_2. We observed a progressive increase in total haemoglobin/myoglobin during the test that supports this hypothesis (e.g. Figure 4.1B).

The final regression model included time-to-minimum StO_2, peak $\dot{V}O_2$ and StO_2 at 1 min, and together these variables explained 64% of the variation in MWD. Time-to-minimum StO_2 appeared to be the most important predictor variable, explaining over half of the variance (Table 4.4). The correlation coefficients for time-to-minimum StO_2 and StO_2 at 1 min are consistent with previous reports (Afaq *et al.*, 2007; Gardner *et al.*, 2008), and these data suggest that patients with greater impairment in exercise performance have faster deoxygenation of haemoglobin in the active musculature during incremental treadmill-walking exercise. The faster haemoglobin deoxygenation responses and greater τ values in those with poorer exercise performance (Table 4.3) suggest that the adaptation of local muscle microvascular blood flow during the transition to exercise is an important determinant of MWD in patients with intermittent claudication (DeLorey *et al.*, 2007). Indeed, this seems conceivable given that PAD is associated with limited limb blood flow during exercise (Green, 2002), reduced capillary-to-fibre ratio (Askew *et al.*, 2005), and macro- and micro-vascular endothelial dysfunction (Brevetti *et al.*, 2003; Rossi and Carpi, 2004), all of which could compromise oxygen delivery to the active skeletal muscles during exercise. However, the importance of skeletal muscle abnormalities to exercise intolerance cannot be dismissed on the basis of the evidence presented here. Further research is needed to quantify the relative contribution of haemodynamic limitations versus changes intrinsic to skeletal muscle to exercise intolerance in PAD patients.

Although resting ABPI and peak calf blood flow were significantly associated with MWD, their correlation coefficients were weak and they were not included in the final regression model (Tables 4.3 and 4.4, respectively). This might appear surprising given that the primary patho-physiological effect of PAD is impaired haemodynamics. However, ABPI reflects pressure in the conduit arteries at rest rather than muscle blood flow during exercise, which is probably a more important determinant of walking performance. Furthermore, resting ABPI is influenced by brachial artery systolic pressure, which probably has little relevance to the blood flow response in the lower limbs. Similarly, peak calf blood flow after an ischaemic challenge appears to be only a

loose indicator of the haemodynamic response during walking, which is of most relevance to patients with intermittent claudication (Green, 2002). Overall, the results support the suggestion made by Green (2002) that measures made during exercise (e.g. StO_2) have a greater explanatory value that those made at rest (e.g. ABPI).

An important feature of this study was the characterisation of peak $\dot{V}O_2$, ventilatory threshold and τ for a representative group of male claudicants. In particular, this is the first PAD study to our knowledge that has reported ventilatory threshold values for incremental treadmill exercise. Previous authors have highlighted that this threshold cannot be detected during walking exercise in many patients because their performance is limited to only a few minutes (Bauer *et al.*, 2004a). To aid threshold detection in the present study, we used an increasing gradient of 1% every 1 min, instead of the traditional '2% every 2 min' protocol commonly used to assess walking distances in patients with intermittent claudication (Labs *et al.*, 1999). This effectively made the test more 'ramp-like' and thus more suitable for detecting ventilatory threshold. This threshold was detected for 56% of the patient cohort, with a mean value of 12 $ml \cdot kg^{-1} \cdot min^{-1}$. This value, while generally only representative for those with better walking performances, is low, and approximately equivalent to 3.5 metabolic equivalents (METs). Thus, the performance of many daily activities (e.g. stair climbing, shopping, gardening: energy requirement ≥3.5 METs) will likely be limited due to substantial requirement of anaerobic metabolism. However, unlike peak $\dot{V}O_2$ and τ, ventilatory threshold was not associated with MWD (Table 4.3). Nevertheless, the correlation coefficients for these other variables generally suggest that patients with poorer cardiopulmonary fitness also have poorer walking performance. Current management guidelines recommend supervised exercise training in this patient group to improve cardiopulmonary fitness, functional capacity and quality of life (Hirsch *et al.*, 2006).

Limitations

The main limitations of this study mostly relate to the NIRS and $\dot{V}O_2$ kinetics data. Although the StO_2 signal reflects primarily haemoglobin saturation, the contribution of myoglobin cannot be definitively excluded. However, any contribution that local myoglobin might have on the calf muscle StO_2 should be minimal beyond the initial phase of exercise (Mancini *et al.*, 1994; Bauer *et al.*, 2004b). Additionally, the

subcutaneous fat thickness might also interfere with the calf muscle StO_2 measurement. However, this is unlikely to have influenced the results because no relationship has been found to exist between the calf skinfold and calf muscle StO_2 during walking in elderly claudicants (Afaq *et al.*, 2007). One might also argue that the inclusion of time-to-minimum StO_2 in the multivariate analysis is inappropriate given that this variable, to a certain degree, is a function of maximum time. However, the inclusion of this variable is supported by the following: (i) time-to-minimum StO_2 occurs well before MWD (Table 6); (ii) time-to-minimum StO_2 is also significantly associated with pain-free walking distance (r = 0.382, P = 0.011); and, (iii) it seems logical that a large, early drop in calf muscle StO_2 is associated with the development of leg pain and cessation of walking.

Limitations of the approach to assessing $\dot{V}O_2$ kinetics include the procedure for phase 1 removal and failure to include a second term in the model that describes the 'slow component'. Regarding the latter, I could not discern and, thus, adequately model a slow component of $\dot{V}O_2$ because the breath-by-breath noise was too high relative to the amplitude of the response. Failure to account for presence of a slow component would lead to an overestimate of τ, and this might explain why the τ values are slightly higher than those of Bauer *et al.* (2004a). Other potential limitations include the large variation in walking performances (which will likely inflate Pearson's r values compared to a more homogeneous patient cohort) and that all the patients were male. Finally, we cannot exclude the possibility that differences in correlation coefficients are explained, at least in part, by differences in reproducibility of measures.

In summary, the physiological variables that best explained the variation in MWD in a group of male claudicants were time-to-minimum StO_2, peak $\dot{V}O_2$ and StO_2 at 1 min. The results suggest that cardiopulmonary fitness and the ability to match O_2 delivery to metabolic demand are important determinants of MWD in this patient population, and that measures made during exercise (e.g. StO_2) generally have a greater explanatory value than those made at rest (e.g. resting ABPI). Our findings also suggest that certain NIRS variables might be useful in studies that are aimed at evaluating the efficacy of clinical interventions, including exercise training, pharmacological therapy or limb revascularisation. Further research is needed to gain a better understanding of the

relationships between the skeletal muscle and haemodynamic changes in PAD and the relative contributions of these changes to the reduced exercise performance.

Chapter 5: Study 2 - Limb-specific and cross-transfer effects of arm-crank exercise training in patients with intermittent claudication

5.1 INTRODUCTION

The main symptom of lower-limb PAD, intermittent claudication, is prevalent in around 5% of people aged 55 to 74 years in Western societies (Fowkes *et al.*, 1991; Bainton *et al.*, 1994). Intermittent claudication is a cramp-like leg pain (usually in the calf region of affected legs) that occurs during walking, when the ability to deliver and utilise oxygen is inadequate to meet the metabolic requirement of the active skeletal muscles (Brass *et al.*, 2004). Intermittent claudication reduces walking performance to about 50% of that seen in healthy individuals of a similar age (Regensteiner *et al.*, 1993), and this impairment can cause a marked reduction in quality of life (Regensteiner *et al.*, 2008).

Lower-limb exercise training such as walking has consistently been shown to improve pain-free and maximum walking distances in claudicants (Gardner and Poehlman, 1995; Leng *et al.*, 2000; Watson *et al.*, 2008). However, since lower-limb exercise can be painful, the desire and ability of these patients to perform such activity might be limited. Indeed, in clinical practice, there is evidence that nearly half of patients refrain from regular walking exercise (Bartelink *et al.*, 2004). As upper-limb arterial disease is over 20 times less frequent than lower-limb arterial disease (Welling *et al.*, 1981), patients are less likely to experience claudication pain during arm exercise. It has previously been shown that arm-crank exercise training is well tolerated by claudicants and can improve walking performance to a similar extent as lower-limb cycle ergometry training (Zwierska *et al.*, 2005), While the improvement in maximum walking distance seems at least partially attributable to an alteration in exercise pain tolerance (Zwierska *et al.*, 2005), the contribution of physiological adaptations remains unclear.

The cross-transfer effect of aerobic exercise training (i.e. increased performance during exercise involving untrained limbs) has largely been used as evidence of central and/or peripheral circulatory adaptations (Loftin *et al.*, 1988; Tordi *et al.*, 2001; Pogliaghi *et al.*, 2006). Such changes would enhance oxygen delivery and underpin improvements in cardiopulmonary fitness variables (i.e. peak $\dot{V}O_2$, ventilatory threshold, $\dot{V}O_2$ kinetics) and skeletal muscle oxygenation recorded during exercise involving untrained limbs. The physiological cross-transfer effects of aerobic exercise training have not previously been investigated in patients with intermittent claudication, and the extent to which

physiological adaptations account for the improvement in walking performance after arm-crank exercise training is unknown. Hence, the main aim of this study was to test the hypothesis that the improvements in walking performance resulting from arm-crank exercise training are attributable, at least in part, to enhanced lower-limb O_2 delivery.

5.2 METHODS

A detailed description of the methods used in this study is provided in Chapter 3.

Participants

Fifty-seven patients with stable intermittent claudication were recruited from the Sheffield Vascular Institute at the Northern General Hospital, Sheffield, UK. They were randomly allocated either to an arm-crank exercise group or a non-exercise control group. Of the 57 patients recruited, two withdrew from the exercise group, and four withdrew from the control group: one patient died of a heart attack; one developed a lower-limb ulcer that required revascularisation surgery; one was identified as having a popliteal artery aneurysm; and, one returned to full-time employment. The remaining two patients cited lack of time as their reason for withdrawal. Demographic data for the two study groups (completers only) are shown in Table 5.1.

Table 5.1 Demographics of the two study groups

Variable	Exercise group ($n = 27$)	Control group ($n = 24$)	P
Age (years)	69 ± 9	70 ± 8	0.671[a]
Body mass (kg)	81.3 ± 11.5	78.4 ± 13.9	0.431[a]
Stature (cm)	174.2 ± 4.4	173.8 ± 5.7	0.779[a]
Body mass index ($kg \cdot m^{-2}$)	26.8 ± 3.5	25.9 ± 3.7	0.377[a]
Resting ABPI	0.68 ± 0.13	0.69 ± 0.12	0.875[a]
Duration of claudication (months)	76 ± 92	44 ± 40	0.114[a]
Previous MI (%)	19	21	1.000[b]
Previous stroke (%)	11	17	0.693[b]
Diabetes (%)	30	8	0.081[b]
Smoking status (%)			0.545[b]
Current	26	33	
Previous	56	58	
Never	18	9	
Medication (%)			
Beta-blockers	15	17	0.578[b]
ACE-inhibitors	33	21	0.248[b]
Calcium blockers	19	25	0.412[b]
Diuretics	19	33	0.187[b]
Nitrates	26	25	0.598[b]
Anti-platelet agents	96	96	1.000[b]
Statins	100	92	0.216[b]

Values are means ± SD unless otherwise stated. ABPI indicates ankle-brachial pressure index; MI, myocardial infarction. ABPI data are for the most symptomatic leg.
[a]Independent t-test; [b]Chi-squared test.

Outcome measures

Outcome measures were assessed over three separate days at baseline and 12 weeks. On day 1, patients performed an incremental arm-crank exercise assessment. On day 2, skin and calf blood flow measurements were obtained before completion of an incremental treadmill-walking test. On day 3, participants completed a physical activity questionnaire as well as a square-wave, moderate-intensity, treadmill-walking protocol to determine pulmonary $\dot{V}O_2$ kinetics.

Statistical analyses

After appropriate verification of underlying assumptions, mixed-model (group-by-time) analyses of covariance were used to detect changes in outcome measures between groups. Statistical significance was set at $P \leq 0.05$.

5.3 RESULTS

Compliance to the supervised exercise programme was 97%. The % predicted maximum heart rate, RPE, and arm pain, at the end of each training session were 74 ± 11%, 13.1 ± 1.5, and 3.3 ± 1.4, respectively (calculated using data from all of the completed training sessions). Training intensity increased from 39 ± 8 W (63 ± 7% peak W) at baseline to 55 ± 11 W (70 ± 8% peak W) at 12 weeks ($P < 0.001$). There were no injuries or adverse events resulting from the exercise training or physiological assessments.

Outcome measures from the incremental arm-crank exercise test

The incremental arm-crank exercise test data are shown in Table 5.2. At baseline, there were no group differences for any of the recorded outcome measures. At 12 weeks, peak values of work rate, $\dot{V}O_2$, and blood lactate were increased in the exercise group compared to the control group. However, there were no changes in either group for ventilatory threshold, or peak values of heart rate, RPE and arm pain.

Table 5.2 Incremental arm-crank exercise test data

	Exercise group		Control group		*P*
	Baseline	12 weeks	Baseline	12 weeks	
Peak work rate (W)	62 ± 14	79 ± 16*	61 ± 18	60 ± 18	<0.001
Peak $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	13.5 ± 2.7	15.2 ± 2.7*	13.3 ± 3.5	13.1 ± 4.4	0.006
Ventilatory threshold ($mL \cdot kg^{-1} \cdot min^{-1}$)	8.4 ± 1.2	8.3 ± 1.4	8.8 ± 1.5	8.0 ± 1.9	0.108
Peak heart rate ($beats \cdot min^{-1}$)	121 ± 23	124 ± 21	116 ± 24	121 ± 21	0.986
Peak blood lactate (mM)	3.94 ± 1.34	4.34 ± 1.12*	3.63 ± 1.28	3.63 ± 0.94	0.011
Peak exertion	15.7 ± 2.4	15.0 ± 2.6	15.6 ± 2.3	15.3 ± 3.0	0.614
Peak arm pain	4.1 ± 2.6	4.8 ± 2.6	3.8 ± 2.8	4.5 ± 2.5	0.972

Values are means ± SD. $\dot{V}O_2$ indicates oxygen consumption.

P values are shown for the group-by-time interaction.

*Significantly different from baseline value ($P < 0.05$).

Outcome measures from the incremental treadmill-walking test

The incremental treadmill test data are shown in Table 5.3 and Figures 5.1 and 5.2. At baseline, there were no group differences for any of the recorded outcome measures. Ventilatory threshold could only be detected at baseline and 12 weeks for 29 of the 51 patients (57%). At 12 weeks, claudication and maximum walking distances were increased in the exercise group (53 and 33%, respectively) compared to the control group (8 and 4%, respectively). Peak values of $\dot{V}O_2$ and blood lactate, and time-to-minimum StO_2 were also increased in the exercise group compared to the control group. In addition, StO_2 was increased at 30, 60, 120 and 180 s after exercise training (Figure 5.1A), with the change in MWD correlated with the change in StO_2 at 180 s ($r = 0.524$, $P = 0.009$) and 240 s ($r = 0.486$, $P = 0.022$). Sub-maximal heart rate was also significantly reduced in the exercise compared to control group (Figure 5.2). There were

no changes in either group for minimum StO_2, end-exercise StO_2, ventilatory threshold, or peak values of heart rate, RPE and leg pain.

Table 5.3 Incremental treadmill-walking test data

	Exercise group		Control group		*P*
	Baseline	12 weeks	Baseline	12 weeks	
Claudication distance (m)	147 ± 125	225 ± 167*	177 ± 160	192 ± 195	0.035
Maximum walking distance (m)	496 ± 250	661 ± 324*	600 ± 300	626 ± 266	0.011
Peak $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	17.2 ± 2.7	18.2 ± 3.4*	18.6 ± 5.1	18.0 ± 4.9	0.038
Ventilatory threshold ($mL \cdot kg^{-1} \cdot min^{-1}$)	11.6 ± 2.3	12.0 ± 2.3	12.5 ± 2.6	11.5 ± 1.7	0.172
Peak heart rate ($beats \cdot min^{-1}$)	115 ± 22	117 ± 20	116 ± 19	112 ± 20	0.100
Peak lactate (mM)	2.80 ± 1.24	3.14 ± 1.25*	2.66 ± 0.99	2.51 ± 1.02	0.048
Peak exertion	16.0 ± 2.7	15.0 ± 3.0	16.5 ± 2.7	16.2 ± 2.8	0.210
Peak leg pain	6.7 ± 3.0	5.7 ± 2.2	6.7 ± 2.3	6.1 ± 2.9	0.405
Time-to-minimum StO_2 (s)	268 ± 305	410 ± 366*	497 ± 372	466 ± 379	<0.001
End-exercise StO_2 (%)	39 ± 14	39 ± 15	38 ± 10	35 ± 11	0.186

Values are means ± SD. $\dot{V}O_2$ indicates oxygen consumption; StO_2, calf muscle oxygen saturation.

P values are shown for the group-by-time interaction.

*Significantly different from baseline value ($P < 0.05$).

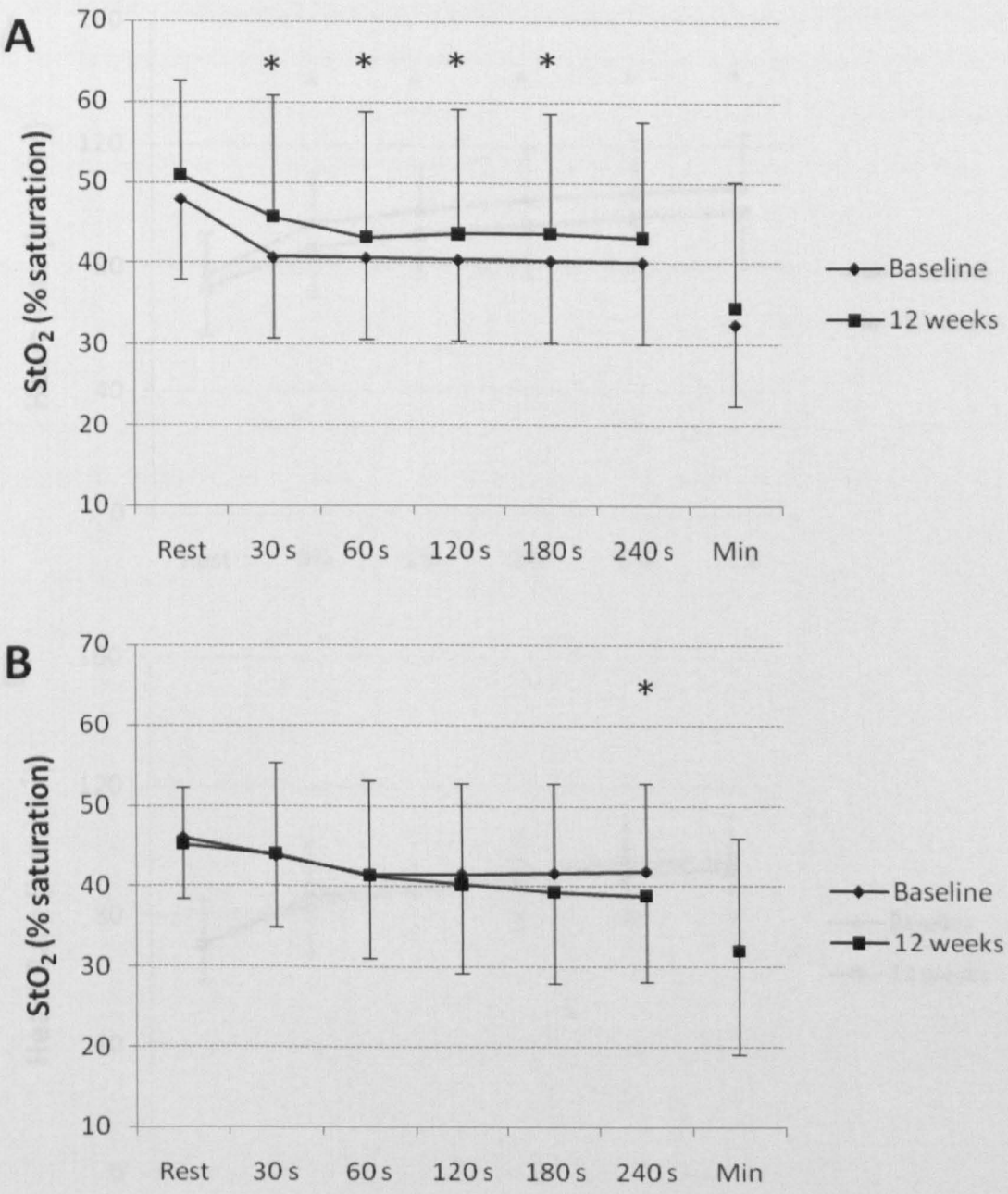

Figure 5.1 Calf muscle oxygen saturation (StO_2) data during the incremental treadmill walking test for A, the arm-crank exercise training group, and B, the control group. Min indicates minimum StO_2 value. *Significantly different from baseline value ($P < 0.05$).

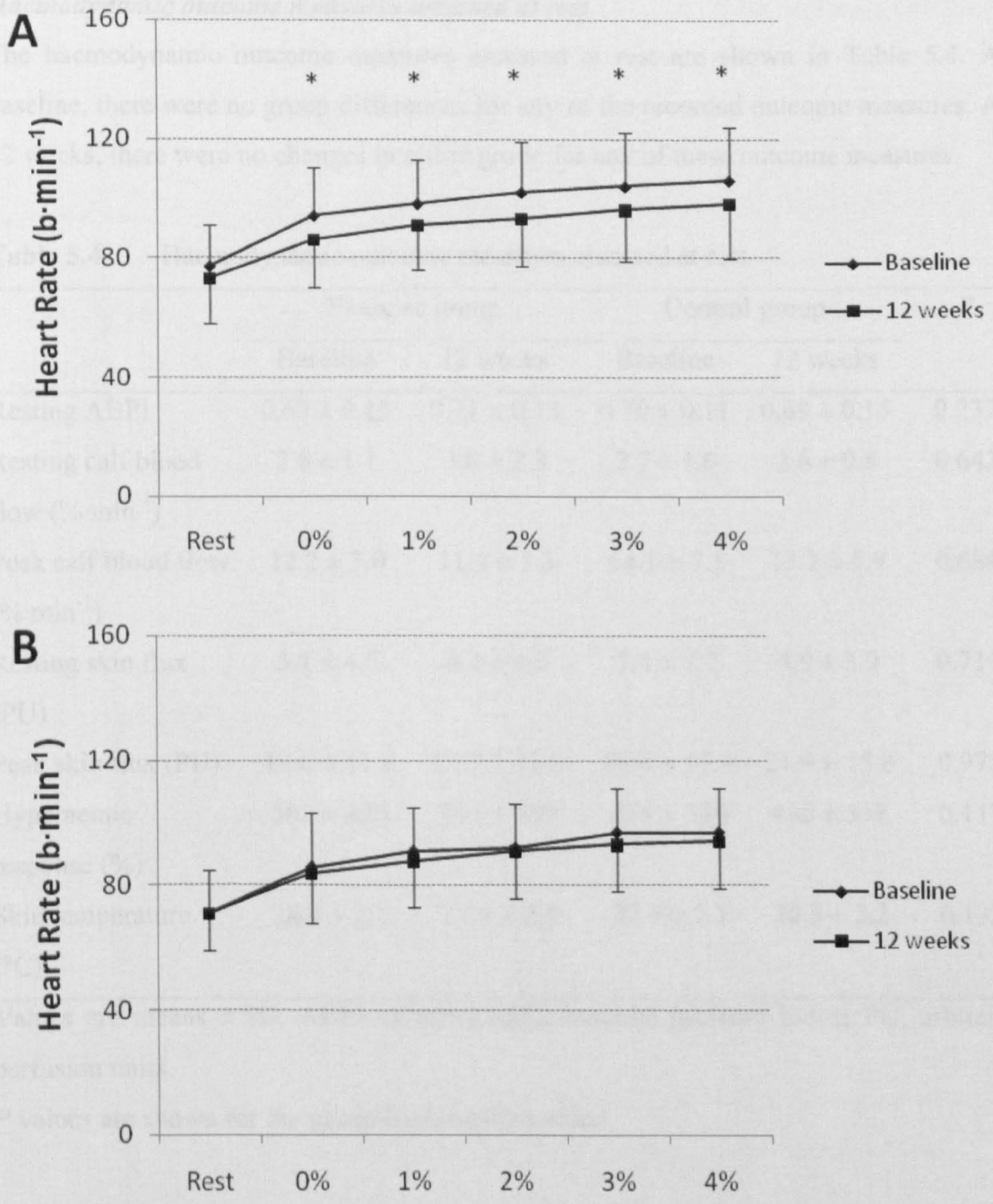

Figure 5.2 Heart rate data during sub-maximal stages of the incremental treadmill walking test for A, the arm-crank exercise training group, and B, the control group. *$P < 0.05$. The speed of the treadmill was fixed at 3.2 km·h^{-1}.

Haemodynamic outcome measures assessed at rest

The haemodynamic outcome measures assessed at rest are shown in Table 5.4. At baseline, there were no group differences for any of the recorded outcome measures. At 12 weeks, there were no changes in either group for any of these outcome measures.

Table 5.4 Haemodynamic outcome measures assessed at rest

	Exercise group		Control group		*P*
	Baseline	12 weeks	Baseline	12 weeks	
Resting ABPI	0.67 ± 0.13	0.71 ± 0.13	0.70 ± 0.11	0.69 ± 0.15	0.237
Resting calf blood flow (%·min^{-1})	2.8 ± 1.1	3.0 ± 2.3	2.7 ± 1.0	2.6 ± 0.8	0.643
Peak calf blood flow (%·min^{-1})	12.2 ± 7.0	11.4 ± 5.3	14.1 ± 7.1	13.3 ± 5.9	0.684
Resting skin flux (PU)	5.1 ± 4.5	4.2 ± 4.3	7.4 ± 7.7	4.9 ± 3.9	0.714
Peak skin flux (PU)	19.6 ± 11.6	21.1 ± 11.8	26.4 ± 17.4	21.9 ± 15.8	0.975
Hyperaemic response (%)	502 ± 420	811 ± 899	478 ± 320	485 ± 338	0.117
Skin temperature (°C)	28.4 ± 2.1	27.8 ± 2.5	27.5 ± 2.1	26.5 ± 2.2	0.132

Values are means ± SD. ABPI indicates ankle-brachial pressure index; PU, arbitrary perfusion units.

P values are shown for the group-by-time interaction.

Outcome measures from the square-wave, moderate-intensity treadmill-walking test

Outcome measures from the square-wave, moderate-intensity treadmill-walking test are shown in Table 5.5. At baseline, there were no group differences for any of the recorded outcome measures. At 12 weeks, the τ and MRT were reduced in the exercise group compared to the control group. In the exercise group, the change in τ was not correlated with the change in MWD (r = -0.164, P = 0.423). There were no changes in either group for the time delay, resting heart rate and $\dot{V}O_2$, or steady state heart rate and $\dot{V}O_2$.

Table 5.5 Outcome measures from the square-wave, moderate-intensity treadmill-walking test

	Exercise group		Control group		*P*
	Baseline	12 weeks	Baseline	12 weeks	
Resting $\dot{V}O_2$ ($mL \cdot min^{-1}$)	307 ± 43	320 ± 41	292 ± 46	277 ± 38	0.190
End-exercise $\dot{V}O_2$ ($mL \cdot min^{-1}$)	915 ± 138	923 ± 179	855 ± 168	822 ± 145	0.144
Time delay (s)	13.1 ± 5.9	12.4 ± 6.6	12.9 ± 4.9	11.0 ± 3.5	0.304
τ (s)	44.7 ± 10.4	41.3 ± 14.4*	44.2 ± 11.1	45.3 ± 11.2	0.032
MRT (s)	57.8 ± 11.6	53.7 ± 13.5*	57.1 ± 9.4	56.3 ± 10.5	0.048
Resting heart rate ($beats \cdot min^{-1}$)	75 ± 13	73 ± 14	71 ± 14	66 ± 10	0.146
Steady state heart rate ($beats \cdot min^{-1}$)	95 ± 20	91 ± 17	87 ± 15	86 ± 15	0.942

Values are means ± SD. $\dot{V}O_2$ indicates oxygen consumption; τ, phase 2 time constant; MRT, mean response time.

P values are shown for the group-by-time interaction.

*Significantly different from baseline value ($P < 0.05$).

Habitual physical activity levels

Habitual physical activity levels (as assessed by the PAD-PAR) were well-balanced between the groups and remained unchanged during the study period (Table 5.6).

Table 5.6 Habitual physical activity data

	Exercise group		Control group		*P*
	Baseline	12 weeks	Baseline	12 weeks	
Occupational activities	25 ± 51	25 ± 44	3 ± 16	4 ± 18	0.266
Household work	71 ± 58	60 ± 37	55 ± 35	64 ± 47	0.236
Leisure activities	107 ± 30	113 ± 33	121 ± 27	119 ± 32	0.211
Total activity	202 ± 56	197 ± 32	180 ± 30	187 ± 41	0.964

Values (in $MET \cdot hour \cdot week^{-1}$) are means ± SD.

P values are shown for the group-by-time interaction.

5.4 DISCUSSION

Overview

The main purpose of this study was to investigate functional and physiological cross-transfer effects of arm-crank exercise training in patients with intermittent claudication. In accordance with previous evidence (Walker *et al.*, 2000; Zwierska *et al.*, 2005), arm-crank exercise training improved walking performance in this patient group. A novel finding was that the three key predictors of MWD outlined in Study 1 (time-to-minimum StO_2, peak $\dot{V}O_2$ and StO_2 at 1 min) were all improved after arm-crank exercise training, providing evidence of enhanced lower-limb O_2 delivery during standardised walking exercise. Furthermore, the improvement in walking performance appears to be explained, at least in part, by enhanced lower-limb O_2 delivery. These findings, together with the excellent training adherence, low drop-out rate and lack of exercise-related complications, lend further support to the use of supervised arm-crank exercise training for improving walking performance and cardiopulmonary fitness in patients with intermittent claudication.

Physiological responses to incremental arm-crank and treadmill-walking exercise in the whole patient cohort

The baseline cardiopulmonary fitness values recorded in both exercise tests were similar to those reported previously for claudicants (Bauer *et al.*, 2004a; Zwierska *et al.*, 2006), and lower than those reported for healthy males of a similar age (Pogliaghi *et al.*, 2006).

At baseline for the whole patient cohort, peak $\dot{V}O_2$ for the arm-crank exercise test was, on average, 76 ± 16% of that measured for the incremental treadmill test, representing an absolute difference of around 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$. This ratio appears higher compared to what has been reported for healthy young and older individuals (~70%; Franklin, 1985). Other peak cardiovascular and metabolic stress responses, such as heart rate and blood lactate concentration, are also lower in healthy individuals for upper-limb exertion than for lower-limb exertion at maximum exercise tolerance (Astrand and Saltin, 1961; Bergh *et al.*, 1976). However, peak responses for heart rate, perceived exertion and blood lactate after incremental upper- and lower-limb exercise were equivalent in our cohort of patients with PAD (Tables 5.2 and 5.3), suggesting upper-limb aerobic exercise could be an equivalent stimulus for evoking cardiovascular

adaptations. These results reflect the impact of impaired perfusion and ischaemic pain on the ability to support moderate-to-high intensity leg exercise in this patient group. This is supported by the level of leg pain experienced at maximal exercise intensity in the incremental treadmill test, which was greater than the level of arm pain experienced in the arm-cranking test, despite similar levels of perceived exertion and capillary-blood lactate.

Limb-specific and cross-transfer effects of arm-crank exercise training on exercise capacity and cardiopulmonary fitness

The improvements in arm-cranking peak work rate (27%) and peak $\dot{V}O_2$ (13%) in the exercise group were similar to those observed previously after arm-crank exercise training in a similar group of claudicants (22 and 13%, respectively; Zwierska *et al.*, 2005). These improvements occurred in the absence of changes in the peak values of heart rate, exertion and arm pain, suggesting that patients exerted themselves to a similar degree at both time points.

The relative improvements in claudication and maximum walking distances in the exercise group of 53 and 33%, respectively, were also consistent with those reported previously in a study using an identical exercise protocol (51 and 29%, respectively; Zwierska *et al.*, 2005). Again, these improvements occurred in the absence of changes in the peak values of heart rate, exertion and arm pain. The changes in walking distances after arm-crank exercise training appear about half the magnitude of those reported after 12-weeks treadmill-walking exercise training in claudicants (100 and 66%, respectively; Keo *et al.*, 2008) and these differences are probably explained by the absence of lower-limb skeletal muscle metabolic adaptations after upper-limb training. Nevertheless, an improvement in MWD of 33% on an incremental walking test is considered clinically meaningful (Hiatt, 1999), and in our patient cohort equated to an absolute improvement of 165 m.

There were also cross-transfer effects of arm-crank exercise training on cardiopulmonary fitness, as evidenced by significant improvements in treadmill-walking peak $\dot{V}O_2$ (6%) and $\dot{V}O_2$ kinetics (τ; 8%). The peak $\dot{V}O_2$ data indicate that about one half of the increase is 'transferable' to a different exercise type, while the

other half is training-specific. The 'transferability' of training benefits has been classically interpreted as indirect evidence of central and/or peripheral circulatory adaptations (Loftin *et al.*, 1988; Tordi *et al.*, 2001; Pogliaghi *et al.*, 2006). Therefore, even though there are no direct measures of adaptive mechanisms, the data could suggest that circulatory and local skeletal muscle adaptations might be equally responsible for the training-induced increase in peak $\dot{V}O_2$ (and perhaps also exercise capacity) in patients with intermittent claudication.

Evidence for improved lower-limb O_2 delivery after arm-crank exercise training

The results from this study support our hypothesis that there is an improvement in lower-limb O_2 delivery after arm-crank exercise training in claudicants. For example, the exercise group exhibited improvements in peak $\dot{V}O_2$ during the incremental treadmill test and $\dot{V}O_2$ kinetics in the square-wave treadmill test. Broadly speaking, these adaptations can occur in response to enhanced O_2 delivery, enhanced O_2 utilisation through localised metabolic adaptations, or a combination of the two. The latter two explanations seem unlikely in this situation because changes in O_2 utilisation are generally confined to exercise-trained skeletal muscles (Tordi *et al.*, 2001). Conversely, an enhancement of O_2 delivery is conceivable, given that reductions in lower-limb muscle blood flow during exercise (Green, 2002), endothelial vasodilator function (Brevetti *et al.*, 2003), and capillary-to-fibre ratio (Askew *et al.*, 2005) could all contribute to an O_2 delivery limitation in claudicants and are potentially modifiable by exercise training.

The speeding of $\dot{V}O_2$ kinetics is particularly interesting since this is the first study to our knowledge that has reported cross-transfer effects of exercise training on this physiological measure. The training-induced change in τ (mean = 3.4 s) was small compared to previous exercise training studies involving other participant groups. For example, Berger *et al.* (2006) reported an 8 to 10 s improvement in τ after 6 weeks of lower-limb exercise training in previously sedentary young adults. This discrepancy is probably largely explained by the fact that cross-transfer effects of exercise training on cardiopulmonary fitness are smaller than limb-specific effects (Tordi *et al.*, 2001, Pogliaghi *et al.*, 2006). The functional significance of this finding is questionable given that the change in τ did not correlate with the change in MWD (r = -0.164, P = 0.423).

However, this lack of association might be explained by the small sample size (n = 27), the small magnitude of change in τ, or the poor reproducibility of τ (TEM = 18%) compared to maximum walking distance (TEM = 6%). Alternatively, $\dot{V}O_2$ kinetics might not be an important determinant of exercise performance in claudicants. Indeed, in Study 1, τ was only weakly correlated to MWD (r = -0.358, P = 0.012). In any case, although a speeding of $\dot{V}O_2$ kinetics is a favourable physiological adaptation (and evidence of improved lower-limb O_2 delivery), the magnitude of change was probably too small to be of large functional significance.

The increase in time-to-minimum StO_2 and sub-maximal StO_2 during the incremental walk also support a post-training enhancement of lower-limb O_2 delivery. Calf muscle StO_2 reflects the balance between O_2 delivery and tissue O_2 utilisation and time-to-minimum StO_2 and sub-maximal StO_2 measures are positively associated with walking performance in patients with intermittent claudication (Gardner *et al.*, 2008). The StO_2 data suggest that after training, patients had a better matching of O_2 delivery to O_2 utilisation in the early stages of the incremental walking test, which likely facilitated improved walking distances by delaying the accumulation of metabolites that cause claudication pain. The moderate correlations between the change in MWD and the change in StO_2 at 180 s (r = 0.524, P = 0.009) and 240 s (r = 0.486, P = 0.022) suggest that the improvement in MWD after arm-crank exercise training is explained, at least in part, by an improvement in lower-limb O_2 delivery.

Potential mechanisms of improved lower-limb O_2 delivery after arm-crank exercise training

The underpinning mechanisms of improved lower-limb O_2 delivery during walking exercise remain unclear. Various central and peripheral circulatory adaptations might be implicated, including an increased stroke volume (cardiac output) and blood volume, and enhanced blood rheology and endothelial function. Indeed, the former seems particularly likely given that arm-crank exercise training has previously been shown to improve stroke volume in young women (Loftin *et al.*, 1988), and to reduce the heart rate response to sub-maximal lower-limb exercise in patients with intermittent claudication, which indicates an increase in stroke volume (Walker *et al.*, 2000). The latter effect was also observed in the present study (Figure 5.2), providing indirect

evidence of enhanced stroke volume. An enhancement of lower-limb endothelial vasodilator function is also feasible given that lower-limb aerobic exercise training has been shown to improve conduit-vessel endothelial function in the relatively untrained upper-limbs in patients with intermittent claudication (Andreozzi *et al.*, 2007). Furthermore, recent evidence suggests that arm-crank exercise training can have an attenuating effect on systemic inflammatory markers (Saxton *et al.*, 2008), which could have a positive impact on systemic endothelial function (Adamopoulos *et al.*, 2001; Andreozzi *et al.*, 2007; Loffredo *et al.*, 2007). Further research is needed to clarify the existence and contribution of these potential mechanisms.

Haemodynamic outcome measures assessed at rest

The ABPI and calf blood flow values (Table 5.4) are typical for patients with intermittent claudication (Brendle *et al.*, 2001; Gardner *et al.*, 2001). At 12 weeks, these outcome measures remained unchanged, which is consistent with most (Gardner and Poehlman, 1995; Tan *et al.*, 2000; Loffredo *et al.*, 2007; Watson *et al.*, 2008), but not all (Brendle *et al.*, 2001; Gardner *et al.*, 2001) previous exercise training studies. This could be interpreted as arm-crank exercise training having no effect on lower-limb haemodynamics, which would stand in contrast to the data outlined above supporting an improvement in lower-limb O_2 delivery. However, resting ABPI and plethysmography-derived calf blood flow measures appear to be, at best, only a loose indicator of muscle blood flow during walking which is likely to be the most important determinant of walking performance in patients with intermittent claudication (Green, 2002). Indeed, in Study 1, there were only weak correlations ($r < 0.4$) between these measures and maximum treadmill walking performance. Thus, it is likely that haemodynamic outcome measures assessed at rest are unsuitable for detecting subtle-but-important changes in lower-limb muscle haemodynamics during exercise after a period of exercise training.

Limitations

A study limitation was that the main investigator was not blinded to group assignment. Therefore, it could be suggested that the patients in the exercise group were provided with extra motivation to perform well during the assessment sessions. However, inspection of the peak heart rate, exertion and pain responses during the incremental exercise tests suggest that patients in each group exerted themselves to a similar extent

at both time points. Nevertheless, many of the outcome measures are independent of motivation (e.g. $\dot{V}O_2$ kinetics, calf muscle StO_2).

Although physical activity levels were well-balanced across both groups during the 12-week study period, it is conceivable that additional physical activity might have consisted primarily of unstructured activities, such as walking in and around the home, which are difficult to discern using standardised physical activity questionnaires since these focus more on structured activities (Gardner *et al.*, 2001). As in previous studies (Gardner *et al.*, 2001, 2005), a further limitation of this study was the select nature of the participants, the large variation in medical conditions and functional capacities, and the absence of female patients. Thus, the results of the study are only applicable to male claudicants with mild-to-moderate PAD and might not be generalisable to females or patients with different symptoms/disease severity. Furthermore, as the patients who participated in this study were volunteers, they might only represent those who were more interested in exercise training, who had better access to transportation to the programme, and who were in relatively better health than those who did not volunteer.

There are other study limitations regarding some of the assessment techniques. For example, the accuracy of the ergometer in the range of 0 to 50 W was unknown. With NIRS, the exact contribution of myoglobin to the StO_2 signal is unclear (Ferreira *et al.*, 2005), and sub-cutaneous fat thickness changes might have influenced our findings. However, the latter is unlikely because no relationship exists between calf skinfold and calf muscle StO_2 during walking in claudicants (Afaq *et al.*, 2007) and significant changes in lower-limb sub-cutaneous fat after a short-term (12-week) programme of upper-limb aerobic exercise are unlikely.

A limitation of our approach to assessing $\dot{V}O_2$ kinetics was the failure to include a second term in the model that describes the 'slow component'. A slow component could not be modelled because the breath-by-breath noise was too high relative to the amplitude of the response. Failure to account for presence of a slow component could lead to an overestimate of τ but inspection of the $\dot{V}O_2$-time plots and steady state $\dot{V}O_2$ values for the constant-intensity walking tests suggests that a slow component was not present for the majority of assessments.

Finally, given that the laser Doppler test of microvascular function had poor reproducibility (the TEM for peak flux was 35%), it was probably too insensitive to detect any subtle but clinically meaningful changes. The use of a laser Doppler imaging system in combination with iontophoretic delivery of vasodilator drugs might have been more appropriate for testing microvascular function. However, this equipment was not available at the time of this study.

In summary, the results of this study support the hypothesis that the improvement in walking performance resulting from arm-crank exercise training in patients with intermittent claudication is explained, at least in part, by enhanced lower-limb O_2 delivery. These findings lend further support to the use of alternative exercise rehabilitation strategies (that avoid the ischaemic pain associated with lower-limb exercise) for improving walking performance and cardiopulmonary fitness in this patient group.

Chapter 6: Summary and future research

Two research studies were described in Section 1 of this thesis. The purpose of Study 1 was to identify key physiological predictors of maximum treadmill-walking performance in patients with intermittent claudication using multiple regression analysis. The purpose of Study 2 was to investigate the limb-specific and cross-transfer effects of arm-crank exercise training in the same patient group.

In Study 1, the physiological variables that best explained the variation in MWD in a group of male claudicants were time-to-minimum StO_2, peak $\dot{V}O_2$ and StO_2 at 1 min. The results suggest that cardiopulmonary fitness and the ability to match O_2 delivery to metabolic demand are important determinants of MWD in this patient population, and that measures made during exercise (e.g. StO_2) generally have a greater explanatory value than those made at rest (e.g. resting ABPI). The results also suggest that certain NIRS variables might be useful in studies that are aimed at evaluating the efficacy of clinical interventions, including exercise training, pharmacological therapy or limb revascularisation.

In Study 2, arm-crank exercise training improved patients' ability to perform both upper-limb (arm-cranking) and lower-limb (walking) exercise. The changes in walking distances at 12 weeks were about half the magnitude of those reported after treadmill-walking exercise training of the same duration (Keo *et al.*, 2008). Thus, while arm-crank exercise training does not appear the optimal exercise modality for improving the walking distances of these patients, it is still a useful alternative, and particularly for those who are unwilling or unable to perform lower-limb exercise or during the early stages of a training programme. The three key predictors of MWD outlined in Study 1 (time-to-minimum StO_2, peak $\dot{V}O_2$ and StO_2 at 1 min) were all improved after arm-crank exercise training, providing evidence of enhanced lower-limb O_2 delivery. Furthermore, the improvement in walking performance appears to be explained, at least in part, by enhanced lower-limb O_2 delivery.

Arm-crank exercise training is a model of alternative systems of aerobic exercise training for claudicants. Given its beneficial effects on walking performance and cardiopulmonary fitness, it is likely that other exercise modalities, such as rowing,

swimming and pole-striding, would be similarly useful. Therefore, I believe that the exercise guidelines described in management documents for these patients need updating to recommend the use of these alternative training systems for those who are unwilling or unable to perform walking exercise. These different exercise modalities could also be used by all claudicants to give variety and facilitate programme adherence.

Future research

In relation to Study 1, further research is needed to gain a better understanding of the relationships between the skeletal muscle and haemodynamic changes in PAD and the relative contributions of these changes to the reduced exercise performance. In relation to Study 2, the precise physiological mechanisms underpinning the observed improvements in lower-limb O_2 delivery cannot be determined from our data. Further research is needed to establish the role of enhanced stroke volume and lower-limb endothelial function in these changes. It would also be interesting to undertake a study in which patients undertake arm-crank exercise training in the early stages and progress to lower-limb exercise (either walking or cycling) to observe whether such a programme further enhances walking performance in these patients. Such a pragmatic programme could have direct clinical implications. In addition, the use of pole-striding as an alternative exercise modality needs further evaluation.

The patient population was heterogeneous, i.e., there was a large variation in medical conditions and functional capacities. It would have been useful to compare the effectiveness of arm-crank exercise training between patients with differing degrees of functional impairment; however, this study was not sufficiently powered to do so. Therefore, future research could address this issue, as well as investigating the impact of medical comorbidities (e.g., diabetes) on the adaptations to exercise training.

Patients in whom PAD progresses develop rest pain and critical limb ischaemia. This often leads to lower-extremity amputation. These patients have extremely low levels of physical activity and are at great risk of having a heart attack or stroke. The usefulness of arm-crank exercise training has yet to be fully established in this patient group. In particular, research is needed to investigate the effects of arm-crank exercise training on cardiovascular health, functional capacity and quality of life in patients with PAD and lower-extremity amputation.

Section 2: Exercise training for post-surgical varicose-vein patients

Chapter 7: General introduction

Chronic venous disease is one of the main vascular pathologies and a substantial cause of morbidity in Western societies (Beebe-Dimmer *et al.*, 2005). The term 'chronic venous disease' is inclusive of all morphological and functional abnormalities of the lower-limb veins (Nicolaides *et al.*, 2008). Symptoms include aching, leg-tiredness, cramps, itching, burning sensations, swelling and the restless leg syndrome, as well as cosmetic dissatisfaction. Signs include telangiectasias (spider veins), reticular and varicose veins, oedema, and skin changes such as pigmentation, lipodermatosclerosis, dermatitis, and ulceration (Eberhardt and Raffetto, 2005). The presence (or absence) of these symptoms and signs varies greatly between chronic-venous-disease patients and is largely dependent on the stage of the disease. As some of the signs, like telangiectasias, are highly prevalent in the adult population, the use of the term 'disease' is often inappropriate. The term chronic venous insufficiency is entrenched in the literature and has sometimes been used to imply functional abnormality (reflux) of the venous system and is usually reserved for patients with more advanced disease including those with oedema, skin changes, or venous ulcers (Nicolaides *et al.*, 2008).

Varicose veins are the most common manifestation of chronic venous disease and are defined as subcutaneous veins of the legs that have become dilated >3 mm when measured in the upright position (Eklof *et al.*, 2004). They are usually tortuous (the Latin root *varix* means twisted), but tubular veins with demonstrated reflux may be classified as varicose (Eklof *et al.*, 2004). Primary varicose veins result from venous dilatation and valve damage, whereas secondary varicose veins are the consequence of venous thrombosis. Varicose veins are common and usually harmless, in that few people with them develop the more serious consequences of chronic venous disease (Campbell, 2006). However, millions of people seek medical attention for their cosmetic appearance annually, and this has a significant impact on healthcare resources (Eberhardt and Raffetto, 2005). Indeed, varicose-vein surgery is the most common elective general surgical operation performed in the UK, with around 40,000 operations performed each year (Winterborn *et al.*, 2004). Aside from cosmetic benefits, varicose-vein surgery can also correct venous hypertension, promote symptomatic relief and facilitate ulcer healing (Howard *et al.*, 2008). However, despite these benefits, the recurrence rates for varicose veins (62% over an 11-yr period; Winterborn *et al.*, 2004)

and venous ulcers (18% over a 5-yr period; Nelzen and Fransson, 2007) are high. A contributory factor could be microvascular endothelial dysfunction that might persist after surgery (Klonizakis *et al.*, 2003, 2006). Therefore, it appears important to identify strategies to improve microvascular endothelial function in this population.

This section describes two studies (Study 3 and Study 4). The main aim of Study 3 was to compare cutaneous microvascular vasodilator function between post-surgical varicose-vein patients and age-matched healthy controls. A secondary aim was to investigate whether any impairment of function could be alleviated by acute lower-limb exercise. The aim of Study 4 was to investigate the effects moderate-intensity exercise training on microvascular vasodilator function in post-surgical varicose-vein patients.

Chapter 8: Literature review - Management of chronic venous disease

8.1 Disease epidemiology

Estimates as to the prevalence of varicose veins in Western societies vary from 10 to 20% in men and from 25 to 33% in women (Nicolaides *et al.*, 2008). The variation in prevalence is probably explained mostly by differences in study methodology, namely variations in disease criteria, use of diagnostic imaging, and population composition with respect to age, race, sex, and geographic location (Beebe-Dimmer *et al.*, 2005). In the Framingham study, the incidence of varicose veins per year was 2.6% for women and 1.9% for men (Brand *et al.*, 1988). The prevalence of oedema and skin changes such as hyperpigmentation and eczema due to chronic venous disease varies from 3 (Coon *et al.*, 1973) to 11% (da Silva *et al.*, 1974) of the population. Venous ulcers (active or healed) are prevalent in around 1% of the adult population (Fowkes *et al.*, 2001). The incidence of leg ulcer has been reported as 0.03 to 2% of the population per year (Valencia *et al.*, 2001). The economic impact of venous ulceration is dramatic, with the treatment of ulcers representing around 1% of the total healthcare budget in Western societies (Eberhardt and Raffetto, 2005).

8.2 Risk factors, aetiology and pathogenesis of chronic venous disease

Table 8.1 provides a summary of established and potential risk factors associated with chronic venous disease based on current evidence. These risk factors include a family history, older age, standing occupations and a history of previous deep venous thrombosis. A number of studies have shown a strong familial tendency for varicose veins (e.g. Hirai *et al.*, 1990; Carpentier, 2000; Berard *et al.*, 2002). Indeed, a variant of the FOXC2 gene and differential expression of human tropomyosin 4 cDNA was recently linked to a genetic predisposition to the development of varicose veins (Ng *et al.*, 2005). Furthermore Hirai *et al.* (1990) reported a positive family history in 42% in Japanese with varicose veins compared with 14% in those without varicosities, and Carpentier *et al.* (2000) observed that a history of varicose veins in first degree relatives and age were the two most important risk factors for varicose veins in both sexes.

Table 8.1 Established and potential risk factors for chronic venous disease (adapted from Beebe-Dimmer *et al.*, 2005)

	Association
Older age	+
Family history	+
Female sex	+/0
Standing occupation	+
Constipation/low fibre intake	+/0
Obesity	+/0
Smoking	+/0
Oral contraceptives/hormone replacement	-/0
therapy	+/0
Hypertension	+/0
Physical inactivity	+/0
Injury	+
History of phlebitis/clot	+

+, positive association; 0, no association; -, negative association

The prevalence of venous disease increases with age presumably because of increased pressure on superficial veins due to the weakening of calf muscles coupled with the gradual deterioration of vessel walls over time (Beebe-Dimmer *et al.*, 2005). Hanemaaijer *et al.* (1993) demonstrated a linear increase in the prevalence of all three grades of varicosities with age. In the United States, data from the Framingham study indicated a chronic-venous-disease prevalence of 1% in men versus 10% in women <30 years of age compared with 57% in men and 77% in women aged >70 years (Brand *et al.*, 1988).

While it is generally believed that chronic venous disease is more prevalent in women than men (Fowkes *et al.*, 2001; Beebe-Dimmer *et al.*, 2005; Eberhardt and Raffetto, 2005), a large UK population study reported age-adjusted prevalences of 40% in men and 32% in women (Lee *et al.*, 2003). Nevertheless, varicose veins do tend to become more prevalent and increase in severity with each successive pregnancy (Naoum *et al.*,

2007). An increase in blood volume, intra-abdominal pressure and secretion of the potent vasodilator relaxin, the increase in venous capacitance induced by oestrogen, and venous relaxation resulting from production of progesterone are among the contributing factors (Naoum *et al.*, 2007).

There is also evidence to suggest that overweight or obese adults have an increased tendency to develop varicose veins (Danielsson *et al.*, 2002; Hulens *et al.*, 2003), although exact reasons for this are largely unknown (Naoum et al. 2007). A few studies suggest that reduced aerobic capacity and a sedentary lifestyle might also contribute to the varicose vein formation (Danielsson *et al.*, 2002; Iannuzzi *et al.*, 2002; Hulens *et al.*, 2003). Occupations associated with either prolonged standing or sitting are also associated with an increase in the prevalence of varicose veins (Fowkes *et al.*, 2001; Beebe-Dimmer *et al.*, 2005). The role of other factors contributing to the occurrence of chronic venous disease including fibre content of diet, cigarette smoking, oral contraceptives, hypertension and diabetes remains to be validated.

8.2.1 Normal venous structure and function

To appreciate the pathophysiology of chronic venous disease, an understanding of normal venous structure and function is necessary. The peripheral venous system acts as a reservoir to store blood and as a conduit to return blood to the heart. Blood that enters into the lower-extremity venous system must travel against gravity and against fluctuating thoraco-abdominal pressures to return to the central circulation in a person in an erect position. Effective venous return from the lower extremities requires the interaction of the heart, a pressure gradient, the peripheral muscle pumps of the legs and competent venous valves.

The veins of the lower extremity are divided into the superficial and deep venous system connected by a series of perforator veins. The superficial venous system is located superficial to the muscle fascia. It comprises an interconnecting network of veins, which drain the cutaneous circulation, and several truncal superficial veins, which function as a conduit to return blood to the deep venous system. The principal named superficial veins of the lower extremity are the small saphenous vein, which runs from the ankle typically to join the popliteal vein at the sapheno-popliteal junction, and the great saphenous vein, which runs from the ankle to join the common femoral vein at the

sapheno-femoral junction (confluence of superficial inguinal veins). Other superficial veins, including the posterior arch, lateral accessory saphenous, and intersaphenous vein, can also develop pathology leading to chronic venous disease (Eberhardt and Raffetto, 2005).

The deep venous system is located deep to the muscle fascia and serves as a transport system for blood outflow from the lower extremities. The deep veins of the lower extremities consist of axial veins, which follow the course of the major arteries, and the intramuscular veins. Thin-walled veins within the leg muscles join to form intramuscular venous networks. Paired calf veins that correspond to the axial arteries merge to form a single large popliteal vein. The popliteal vein, on passing through the adductor canal, is subsequently known as the femoral vein. The femoral vein is joined by the profunda femoris (or deep femoral) vein in the upper thigh to form the major outflow of the leg, the common femoral and eventually the external iliac vein. The superficial and deep venous systems are connected by perforating veins that pass through the muscle fascia.

A series of bicuspid valves located throughout the deep and superficial veins facilitate blood transport towards the heart during upright posture. The first of these lower-extremity valves is usually located in the common femoral vein or less commonly in the external iliac vein (Eberhardt and Raffetto, 2005). The frequency of venous valves increases from the proximal to the distal leg to help prevent an increase in pressure within the distal veins resulting from the effects of gravity. Perforating veins also contain one-way valves that prevent reflux of blood from the deep veins into the superficial system.

The valves function in concert with venous muscle pumps to facilitate the return of blood to the heart. The structural configuration of veins (intima, media, and adventitia) also provides contractile efficiency, thus facilitating venous return (Naoum *et al.*, 2007). Contraction of the muscle pumps primarily in the calf, but also in the foot and thigh, forces blood into the deep venous system and towards the heart. The valve system prevents blood from being forced distally within the deep venous system or through the perforator system into the superficial system. Relaxation of the muscle pump allows blood to return to the deep venous system via arterial inflow through the superficial and

the distal deep venous systems. With prolonged standing, the veins slowly fill and become distended, allowing the valves to open and eventually increase pressure that is directly related to the height of the column of blood. Contraction of the muscle pump will again empty the veins and reduce venous pressure.

8.2.2 Venous pathophysiology and dysfunction

Venous pathology develops when venous pressure is increased and when normal venous blood transport is disturbed (Beebe-Dimmer *et al.*, 2005). These effects can result from valvular incompetence of the axial deep or superficial veins, perforator valve incompetence, venous obstruction, or a combination of these (Eberhardt and Raffetto, 2005). These factors are exacerbated by muscle pump dysfunction in the lower-extremity, and collectively serve to produce blood reflux and venous hypertension during ambulation. Indeed, while normal lower-limb ambulatory venous pressure is 20 to 30 mmHg, it is commonly 80 to 100 mmHg in patients with chronic venous disease (Rajendran *et al.*, 2007). Ambulatory venous hypertension (a failure to reduce venous pressure with exercise) is thought to underlie many, and possibly all, of the clinical manifestations of chronic venous disease (Bergan *et al.*, 2008).

Valvular incompetence is the most important cause of venous hypertension (Nicolaides, 2005). Recent findings suggest that elevated venous pressure and low/zero flow, as typically occurs during periods of prolonged standing or physical inactivity, can trigger inflammatory cascades in the vein wall and venous valves that can cause progressive valvular incompetence and the development of varicose veins (Nicolaides, 2005; Bergan *et al.*, 2008). Indeed, Ono *et al.* (1998) observed infiltration of valve leaflets and the venous wall by leucocytes (monocytes and tissue macrophages) in all valve specimens from patients with chronic venous disease and none from controls. Incompetence at the sapheno-femoral junction in the groin is the commonest cause of reflux from the deep to superficial venous systems (Campbell, 2006). The main superficial veins of the legs that are commonly affected by varicose veins are highlighted in Figure 8.1.

Figure 8.1 Main superficial veins of the legs commonly affected by varicose veins (Campbell, 2006)

Changes in the haemodynamics of the large veins of the lower extremity often lead to chronic damage and dysfunction of the microcirculation (Eberhardt and Raffetto, 2005). According to Partsch (1985), ambulatory venous hypertension is associated with pressure peaks in the veins that recur with every contraction of the leg muscles and are transmitted across defective valves into the capillary network. The microcirculatory impairment caused by chronic venous hypertension is one of the earliest signs of chronic venous disease and is frequently associated with the severity of the disease (Virgini-Magalhaes *et al.*, 2006). Cutaneous capillaries can become progressively enlarged and tortuous, forming bulks described in the literature as "glomerulus-like" capillaries (Junger *et al.*, 2000). Swollen endothelial cells with enlarged inter-endothelial spaces make the capillary lumen irregular (Cheatle *et al.*, 1991) and cause an increase in macromolecular permeability with plasma, red cells and fibrinogen leakage, which could impair nutrient exchange (Burnand *et al.*, 1982; Cheatle *et al.*, 1991). The continuation of venous stasis and hypertension lead to a chronic inflammatory process of the capillary bed and surrounding tissues (Schmid-Schonbein *et al.*, 2001) and oedema formation (Agren *et al.*, 2000). The reduction in the number of capillaries leads to trophic disorders and leg ulceration (Junger *et al.*, 2000).

In addition to changes in the blood vessels and connective tissue, alteration of the lymphatic network and nervous system might occur (Eberhardt and Raffetto, 2005). Fragmentation and destruction of microlymphatics might further impair drainage from the extremity, whereas dysfunction of local nerve fibres might alter regulatory mechanisms. Nutritive blood flow in the more advanced stages of chronic venous disease is further impaired by abnormal blood rheology (Junger *et al.*, 2000).

Several mechanisms linking venous hypertension to macro- and micro-circulatory abnormalities have been postulated, including fibrin cuff formation, growth factor trapping and leucocyte-endothelium interaction (Eberhardt and Raffetto, 2005). The latter of these is currently the most credible (Nicolaides, 2005; Smith, 2006). This mechanism involves valve and venous wall infiltration by monocytes and macrophages, leading to valve destruction and varicose remodelling. In the microcirculation, occlusion of capillaries by plugs of white blood cells creates distal ischaemia (Smith, 2006). The trapped white cells release agents which damage the endothelium, increasing capillary permeability and promoting the formation of a fibrin cuff. It has been suggested that it is

the infiltration of the skin by white cell products alone that mediates tissue destruction (Smith, 2006).

8.2.3 Clinical manifestations

The major clinical features of chronic venous disease are varicose veins, oedema, leg pain, skin changes and ulceration (Eberhardt and Raffetto, 2005). This spectrum of clinical effects means that the impact of chronic venous disease on health-related quality of life, the benefit from different kinds of treatment, and the cost-effectiveness of interventions might vary greatly. Patients with chronic venous disease are usually classified using the Clinical-Etiology-Anatomy-Pathophysiology (CEAP) system (Table 8.2).

Table 8.2 Classification of chronic venous disease: The Clinical-Etiology-Anatomy-Pathophysiology (CEAP) classification system (adapted from Eklof *et al.*, 2004)

Classification	Description/Definition
C, Clinical (sub-divided into A for asymptomatic, S for symptomatic)	
0	No venous disease
1	Telangiectases
2	Varicose veins
3	Oedema
4a	Pigmentation or eczema
4b	Lipodermatosclerosis or white atrophy
5	Healed venous ulcer
6	Active venous ulcer
E, Etiologic classification	
Congenital	Present since birth
Primary	Undetermined etiology
Secondary	Post-thrombotic
A, Anatomic distribution	
Superficial	Great and short saphenous veins
Deep	Cava, iliac, gonadal, femoral, profunda,
Perforator	popliteal, tibial, and muscular veins
P, Pathophysiology	
Reflux	Axial and perforating veins
Obstruction	Acute and chronic
Combination of both	Valvular dysfunction and thrombus

Varicose veins are mainly found in at the lower extremities of the body, especially on the back of the calf or on the inside of the thighs. Most people with varicose veins will suffer no medical harm from them throughout their lifetime (Campbell, 2006). The commonest complaint is their unsightliness, and cosmetic motives probably underlie many requests for treatment on the grounds of minor symptoms (Michaels *et al.*, 2006b). In addition, people are worried about the spectre of complications from their varicose veins, specifically in the context of a family history of ulcers or other leg problems, and also in relation to the risk of deep vein thrombosis (Palfreyman *et al.*, 2004), promoted by recent media reports of the dangers of air travel and venous thrombo-embolism.

Apart from cosmetic embarrassment and concern about the future, the commonest symptom from varicose veins is discomfort (Bradbury *et al.*, 1999). This can take a variety of forms, typically described by patients as aching, heaviness or itching. These, and other leg pains, are often present in people with varicose veins as a result of other conditions, including arthritis and muscular problems (Bradbury *et al.*, 1999), but a careful history might help to identify symptoms related to varicose veins. Discomfort after prolonged standing, relief by elevation of the leg or by wearing support hosiery, and symptoms over the varicose veins are pointers towards a venous cause, but this is an area of uncertainty (Michaels *et al.*, 2006b). Ankle swelling as a result of oedema is another complaint which might result from varicose veins (Speakman and Collin, 1986), but which has other common causes.

Patients with the above complaints might be said to have 'medically-uncomplicated varicose veins' because their veins are causing no damage or threat to their legs. It is in these patients that the greatest uncertainty exists about the benefits and cost-effectiveness of treatment (Michaels *et al.*, 2006b). The 'medical complications' of varicose veins are thrombo-phlebitis, bleeding, eczema, lipodermatosclerosis (the latter two are often conveniently called 'skin changes') and ulceration.

Superficial thrombo-phlebitis is pain and inflammation in a vein just under the skin (superficial vein), which is usually caused by a blood clot (thrombus). Treatment of the varicose veins might be considered if they are causing other symptoms or if phlebitis is recurrent (sometimes varicose veins become permanently occluded as a result of phlebitis, and then there is no need to consider definitive treatment). Reports of

extension of thrombus into the deep veins have raised concerns about this risk (Bergqvist and Jaroszewski, 1986; Skillman *et al.*, 1990), but deep vein thrombosis is very uncommon (Campbell, 2006) and most cases of superficial thrombo-phlebitis are dealt with in primary care (Michaels *et al.*, 2006b).

External bleeding is an uncommon consequence of varicose veins and almost always occurs through an area of obviously compromised skin overlying a varicosity in the lower leg (Campbell, 2006). It is alarming, poses a potential threat to life, and is an indication for early referral and treatment (Michaels *et al.*, 2006b).

The venous hypertension caused by varicose veins is an important cause of damage to the skin and subcutaneous tissues of the lower leg (Smith, 2006). This usually starts with eczema or pigmentation and then progresses through varying severities of lipodermatosclerosis to ulceration (Michaels *et al.*, 2006b). Such changes usually occur in the peri-malleolar region, also known as the gaiter area (Agren *et al.*, 2000). The chance of any individual with varicose veins developing skin damage is both uncertain and small (Iafrati *et al.*, 1994). Among those who do develop skin changes, the risk of ulceration is also unpredictable, but any signs of venous damage to the skin of the leg are usually regarded as an indication to consider preventative measures in the form of either compression therapy or interventional treatment (Michaels *et al.*, 2006b).

8.3 Current management of chronic venous disease

The initial treatment of chronic venous disease involves conservative measures to reduce symptoms and help prevent the development of secondary complications and the progression of disease. Behavioural measures such as elevating the legs to minimise oedema and reducing intra-abdominal pressure should be advocated. The use of compressive stockings is the mainstay of conservative treatment and described in more detail below. If conservative measures fail or provide an unsatisfactory response, then further treatment should be considered based on anatomic and pathophysiological features (Figure 8.2).

Figure 8.2 A simplified overview for the diagnosis and treatment of chronic venous disease based on pathophysiological mechanisms (Eberhardt and Raffetto, 2005). Multiple pathophysiological mechanisms might contribute to chronic venous disease within the same patient and require a combination of treatment options. APG, air plethysmography; SEPS, sub-fascial endoscopic perforator surgery.

Regarding the treatment of chronic venous disease, the practitioner should be able to recognise the manifestations of chronic venous disease and use confirmatory testing such as venous duplex reflux studies and perhaps air plethysmography (Eberhardt and Raffetto, 2005). Specific treatment is based on severity of disease, with CEAP clinical classes 4 to 6 (Table 8.2) often requiring invasive treatment. Referral to a vascular specialist should be made for patients with CEAP classes 4 to 6 (and probably for CEAP class 3 with extensive oedema). These patients with uncorrected advanced chronic venous disease are at risk for ulceration, recurrent ulceration, and non-healing venous ulcers with progressive infection and lymphoedema (Eberhardt and Raffetto, 2005).

8.3.1 Surgery

Various surgical procedures might be considered in patients with moderate-to-severe chronic venous disease who are unable to comply with compression therapy or experience recurrent varicose veins. The goals of surgery are to relieve presenting symptoms, prevent adverse effects of chronic venous hypertension and normalise venous physiology by eradicating main stem reflux and removing visible varicosities. Conventional surgery usually means sapheno-femoral ligation with stripping of the long saphenous vein and phlebectomies (Campbell, 2006), although other surgical approaches include venous bypass procedures, balloon angioplasty with or without stenting, superficial endoscopic perforator surgery (SEPS) and venous valve reconstruction. There is no indication for surgery in patients in CEAP classes 0 or 1 (Nicolaides *et al.*, 2008).

8.3.1.1 Ligation and stripping and venous phlebectomy

The most common operation for patients in CEAP classes 2 to 6 with superficial venous reflux involves tying-off and pulling-out the long saphenous vein. This procedure, called ligation and stripping, eliminates venous reflux during exercise allowing the calf muscle pump to reduce superficial venous pressure to near-normal levels (Subramonia and Lees, 2007). Large varicose-vein clusters that communicate with the incompetent saphenous vein can also be avulsed at the same setting by a technique called stab phlebectomy. This is an out-patient procedure that involves removal of superficial veins through small, slit-like incisions in the skin.

Ligation and stripping of the saphenous vein has been shown to result in significant improvements in venous haemodynamics, elimination of concomitant deep venous reflux, symptomatic relief, and facilitated ulcer healing (Padberg *et al.*, 1996, MacKenzie *et al.*, 2004). Recent evidence also indicates that varicose-vein surgery is more clinically- and cost-effective than sclerotherapy (Michaels *et al.*, 2006b). However, surgical treatment involves a day-case or in-patient operation, general anaesthesia (usually), often a period of recuperation and time off work, and a possibility of complications such as bleeding, pain, nerve injury, scarring and thrombosis (Subramonia and Lees, 2007). Varicose veins recurrence rates are also quite high, ranging from 20 to 80% between five and 20 years after surgery (Perrin *et al.*, 2000).

8.3.1.2 Relief of obstruction (bypass surgery or stenting)

Obstruction is the principal cause of symptoms in about one-third of post-thrombotic limbs (Nicolaides *et al.*, 2008). It is associated with reflux in 55% of symptomatic patients with chronic venous disease (Neglen *et al.*, 2003). Reflux plus obstruction leads to the highest levels of venous hypertension and the most severe symptoms as compared to either reflux or obstruction alone (Nicolaides *et al.*, 1993). Proximal obstruction, especially in the iliac vein, is more likely to cause symptoms than lower segmental blockages (Mavor and Galloway, 1969). After ilio-femoral deep vein thrombosis, only 20 to 30% of iliac veins completely recanalise spontaneously, while the remaining veins have residual obstruction and varying degrees of collaterals (Mavor and Galloway, 1967; Plate *et al.*, 1990). The main aim from intervention is to relieve proximal outflow obstruction using venous bypass grafting or balloon angioplasty with or without stenting.

Bypass surgery involves grafting a piece of surgically-excised vein or a synthetic replacement so that venous stenoses/occlusions are circumnavigated. Although autogenous bypass procedures appear to be less thrombogenic with better patency than prosthetic grafts (Palma and Esperon, 1960; Jost *et al.*, 2001), bypass grafting generally appears to have relatively poor patency results in the treatment of chronic venous disease. For example, the 7-year patency rates for patients having crossover femoral saphenous vein or sapheno-popliteal vein bypass surgery have been reported as 75 and 56%, respectively (AbuRahma *et al.*, 1991). Reasons for such poor results included low velocity flow, external compression of the low pressure bypass, inherent

thrombogenicity of non-saphenous graft material, and poor distal inflow due to extensive distal disease (Nicolaides *et al.*, 2008).

The introduction of percutaneous balloon angioplasty and stenting has expanded the scope of treatment for patients with venous stenoses. Balloon angioplasty is a catheter-based procedure used to flatten the fatty plaque/thrombus against the blood vessel wall, thus increasing the vessel lumen and facilitating blood flow. In most cases, a stent (wire mesh tube) will then be permanently implanted to hold the vessel open. Complications are minimal and to date mortality has been nil (Nicolaides *et al.*, 2008).

In a study by Neglen and Raju (2004), severe in-stent recurrent stenosis (iliac vein), defined as >50% diameter decrease on single-plane antero-posterior venogram, occurred in only 15% of patients at 42 months. Higher rates of in-stent recurrent stenosis were found in thrombotic compared to non-thrombotic limbs, reported as 23 and 4%, respectively. Long stents (>13 cm) and extension of the stent below the inguinal ligament had a cumulative rate of severe in-stent recurrent stenosis of 25% at 36 months and 40% at 24 months, respectively.

The incidence of ulcer healing after iliac vein balloon dilation and stent placement in 304 limbs with active ulcer has been reported as 68%, with 62% of these remaining ulcer-free at 2 years (Raju *et al.*, 2002). Median swelling and pain severity scores decreased significantly. The frequency of limbs with any swelling decreased from 88 to 53% and limbs with any pain from 93 to 29%. Using a quality of life questionnaire assessing subjective pain, sleep disturbance, morale and social activities, and routine or strenuous physical activities, patients indicated significant improvement on all major categories after venous stenting.

Because long-term effects of stents in the venous system are not fully known, monitoring for several more years is required to assess efficacy and safety. However, at present, iliocaval angioplasty and stenting is the primary treatment for proximal iliofemoral venous obstruction, with bypass surgery assuming a secondary role (Meissner *et al.*, 2007).

8.3.1.3 Sub-fascial endoscopic perforator surgery (SEPS)

The importance of the incompetent perforator vein to the manifestations of chronic venous disease and its more advanced forms has been appreciated for many years. A surgical principle has been to ligate perforator veins that might contribute to the focal high pressure within the superficial veins with advanced chronic venous disease. This might present difficulties with traditional surgical techniques because of the pre-existing tissue damage in the affected area. Sub-fascial endoscopic perforator surgery (SEPS), however, provides a means to ligate incompetent perforator veins by gaining access from a remote site on the leg that is away from the area with lipodermatosclerosis or ulcers. A multi-centre study involving 148 SEPS procedures in patients with active and healed venous ulcers demonstrated accumulative ulcer healing of 88% at 1 year and an ulcer recurrence of 28% at 2 years (Gloviczki *et al.*, 1999). SEPS can also be used in conjunction with superficial vein ablation with 91% ulcer healing at a mean of 2.9 months, and is accompanied by a significant improvement in the clinical severity and venous disability scores (Bianchi *et al.*, 2003). In a large study evaluating 832 patients in CEAP classes 4 to 6 for 9 years, 55% of patients underwent both SEPS and ligation and stripping of the superficial venous segments. In this group, 92% of ulcers healed overall with only 4% recurrence rates, 3% non-fatal complication rate, and a significant improvement in venous haemodynamics (Tawes *et al.*, 2003). This study underscores the importance of surgical interruption of incompetent superficial and perforator veins in patients with advanced chronic venous disease.

8.3.1.4 Venous-valve reconstruction

Venous-valve injury or dysfunction might contribute to the development and progression of chronic venous disease. Venous valve reconstruction of the deep vein valves has been performed in selected patients with advanced chronic venous disease who have recurrent ulceration with severe and disabling symptoms. An open technique for repairing the femoral vein valve that renders the valve leaflets competent has been described (Kistner, 1975). This technique of open valvuloplasty has been refined, and closed techniques for venous repair developed with transcommissural valvuloplasty (Raju *et al.*, 2000). Venous valvuloplasty has been shown to provide 59% competency and 63% ulcer-free recurrence at 30 months. Complications from valvuloplasty include bleeding because patients need to remain anti-coagulated, deep vein thrombosis, pulmonary embolism, ulcer recurrence, and wound infections. The technique is not

routinely performed and is considered only in selected patients. Other procedures for reconstructing non-functioning venous valves resulting from post-thrombotic valve destruction (not amenable to valvuloplasty) include transposition of the profunda femoris vein or saphenous vein valve and axillary vein valve transplantation to the popliteal or femoral vein segments (Eberhardt and Raffetto, 2005). Cryo-preserved vein valve allografts also have been used; however, early thrombosis, poor patency and competency, and a high patient morbidity have precluded their use as a primary intervention (Neglen and Raju, 2003).

8.3.2 Non-surgical interventional treatment

8.3.2.1 Conventional sclerotherapy

Sclerotherapy is a treatment used for obliterating telangiectases, varicose veins and venous segments with reflux. Sclerotherapy can be used as a primary treatment or in conjunction with surgical procedures in the management of chronic venous disease. Sclerotherapy is suitable for a variety of conditions including spider veins (<1 mm), varicose veins of 1 to 4 mm in diameter, and bleeding varicosities (Eberhardt and Raffetto, 2005).

Conventional sclerotherapy involves injection of a sclerosant, commonly sodium tetradecyl or polidocanol, into the diseased vein, followed by a period of compression bandaging and/or compression hosiery. There is little good evidence on how long compression needs to be worn and advice varies from a few days to three or four weeks (Campbell, 2006). The main risk of sclerotherapy is injection outside the vein, which can result in local tissue necrosis and scarring (Campbell, 2006).

Conventional sclerotherapy is a clinically- and cost-effective treatment for smaller varicose veins, particularly those that are not subject to upstream incompetence and those below the knee (Michaels *et al.*, 2006a, 2006b). However, its results are not long lasting in the presence of sapheno-femoral reflux (the most usual situation for varicose veins with troublesome symptoms): a randomised controlled trial found that most varicose veins recur within five years (Hobbs, 1974). Sclerotherapy became popular in the 1970s, but its use then declined because so many varicose veins recurred (Campbell, 2006).

8.3.2.2 Foam sclerotherapy

Foam sclerotherapy involves mixing sclerosant with a small quantity of air or carbon dioxide to produce foam that spreads rapidly and widely through the veins, pushing the blood aside and causing the veins to go into spasm. This is believed to increase the effectiveness of the sclerosant in obliterating long segments of superficial veins. Indeed, the superiority of foam over liquid sclerosant has been clearly demonstrated (Hamel-Desnos *et al.*, 2003; Yamaki *et al.*, 2004). Duplex ultrasonography is used to guide placement of the injecting cannula in the chosen vein and to monitor spread of sclerosant through the veins. The treated leg is bandaged, and compression hosiery is advised for up to a month after treatment. After treatment, larger varicose veins are commonly hard and prominent for many weeks before they gradually shrivel. Further sessions of foam treatment might be required for extensive or bilateral varicose veins.

With foam sclerotherapy, immediate or early closure in medium-to-large veins is achieved in 85 to 99% of cases (Tessari *et al.*, 2001; Frullini and Cavezzi, 2002; Barrett *et al.*, 2004; Belentsov 2007), and around 90% of these remain occluded 2 years post-treatment (Belentsov, 2007). High patient satisfaction and significant improvement in symptoms and quality of life has also been reported at a mean follow-up of 2 years after foam sclerotherapy (Barrett *et al.*, 2004). Furthermore, a recent randomised controlled trial described that foam treatment combined with sapheno-femoral ligation was more successful in the short-term compared with conventional surgery (Bountouroglou *et al.*, 2006).

There has been concern about the possibility of foam entering the deep veins and causing venous thrombo-embolism: Frullini and Cavezzi (2002) reported five cases of deep vein thrombosis after foam sclerotherapy treatment. Visual disturbances have also been reported, particularly in individuals prone to migraine, and these might be due to vasospasm (Subramonia and Lees, 2007). Of greater concern is the possibility of foam passing through a patent foramen ovale (present in many people) to enter small arteries in the eye or brain. A recent report of a stroke attributed to foam treatment, albeit after injection of an unusually large volume of foam, must sound a note of caution (Forlee *et al.*, 2006). Nevertheless, the popularity of foam sclerotherapy continues to increase among both patients and specialists, and it looks set to become an important treatment for varicose veins. However, the correct indications, the best sclerosant and the most

effective technique are still unclear. Long-term data on quality of life and symptomatic, clinical- and cosmetic-improvement are also lacking.

8.3.2.3 Endovascular ablation therapy

Various electro-surgical devices have been used in an endeavour to develop a minimally invasive alternative to conventional surgery for treating varicose veins. In recent years, radiofrequency and laser ablation techniques have evolved for endoluminal obliteration for long saphenous vein reflux. Both methods involve introduction of a catheter through a venepuncture at the ankle or knee level under ultrasound guidance and then ablation of the diseased vein (usually a straight long saphenous vein with no tortuous or thrombosed sections). Radiofrequency ablation induces resistive heating (85°C) causing contraction of collagen fibres with associated circumferential endothelial denudation and muscle necrosis (Fassiadis *et al.*, 2002). Laser ablation uses thermal energy to boil blood producing thermo-chemical destruction of the venous wall (Proebstle *et al.*, 2002).

Single-centre studies have reported a long saphenous vein occlusion rate at 1 and 2 years between 90 and 99% after radiofrequency ablation (Weiss and Weiss, 2002; Fassiadis *et al.*, 2003; Lurie *et al.*, 2005). One multi-centre study involving 1222 limbs from 34 clinical sites achieved complete occlusion of the long saphenous vein in 87% of 117 patients at 5 years (Merchant *et al.*, 2005). Two randomised controlled trials demonstrated that endovenous obliteration by radiofrequency ablation with additional phlebectomies or sclerotherapy appears to provide a safe and effective minimally invasive method avoiding the morbidity of traditional surgical approaches (Lurie *et al.*, 2003, 2005). It is also associated with reduced post-operative pain and a shorter return to work and to normal daily activities in comparison to conventional varicose-vein surgery (Rautio *et al.*, 2002; Lurie *et al.*, 2003, 2005). However, other studies have indicated poorer short-term results compared with conventional surgery (Perala *et al.*, 2005), and the cost-effectiveness of this treatment is unclear (Subramonia and Lees, 2007).

Current safety and efficacy data appear to support the use of laser ablation, but long-term data are lacking (Subramonia and Lees, 2007). In a systematic review article involving 18 clinical studies for treatment of varicose veins by laser ablation, occlusion of the saphenous vein abolished venous reflux in 88 to 100% of limbs, with low rates of

recanalisation and re-treatment (Mundy *et al.*, 2005). Other studies have also shown that laser ablation is safe and well tolerated, with results comparable to conventional surgery (de Medeiros and Luccas, 2005; Rasmussen *et al.*, 2007). However, 1-year saphenous vein occlusion rates appear higher after radiofrequency ablation than after laser ablation (Morrison, 2005).

Transient sensory disturbances are the most common problem after endovascular treatment, although the rate can be reduced by ultrasound-guided tumescent infiltration of especially superficial segments of the long saphenous vein that reduces the thermal insult to peri-venous tissue during treatment (Nicolaides *et al.*, 2008). Recanalisation of the saphenous vein, treatment failure, skin burns and common femoral vein stenosis are potential complications but should not occur in the hands of an experienced operator (Nicolaides *et al.*, 2008). Deep vein thrombosis is an uncommon post-operative complication (Subramonia and Lees, 2007), although Merchant *et al.* (2002) reported an incidence of 16% after radiofrequency ablation.

8.3.3 Conservative treatment

8.3.3.1 Compression therapy systems

A preliminary therapeutic consideration for all CEAP clinical classes of chronic venous disease, except for those with concomitant arterial occlusive disease (Meissner *et al.*, 2007), is compression therapy. The objective is to provide graded external compression to the leg and oppose the hydrostatic forces of venous hypertension. Compression therapy is thought to reduce and suppress superficial and deep venous reflux, restore valvular competence, reduce oedema, soften lipodermatosclerotic skin, aid venous return, increase arterial flow, enhance lymph drainage and improve microcirculation (Rajendran *et al.*, 2007). A number of compression delivery systems are available, including elasticated hosiery, compression bandages and intermittent pneumatic pumps.

The use of graded compressive garments (with 20 to 50 mmHg of tension) is well established in the treatment of chronic venous disease. Treatment with 30 to 40 mmHg compression stockings results in significant improvement in pain, swelling, skin pigmentation, activity and well-being if compliance of 70 to 80% is achieved (Motykie *et al.*, 1999). In patients with venous ulcers, graded compression stockings and other compressive bandaging modalities are effective in both healing and preventing

recurrences of ulceration (Nicolaides *et al.*, 2008). With a structured regimen of compression therapy 93% of patients with venous ulcers can achieve complete healing at a mean of 5.3 months (Mayberry *et al.*, 1991). Several studies have also investigated the haemodynamic benefits of compression therapy in patients with chronic venous disease. Compression stockings that exert external pressures of up to 40 mmHg appear effective in increasing blood flow velocity in the supine position and reducing oedema after extended periods of sitting and standing (Rajendran *et al.*, 2007). In addition, short-stretch and multi-layer compression bandages that exert pressures of over 40 mmHg can reduce venous hypertension during walking and improve venous pumping function (Rajendran *et al.*, 2007).

While compressive leg garments generally appear beneficial in most stages of chronic venous disease, they do not always work, and some people are not willing or able to wear them. For example, many elderly patients have reduced strength and dexterity that makes it difficult to put on the stockings. Alternatively, compression to the limb can be provided with intermittent pneumatic compression devices. These comprise an air pump and sleeve that surrounds the leg. The sleeve usually contains a series of bladders that are periodically inflated and deflated by the air pump. This technique has been used to treat oedema and promote venous leg ulcer healing in patients with chronic venous disease (Falanga and Eaglstein, 1986; Berliner *et al.*, 2003). In a review of the available literature, Berlinger *et al.* (2003) concluded that intermittent pneumatic compression therapy increases ulcer healing rates compared with no compression. However, at present there appears insufficient evidence to suggest that these devices improve ulcer healing when compared with compression garments alone or when added to a standard regimen of compression/hosiery (Nelson *et al.*, 2008).

8.3.3.2 Wound and skin care

Because progressive chronic venous disease might lead to compromised skin integrity, it is important to keep the affected area well moisturised to reduce the risk of skin breakdown and possibility of infection. The development of stasis dermatitis needs to be treated with a topical steroid. With venous ulcers, bacterial overgrowth control and aggressive wound care are required to minimise infectious complications. A variety of hydrocolloids and foam dressings are available to control wound fluid drainage and resultant maceration of the adjacent skin. In the presence of an infected ulcer bed,

silver-impregnated dressings have been effective in controlling infection and restoring tissue integrity (Karlsmark *et al.*, 2003; Jones *et al.*, 2004).

8.3.3.3 Pharmacological therapy

A small number of drugs have been evaluated in the treatment of chronic venous disease including coumarins (α-benzopyrones), flavonoids (γ-benzopyrones), saponosides (horse chestnut extracts), and other plant extracts. These drugs have veno-active properties and are often used in Europe. The principle for the use of veno-active drugs in chronic venous disease is to improve venous tone and capillary permeability, although a precise mechanism of action of these drugs is not known. It is thought that the flavonoids affect leucocytes and the endothelium by modifying the degree of inflammation and reducing oedema (Eberhardt and Raffetto, 2005). A micronised purified flavonoid fraction, Daflon, has been shown to reduce oedema-related symptoms as either primary treatment or in conjunction with surgical therapy (Nicolaides, 2003). A trial of 231 patients with chronic venous disease found a combination of coumarin and troxerutin (a flavonoid) with compression garments given for 12 weeks resulted in less oedema and pain as compared with placebo (Vanscheidt *et al.*, 2002). Horse chestnut seed extract has been found to be as effective as compression stockings in the short-term at reducing leg oedema and pain from chronic venous disease, but the long-term safety and efficacy has not been established (Pittler and Ernst, 2004).

Other agents have been used in the treatment of advanced venous disease with ulceration. Pentoxifylline is a haemo-rheological agent that is known to influence microcirculatory blood flow and oxygenation of ischaemic tissues, although the actual mechanism of action is uncertain (Jull *et al.*, 2007). On the basis of current evidence, Jull *et al.* (2007) concluded that pentoxifylline is an effective treatment for venous leg ulcers, either as an adjuvant to compression, or alone where compression cannot be used. Although gastro-intestinal side effects were common, they were tolerated by participants. The use of other agents such as aspirin and platelet-derived growth factor in promoting the healing or preventing the recurrence of venous ulceration has also been reported (Layton *et al.*, 1994; Stacey *et al.*, 2000); however, there have been no large randomised studies.

8.3.3.4 Exercise training

Although exercise training is generally recommended for patients with chronic venous disease, evidence of effectiveness is lacking and provision of specific advice is sporadic (Michaels *et al.*, 2006b).

Exercise training might benefit patients with advanced chronic venous disease by facilitating efficient calf-muscle pump function and increasing ankle range of motion. Indeed, calf muscle pump failure is considered a main aetiological factor in venous ulceration (Eberhardt and Raffetto, 2005) and decreased ankle mobility is associated with delayed healing of venous ulcers (Davies *et al.*, 2007). In a small controlled study, 31 patients with CEAP class 4 to 6 chronic venous disease were randomised to structured calf muscle exercise or routine daily activities (Padberg *et al.*, 2004). Venous haemodynamics was assessed with duplex ultrasound and air plethysmography, and muscle strength was assessed with an isokinetic dynamometer. After 6 months, patients receiving the calf muscle exercise regimen had normalised their calf muscle pump function parameters (e.g. venous ejection fraction) but experienced no change in the amount of reflux or severity scores. The authors concluded that structured exercise to re-establish calf muscle pump function in chronic venous disease might prove beneficial as a supplemental therapy to medical and surgical treatment in advanced disease. In a small, single-arm pilot study, eleven patients with long-standing venous ulcers were encouraged to undertake a thrice-weekly ankle exercise programme for 24 weeks. Exercise training was well-adhered-to and resulted in improved ankle range of motion and decreased ankle pain. Unfortunately, no data were collected to show if exercise training had a positive effect on ulcer healing.

Regular exercise training might also help correct some of the microvascular abnormalities caused by venous disease. There is evidence that varicose-vein patients have microvascular endothelial dysfunction (Klonizakis *et al.*, 2003), and that this persists after venous surgery (Klonizakis *et al.*, 2006). Microvascular endothelial dysfunction might occur in these patients because of venous stasis in the microcirculation that reduces the shear rate on the endothelial cells resulting in a reduction in cellular levels of NO (Naoum *et al.*, 2007). This favours leucocyte adhesion and neutrophil and monocyte activation, which ultimately results in endothelial injury. Several factors might affect tissue viability and susceptibility to ulceration, but the

structural and functional integrity of the microcirculation to maintain blood flow, tissue oxygenation and nutrient delivery might be particularly important (Iabichella *et al.*, 2006). Therefore, microvascular endothelial dysfunction might be an important risk factor for venous ulceration. Regular exercise training has been shown to enhance microvascular endothelial function in diabetics (Colberg *et al.*, 2002) and older sedentary adults (Black *et al.*, 2008). Such an improvement would also be likely after exercise training in both pre- and post-surgical varicose-vein patients. Therefore, an improvement in microvascular endothelial function after exercise training might be clinically meaningful with respect to lowering the risk of venous ulceration. However, further research is needed to clarify this.

Exercise training might also help prevent varicose-vein formation/recurrence by facilitating weight management. Indeed, some studies have shown an association between obesity and varicose veins for women (Abramson *et al.*, 1981; Lee *et al.*, 2003). However, this is not a consistent finding (Guberan *et al.*, 1973; Hirai *et al.*, 1990) and no such association has been demonstrated for men (Callam, 1994).

The type of exercise performed appears very important. Dynamic exercise with little impact and gravity effect is usually advocated for patients with chronic venous disease (Berard *et al.*, 2002). This approach is based on evidence that vigorous exercise (e.g. running, football, basketball, tennis) increases the likelihood of venous ulceration (Berard *et al.*, 2002), probably because of an increased risk of impact-related injuries. Walking might be an appropriate exercise modality for chronic venous disease patients, since it activates (and potentially trains) the calf muscle pump and is relatively low impact. Calf muscle strengthening and flexibility exercises might be similarly appropriate.

In summary, exercise training might facilitate reduced pain and ulcer healing in patients with advanced chronic venous disease and prevent varicose vein and venous ulcer formation in at-risk individuals. Relevant beneficial physiological effects of exercise training include improved calf muscle pump function, weight loss/management, enhanced ankle range of motion, and attenuation of microvascular endothelial dysfunction. Appropriate exercise programmes for chronic venous disease patients might include walking as well as ankle mobility and heel-rising (plantarflexion)

exercises. Further research is clearly needed in the area of exercise training for chronic-venous-disease patients.

8.4 Aims of the studies

Cutaneous microvascular function after varicose-vein surgery is poorly characterised and there were no published data about the effects of acute or chronic exercise on microvascular function. Therefore, the aims of the studies described in Section 2 of this thesis were to compare cutaneous microvascular function between patients who have recently had varicose-vein surgery and age-matched healthy controls, and to investigate whether any impairment of function could be alleviated by acute or chronic lower-limb exercise. We hypothesised that, before acute/chronic exercise, microvascular endothelial function would be relatively depressed in patients compared to controls. Secondly, we hypothesised that any baseline impairment of microvascular endothelial vasodilator function would be attenuated by both acute and chronic lower-limb exercise.

Chapter 9: Methods

9.1 Overview of Studies 3 and 4

Study 3 compared cutaneous microvascular function between patients who had recently had varicose-vein surgery and age-matched healthy controls. A secondary aim was to investigate whether any impairment of function could be alleviated by acute lower-limb exercise. Twenty-four post-surgical varicose-vein patients and 12 age-matched healthy participants were recruited. Cutaneous microvascular function of the gaiter area was assessed in supine and standing positions before and after a 25-min moderate-intensity walk using laser Doppler fluximetry combined with incremental-dose iontophoretic administration of vasodilator drugs. Group differences in cutaneous flux responses before acute exercise were assessed using mixed-model (group-by-dose) analyses of variance. The acute effects of exercise on peak cutaneous flux responses in each group were assessed using mixed-model (group-by-time) analyses of variance.

Study 4 investigated the effects of eight weeks moderate-intensity lower-limb exercise training on cutaneous microvascular function in post-surgical varicose-vein patients. Sixteen patients were included in this study: eight were randomly allocated to a treadmill-walking exercise training group and eight to a non-exercise control group. The exercise group trained twice weekly for eight weeks. At baseline and eight weeks, cutaneous microvascular function of the gaiter area was assessed using laser Doppler fluximetry combined with incremental-dose, iontophoretic administration of vasodilator drugs. Mixed-model (group-by-time) analyses of covariance were used to detect changes in outcome measures between groups.

9.2 Patient recruitment

9.2.1 Selection and recruitment

Patients who had recently (four-to-five weeks) had varicose-vein surgery (unilateral sapheno-femoral ligation and partial stripping) were selected and recruited using clinical notes at the Sheffield Vascular Institute, Northern General Hospital, Sheffield, UK. Selection was based on patient's medical history, previous physical examination, and on the inclusion and exclusion criteria dictated by this study (see below). Healthy age- and body mass-matched participants were also recruited from Sheffield Hallam University to provide baseline data for comparison.

Patients satisfying the study criteria were sent a letter (Appendix 6), with an attached patient information sheet (Appendix 7) and a leaflet describing the facilities and directions to The Centre for Sport and Exercise Science at Sheffield Hallam University. The letter clearly stated that there was no obligation or pressure to participate in this study. If patients did not wish to participate, their future medical care would not be jeopardised. Patients declining to be contacted were requested to leave an answer-phone message by a specific date.

Patients who had not left an answer-phone message declining to take part were presumed to have a possible interest in study participation. These patients were contacted via telephone, and vetted to ascertain that they still fulfilled the study criteria. Patients' initial questions were answered. Patients satisfying the study criteria were invited to The Centre for Sport and Exercise Science for an initial consultation session to view the facilities and to discuss all aspects of the study.

The composition of the study groups, the randomisation procedure, potential benefits of the exercise training programme, the required commitment to the exercise sessions and the measurements that were to be taken during the assessment sessions were all discussed, as were the requirements from patients if randomised to the control group. Suitability was re-assessed on the basis of previous history, in accordance with the study inclusion and exclusion criteria.

9.2.2 Inclusion criteria

Patients were included in this study on the basis of:

- Age ≥40 years
- Varicose-vein surgery (unilateral sapheno-femoral ligation and partial stripping) received within the previous four-to-five weeks
- Ability to undertake exercise

9.2.3 Exclusion criteria

Patients were excluded on the basis of:

- Presence of trophic skin changes
- Presence of diabetes or cardiovascular disease

- Presence of hypertension or hypercholesterolaemia
- Taking vasodilatory medication
- Major surgery within the previous 12 months
- Inability to meet the physical requirements of the study

This research was carried out in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the North Sheffield Research Ethics Committee (Appendix 8). Written informed consent was obtained from each patient prior to investigation.

9.2.4 Power calculations for determination of sample size

The primary outcome variable for the calculation of sample size (Study 4) was peak cutaneous flux measured during the laser Doppler fluximetry microvascular functioning tests. Sample size calculation was made using data from similar research (Klonizakis *et al.*, 2006). An improvement in peak cutaneous flux of 30 perfusion units after exercise training was considered to be clinically important in post-surgical varicose-vein patients. Using a mean standard deviation of 17.3 perfusion units, and taking into account a possible patient drop-out of 10% over the eight-week intervention period, recruitment of eight patients for each group yielded a 90% power to detect an increase in peak cutaneous flux of this magnitude at the alpha value of 0.05. Therefore, 16 patients were recruited for Study 4 because it involved two treatment groups.

9.2.5 Medical examination

Prior to entering the study, all patients underwent a medical examination performed by Dr Markos Klonizakis (Clinical Exercise Scientist, Northern General Hospital, Sheffield, UK). During this examination, details of surgical history, co-morbid conditions, risk factors, and current medication details were confirmed. Patients on long-term medication continued on their treatment. Blood pressure was taken (manual sphygmomanometer) and a resting 12-lead electrocardiogram (Cardioperfect, Welch Allyn, USA) was performed with the patient in the supine position, to identify evidence of arrhythmias, previous myocardial infarction, or ischaemia. Patients with abnormal electrocardiogram or blood pressure readings were excluded from participation in the study (as explained in Chapter 3). All sessions were undertaken in the presence of

personnel who had received training in advanced-life-support procedures. All patients' general practitioners were notified in writing of study participation.

9.3 Assessment of cutaneous microvascular function

Patients were fully accustomed with the assessment protocols prior to baseline data collection. Cutaneous microvascular function was assessed at baseline for all participants and also at eight weeks for those patients participating in Study 4. Procedures were randomly checked for consistency by the lead investigator at Sheffield Hallam University, who was blinded to group assignment.

Cutaneous microvascular function was assessed using laser Doppler fluximetry combined with iontophoretic delivery of vasodilator drugs. The microcirculation comprises the arterioles (of diameter <300 μm), capillaries and venules. These vascular beds are not easily accessible in humans. However, the skin is a highly vascular organ covering the whole body and therefore has been the subject of many studies attempting to elucidate its regulatory control mechanisms. The skin vascular bed is considered a creditable 'window', reflecting vascular beds elsewhere in the body (Holowatz *et al.*, 2008), which makes assessment of skin microcirculation a very useful and much explored avenue of research. In a review of microcirculatory disturbances in chronic venous disease patients, Wollina *et al.* (2006) believed that small-vessel abnormalities are implicated in most of the clinical manifestations of chronic venous disease, including pigmentation, lipodermatosclerosis and ulceration. Since markers of microcirculatory dysfunction might be apparent before the onset of clinical symptoms, identification of this dysfunction might provide a precursory indication of those patients who are greatest risk.

Skin microvessels can be separated into two distinct types; those that serve thermoregulatory requirements, and those that provide nutritional flow. Furthermore, the relative composition of glabrous and non-glabrous skin of these vessels is significantly different, which probably reflects their different haemodynamic responsive to certain stimuli. Non-glabrous skin is composed almost entirely of nutritional capillary vessels, whereas the majority of glabrous skin blood flow is thought to be provided by numerous arterio-venous anastomoses. These anastomoses or shunts are mainly present in the hands, feet, ears, and the nail beds, where they allow the movement of skin blood

flow directly from arterioles to venules, bypassing the capillary network. Arterio-venous shunts are densely innervated and have a thick layer of vascular smooth muscle in their wall, which confers the ability to participate in peripheral vascular reactivity. For this reason, glabrous skin is prone to wide fluctuations in blood flow.

Unlike flow-mediated dilation of the brachial artery to assess conduit vessel structure and function (Corretti *et al.*, 2002; Tinken *et al.*, 2008), the assessment of microvascular structure and function is not standardised (Cracowski *et al.*, 2006). Of the techniques that have been developed to quantify microvascular structure and/or function, the most exploited appears to be laser Doppler fluximetry.

The basic theory of laser Doppler fluximetry is described in Chapter 3. Skin perfusion assessed under baseline conditions using laser Doppler fluximetry has been documented to be normal in patients with uncomplicated chronic venous disease (Cheatle *et al.*, 1991; Klonizakis *et al.*, 2003), and increased in patients with complicated chronic venous disease, such as those with lipodermatosclerosis or ulceration (Malanin *et al.*, 2004; Mlacak *et al.*, 2005). The increase in resting skin perfusion of those with more severe chronic venous disease has been attributed to the dilated and tortuous microvessels that result from persistent venous hypertension (Kelechi and Michel, 2007). Despite this relatively high resting skin perfusion, the nutritive capillary bed is compromised (Fagrell, 1982). Thus, any increase in metabolic demand, as is the case during ulcer healing, is unlikely to be met with adequate blood (= oxygen) delivery.

The use of provocation tests, such as post-occlusive reactive hyperaemia or iontophoretic delivery of vasodilatory agents, also highlights microcirculatory abnormalities in patients with chronic venous disease. For example, the skin hyperaemic response to thigh cuff arterial occlusion, a common test of microvascular function (Cracowski *et al.*, 2006), has been shown to be blunted in patients with advanced chronic venous disease. Junger *et al.* (2000) reported that the reactive hyperaemia response measured at skin next to an ulcer was diminished compared to that measured in the gaiter area of healthy controls (20 ± 41 vs. 721 ± 490%, respectively). Furthermore, Cheatle *et al.* (1991) demonstrated that a short-term period of experimental venous hypertension (proximal cuff inflation to 80 mmHg for 30 min)

reduced reactive hyperaemia indices in healthy adults, consistent with the theory of leucocyte activation and capillary plugging.

Iontophoresis is a technique that uses a weak electrical current to transport drug ions across the skin barrier, with changes in skin perfusion measured by laser Doppler fluximetry. Iontophoretic delivery of acetylcholine (ACh) and sodium nitroprusside (SNP) is commonly used for assessing microvascular endothelial-dependent and -independent vasodilator function, respectively (Turner *et al.*, 2008). ACh binds to M2 muscarinic receptors on the endothelial surface and elicits an increase in cutaneous blood flow by a direct receptor-mediated effect (Berghoff *et al.*, 2002). Acting via a G-protein-coupled receptor and catalysed by constitutively expressed endothelial NO synthase, ACh stimulates the production of NO. NO is a potent mediator of endothelium-dependent vasodilation (Furchgott and Zawadzki, 1980). ACh might also induce the release of other vasodilating substances including prostaglandins and endothelium-derived hyperpolarising factor (Rubanyi, 1991; Feletou and Vanhoutte, 1999). Therefore, although ACh iontophoresis combined with laser Doppler fluximetry does represent a test of 'endothelial function', this function is not limited to the NO system in human skin. In contrast, SNP is a NO donor. The NO activates soluble guanylyl cyclase in vascular smooth muscle cells, which initiates the transformation of guanosine triphosphate to cyclic guanosine monophosphate (cGMP) (Hojs *et al.*, 2009). The activation of cGMP-dependent protein kinase G is followed by the removal of cytosolic calcium from the cell and dephosphorilation of the light chains of myosin (Stankevicius *et al.*, 2003). This leads to the inhibition of contractile apparatus and consequently to vasodilation.

Klonizakis *et al.* (2003) used iontophoresis of ACh and SNP combined with laser Doppler fluximetry to compare microvascular function between patients with isolated superficial venous insufficiency and age-matched healthy controls. In the standing position, peak cutaneous flux responses to ACh were lower in patients than controls (68 ± 8 vs. 109 ± 11 PU). In contrast, there were no differences in flux responses to SNP. The authors concluded that the patients had impaired microvascular endothelial function and, because of this, an increased risk of venous ulceration. In a later study, the same research group observed that superficial venous surgery improved skin endothelial-independent function, with unchanged responses to ACh (Klonizakis *et al.*, 2006). Thus,

it appears that microvascular endothelial dysfunction persists in patients who have had surgery for varicose veins. Microvascular endothelial dysfunction might be a contributory factor towards the high recurrence rate for venous ulceration in this group (Iabichella *et al.*, 2006).

For the cutaneous microvascular function tests performed for this thesis, participants were instructed not to perform any vigorous exercise in the 24 hours before an assessment and to abstain from caffeine and nicotine intake for at least 2 hours before an assessment. All assessments were performed in a temperature controlled room (range 22 to 24°C) after an acclimatisation period ≥15 min. With the participant lying supine, the gaiter area of the leg to be studied (the leg that had been operated on for the patients or the left leg for the control group) was cleaned with an alcohol wipe and allowed to dry before applying two drug delivery electrodes (PF383; Perimed AB, Järfälla, Sweden) to the surface of the leg 4 to 8 cm proximal to the medial malleolus. The drug delivery electrodes were positioned over healthy looking skin, approximately 4 cm apart with one containing 80 µl of 1% ACh (Miochol-E, Novartis, Stein, Switzerland; solvent = deionised water) and the other 80 µl of 1% SNP (Fagron, Hoogeveenenweg, The Netherlands; solvent = deionised water). To obtain an index of skin blood flow, cutaneous red cell flux was measured by placing an iontophoresis laser-Doppler probe (PF481-1; Perimed AB), connected to a laser Doppler fluxmeter (PF5001; Perimed AB), in the centre of each drug delivery electrode. The laser-Doppler probe signals were continuously monitored via an online software chart recorder (PSW; Perimed AB). The experimental set-up is depicted in Figure 9.1.

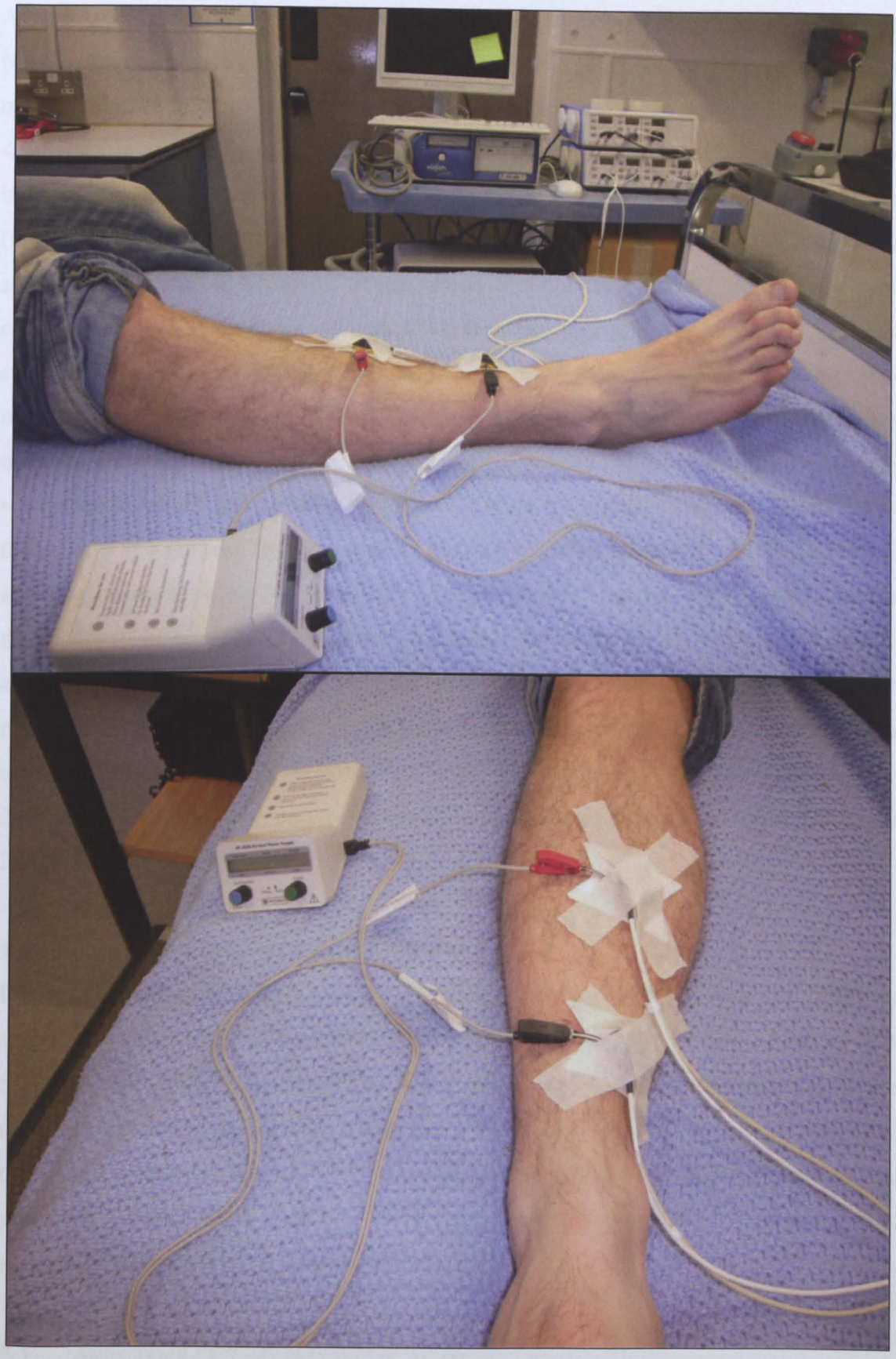

Figure 9.1 Cutaneous microvascular function experimental set-up

A battery-powered iontophoresis controller (PeriIont PF382b; Perimed AB) was used to provide the charge needed for ACh and SNP delivery. The anodal (positive) current was used to transfer ACh, with the cathodal (negative) current used to transfer SNP. After a 4-min stable recording of baseline flux, dose-response curves for ACh- and SNP-induced vasodilation were characterised using the following protocol: 0.2 mA for 10 s (i.e. 2 mC), 0.2 mA for 15 s (i.e. 3 mC), 0.2 mA for 20 s (i.e. 4 mC), and 0.3 mA for 20 s (i.e. 6 mC), with a 4-min recording period between each dose. The protocol was chosen as it is sufficient to provide effective ACh and SNP delivery but avoids the non-specific vasodilation observed with higher electrical charges (Droog *et al.*, 2004). This protocol was then repeated with the participant in a standing position to assess the effect of posture on cutaneous microvascular function. Participants then completed a 25-min self-paced treadmill walk (Patients: 4.9 ± 0.1 $km \cdot hr^{-1}$; Controls: 5.0 ± 0.1 $km \cdot hr^{-1}$; P = 0.619) on a 0% gradient before repeating the iontophoresis protocols in both supine and standing positions to assess the effects of acute exercise on cutaneous microvascular sensitivity to ACh and SNP in different postures. The probes were moved to fresh skin areas for each body position. The peak cutaneous flux responses to ACh and SNP, measured in conventional perfusion units (PU), were used as measures of microvascular endothelial-dependent and -independent function, respectively (Klonizakis *et al.*, 2003, 2006). The technical error of measurement for drug-induced peak flux responses in our laboratory is 15%.

9.4 Randomisation process for Study 4

Upon completing all baseline assessments, 16 patients were randomised (random selection without replacement) using a computer programme (nQuery Advisor 6.0, Statistical Solutions, Ireland) to either a treadmill-walking exercise training group or a non-exercise control group. The randomisation procedure was conducted by a member of the research team not involved in the recruitment, accustomisation or assessment processes. Patients were randomised into the groups regardless of sex, age, severity of symptoms, current medication or smoking status.

9.5 Supervised exercise sessions in Study 4

Patients allocated to the exercise group were invited to complete twice weekly supervised training sessions for eight weeks. Training was usually undertaken on a group basis (maximum of three patients), with the sessions equally balanced during the

week; sessions were normally organised for Mondays and Thursdays. This provided a structure and allowed patients time to recover between sessions. Alternative training sessions were offered, if needed, to maintain twice-weekly attendance.

The training sessions were performed using standard treadmills (EC-T220, Cateye Fitness, UK). Each session began with a 2-min warm-up period consisting of low-intensity walking. Patients then trained in cycles of 2 min exercise, followed by 2 min rest, for a total exercise time of 20 min in a 40-minute session. This strategy enables a greater volume of higher intensity exercise to be performed in a given amount of time than can be achieved using continuous exercise of a similar nature, and therefore optimises the stimulus for cardiovascular adaptations. The intensity of the exercise was set to elicit a RPE of 11 to 13 ('light' to 'somewhat-hard') using Borg's 6 to 20 RPE scale (Borg, 1998). Heart rate and RPE were monitored throughout each training session. Each session finished with a 2-min cool-down period consisting of low-intensity walking.

Patients allocated to the control group were informed of the benefits of an active lifestyle but did not undertake any supervised exercise. This was reinforced by telephone every fortnight during the study period. Control patients undertook assessments at identical time points as those in the exercise group (i.e. at baseline and eight weeks).

9.6 Statistical analyses

9.6.1 Study 3

Outcome measures were first tested for normal distribution using the Kolmogorov-Smirnov goodness-of-fit test. Histograms of all outcome measures at all time-points were obtained to confirm normality of distribution. Homogeneity-of-variance checks were performed using Levene's test. The majority of outcome measures, including the peak flux responses with each drug, body position and time-point, were non-normally distributed. These variables were log-transformed before further analysis.

Differences in characteristics between patients and controls were assessed using independent *t*-tests and χ-squared tests. Group differences in cutaneous flux responses before acute exercise were assessed using mixed-model (group-by-dose) analyses of

variance for each body position and drug, with independent *t*-tests used to interpret significant interaction effects. The acute effects of exercise on peak cutaneous flux responses in each group were assessed using mixed-model (group-by-time) analyses of variance for each position and drug, with paired-samples *t*-tests used to interpret significant interaction effects.

Effect sizes (Cohen's *d*) were calculated for exercise-induced changes in peak cutaneous flux responses, with 0.2, 0.5 and 0.8 representing small, medium and large effects, respectively (Mullineaux *et al.*, 2001). All statistical analyses were performed using SPSS for Windows version 16 (SPSS Ltd, Woking, UK), with significance set at $P \leq 0.05$. Data are presented as mean ± SD unless otherwise stated.

9.6.2 Study 4

Outcome measures were first tested for normal distribution using the Kolmogorov-Smirnov goodness of fit test. Histograms of all outcome measures at all time-points were obtained to confirm normality of distribution. Homogeneity-of-variance checks were performed using Levene's test. The majority of outcome measures, including the peak flux responses with each drug, body position, and time-point, were non-normally distributed. These variables were log-transformed before further analysis.

Differences in characteristics between patients in the exercise and control groups were assessed using independent *t*-tests and χ-squared tests. Mixed-model (group-by-time) analyses of covariance were used to detect changes in outcome measures between groups, with baseline data used as the covariate (Vickers and Altman, 2001). Effect sizes (Cohen's *d*) were calculated for the exercise group data, with 0.2, 0.5, and 0.8 representing small, medium, and large effects, respectively (Mullineaux *et al.*, 2001). All statistical analyses were performed using SPSS for Windows version 16 (SPSS Ltd, Woking, UK), with significance set at $P \leq 0.05$. Data are presented as mean ± SD unless otherwise stated.

Chapter 10: Study 3 - Impaired microvascular endothelial function is restored by acute lower-limb exercise in post-surgical varicose-vein patients

10.1 INTRODUCTION

Chronic venous disease with skin changes of the leg is a common condition affecting up to 1 in 20 people in Western societies (Smith, 2006). Dilatation and incompetence of the deep, superficial, or perforating veins leads to impairment of the venous muscle pumps in the lower limb, often resulting in venous hypertension with upright posture (Naoum *et al.*, 2007). Venous hypertension can activate neutrophils and monocytes, which can cause injury to the endothelium of lower-limb microvessels (Smith, 2006). Chronic injury to the endothelium can lead to a chronic inflammatory condition of the skin known clinically as lipodermatosclerosis, and skin in this state also has the potential to ulcerate in response to minor injury (Smith, 2006).

Patients with chronic venous disease often benefit from varicose-vein surgery, e.g. sapheno-femoral ligation and stripping (Campbell, 2006). Indeed, venous surgery typically results in haemodynamic benefit for the legs (Gohel *et al.*, 2005), facilitated ulcer healing (Obermayer *et al.*, 2008), and reduced risk of ulcer recurrence (Gohel *et al.*, 2007). Despite these benefits, the recurrence rates for varicose veins (62% over an 11-year period; Winterborn *et al.*, 2004) and venous ulcers (18% over a 5-year period; Nelzen and Fransson, 2007) remain relatively high. A contributory factor could be microvascular endothelial dysfunction that persists after surgery (Klonizakis *et al.*, 2003, 2006). Therefore, it appears important to identify strategies to improve microvascular endothelial function in this population.

At present, cutaneous microvascular function after varicose-vein surgery is poorly characterised and there are no data regarding the effects of acute exercise on microvascular endothelial-dependent and -independent vasodilator function. Therefore, the aim of this study was to compare cutaneous microvascular vasodilator function between patients who have recently had varicose-vein surgery and age-matched controls. A secondary aim was to investigate whether any impairment of function can be alleviated by acute lower-limb exercise. In view of the existing literature (Klonizakis *et al.*, 2003, 2006), we hypothesised that, before acute exercise, microvascular endothelial function would be relatively depressed in patients compared to controls. Secondly, we hypothesised that any baseline impairment of microvascular endothelial vasodilator function would be attenuated by acute lower-limb exercise.

10.2 METHODS

A detailed description of the methods used in this study is provided in Chapter 9.

Participants

Twenty-four post-surgical varicose-vein patients and 12 age-matched healthy controls were recruited. Participant characteristics for both groups are shown in Table 10.1.

Table 10.1 Characteristics of the patients and age-matched controls

	Patients	Controls	*P*
Sex	4 male, 20 female	5 male, 7 female	0.102[a]
Age (years)	54 ± 11	50 ± 6	0.229[b]
Body mass (kg)	77.3 ± 12.1	78.9 ± 21.4	0.772[b]
Stature (cm)	169.0 ± 9.6	169.0 ± 12.5	0.990[b]

Values are means ± SD. [a]Chi-squared tests, [b]independent *t*-test

Outcome measures

Cutaneous microvascular function of the gaiter area was assessed in supine and standing positions before and after a 25-min moderate-intensity walk using laser Doppler fluximetry combined with incremental-dose iontophoretic administration of ACh and SNP.

Statistical analyses

Group differences in cutaneous flux responses before acute exercise were assessed using mixed-model (group-by-dose) analyses of variance. The acute effects of exercise on peak cutaneous flux responses in each group were assessed using mixed-model (group-by-time) analyses of variance. Statistical significance was set at $P \leq 0.05$. Effect sizes (Cohen's *d*) were calculated for the exercise group data, with 0.2, 0.5 and 0.8 representing small, medium and large effects, respectively.

10.3 RESULTS

Group differences in cutaneous flux responses before acute exercise

Typical cutaneous red cell flux responses to incremental dose administration of ACh and SNP are shown in Figure 10.1. Cutaneous flux responses before acute exercise in the supine and standing positions are shown in Figures 10.2 and 10.3, respectively. Resting (unstimulated) cutaneous flux in the gaiter area, both in the supine and standing positions, was not different between patients and controls. In the supine position, there no differences between patients and controls for the cutaneous flux responses to ACh or SNP (Figure 10.2). In the standing position, the peak cutaneous flux response to ACh was significantly higher in controls compared to patients (96 ± 96 vs. 48 ± 52 PU; P = 0.032; Figure 10.3 upper panel), indicating a relatively depressed endothelial-dependent function in post-surgical varicose-vein patients. In contrast, the cutaneous flux responses to SNP were generally higher in patients than controls, and this reached statistical significance for the 3 mC dose (24 ± 18 vs. 10 ± 9 PU; P = 0.023; Figure 10.3 lower panel), indicating a relatively enhanced endothelial-independent function in post-surgical varicose-vein patients.

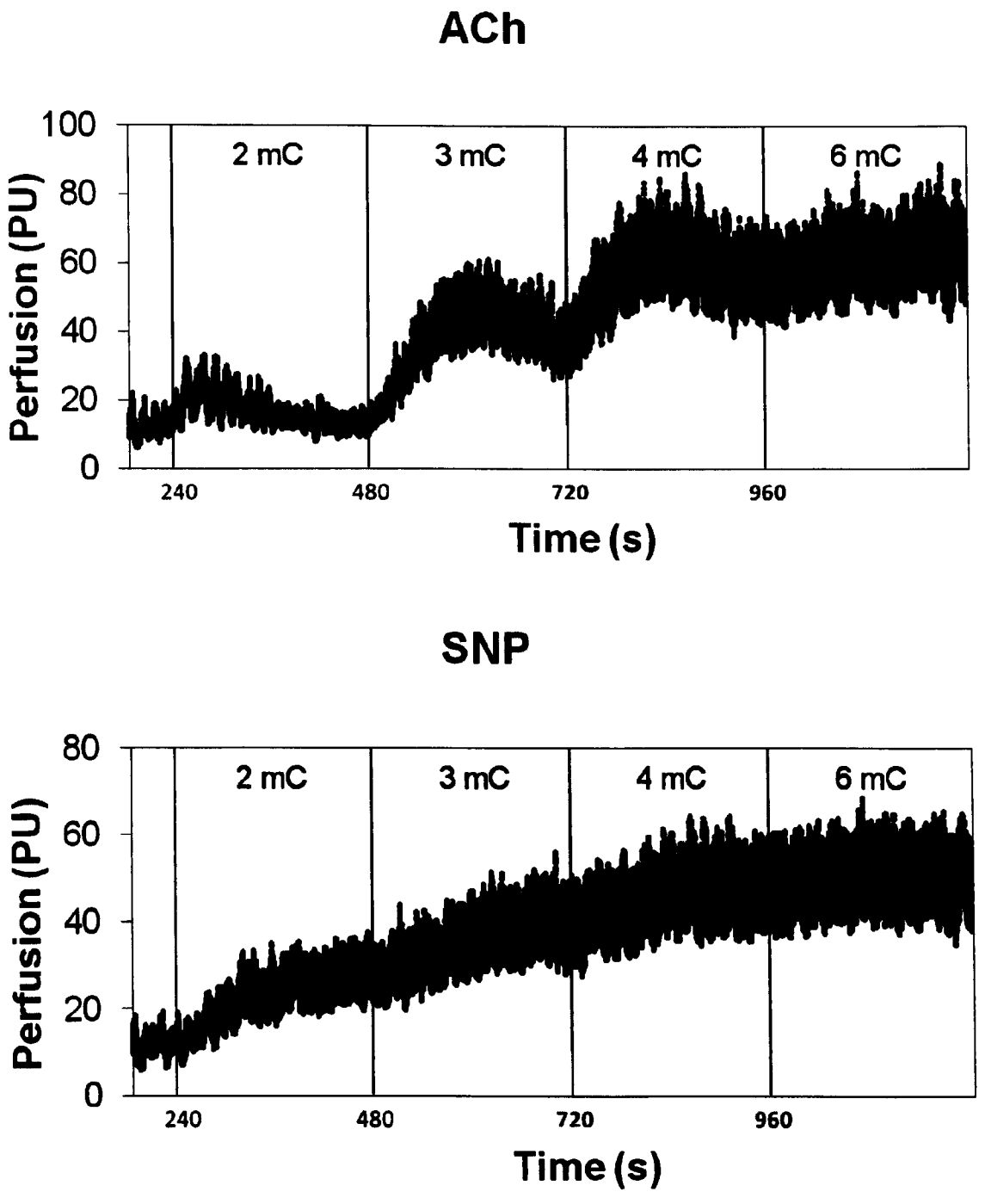

Figure 10.1 Example laser Doppler perfusion responses to increasing concentrations of ACh (upper panel) and SNP (lower panel). The dose-response curves were made up by using charges of 2 mC, 3 mC, 4 mC, and 6 mC. The response measuring period for each dose was 4 min.

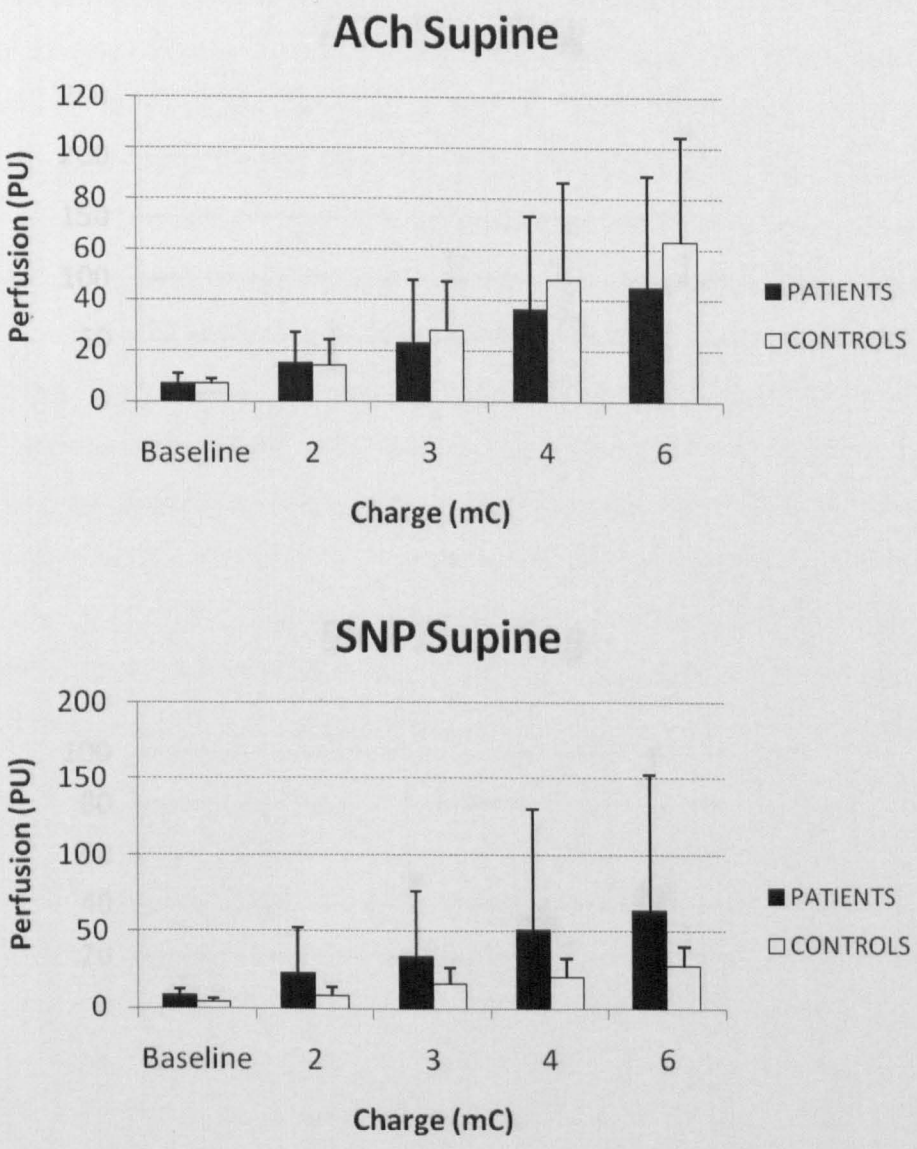

Figure 10.2 Pre-acute exercise cutaneous flux responses to incremental doses of ACh (upper panel) and SNP (lower panel) in post-surgical varicose-vein patients and age-matched healthy controls in the supine position. Data are presented as means + SD. $P > 0.05$ for both drugs at all doses.

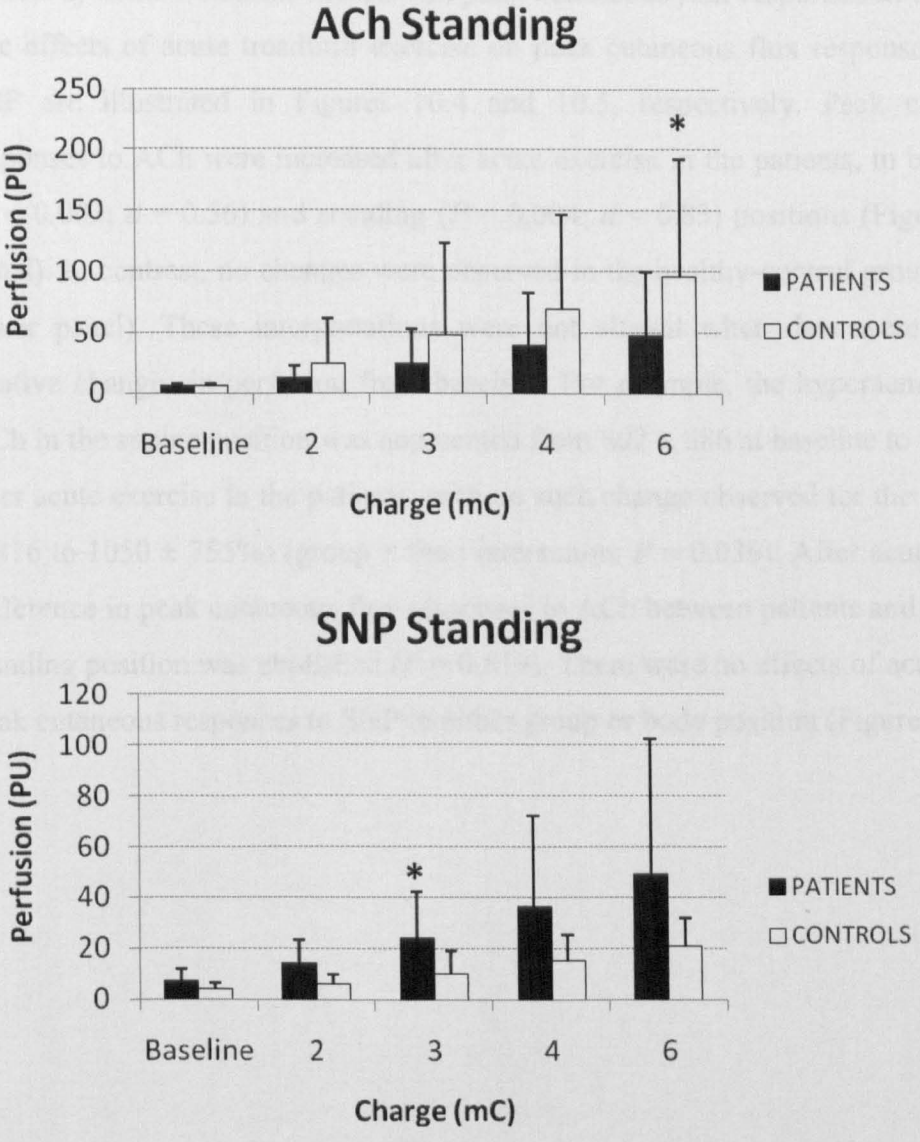

Figure 10.3 Pre-acute exercise cutaneous flux responses to incremental doses of ACh (upper panel) and SNP (lower panel) in post-surgical varicose-vein patients and age-matched healthy controls in the standing position. Data are presented as means + SD. *$P < 0.05$ between groups.

Effects of acute treadmill exercise on peak cutaneous flux responses in each group

The effects of acute treadmill exercise on peak cutaneous flux responses to ACh and SNP are illustrated in Figures 10.4 and 10.5, respectively. Peak cutaneous flux responses to ACh were increased after acute exercise in the patients, in both the supine (P = 0.003; d = 0.56) and standing (P = 0.004; d = 0.83) positions (Figure 10.4 upper panel). In contrast, no changes were observed in the healthy-control group (Figure 10.4 lower panel). These interpretations were not altered when data were expressed as relative changes in perfusion from baseline. For example, the hyperaemic response to ACh in the supine position was augmented from 802 ± 886 at baseline to 1273 ± 1078% after acute exercise in the patients, with no such change observed for the controls (1156 ± 816 to 1050 ± 755%) (group × time interaction: P = 0.039). After acute exercise, the difference in peak cutaneous flux responses to ACh between patients and controls in the standing position was abolished (P = 0.819). There were no effects of acute exercise on peak cutaneous responses to SNP in either group or body position (Figure 10.5).

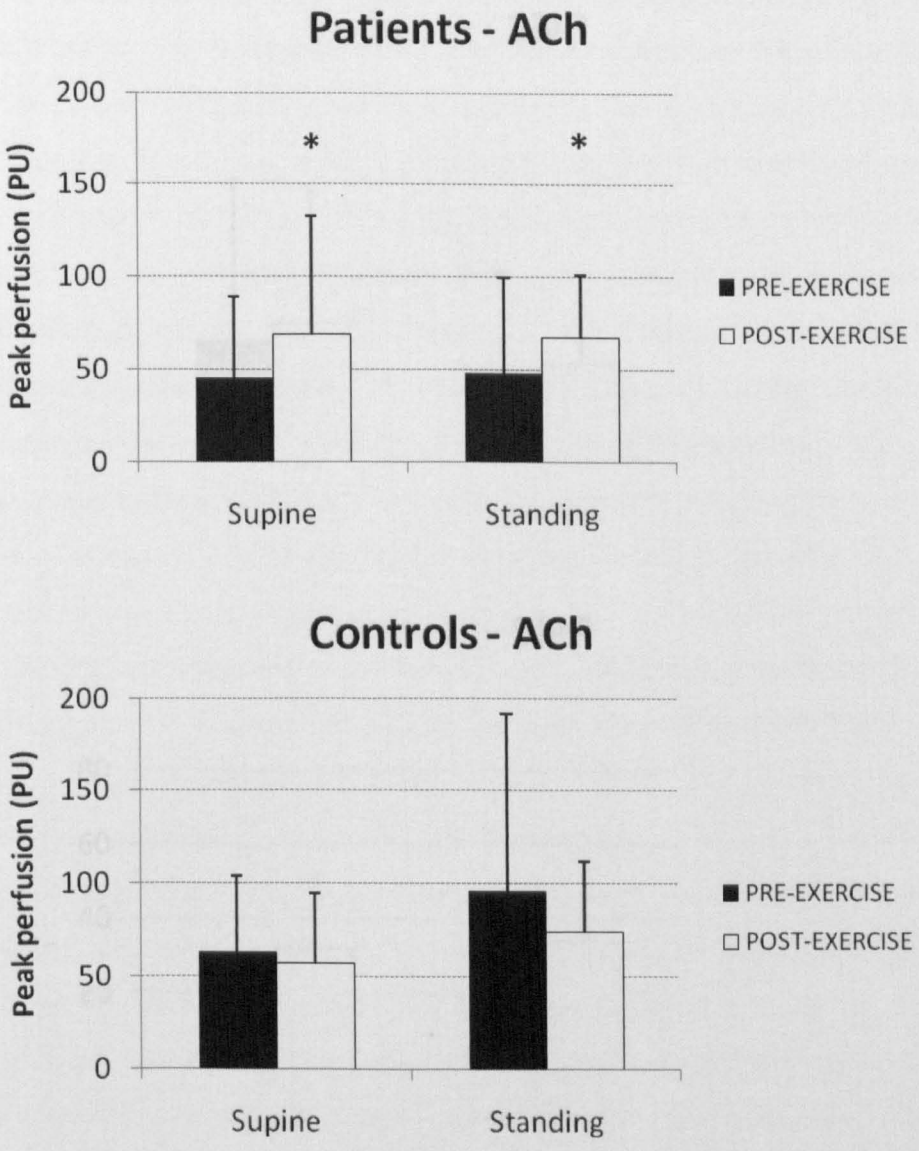

Figure 10.4 Comparison of pre- and post-acute exercise peak cutaneous flux responses to ACh in post-surgical varicose-vein patients (upper panel) and age-matched healthy controls (lower panel). Data are presented as means + SD. *$P \leq 0.004$ between pre- and post-acute exercise data.

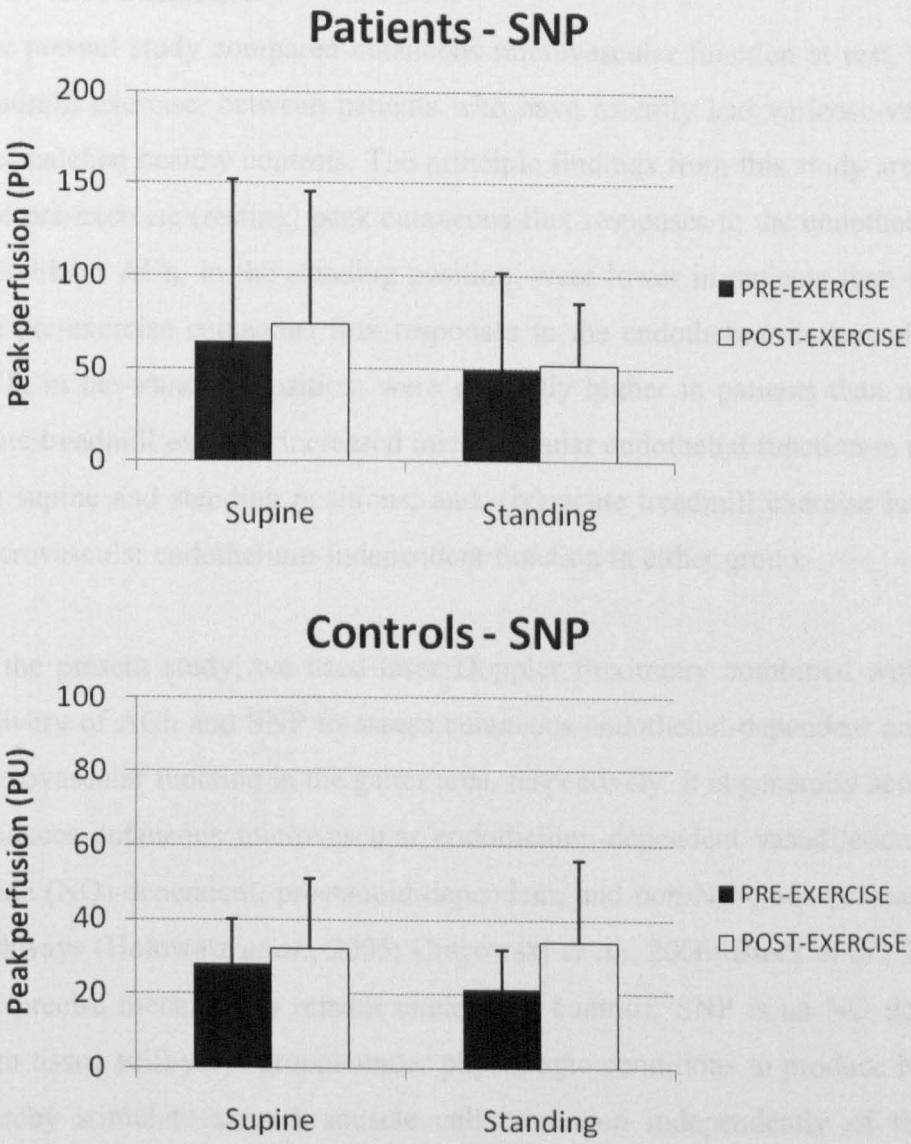

Figure 10.5 Comparison of pre- and post-acute exercise peak cutaneous flux responses to SNP in post-surgical varicose-vein patients (upper panel) and age-matched healthy controls (lower panel). Data are presented as means + SD. $P > 0.05$ for both groups and positions.

10.4 DISCUSSION

The present study compared cutaneous microvascular function at rest, and after acute treadmill exercise, between patients who have recently had varicose-vein surgery and age-matched healthy controls. The principle findings from this study are as follows: (i) The pre-exercise (resting) peak cutaneous flux responses to the endothelium-dependent vasodilator ACh, in the standing position, were lower in patients than in controls; (ii) the pre-exercise cutaneous flux responses to the endothelium-independent vasodilator SNP, in the standing position, were generally higher in patients than in controls; (iii) acute treadmill exercise increased microvascular endothelial function in patients in both the supine and standing positions; and, (iv) acute treadmill exercise had no effect on microvascular endothelium-independent function in either group.

In the present study, we used laser Doppler fluximetry combined with iontophoretic delivery of ACh and SNP to assess cutaneous endothelial-dependent and -independent microvascular function in the gaiter area, respectively. It is generally accepted that ACh produces cutaneous microvascular endothelium-dependent vasodilation through nitric oxide (NO)-dependent, prostanoid-dependent, and non-NO-, non-prostanoid-dependent pathways (Holowatz *et al.*, 2005; Cracowski *et al.*, 2006; Black *et al.*, 2008), although the precise mechanisms remain unclear. In contrast, SNP is an NO donor that reacts with tissue sulfhydryl groups under physiologic conditions to produce NO directly and thereby stimulate smooth muscle cell relaxation independently of the endothelium (Turner *et al.*, 2008). The main limitation with iontophoretic drug delivery is that non-specific microvascular responses, associated with drug concentration and charge and vehicle characteristics, are common with most protocols used (Droog *et al.*, 2004). Although we cannot exclude the possibility that our protocol elicited non-specific vasodilatory effects, the use of 10 $g \cdot l^{-1}$ drug concentrations and currents ≤ 0.3 mA should have at least minimised such effects (Droog *et al.*, 2004). In addition, as the protocol was consistent between groups before and after acute lower-limb exercise, it is unlikely that any observed differences/changes in microvascular responses are due to non-specific, protocol-related vasodilatory effects.

Group differences in cutaneous flux responses before exercise

The peak endothelium-dependent responses to ACh in the standing position were lower in patients than in controls (Figure 10.3 upper panel). This finding is consistent with

previous reports that microvascular endothelial function in the standing position is relatively depressed in patients with isolated superficial venous insufficiency compared to healthy age-matched controls (Klonizakis *et al.*, 2003), and that venous surgery has no effect on microvascular endothelial function (Klonizakis *et al.*, 2006). Vascular integrity in the healthy endothelium is maintained through the release of a variety of paracrine factors, such as NO. In venous disease, venous stasis in the microcirculation reduces the shear rate on the endothelial cells resulting in a reduction in cellular levels of NO (Naoum *et al.*, 2007). This favours leucocyte adhesion and neutrophil and monocyte activation, which results in endothelial injury and dysfunction in lower-limb microvessels (Smith, 2006). In post-surgical varicose vein patients, there are high recurrence rates for varicose veins (Winterborn *et al.*, 2004) and venous ulcers (Nelzen and Fransson, 2007). A contributory factor for the latter might be microvascular endothelial dysfunction that persists after surgery. Therefore, it appears important to develop strategies to improve microvascular endothelial function in this population.

In contrast, the endothelium-independent responses to SNP in the standing position were generally higher in patients than controls, and significantly so for the 3 mC dose (Figure 10.3 lower panel). These findings are consistent with previous reports that microvascular endothelial-independent function in the standing position is similar between varicose-vein patients and age-matched healthy controls (Klonizakis *et al.*, 2003), and that varicose-vein surgery enhances cutaneous responsiveness to SNP (Klonizakis *et al.*, 2006). The higher SNP responses of patients in the standing position are consistent with a reduction in the venoarteriolar reflex after surgery, i.e. less activation of local neurovascular pathways that cause vascular smooth muscle contraction and oppose the effects of SNP. A reduction in the venoarteriolar reflex might be explained by differences in venous pressure as a result of surgery, or by changes in the sensory or effector arms of the reflex pathway, including down-regulation of cutaneous vasoconstrictor mechanisms (Klonizakis *et al.*, 2006). Extracellular matrix remodelling is one of several mechanisms that might affect the balance of vasodilator and vasoconstrictor responses of the blood vessel wall (Kowalewski *et al.*, 2004). Alternatively, the lower SNP responsiveness in controls might be explained by a relative nitrate tolerance compared to patients (Laursen *et al.*, 1996).

Effects of acute treadmill exercise on peak cutaneous flux responses

After a 25-min moderate-intensity treadmill walk, the peak cutaneous flux responses to ACh were increased in patients in both the supine and standing positions (Figure 10.4 upper panel). This is the first study to report an improvement in microvascular endothelial function after acute lower-limb exercise in patients who have recently undergone surgical varicose-vein treatment. The moderate-to-large effect sizes that were observed in the supine (Cohen's d = 0.56) and standing positions (Cohen's d = 0.83) suggests that these changes could potentially be clinically meaningful with respect to risk of venous ulceration. No such changes were observed in the control group (Figure 10.4 lower panel), which is consistent with some (Rossi *et al.*, 2002; Colberg *et al.*, 2006), but not all (Kvernmo *et al.*, 1998), previous studies. As a result, the impairment of microvascular endothelial vasodilator function that was observed in the patients before exercise was abolished by acute lower-limb exercise, such that there was no difference in the peak perfusion responses to ACh between patients and controls. Finally, there was no effect of acute exercise on SNP responsiveness in either group or position, which is consistent with the literature (Kvernmo *et al.* 1998; Rossi *et al.*, 2002). These findings suggest that moderate-intensity lower-limb aerobic exercise is an effective stimulus for improving microvascular endothelial function in post-surgical varicose-vein patients; however, further research is needed to clarify the clinical relevance of these findings, i.e. whether chronic exercise training reduces recurrence rates for venous ulcers.

Various mechanisms might explain the increased microvascular endothelial function after acute exercise in the patient group. Firstly, during exercise, increased microcirculatory flow and the corresponding increase in shear stress to the vessel walls are stimuli that elicit endothelial-dependent vasodilation (Green *et al.*, 2004). Mechanical alteration/deformation of the endothelium during exercise as a result of increased pulsatile flow could also contribute to increased release of endothelium-derived hyperpolarising factor (EDHF) and endothelial NO synthase upregulation (Green *et al.*, 2004). Therefore, increased cutaneous blood flow during exercise might alter endothelial function through increased EDHF and NO bioavailability. Another potential mechanism is reduced sympathetic nervous system (SNS) activity. Neurogenic sympathetic regulation is one of the main mechanisms responsible for maintenance of peripheral vessel tone (Krupatkin, 2006). Given that SNS activity is decreased for

several hours after a bout of exercise (Pober *et al.*, 2004), it is possible that the observed improvements in microvascular endothelial vasodilator function are partly explained by attenuation of sympathetic outflow. Further research is needed to clarify the exact role of each of these potential mechanisms.

Limitations

Although we questioned participants about their health status, we did not take any measures of body fatness, blood pressure, blood cholesterol, or hormonal status. Therefore, it is possible that these confounding influences on microvascular function might have impacted upon the results of this study. Another limitation was that we did not measure blood pressure or maximal perfusion responses during the microvascular function test. This prevented us from presenting data as cutaneous vascular conductance normalised to maximal perfusion, which Cracowski *et al.* (2006) believe is the optimal method of data presentation in laser Doppler studies. However, as autoregulatory processes (e.g. the myogenic response) exist to maintain a relatively constant microcirculatory flow (Schubert and Mulvany, 1999), changes in brachial artery blood pressure are unlikely to be representative of changes in microcirculatory blood pressure. We did not measure maximal perfusion because this is usually achieved through sub-dermal infusion of SNP and/or by local heating at 42 to 44 °C for 30 min (Cracowski *et al.*, 2006), neither of which were appropriate to our acute exercise experimental model. Nevertheless, we believe that this does not detract from our findings in any way. Another potential limitation is that the relative intensity of exercise for each group was unknown. Therefore, the contrasting effects of acute exercise on microvascular responsiveness might be due to group differences in the relative intensity of exercise. However, this is unlikely given that absolute walking speeds were similar and that participants were well-matched for age, body mass, stature, and activity status. Finally, further research with post-surgical varicose-vein patients would be needed to clarify the duration for which microvascular endothelial function is enhanced after acute exercise, and the effects of exercise characteristics (i.e. mode, intensity, duration) on microvascular function.

In summary, we observed an impairment of microvascular endothelial function in the gaiter area of post-surgical varicose-vein patients in the standing position which was abolished after a 25-min moderate-intensity treadmill walk. Although, the mechanisms

of improved microvascular endothelial function after acute exercise are unclear, they are likely to be associated with enhanced NO bioavailability and/or attenuation of SNS outflow. Further research on the acute and chronic effects of exercise for post-surgical varicose-vein patients is warranted to establish the clinical relevance of these findings.

Chapter 11: <u>Study 4</u> - Exercise training improves cutaneous microvascular endothelial function in post-surgical varicose-vein patients

11.1 INTRODUCTION

Chronic venous disease with skin changes of the leg is a common condition affecting up to 1 in 20 people in Western societies (Smith, 2006). Venous dilatation and valve damage allows reflux of blood and increased venous pressures (Eberhardt and Raffetto, 2005). Ambulatory venous hypertension causes microcirculatory changes that underlie many, and possibly all, of the clinical manifestations of chronic venous disease, including eczema, lipodermatosclerosis, and ulceration (Iabichella *et al.*, 2006; Bergan *et al.*, 2008).

Patients with chronic venous disease often benefit from venous surgery, e.g. saphenofemoral ligation with stripping of the long saphenous vein (Campbell, 2006). Venous surgery typically results in correction of venous hypertension, symptomatic relief and facilitated ulcer healing (Howard *et al.*, 2008). Despite these benefits, the recurrence rates for varicose veins (62% over an 11-yr period; Winterborn *et al.*, 2004) and venous ulcers (18% over a 5-yr period; Nelzen and Fransson, 2007) are high. A contributory factor could be microvascular endothelial dysfunction that persists after surgery (Klonizakis *et al.*, 2003, 2006). Therefore, it appears important to identify strategies to improve microvascular endothelial function in this population.

Regular exercise training has been found to enhance microvascular endothelial function in diabetics (Colberg *et al.*, 2002) and older sedentary adults (Black *et al.*, 2008). Study 1 also provided evidence that acute moderate-intensity walking exercise augments microvascular endothelial function in post-surgical varicose-vein patients. However, no studies have described the effects of chronic exercise training on microvascular function in this patient group. Hence, the aim of this study was to investigate the effects moderate-intensity exercise training on microvascular vasodilator function in post-surgical varicose-vein patients. We hypothesised that microvascular endothelial-dependent vasodilator function would be improved after exercise-training.

11.2 METHODS

A detailed description of the methods used in this study is provided in Chapter 9.

Participants

Sixteen post-surgical varicose-vein patients were recruited from the Sheffield Vascular Institute at the Northern General Hospital, Sheffield, UK. They were randomly allocated either to a treadmill-walking exercise group or a non-exercise control group. The exercise group trained twice weekly for eight weeks. The groups were well-balanced for demographic variables (Table 11.1).

Table 11.1 Demographic data

	Exercise group	Control group	*P*
Sex	1 male, 7 female	1 male, 7 female	1.000[a]
Age (y)	57 ± 9	51 ± 9	0.949[b]
Body mass (kg)	71.8 ± 12.2	81.4 ± 10.4	0.652[b]
Stature (cm)	168.2 ± 5.0	166.2 ± 8.9	0.141[b]

Values are means ± SD. [a]Chi-squared tests, [b]independent *t*-test

Outcome measures

Cutaneous microvascular function of the gaiter area was assessed at baseline and 8 weeks using laser Doppler fluximetry combined with incremental-dose, iontophoretic administration of ACh and SNP.

Statistical analyses

Mixed-model (group-by-time) analyses of covariance were used to detect changes in outcome measures between groups. Statistical significance was set at $P \leq 0.05$. Effect sizes (Cohen's *d*) were calculated for the exercise group data, with 0.2, 0.5 and 0.8 representing small, medium and large effects, respectively.

11.3 RESULTS

Compliance to the twice weekly exercise sessions was 100% and there were no drop-outs or exercise-related complications. The average % predicted maximum heart rate and RPE at the end of each training session were 81.3 ± 4.6% and 11.5 ± 1.5, respectively.

Incremental administration of ACh and SNP produced dose-dependent increases in cutaneous flux for both groups and both body positions ($P < 0.001$). Resting (unstimulated) cutaneous flux for each body position, probe site (ACh or SNP) and time point, was not different between groups ($P > 0.05$). For example, resting cutaneous flux for the ACh probe in the supine position, prior to exercise training was 6 ± 3 PU in the exercise group versus 7 ± 5 PU in the control group ($P = 0.449$). Furthermore, there were no group differences at baseline in the cutaneous flux responses to ACh or SNP in either body position ($P > 0.05$), and posture had no effect on microvascular function in either group ($P > 0.05$).

At eight weeks, peak responses to ACh in the supine position were increased in the exercise group ($P = 0.03$, $d = 0.63$), with a similar trend in the standing position ($P = 0.08$, $d = 1.37$) (Figure 11.1A). There were no such changes in the control group ($P > 0.05$; Figure 11.1B). Peak cutaneous flux responses to SNP were unchanged in both groups and both body positions ($P = 0.07$; Figure 11.2).

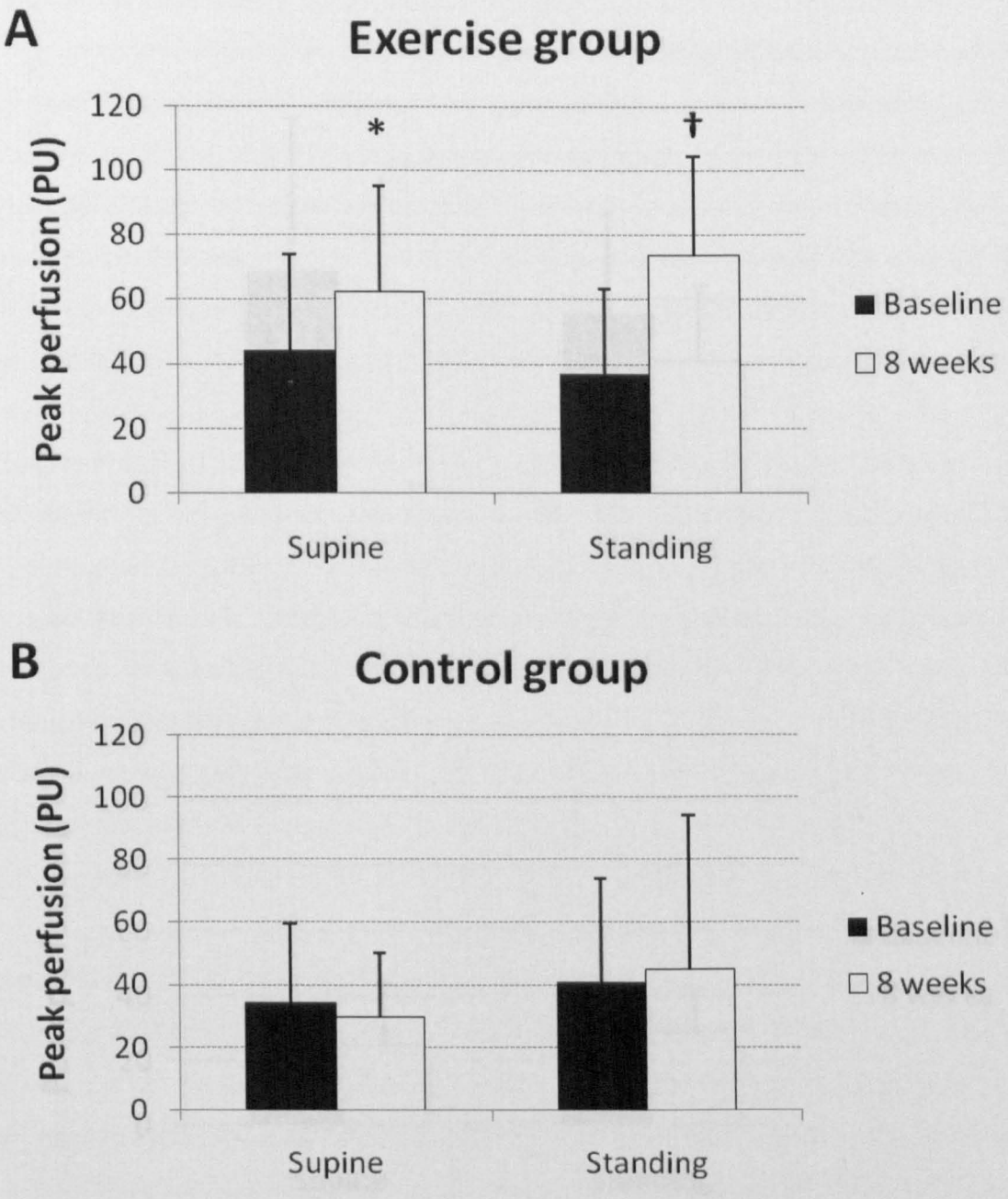

Figure 11.1 Comparison of peak cutaneous flux responses to ACh in A, the exercise group, and B, the control group, at baseline and eight weeks. Data are presented as means + SD. *$P = 0.03$; †$P = 0.08$.

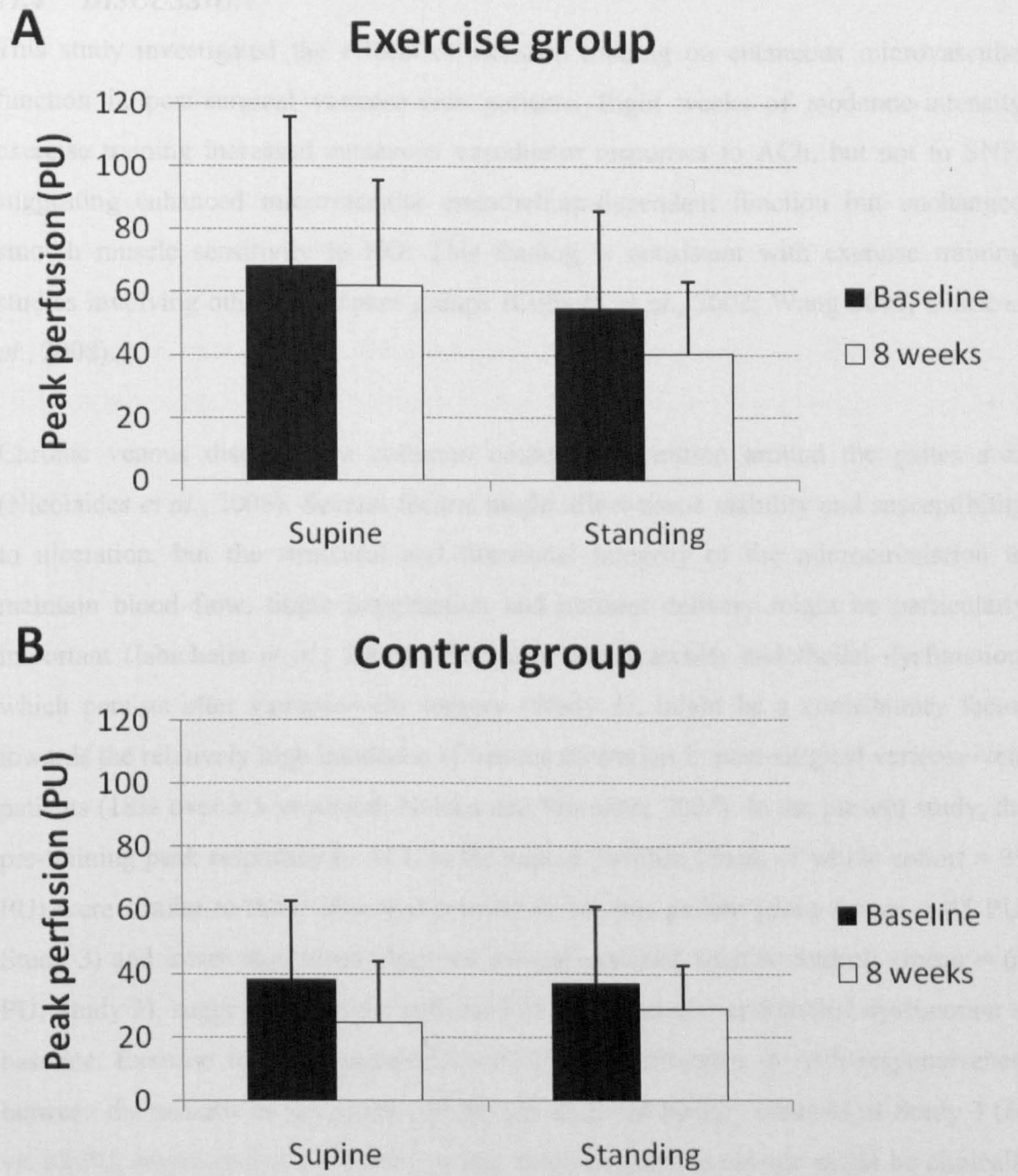

Figure 11.2 Comparison of peak cutaneous flux responses to SNP in A, the exercise group, and B, the control group, at baseline and eight weeks. Data are presented as means + SD. $P > 0.05$ throughout.

11.4 DISCUSSION

This study investigated the effects of exercise training on cutaneous microvascular function in post-surgical varicose-vein patients. Eight weeks of moderate-intensity exercise training increased cutaneous vasodilator responses to ACh, but not to SNP, suggesting enhanced microvascular endothelium-dependent function but unchanged smooth muscle sensitivity to NO. This finding is consistent with exercise training studies involving other participant groups (Colberg *et al.*, 2002; Wang 2005; Black *et al.*, 2008).

Chronic venous disease is a common cause of ulceration around the gaiter area (Nicolaides *et al.*, 2008). Several factors might affect tissue viability and susceptibility to ulceration, but the structural and functional integrity of the microcirculation to maintain blood flow, tissue oxygenation and nutrient delivery might be particularly important (Iabichella *et al.*, 2006). Therefore, microvascular endothelial dysfunction, which persists after varicose-vein surgery (Study 3), might be a contributory factor towards the relatively high incidence of venous ulceration in post-surgical varicose-vein patients (18% over a 5-yr period; Nelzen and Fransson, 2007). In the present study, the pre-training peak responses to ACh in the supine position (mean of whole cohort = 39 PU) were similar to those observed previously for this patient group (mean = 45 PU; Study 3) and lower than those observed for age-matched healthy controls (mean = 63 PU; Study 3), suggesting that the patients had microvascular endothelial dysfunction at baseline. Exercise training appeared to abolish the difference in ACh-responsiveness between the patients in this study and the age-matched healthy controls of Study 3 (62 vs. 63 PU, respectively; $P = 0.841$), which suggests that this change might be clinically meaningful with respect to risk of venous ulceration. Indeed, the moderate-to-large effect sizes for the ACh data (Cohen's $d = 0.63$ and 1.37 for the supine and standing positions, respectively) support this interpretation. However, a longer-term study with clinical end-points is needed to clarify this.

The mechanisms responsible for the improvement in ACh-responsiveness remain unclear, although several possibilities exist. It is generally accepted that a large part of the ACh response in microvessels is NO-dependent, whilst other mediators such as prostanoids, endothelium-derived hyperpolarising factor, and sensory nerves might also contribute (Berghoff *et al.*, 2002; Black *et al.*, 2008). Therefore, a change in ACh-

responsiveness could occur via an alteration of any/all of these mediators. An increase in NO bioavailability might be a particularly plausible contributory factor to the improved ACh-responsiveness given that exercise training has been shown to increase the NO-component of ACh-induced vasodilation in previously sedentary, healthy older adults (Black *et al.*, 2008). During exercise, increased microcirculatory flow and the corresponding increase in shear and/or circumferential stress to the vessel walls are stimuli that could lead to enhanced NO bioavailability via endothelial NO synthase upregulation (Green *et al.*, 2004). Alternatively, exercise training might increase NO bioavailability via reduced production of free radical species (Leeuwenburgh and Heinecke, 2001) and/or enhanced anti-oxidant defences (Sen, 1995). An attenuation of sympathetic outflow and an enhancement of lipid/lipoprotein profiles are other potential mechanisms of increased ACh-responsiveness that cannot be excluded on the basis of our data. Further research is needed to clarify the exact role of each of these potential mechanisms.

Limitations

Given that the effects of vasoactive substances on vascular tone/blood flow depend on a balance between vasodilators and vasoconstrictors (Thijssen *et al.*, 2008), and that we only assessed changes in microvascular responses to vasodilators (ACh and SNP), we cannot exclude the possibility that attenuation of vasoconstrictor pathways contributed to the increase in ACh-induced vasodilation after exercise training. Furthermore, as we did not take any measures of body fatness, blood pressure, blood cholesterol, or hormonal status, it is also possible that these confounding influences on microvascular function might have impacted upon the results of this study. In addition, a limitation with iontophoretic drug delivery is that non-specific microvascular responses, associated with drug concentration and charge and vehicle characteristics, are common with most protocols used (Droog *et al.*, 2004). Although we cannot exclude the possibility that our protocol elicited non-specific vasodilatory effects, the use of 10 $g \cdot l^{-1}$ drug concentrations and currents ≤ 0.3 mA should have at least minimised such effects (Droog *et al.*, 2004). In addition, as the protocol was consistent between groups, it is unlikely that any observed differences/changes in microvascular responses are due to non-specific, protocol-related vasodilatory effects. Finally, despite careful probe positioning, it is likely our microvascular measures were taken from different areas of skin pre- and post-training. Nevertheless, our randomised controlled design provides

reassurance that the observed changes are real and not an artefact of this experimental limitation, as it could be expected that experimental error would be similar between the groups.

In summary, the results suggest that moderate-intensity exercise training improves microvascular endothelial vasodilator function in post-surgical varicose-vein patients. Attenuation of microvascular abnormalities might be important for reducing the risk of venous ulceration in this patient group. However, further research is required to substantiate this and to explore the feasibility of more wide-spread exercise rehabilitation in the clinical setting for this patient group.

Chapter 12: Summary and future research

Two research studies were described in Section 2 of this thesis. The purpose of these studies was to investigate if post-surgical varicose-vein patients have cutaneous microvascular dysfunction compared with age-matched healthy controls and to see if any impairment of cutaneous microvascular function could be attenuated by either acute or chronic moderate-intensity treadmill-walking exercise.

The principle findings of these two studies were as follows: (i) The baseline (pre-acute/chronic exercise) peak cutaneous flux responses to the endothelium-dependent vasodilator ACh, in the standing position, were lower in post-surgical varicose-vein patients than age-matched healthy controls; (ii) the baseline (pre-acute/chronic exercise) cutaneous flux responses to the endothelium-independent vasodilator SNP, in the standing position, were generally higher in patients than in controls; (iii) acute moderate-intensity exercise improved microvascular endothelial function in patients in both the supine and standing positions; (iv) 8-weeks of moderate-intensity exercise training improved microvascular endothelial function in patients in the supine position with a similar trend in the standing position; and, (v) both acute and chronic moderate-intensity exercise had no effect on microvascular endothelium-independent function.

Given that microvascular endothelial dysfunction is a risk factor for venous ulceration, the results of these studies suggest that moderate-intensity treadmill-walking exercise training is a useful adjunct therapy for post-surgical varicose-vein patients.

Future research

Further research is needed to identify the exact mechanisms of improved microvascular endothelial function after both acute and chronic exercise, to assess the effects of longer-term exercise training on varicose vein and venous ulcer formation/recurrence, and to clarify the association between microvascular reactivity and venous ulceration. In addition, further research is needed to clarify the duration for which microvascular endothelial function is enhanced after exercise training in post-surgical varicose-vein patients, and the effects of exercise characteristics (i.e. mode, intensity, duration) on microvascular function. Regarding the latter, it would be particularly useful to investigate the efficacy of alternative exercise modalities, such as arm-cranking, given

that some patients might have lower-limb problems that limit their walking ability. It might also be interesting to assess changes in both vasodilatory and vasoconstrictory function after a period of exercise training.

Although physical activity is usually recommended for patients with chronic venous disease, evidence of effectiveness is lacking. Thus, it seems important to assess the effects of different exercise training programmes on different markers of disease status in patients in different clinical classes. Further studies are also required to explore the feasibility and cost-effectiveness of wide-spread exercise rehabilitation in patients with chronic venous disease.

Chapter 13: Thesis summary and conclusions

This chapter provides a summary and conclusions for the thesis as a whole and, for the purposes of clarity, is sub-divided into three sections: overview of findings and contribution to knowledge; exercise for patients with peripheral vascular disease - take-home messages; and, implications for clinical practice.

Overview of findings and contribution to knowledge

As the primary determinants of walking impairment in patients with intermittent claudication are poorly understood, the main aim of Study 1 was to identify key physiological predictors of MWD in these patients using multiple regression analysis. The final regression model comprised peak $\dot{V}O_2$, StO_2 at 1 min, and time-to-minimum StO_2, and explained 64% of the variance in MWD. The results suggest that cardiopulmonary fitness and the ability to match skeletal muscle microvascular blood flow to metabolic demand are important determinants of walking performance, and that measures made during exercise (e.g., StO_2) generally have a greater explanatory value than those made at rest (e.g., resting ABI). The findings also suggest that specific NIRS variables might be useful in studies that are aimed at evaluating the efficacy of clinical interventions, including exercise training, pharmacological therapy, or limb revascularisation. Although previous studies had described univariate relationships between walking performance and various haemodynamic, NIRS, and cardiopulmonary fitness measurements in claudicants, none had used multiple regression analysis to investigate which combination of these variables explains most of the variation in walking performance in the same cohort of patients. Another novel aspect of this study was the characterisation of ventilatory threshold for a representative group of male claudicants.

Study 2 built on previous research that had shown arm-crank exercise training to be useful for improving walking performance in patients with intermittent claudication (Walker *et al.*, 2000; Zwierska *et al.*, 2005). As the mechanisms of such an improvement were poorly understood, the main aim of this study was to investigate the effects of arm-crank exercise training on lower-limb O_2 delivery in these patients. Arm-crank exercise training induced favourable changes in various NIRS (time-to-minimum StO_2 and sub-maximal StO_2) and cardiopulmonary fitness (peak $\dot{V}O_2$ and $\dot{V}O_2$ kinetics)

variables assessed during treadmill walking exercise, thus providing evidence of enhanced lower-limb O_2 delivery. This is the first study to demonstrate that there is a physiological basis for the improvement in walking performance after arm-crank exercise training in patients with intermittent claudication.

In Part 2 of this thesis, I described two studies (Studies 3 and 4) involving post-surgical varicose-vein patients. Given that cutaneous microvascular function after varicose-vein surgery was poorly characterised, the purpose of Study 3 was to compare cutaneous microvascular function between post-surgical varicose-vein patients and age-matched healthy controls. A secondary aim was to investigate whether or not any impairment of function could be alleviated by acute lower-limb exercise. Before exercise, patients generally had depressed skin vasodilator responses to iontophoretically-administered ACh compared to controls, suggesting that they had microvascular endothelial dysfunction. Acute walking exercise abolished this difference suggesting that lower-limb aerobic exercise training might be a useful intervention for reducing the risk of venous ulceration in this patient group. This is the first study to characterise lower-limb cutaneous microvascular function in post-surgical varicose-vein patients and the first to demonstrate an augmentation in ACh-responsiveness in these patients after acute lower-limb exercise.

The purpose of Study 4 was to investigate the effects of moderate-intensity exercise training on cutaneous microvascular function in post-surgical varicose-vein patients. Consistent with Study 3, exercise training resulted in a moderate-to-large improvement in microvascular endothelial function, with no change in vascular smooth muscle sensitivity to NO (SNP-responsiveness). This is a novel finding and lends further support to the notion that lower-limb aerobic exercise training might be a useful intervention for reducing the risk of venous ulceration in this patient group.

Exercise for patients with peripheral vascular disease: take-home messages

The findings from Study 2 indicate that moderate-intensity arm-crank exercise training improves walking performance in patients with intermittent claudication. The changes in walking distances were about half the magnitude of those previously reported after the same duration of treadmill-walking exercise training (Keo *et al.*, 2008). Therefore, although arm-cranking appears useful, it is probably not the optimal mode of exercise

for improving functional capacity in these patients. However, this is not to say that arm-cranking should be avoided in claudication exercise programmes. Indeed, arm-cranking might be useful for patients who are unwilling or unable to perform walking exercise because of the pain encountered. It might also facilitate adherence to an exercise programme by providing variety. Unfortunately, a major problem with arm-crank exercise training is that arm-crank ergometers are expensive and many exercise centres do not have them.

Although exercise training is generally recommended for patients with chronic venous disease, evidence of effectiveness is lacking and provision of specific advice is sporadic (Michaels *et al.*, 2006b). As discussed in Chapter 8, exercise training might facilitate reduced pain and ulcer healing in patients with advanced chronic venous disease and prevent varicose vein and venous ulcer formation in at-risk individuals. Relevant beneficial physiological effects of exercise training might include improved calf muscle pump function, weight loss/management, enhanced ankle range of motion, and attenuation of microvascular endothelial dysfunction. The findings from Part 2 of this thesis relate to the latter of these and suggest that regular walking exercise might reduce the risk of venous ulceration in post-surgical varicose-vein patients via attenuation of microvascular endothelial dysfunction. Although I did not compare different modes of exercise, I believe that walking should be the main exercise of choice for these patients, as well as for those in the other stages of chronic venous disease. This is because walking is probably the most appropriate exercise for maintaining/developing good calf muscle pump function, for evoking lower-limb blood flow conditions that will stimulate favourable vascular adaptations (e.g. improved microvascular endothelial function), and for providing a whole-body stimulus for improved cardiopulmonary fitness. Furthermore, walking is a cheap, accessible and practical mode of exercise. More research is clearly needed in the area of exercise training for chronic-venous-disease patients.

Implications for clinical practice

I believe that there are two main implications for clinical practice from Part 1 of this thesis. Firstly, I believe that the exercise guidelines described in management documents for PAD (e.g., Hirsch *et al.*, 2006; Norgren *et al.*, 2007) need updating to reflect the beneficial effects of arm-cranking and other, non-traditional modes of

exercise (e.g., cycling, rowing, pole-striding). At present, management guidelines typically only recommend walking exercise and do not acknowledge that many claudicants might refrain from such activity because of the pain experienced. Secondly, I think that supervised exercise should be made available as part of the initial treatment for all patients with PAD. There is evidence that only around one quarter of vascular surgeons in the UK have access to supervised exercise (Stewart and Lamont, 2001). Although the offer of information about exercise either verbally or via leaflet is commended, this has been shown as inferior to supervised exercise (Bendermacher *et al.*, 2006). Indeed, the control patients in Study 2, who were informed about the benefits of regular exercise training, showed no changes in walking performance, cardiopulmonary fitness or physical activitiy status at 12 weeks.

Relating to Part 2 of this thesis, vascular surgeons need to recognise that although venous surgery typically corrects large-blood-vessel (venous) abnormalities, microvascular problems (i.e., microvascular endothelial dysfunction) often persist. Therefore, to reduce the risk of venous ulceration, additional steps need to be taken to attenuate microvascular endothelial dysfunction in post-surgical venous-disease patients. Regular supervised walking exercise might be useful (Studies 3 and 4), and probably more so than unsupervised walking or other modes of exercise. I believe that the development of clear exercise guidelines and pathways to supervised exercise for these patients would likely improve their clinical management markedly.

References

Abramson, J.H., Hopp, C. and Eptein, L.M. (1981). The epidemiology of varicose veins. A survey in western Jerusalem. *Journal of Epidemiology and Community Health*, **35**, 213-217.

Aburahma, A. F., Robinson, P. A. and Boland, J. P. (1991). Clinical, hemodynamic, and anatomic predictors of long-term outcome of lower extremity venovenous bypasses. *Journal of Vascular Surgery*, **14**, 635-644.

Adam, D.J., Beard, J.D., Cleveland, T., Bell, J., Bradbury, A.W., Forbes, J.F., Fowkes, F.G., Gillepsie, I., Ruckley, C.V., Raab, G. and Storkey, H. (2005). Bypass versus angioplasty in severe ischaemia of the leg (BASIL): Multicentre, randomised controlled trial. *Lancet*, **366**, 1925-1934.

Adamopoulos, S., Parissis, J., Kroupis, C., Georgiadis, M., Karatzas, D., Karavolias, G., Koniavitou, K., Coats, A.J. and Kremastinos, D.T. (2001). Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. *European Heart Journal*, **22**, 791-797.

Afaq, A., Montgomery, P.S., Scott, K.J., Blevins, S.M., Whitsett, T.L. and Gardner, A.W. (2007). The effect of current cigarette smoking on calf muscle hemoglobin oxygen saturation in patients with intermittent claudication. *Vascular Medicine*, **12**, 167-173.

Agren, M.S., Eaglstein, W.H., Ferguson, M.W., Harding, K.G., Moore, K., Saarialho-Kere, U.K. and Schultz, G.S. (2000). Causes and effects of the chronic inflammation in venous leg ulcers. *Acta Dermato-Venereologica*, **210**, 3-17.

Ali, A., Mehra, M.R., Lavie, C.J., Malik, F.S., Murgo, J.P., Lohmann, T.P., Li, S., Lin, H.C. and Milani, R.V. (1998). Modulatory impact of cardiac rehabilitation on hyperhomocysteinemia in patients with coronary artery disease and "normal" lipid levels. *The American Journal of Cardiology*, **82**, 1543-5.

Almahameed, A. and Bhatt, D.L. (2006). Contemporary management of peripheral arterial disease: III. endovascular and surgical management. *Cleveland Clinic Journal of Medicine*, **73**, S45-51.

Alpert, J.S., Larsen, O.A. and Lassen, N.A. (1969). Exercise and intermittent claudication. Blood flow in the calf muscle during walking studied by the xenon-133 clearance method. *Circulation*, **39**, 353-359.

Ambrose, J.A. and Barua, R.S. (2004). The pathophysiology of cigarette smoking and cardiovascular disease: An update. *Journal of the American College of Cardiology*, **43**, 1731-1737.

American College of Sports Medicine (2000). *Guidelines for exercise testing and prescription*, 6th ed. Lippincott, Williams & Wilkins, Maryland, USA.

Andreozzi, G.M., Leone, A., Laudani, R., Deinite, G. and Martini, R. (2007). Acute impairment of the endothelial function by maximal treadmill exercise in patients with intermittent claudication, and its improvement after supervised physical training. *International Angiology*, **26**, 12-17.

Antithrombotic Trialists' Collaboration (2002). Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *British Medical Journal*, **324**, 71-86.

Aquino, R., Johnnides, C., Makaroun, M., Whittle, J.C., Muluk, V.S., Kelley, M.E. and Muluk, S.C. (2001). Natural history of claudication: Long-term serial follow-up study of 1244 claudicants. *Journal of Vascular Surgery*, **34**, 962-970.

Aronow, W.S., Nayak, D., Woodworth, S. and Ahn, C. (2003). Effect of simvastatin versus placebo on treadmill exercise time until the onset of intermittent claudication in older patients with peripheral arterial disease at six months and at one year after treatment. *The American Journal of Cardiology*, **92**, 711-712.

Askew, C.D., Green, S., Hou, X.Y. and Walker, P.J. (2002). Physiological and symptomatic responses to cycling and walking in intermittent claudication. *Clinical Physiology and Functional Imaging*, **22**, 348-355.

Askew, C.D., Green, S., Walker, P.J., Kerr, G.K., Green, A.A., Williams, A.D. and Fabbraio, M.A. (2005). Skeletal muscle phenotype is associated with exercise tolerance in patients with peripheral arterial disease. *Journal of Vascular Surgery*, **41**, 802-807.

Astrand, P.O. and Saltin, B. (1961). Maximal oxygen uptake and heart rate in various types of muscular activity. *Journal of Applied Physiology*, **16**, 977-981.

Bainton, D., Sweetnam, P., Baker, I. and Elwood, P. (1994). Peripheral vascular disease: Consequence for survival and association with risk factors in the speedwell prospective heart disease study. *British Heart Journal*, **72**, 128-132.

Baker, J.D. and Dix, D.E. (1981). Variability of Doppler ankle pressures with arterial occlusive disease: An evaluation of ankle index and brachial-ankle pressure gradient. *Surgery*, **89**, 134-137.

Balady, G.J. (1993). Types of exercise: arm-leg and static-dynamic. *Cardiology Clinics*, 11, 297-308.

Barker, G.A., Green, S., Askew, C.D., Green, A.A. and Walker, P.J. (2001). Effect of propionyl-L-carnitine on exercise performance in peripheral arterial disease. *Medicine and Science in Sports and Exercise*, **33**, 1415-1422.

Barrett, J. M., Allen, B., Ockelford, A. and Goldman, M.P. (2004). Microfoam ultrasound-guided sclerotherapy of varicose veins in 100 legs. *Dermatologic Surgery*, **30**, 6-12.

Barstow, T.J., Lamarra, N. and Whipp, B.J. (1990). Modulation of muscle and pulmonary O_2 uptakes by circulatory dynamics during exercise. *Journal of Applied Physiology*, **68**, 979-989.

Bartelink, M.L., Stoffers, H.E., Biesheuvel, C.J. and Hoes, A.W. (2004). Walking exercise in patients with intermittent claudication. Experience in routine clinical practice. *The British Journal of General Practice*, **54**, 196-200.

Bauer, T.A., Brass, E.P., Barstow, T.J. and Hiatt, W.R. (2007). Skeletal muscle StO_2 kinetics are slowed during low work rate calf exercise in peripheral arterial disease. *European Journal of Applied Physiology*, **100**, 143-151.

Bauer, T.A., Brass, E.P., Nehler, M., Barstow, T.J. and Hiatt, W.R. (2004a). Pulmonary VO_2 dynamics during treadmill and arm exercise in peripheral arterial disease. *Journal of Applied Physiology*, **97**, 627-634.

Bauer, T.A., Brass, E.P. and Hiatt, W.R. (2004b). Impaired muscle oxygen use at onset of exercise in peripheral arterial disease. *Journal of Vascular Surgery*, **40**, 488-493.

Bauer, T.A., Regensteiner, J.G., Brass, E.P. and Hiatt, W.R. (1999). Oxygen uptake kinetics during exercise are slowed in patients with peripheral arterial disease. *Journal of Applied Physiology*, **87**, 809-816.

Beaver, W.L., Wasserman, K. and Whipp, B.J. (1986). A new method for detecting anaerobic threshold by gas exchange. *Journal of Applied Physiology*, **60**, 2020-2027.

Beebe-Dimmer, J.L., Pfeifer, J.R., Engle, J.S. and Schottenfeld, D. (2005). The epidemiology of chronic venous insufficiency and varicose veins. *Annals of Epidemiology*, **15**, 175-184.

Belentsov, S.M. (2007). The use of echo-foam-sclerotherapy for pathological refluxes in patients with lower limb varicose veins: Early and long-term results. *Angiology and Vascular Surgery*, **13**, 57-60.

Bendermacher, B.L., Willigendael, E.M., Teijink, J.A. and Prins, M.H. (2006). Supervised exercise therapy versus non-supervised exercise therapy for intermittent claudication. *Cochrane Reviews*, CD005263.

Berard, A., Abenhaim, L., Platt, R., Kahn, S.R. and Steinmetz, O. (2002). Risk factors for the first-time development of venous ulcers of the lower limbs: The influence of heredity and physical activity. *Angiology*, **53**, 647-657.

Bergan, J.J., Pascarella, L. and Schmid-Schonbein, G.W. (2008). Pathogenesis of primary chronic venous disease: Insights from animal models of venous hypertension. *Journal of Vascular Surgery*, **47**, 183-192.

Berger, N.J., Tolfrey, K., Williams, A.G. and Jones, A.M. (2006). Influence of continuous and interval training on oxygen uptake on-kinetics. *Medicine and Science in Sports and Exercise*, **38**, 504-512.

Bergh, U., Kanstrup, I.L. and Ekblom, B. (1976). Maximal oxygen uptake during exercise with various combinations of arm and leg work. *Journal of Applied Physiology*, **41**, 191-196.

Berghoff, M., Kathpal, M., Kilo, S., Hilz, M.J. and Freeman, R. (2002). Vascular and neural mechanisms of ACh-mediated vasodilation in the forearm cutaneous microcirculation. *Journal of Applied Physiology*, **92**, 780-788.

Bergqvist, D. and Jaroszewski, H. (1986). Deep vein thrombosis in patients with superficial thrombophlebitis of the leg. *British Medical Journal*, **292**, 658-659.

Berliner, E., Ozbilgin, B. and Zarin, D.A. (2003). A systematic review of pneumatic compression for treatment of chronic venous insufficiency and venous ulcers. *Journal of Vascular Surgery*, **37**, 539-544.

Bianchi, C., Ballard, J.L., Abou-Zamzam, A.M. and Teruya, T.H. (2003). Subfascial endoscopic perforator vein surgery combined with saphenous vein ablation: Results and critical analysis. *Journal of Vascular Surgery*, **38**, 67-71.

Binnie, A., Perkins, J. and Hands, L. (1999). Exercise and nursing therapy for patients with intermittent claudication. *Journal of Clinical Nursing*, **8**, 190-200.

Black, M.A., Green, D.J. and Cable, N.T. (2008). Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. *The Journal of Physiology*, **586**, 3511-3524.

Borg, G.A.V. (1998). *Borg's rating of perceived exertion and pain scales*. Human Kinetics, Leeds, UK

Bountouroglou, D.G., Azzam, M., Kakkos, S.K., Pathmarajah, M., Young, P. and Geroulakos, G. (2006). Ultrasound-guided foam sclerotherapy combined with sapheno-femoral ligation compared to surgical treatment of varicose veins: Early results of a randomised controlled trial. *European Journal of Vascular and Endovascular Surgery*, **31**, 93-100.

Boushel, R., Langberg, H., Olesen, J., Gonzales-Alonzo, J., Bulow, J. and Kjaer, M. (2001). Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease. *Scandinavian Journal of Medicine and Science in Sports*, **11**, 213-222.

Bradbury, A., Evans, C., Allan, P., Lee, A., Ruckley, C.V. and Fowkes, F.G. (1999). What are the symptoms of varicose veins? Edinburgh Vein Study cross-sectional population survey. *British Medicial Journal*, **318**, 353-356.

Brnad, F.N., Dannenberg, A.L., Abbott, R.D. and Kannel, W.B. (1988). The epidemiology of varicose veins: The Framingham Study. *American Journal of Preventive Medicine*, **4**, 96-101.

Brass, E.P. and Hiatt, W.R. (2000). Acquired skeletal muscle metabolic myopathy in atherosclerotic peripheral arterial disease. *Vascular Medicine*, **5**, 55-59.

Brass, E.P., Hiatt, W.R. and Green, S. (2004). Skeletal muscle metabolic changes in peripheral arterial disease contribute to exercise intolerance: A point-counterpoint discussion. *Vascular Medicine*, **9**, 293-301.

Breek, J.C., De Vries, J. and Hamming, J.F. (2007). The Oslo Balloon Angioplasty versus Conservative Treatment study (OBACT) - The 2-years results of a single centre, prospective, randomised study in patients with intermittent claudication. *European Journal of Vascular and Endovascular Surgery*, **34**, 378.

Brendle, D.C., Joseph, L.J., Corretti, M.C., Gardner, A.W. and Katzel, L.I. (2001). Effects of exercise rehabilitation on endothelial reactivity in older patients with peripheral arterial disease. *The American Journal of Cardiology*, **87**, 324-329.

Brevetti, G., Silvestro, A., Di Giacomo, S., Bucur, R., Di Donato, A., Schiano, V. and Scopacasa, F. (2003). Endothelial dysfunction in peripheral arterial disease is related to increase in plasma markers of inflammation and severity of peripheral circulatory impairment but not to classic risk factors and atherosclerotic burden. *Journal of Vascular Surgery*, **38**, 374-379.

Brevetti, G., Diehm, C. and Lambert, D. (1999). European multicenter study on propionyl-L-carnitine in intermittent claudication. *Journal of the American College of Cardiology*, **34**, 1618-1624.

Brevetti, G., Perna, S., Sabba, C., Martone, V.D. and Condorelli, M. (1995). Propionyl-L-carnitine in intermittent claudication: Double-blind, placebo-controlled, dose titration, multicenter study. *Journal of the American College of Cardiology*, **26**, 1411-1416.

Brevetti, G., Perna, S., Sabba, C., Rossini, A., Scotto di Uccio, V., Berardi, E. and Godi, L. (1992). Superiority of L-propionylcarnitine vs L-carnitine in improving walking capacity in patients with peripheral vascular disease: An acute, intravenous, double-blind, cross-over study. *European Heart Journal*, **13**, 251-255.

Brooks, B., Dean, R., Patel, S., Wu, B., Molyneaux, L. and Yue, D.K. (2001). TBI or not TBI: That is the question. Is it better to measure toe pressure than ankle pressure in diabetic patients? *Diabetic Medicine*, **18**, 528-532.

Buchwald, H., Bourdages, H.R., Campos, C.T., Nguyen, P., Williams, S.E. and Boen, J.R. (1996). Impact of cholesterol reduction on peripheral arterial disease in the Program on the Surgical Control of the Hyperlipidemias (POSCH). *Surgery*, **120**, 672-679.

Burnand, K.G., Whimster, I., Naidoo, A. and Browse, N.L. (1982). Pericapillary fibrin in the ulcer-bearing skin of the leg: The cause of lipodermatosclerosis and venous ulceration. *British Medical Journal*, **285**, 1071-1072.

Cahalin, L.P., Mathier, M.A., Semigran, M.J., Dec, G.W. and DiSalvo, T.G. (1996). The six-minute walk test predicts peak oxygen uptake and survival in patients with advanced heart failure. *Chest*, **110**, 325-332.

Callam, M.J. (1994). Epidemiology of varicose veins. *The British Journal of Surgery*, **81**, 167-173.

Campbell, B. (2006). Varicose veins and their management. *British Medical Journal*, **333**, 287-292.

Carpentier, P.H. (2000). Epidemiology and physiopathology of chronic venous leg diseases. *La Revue du Praticien*, **50**, 1176-1181.

Casillas, J.M., Gremeaux, V., Damak, S., Feki, A. and Perennou, D. (2007). Exercise training for patients with cardiovascular disease. *Annales de Readaptation et de Medecine Physique*, **50**, 403-18.

Caslake, M.J., Packard, C.J., Suckling, K.E., Holmes, S.D., Chamberlain, P. and Macphee, C.H. (2000). Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase: A potential new risk factor for coronary artery disease. *Atherosclerosis*, **150**, 413-419.

Cassar, K., Bachoo, P. and Brittenden, J. (2003). The effect of peripheral percutaneous transluminal angioplasty on quality of life in patients with intermittent claudication. *European Journal of Vascular and Endovascular Surgery*, **26**, 130-136.

Cassar, K., Bachoo, P., Ford, I., Greaves, M. and Brittenden, J. (2003). Platelet activation is increased in peripheral arterial disease. *Journal of Vascular Surgery*, **38**, 99-103.

Cheatle, T.R., Chittenden, S.J., Coleridge-Smith, P.D. and Scurr, J.H. (1991). The effect on skin blood flow of short-term venous hypertension in normal subjects. *Angiology*, **42**, 114-122.

Cheatle, T.R., Coleridge-Smith, P.D. and Scurr, J.H. (1991). Skin microcirculatory responses in chronic venous insufficiency: The effect of short-term venous hypertension. *Vasa*, **20**, 63-69.

Cheatle, T.R., Chittenden, S.J., Coleridge-Smith, P.D. and Scurr, J.H. (1991). The pathogenesis of skin damage in venous disease: A review. *European Journal of Vascular Surgery*, **5**, 115-123.

Chi, Y.W. and Jaff, M.R. (2008). Optimal risk factor modification and medical management of the patient with peripheral arterial disease. *Catheterization and Cardiovascular Interventions*, **71**, 475-489.

Chien, S., Usami, S., Taylor, H.M., Lundberg, J.L. and Gregersen, M.I. (1966). Effects of hematocrit and plasma proteins on human blood rheology at low shear rates. *Journal of Applied Physiology*, **21**, 81-87.

Chong, P.F., Golledge, J., Greenhalgh, R.M. and Davies, A.H. (2000). Exercise therapy or angioplasty? A summation analysis. *European Journal of Vascular and Endovascular Surgery*, **20**, 4-12.

Cimminiello, C. (2002). PAD. Epidemiology and pathophysiology. *Thrombosis Research*, **106**, V295-301.

Cipolla, M.J., Nicoloff, A., Rebello, T., Amato, A. and Porter, J.M. (1999). Propionyl-L-carnitine dilates human subcutaneous arteries through an endothelium-dependent mechanism. *Journal of Vascular Surgery*, **29**, 1097-1103.

Colberg, S.R., Parson, H.K., Nunnold, T., Holton, D.R. and Vinik, A.I. (2006). Effect of a single bout of prior moderate exercise on cutaneous perfusion in type 2 diabetes. *Diabetes Care*, **29**, 2316-2318.

Colberg, S.R., Stansberry, K.B., McNitt, P.M. and Vinik, A.I. (2002). Chronic exercise is associated with enhanced cutaneous blood flow in type 2 diabetes. *Journal of Diabetes and its Complications*, **16**, 139-145.

Collins, E.G., Langbein, W.E., Orebaugh, C., Bammert, C., Hanson, K., Reda, D., Edwards, L.C. and Littooy, F.N. (2005). Cardiovascular training effect associated with polestriding exercise in patients with peripheral arterial disease. *The Journal of Cardiovascular Nursing*, **20**, 177-185.

Collins, P. and Brittenden, J. (2004). Lipid-lowering therapy in patients with peripheral arterial disease. *Vascular Medicine*, **9**, 303-306.

Comerota, A.J., Throm, R.C., Kelly, P. and Jaff, M. (2003). Tissue (muscle) oxygen saturation (StO2): A new measure of symptomatic lower-extremity arterial disease. *Journal of Vascular Surgery*, **38**, 724-729.

Coon, W.W., Willis, P.W., and Keller, J.B. (1973). Venous thromboembolism and other venous disease in the Tecumseh community health study. *Circulation*, **48**, 839-846.

Corretti, M.C., Anderson, T.J., Benjamin, E.J., Celermajer, D., Charbonneau, F., Creager, M.A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., Vallance, P., Vita, J. and Vogel, R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the international brachial artery reactivity task force. *Journal of the American College of Cardiology*, **39**, 257-265.

Cracowski, J.L., Minson, C.T., Salvat-Melis, M. and Halliwill, J.R. (2006). Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends in Pharmacological Sciences*, **27**, 503-508.

Criqui, M.H., Langer, R.D., Fronek, A., Feigelson, H.S., Klauber, M.R., McCann, T.J. and Browner, D. (1992). Mortality over a period of 10 years in patients with peripheral arterial disease. *The New England Journal of Medicine*, **326**, 381-386.

Criqui, M.H., Fronek, A., Barrett-Connor, E., Klauber, M.R., Gabriel, S. and Goodman, D. (1985). The prevalence of peripheral arterial disease in a defined population. *Circulation*, **71**, 510-515.

Crowther, R.G. Spinks, W.L., Leicht, A.S., Sangla, K., Quigley, F. and Golledge, J. (2008). Effects of a long-term exercise program on lower limb mobility, physiological responses, walking performance, and physical activity levels in patients with peripheral arterial disease. *Journal of Vascular Surgery*, **47**, 303-309.

Da Silva, A., Widmer, L.K., Martin, H., Mall, T., Glaus, L. and Schneider, M. (1974). Varicose veins and chronic venous insufficiency. *Vasa*, **3**, 118-125.

Dakak, N., Husain, S., Mulcahy, D., Andrews, N.P., Panza, J.A., Waclawiw, M., Schenke, W. and Quyyumi, A.A. (1998). Contribution of nitric oxide to reactive hyperemia: Impact of endothelial dysfunction. *Hypertension*, **32**, 9-15.

Danielsson, G., Eklof, B., Grandinetti, A. and Kistner, RL. (2002). The influence of obesity on chronic venous disease. *Vascular and Endovascular Surgery*, **36**, 271-276.

Davies, J.A., Bull, R.H., Farrelly, I.J. and Wakelin, M.J. (2007). A home-based exercise programme improves ankle range of motion in long-term venous ulcer patients. *Phlebology*, **22**, 86-89.

De Backer, T.L., Vander-Stichele, R., Lehert, P. and Van Bortel, L. (2008). Naftidrofuryl for intermittent claudication. *Cochrane Database of Systematic Reviews*, CD001368.

De Medeiros, C.A. and Luccas, G.C. (2005). Comparison of endovenous treatment with an 810 nm laser versus conventional stripping of the great saphenous vein in patients with primary varicose veins. *Dermatologic Surgery*, **31**, 1685-94.

De Vries, S.O. and Hunink, M.G. (1997). Results of aortic bifurcation grafts for aortoiliac occlusive disease: A meta-analysis. *Journal of Vascular Surgery*, **26**, 558-569.

Delis, K.T. (2005). The case for intermittent pneumatic compression of the lower extremity as a novel treatment in arterial claudication. *Perspectives in Vascular Surgery and Endovascular Therapy,* **17**, 29-42.

Delis, K.T. and Nicolaides, A.N. (2005). Effect of intermittent pneumatic compression of foot and calf on walking distance, hemodynamics, and quality of life in patients with arterial claudication: A prospective randomized controlled study with 1-year follow-up. *Annals of Surgery*, **241**, 431-441.

Delis, K.T., Nicolaides, A.N., Wolfe, J.H. and Stansby, G. (2000). Improving walking ability and ankle brachial pressure indices in symptomatic peripheral vascular disease with intermittent pneumatic foot compression: A prospective controlled study with one-year follow-up. *Journal of Vascular Surgery*, **31**, 650-661.

Delorey, D.S., Paterson, D.H. and Kowalchuk, J.M. (2007). Effects of ageing on muscle O_2 utilization and muscle oxygenation during the transition to moderate-intensity exercise. *Applied Physiology, Nutrition, and Metabolism*, **32**, 1251-1262.

Demarle, A.P., Slawinski, J.J., Laffite, L.P., Bocquet, V.G., Koralsztein, J.P. and Billat, V.L. (2001). Decrease of O_2 deficit is a potential factor in increased time to exhaustion after specific endurance training. *Journal of Applied Physiology*, **90**, 947-953.

D'Hooge, D., Lehert, P. and Clement, D.L. (2001). Naftidrofuryl in Quality of Life (NIQOL). A Belgian study. *International Angiology*, **20**, 288-294.

Dormandy, J.A. and Murray, G.D. (1991). The fate of the claudicant--a prospective study of 1969 claudicants. *European Journal of Vascular Surgery*, **5**, 131-133.

Dormandy, J.A. and Rutherford, R.B. (2000). Management of peripheral arterial disease (PAD). TASC working group. TransAtlantic inter-society consensus (TASC). *Journal of Vascular Surgery*, **31**, S1-S296.

Drinkard, B., McDuffie, J., McCann, S., Uwaifo, G.I., Nicholson, J. and Yanovski, J.A. (2001). Relationships between walk/run performance and cardiorespiratory fitness in adolescents who are overweight. *Physical Therapy*, **81**, 1889-1896.

Droog, E.J., Henricson, J., Nilsson, G.E. and Sjoberg, F. (2004). A protocol for iontophoresis of acetylcholine and sodium nitroprusside that minimises nonspecific vasodilatory effects. *Microvascular Research*, **67**, 197-202.

Eberhardt, R.T. and Raffetto, J.D. (2005). Chronic venous insufficiency. *Circulation*, **111**, 2398-2409.

Eklof, B., Rutherford, R.B., Bergan, J.J., Carpentier, P.H., Gloviczki, P., Kistner, R.L., Meissner, M.H., Moneta, G.L., Myers, K., Padberg, F.T., Perrin, M., Ruckley, C.V., Smith, P.C. and Wakefield, T.W. (2004). Revision of the CEAP classification for chronic venous disorders: Consensus statement. *Journal of Vascular Surgery*, **40**, 1248-1252.

Engelke, K.A., Halliwill, J.R., Proctor, D.N., Dietz, N.M. and Joyner, M.J. (1996). Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. *Journal of Applied Physiology*, **81**, 1807-1814.

Ernst, E.E. and Matrai, A. (1987). Intermittent claudication, exercise, and blood rheology. *Circulation*, **76**, 1110-1114.

Fagard, R.H. (1997). Impact of different sports and training on cardiac structure and function. *Cardiology Clinics*, **15**, 397-412.

Fagrell, B. (1982). Microcirculatory disturbances - the final cause for venous leg ulcers? *Vasa*, **11**, 101-103.

Falanga, V. and Eaglstein, W.H. (1986). A therapeutic approach to venous ulcers. *Journal of the American Academy of Dermatology*, **14**, 777-784.

Fassiadis, N., Kianifard, B., Holdstock, J.M. and Whiteley, M.S. (2002). A novel approach to the treatment of recurrent varicose veins. *International Angiology*, **21**, 275-276.

Fassiadis, N., Holdstock, J.M. and Whiteley, M.S. (2003). Endoluminal radiofrequency ablation of the long saphenous vein (VNUS closure) -- a minimally invasive management of varicose veins. *Minimally Invasive Therapy & Allied Technologies*, **12**, 91-94.

Feletou, M. and Vanhoutte, P.M. (1999). The third pathway: Endothelium-dependent hyperpolarization. *Journal of Physiology and Pharmacology*, **50**, 525-534.

Fellmann, N. (1992). Hormonal and plasma volume alterations following endurance exercise. A brief review. *Sports Medicine*, **13**, 37-49.

Ferreira, L.F., Townsend, D.K., Lutjemeier, B.J. and Barstow, T.J. (2005). Muscle capillary blood flow kinetics estimated from pulmonary O_2 uptake and near-infrared spectroscopy. *Journal of Applied Physiology*, **98**, 1820-1828.

Fitts, R.H. (1994). Cellular mechanisms of muscle fatigue. *Physiological Reviews*, **74**, 49-94.

Forlee, M.V., Grouden, M., Moore, D.J. and Shanik, G. (2006). Stroke after varicose vein foam injection sclerotherapy. *Journal of Vascular Surgery,* **43**, 162-164.

Fowkes, F.G. (2001). Epidemiological research on peripheral vascular disease. *Journal of Clinical Epidemiology*, **54**, 863-868.

Fowkes, F.G. (1988). The measurement of atherosclerotic peripheral arterial disease in epidemiological surveys. *International Journal of Epidemiology*, **17**, 248-254.

Fowkes, F.G., Evans, C.J. and Lee, A.J. (2001). Prevalence and risk factors of chronic venous insufficiency. *Angiology*, **52**, S5-15.

Fowkes, F.G., Housley, E., Cawood, E.H., Macintyre, C.C., Ruckley, C.V. and Prescott, R.J. (1991). Edinburgh Artery Study: Prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *International Journal of Epidemiology*, **20**, 384-392.

Franklin, B.A. (1985). Exercise testing, training and arm ergometry. *Sports Medicine*, **2**, 100-119.

Frullini, A. and Cavezzi, A. (2002). Sclerosing foam in the treatment of varicose veins and telangiectases: History and analysis of safety and complications. *Dermatologic Surgery*, **28**, 11-15.

Furchgott, R.F. and Zawadzki, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373-376.

Garcia, L.A. (2006). Epidemiology and pathophysiology of lower extremity peripheral arterial disease. *Journal of Endovascular Therapy*, **13**, 3-9.

Gardner, A.W., Forrester, L. and Smith, G.V. (2001). Altered gait profile in subjects with peripheral arterial disease. *Vascular Medicine*, **6**, 31-34.

Gardner, A.W., Katzel, L.I., Sorkin, J.D., Bradham, D.D., Hochberg, M.C., Flinn, W.R. and Goldberg, A.P. (2001). Exercise rehabilitation improves functional outcomes and peripheral circulation in patients with intermittent claudication: A randomized controlled trial. *Journal of the American Geriatrics Society*, **49**, 755-762.

Gardner, A.W. and Killewich, L.A. (2001). Lack of functional benefits following infrainguinal bypass in peripheral arterial occlusive disease patients. *Vascular Medicine*, **6**, 9-14.

Gardner, A.W., Montgomery, P.S., Flinn, W.R. and Katzel, L.I. (2005). The effect of exercise intensity on the response to exercise rehabilitation in patients with intermittent claudication. *Journal of Vascular Surgery*, **42**, 702-709.

Gardner, A. W., Parker, D.E., Webb, N., Montgomery, P.S., Scott, K.J. and Blevins, S.M. (2008). Calf muscle hemoglobin oxygen saturation characteristics and exercise performance in patients with intermittent claudication. *Journal of Vascular Surgery*, **48**, 644-649.

Gardner, A.W. and Poehlman, E.T. (1995). Exercise rehabilitation programs for the treatment of claudication pain. A meta-analysis. *Journal of the American Medical Association*, **274**, 975-980.

Gardner, A.W., Skinner, J.S., Cantwell, B.W. and Smith, L.K. (1992). Prediction of claudication pain from clinical measurements obtained at rest. *Medicine and Science in Sports and Exercise*, **24**, 163-170.

Gartenmann, Ch, Kirchberger, I., Herzig, M., Baumgartner, I., Saner, H., Mahler, F. and Meyer, K. (2002). Effects of exercise training program on functional capacity and quality of life in patients with peripheral arterial occlusive disease. Evaluation of a pilot project. *Vasa*, **31**, 29-34.

Ghosh, R., Walsh, S.R., Tang, T.Y., Noorani, A. and Hayes, P.D. (2008). Gene therapy as a novel therapeutic option in the treatment of peripheral vascular disease: Systematic review and meta-analysis. *International Journal of Clinical Practice*, **62**, 1383-1390.

Girolami, B., Bernardi, E., Prins, M.H., Ten Cate, J.W., Hettiarachchi, R., Prandoni, P., Girolami, A. and Büller, H.R. (1999). Treatment of intermittent claudication with physical training, smoking cessation, pentoxifylline, or nafronyl: A meta-analysis. *Archives of Internal Medicine*, **159**, 337-345.

Gloviczki, P., Bergan, J.J., Rhodes, J.M., Canton, L.G., Harmsen, S. and Ilstrup, D.M. (1999). Mid-term results of endoscopic perforator vein interruption for chronic venous insufficiency: Lessons learned from the North American subfascial endoscopic perforator surgery registry. The North American study group. *Journal of Vascular Surgery*, **29**, 489-502.

Gohel, M.S., Barwell, J.R., Earnshaw, J.J., Heather, B.P., Mitchell, D.C., Whyman, M.R. and Poskitt, K.R. (2005). Randomized clinical trial of compression plus surgery versus compression alone in chronic venous ulceration (ESCHAR study)--haemodynamic and anatomical changes. *The British Journal of Surgery*, **92**, 291-297.

Gohel, M.S., Barwell, J.R., Taylor, M., Chant, T., Foy, C., Earnshaw, J.J., Heather, B.P., Mitchell, D.C., Whyman, M.R. and Poskitt, K.R. (2007). Long term results of compression therapy alone versus compression plus surgery in chronic venous ulceration (ESCHAR): Randomised controlled trial. *British Medical Journal*, **335**, 83.

Gore, C.J. (2000). *Physiological tests for elite athletes*. Human Kinetics, Champaign, Illinois.

Gowdak, L.H., Poliakova, L., Wang, X., Kovesdi, I., Fishbein, K.W., Zacheo, A., Palumbo, R., Straino, S., Emanueli, C., Marrocco-Trischitta, M., Lakatta, E.G., Anversa, P., Spencer, R.G., Talan, M. and Capogrossi, M.C. (2000). Adenovirus-mediated VEGF(121) gene transfer stimulates angiogenesis in normoperfused skeletal muscle and preserves tissue perfusion after induction of ischemia. *Circulation*, **102**, 565-571.

Grassi, B. (2006). Oxygen uptake kinetics: Why are they so slow and what do they tell us? *Journal of Physiology and Pharmacology*, **57**, 53-65.

Grassi, B., Poole, D.C., Richardson, R.S., Knight, D.R., Erickson, B.K. and Wagner, P.D. (1996). Muscle O_2 uptake kinetics in humans: Implications for metabolic control. *Journal of Applied Physiology*, **80**, 988-998.

Green, D.J., Maiorana,A., O'Driscoll, G. and Taylor, R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *The Journal of Physiology*, **561**, 1-25.

Green, D.J., Maiorana, A.J. and Cable, N.T. (2008). Point: Exercise training does induce vascular adaptations beyond the active muscle beds. *Journal of Applied Physiology*, **105**, 1002-4.

Green, S. (2002). Haemodynamic limitations and exercise performance in peripheral arterial disease. *Clinical Physiology and Functional Imaging*, **22**, 81-91.

Guberan, E., Widmer, L.K., Glaus, L., Muller, R., Rougemont, A., DaSilva, A. and Gendre, F. (1973). Causative factors of varicose veins: Myths and facts. An epidemiological study of 610 women. *Vasa*, **2**, 115-120.

Hamel-Desnos, C., Desnos, P., Wollmann, J.C., Ouvry, P., Mako, S. and Allaert, F.A. (2003). Evaluation of the efficacy of polidocanol in the form of foam compared with liquid form in sclerotherapy of the greater saphenous vein: Initial results. *Dermatologic Surgery*, **29**, 1170-5.

Hanemaaijer, R., Koolwijk, P., le Clercq, L., de Vree, W.J. and van Hinsbergh, V.W. (1993). Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. *The Biochemical Journal*, **296**, 803-809.

Hiatt, W.R. (2004). Carnitine and peripheral arterial disease. *Annals of the New York Academy of Sciences*, **1033**, 92-98.

Hiatt, W.R. (1999). Functional assessment of the claudicant importance of treatment and follow-up strategies. *Minerva Cardioangiologica*, **47**, 385-386.

Hiatt, W.R., Hoag, S. and Hamman, R. F. (1995a). Effect of diagnostic criteria on the prevalence of peripheral arterial disease. The San Luis Valley Diabetes Study. *Circulation*, **91**, 1472-1479.

Hiatt, W.R., Hirsch, A.T., Regensteiner, J.G. and Brass, E.P. (1995b). Clinical trials for claudication. Assessment of exercise performance, functional status, and clinical end points. Vascular Clinical Trialists. *Circulation*, **92**, 614-621.

Hiatt, W.R., Nawaz, D. and Brass, E.P. (1987). Carnitine metabolism during exercise in patients with peripheral vascular disease. *Journal of Applied Physiology*, **62**, 2383-2387.

Hiatt, W.R., Regensteiner, J.G., Creager, M.A., Hirsch, A.T., Cooke, J.P., Olin, J.W., Gorbunov, G.N., Isner, J., Lukjanov, Y.V., Tsitsiashvili, M.S., Zabelskaya, T.F. and Amato, A. (2001). Propionyl-L-carnitine improves exercise performance and functional status in patients with claudication. *The American Journal of Medicine*, **110**, 616-622.

Hiatt, W.R., Regensteiner, J.G., Hargarten, M.E., Wolfel, E.E. and Brass, E.P. (1990). Benefit of exercise conditioning for patients with peripheral arterial disease. *Circulation*, **81**, 602-609.

Hiatt, W.R., Regensteiner, J.G., Wolfel, E.E., Carry, M.R. and Brass, E.P. (1996). Effect of exercise training on skeletal muscle histology and metabolism in peripheral arterial disease. *Journal of Applied Physiology*, **81**, 780-788.

Higashi, Y. and Yoshizumi, M. (2003). New methods to evaluate endothelial function: Method for assessing endothelial function in humans using a strain-gauge plethysmography: Nitric oxide-dependent and -independent vasodilation. *Journal of Pharmacological Sciences*, **93**, 399-404.

Hirai, M., Naiki, K. and Nakayama, R. (1990). Prevalence and risk factors of varicose veins in Japanese women. *Angiology*, **41**, 228-232.

Hirsch, A.T., Haskal, Z.J., Hertzer, N.R., Bakal, C.W., Creager, M.A., Halperin, J.L., Hiratzka, L.F., Murphy, W.R., Olin, J.W., Puschett, J.B., Rosenfield, K.A., Sacks, D., Stanley, J.C., Taylor, L.M., White, C.J., White, J., White, R.A., Antman, E.M., Smith, S.C., Adams, C.D., Anderson, J.L., Faxon, D.P., Fuster, V., Gibbons, R.J., Hunt, S.A., Jacobs, A.K., Nishimura, R., Ornato, J.P., Page, R.L. and Riegel, B. (2006). ACC/AHA 2005 practice guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic). *Circulation*, **113**, e463-654.

Hobbs, J.T. (1974). Surgery and sclerotherapy in the treatment of varicose veins. A random trial. *Archives of Surgery*, **109**, 793-796.

Hojs, N., Strucl, M. and Cankar, K. (2009). The effect of glibenclamide on acetylcholine and sodium nitroprusside induced vasodilatation in human cutaneous microcirculation. *Clinical Physiology and Functional Imaging*, **29**, 38-44.

Holloszy, J.O. and Coyle, E.F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *Journal of Applied Physiology*, **56**, 831-838.

Holm, J., Dahllof, A.G., Bjorntorp, P. and Schersten, T. (1973). Enzyme studies in muscles of patients with intermittent claudication. Effect of training. *Scandinavian Journal of Clinical and Laboratory Investigation*, **128**, 201-205.

Holowatz, L.A., Thompson, C.S., Minson, C.T. and Kenney, W.L. (2005). Mechanisms of acetylcholine-mediated vasodilatation in young and aged human skin. *The Journal of Physiology*, **563**, 965-973.

Holowatz, L.A., Thompson-Torgerson, C.S. and Kenney, W.L. (2008). The human cutaneous circulation as a model of generalized microvascular function. *Journal of Applied Physiology,* **105**, 370-372.

Holtzman, J., Caldwell, M., Walvatne, C. and Kane, R. (1999). Long-term functional status and quality of life after lower extremity revascularization. *Journal of Vascular Surgery*, **29**, 395-402.

Hoppeler, H. (1986). Exercise-induced ultrastructural changes in skeletal muscle. *International Journal of Sports Medicine*, **7**, 187-204.

Howard, D.P., Howard, A., Kothari, A., Wales, L., Guest, M. and Davies, A.H. (2008). The role of superficial venous surgery in the management of venous ulcers: A systematic review. *European Journal of Vascular and Endovascular Surgery*, **36**, 458-465.

Hulens, M., Vnsant, G., Claessens, A.L., Lysens, R. and Muls, E. (2003). Predictors of 6-minute walk test results in lean, obese and morbidly obese women. *Scandinavian Journal of Medicine and Science in Sports*, **13**, 98-105.

Iabichella, M.L., Melillo, E. and Mosti, G. (2006). A review of microvascular measurements in wound healing. *The International Journal of Lower Extremity Wounds*, **5**, 181-199.

Iafrati, M.D., Welch, H., O'Donnell, T.F., Belkin, M., Umphrey, S. and McLaughlin, R. (1994). Correlation of venous noninvasive tests with the Society for Vascular Surgery/International Society for Cardiovascular Surgery clinical classification of chronic venous insufficiency. *Journal of Vascular Surgery*, **19**, 1001-1007.

Iannuzzi, A., Panico, S., Ciardullo, A.V., Bellati, C., Cioffi, V., Iannuzzo, G., Celentano, E., Berrino, F. and Rubba, P. (2002). Varicose veins of the lower limbs and venous capacitance in postmenopausal women: Relationship with obesity. *Journal of Vascular Surgery*, **36**, 965-968.

Izquierdo-Porrera, A.M., Gardner, A.W., Powell, C.C. and Katzel, L.I (2000). Effects of exercise rehabilitation on cardiovascular risk factors in older patients with peripheral arterial occlusive disease. *Journal of Vascular Surgery*, **31**, 670-677.

Jamsen, T., Tulla, H., Manninen, H., Raisanen, H., Lahtinen, S., Aittola, V. and Jaakkola, P. (2001). Results of infrainguinal bypass surgery: An analysis of 263 consecutive operations. *Annales Chirurgiae et Gynaecologiae*, **90**, 92-99.

Johnson, E.C., Voyles, W.F., Atterbom, H.A., Pathak, D., Sutton, M.F. and Greene, E.R. (1989). Effects of exercise training on common femoral artery blood flow in patients with intermittent claudication. *Circulation*, **80**, 59-72.

Jonason, T. and Bergstrom, R. (1987). Cessation of smoking in patients with intermittent claudication. Effects on the risk of peripheral vascular complications, myocardial infarction and mortality. *Acta Medica Scandinavica*, **221**, 253-260.

Jones, A.M. and Poole, D.C. (2005). Oxygen uptake dynamics: From muscle to mouth--an introduction to the symposium. *Medicine and Science in Sports and Exercise*, **37**, 1542-1550.

Jones, S.A., Bowler, P.G., Walker, M. and Parsons, D. (2004). Controlling wound bioburden with a novel silver-containing hydrofiber dressing. *Wound Repair and Regeneration*, **12**, 288-294.

Jost, C.J., Gloviczki, P., Cherry, K.J., McKusick, M.A., Harmsen, W.S., Jenkins, G.D. and Bower, T.C. (2001). Surgical reconstruction of iliofemoral veins and the inferior vena cava for nonmalignant occlusive disease. *Journal of Vascular Surgery*, **33**, 320-7.

Joyner, M.J., Dietz, N.M. and Shepherd, J.T. (2001). From Belfast to Mayo and beyond: The use and future of plethysmography to study blood flow in human limbs. *Journal of Applied Physiology*, **91**, 2431-2441.

Jull, A., Waters, J. and Arroll, B. (2007). Pentoxifylline for treating venous leg ulcers. *Cochrane Database of Systematic Reviews*, CD001733.

Junger, M., Steins, A., Hahn, M. and Hafner, H.M. (2000). Microcirculatory dysfunction in chronic venous insufficiency (CVI). *Microcirculation*, **7**, S3-12.

Kakkos, S.K., Geroulakos, G. and Nicolaides, A.N. (2005). Improvement of the walking ability in intermittent claudication due to superficial femoral artery occlusion with supervised exercise and pneumatic foot and calf compression: A randomised controlled trial. *European Journal of Vascular and Endovascular Surgery*, **30**, 164-175.

Kandarpa, K., Becker, G.J., Hunink, M.G., McNamara, T.O., Rundback, J.H., Trost, D.W., Sos, T.A., Poplausky, M.R., Semba, C.P. and Landow, W.J. (2001). Transcatheter interventions for the treatment of peripheral atherosclerotic lesions: Part I. *Journal of Vascular and Interventional Radiology*, **12**, 683-695.

Kannel, W.B. and McGee, D.L. (1985). Update on some epidemiologic features of intermittent claudication: The Framingham Study. *Journal of the American Geriatrics Society*, **33**, 13-18.

Karlsmark, T., Agerslev, R.H., Bendz, S.H., Larsen, J.R., Roed-Petersen, J. and Andersen, K.E. (2003). Clinical performance of a new silver dressing, Contreet Foam, for chronic exuding venous leg ulcers. *Journal of Wound Care*, **12**, 351-354.

Kashyap, V.S., Pavkov, M.L., Bena, J.F., Sarac, T.P., O'Hara, P.J., Lyden, S.P. and Clair, D.G. (2008). The management of severe aortoiliac occlusive disease: Endovascular therapy rivals open reconstruction. *Journal of Vascular Surgery*, **48**, 1451-7.

Kelechi, T.J. and Michel, Y. (2007). A descriptive study of skin temperature, tissue perfusion, and tissue oxygen in patients with chronic venous disease. *Biological Research for Nursing*, **9**, 70-80.

Keo, H., Grob, E., Guggisberg, F., Widmer, J., Baumgartner, I., Schmid, J.P., Kalka, C. and Saner, H. (2008). Long-term effects of supervised exercise training on walking capacity and quality of life in patients with intermittent claudication. *Vasa*, **37**, 250-256.

Kilding, A.E., Winter, E.M. and Fysh, M. (2006). Moderate-domain pulmonary oxygen uptake kinetics and endurance running performance. *Journal of Sports Sciences*, **24**, 1013-1022.

Kistner, R.L. (1975). Surgical repair of the incompetent femoral vein valve. *Archives of Surgery*, **110**, 1336-1342.

Klonizakis, M., Yeung, J.M., Lingam, K., Nash, J.R., Manning, G. and Donnelly, R. (2006). Contrasting effects of varicose vein surgery on endothelial-dependent and -independent cutaneous vasodilation in the perimalleolar region. *European Journal of Vascular and Endovascular Surgery*, **31**, 434-438.

Klonizakis, M., Yeung, J.M., Nash, J.R., Lingam, K., Manning, G. and Donnelly, R. (2003). Effects of posture and venous insufficiency on endothelial-dependent and -independent cutaneous vasodilation in the perimalleolar region. *European Journal of Vascular and Endovascular Surgery*, **26**, 100-104.

Koppo, K., Bouckaert, J. and Jones, A.M. (2004). Effects of training status and exercise intensity on phase II VO_2 kinetics. *Medicine and Science in Sports and Exercise*, **36**, 225-232.

Kowalewski, R., Sobolewski, K., Wolanska, M. and Gacko, M. (2004). Matrix metalloproteinases in the vein wall. *International Angiology*, **23**, 164-169.

Krupatkin, A.I. (2006). The influence of the sympathetic innervation on the skin microvascular tone and blood flow oscillations. *Fiziologiia Cheloveka*, **32**, 95-103.

Kvernmo, H.D., Stefanovska, A., Kirkeboen, K.A., Osterud, B. and Kvernebo, K. (1998). Enhanced endothelium-dependent vasodilatation in human skin vasculature induced by physical conditioning. *European Journal of Applied Physiology*, **79**, 30-36.

Labs, K.H., Dormandy, J.A., Jaeger, K.A., Stuerzebecher, C.S. and Hiatt, W.R. (1999). Transatlantic conference on clinical trial guidelines in peripheral arterial disease:

Clinical trial methodology. Basel PAD clinical trial methodology group. *Circulation*, **100**, e75-81.

Lamarra, N., Whipp, B.J., Ward, S.A. and Wasserman, K. (1987). Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *Journal of Applied Physiology*, **62**, 2003-2012.

Laursen, J.B., Boesgaard, S., Poulsen, H.E. and Aldershvile, J. (1996). Nitrate tolerance impairs nitric oxide-mediated vasodilation in vivo. *Cardiovascular Research*, **31**, 814-819.

Layton, A.M., Ibbotson, S.H., Davies, J.A. and Goodfield, M.J. (1994). Randomised trial of oral aspirin for chronic venous leg ulcers. *Lancet*, **344**, 164-165.

Lazarous, D.F., Unger, E.F., Epstein, S.E., Stine, A., Arevalo, J.L., Chew, E.Y. and Quyyumi, A.A. (2000). Basic fibroblast growth factor in patients with intermittent claudication: Results of a phase I trial. *Journal of the American College of Cardiology*, **36**, 1239-1244.

Lederman, R.J., Mendelsohn, F.O., Anderson, R.D., Saucedo, J.F., Tenaglia, A.N., Hermiller, J.B., Hillegass, W.B., Rocha-Singh, K., Moon, T.E., Whitehouse, M.J. and Annex, B.H. (2002). Therapeutic angiogenesis with recombinant fibroblast growth factor-2 for intermittent claudication (the TRAFFIC study): A randomised trial. *Lancet*, **359**, 2053-2058.

Lee, A.J., Evans, C.J., Allan, P.L., Ruckley, C.V. and Fowkes, F.G. (2003). Lifestyle factors and the risk of varicose veins: Edinburgh Vein Study. *Journal of Clinical Epidemiology*, **56**, 171-179.

Leeuwenburgh, C. and Heinecke, J.W. (2001). Oxidative stress and antioxidants in exercise. *Current Medicinal Chemistry*, **8**, 829-838.

Lehert, P., Riphagen, F.E. and Gamand, S. (1990). The effect of naftidrofuryl on intermittent claudication: A meta-analysis. *Journal of Cardiovascular Pharmacology*, **16**, S81-6.

Leng, G.C., Fowler, B. and Ernst, E. (2000). Exercise for intermittent claudication. *Cochrane Database of Systematic Reviews*, CD000990.

Libby, P. (2002). Inflammation in atherosclerosis. *Nature*, **420**, 868-874.

Libby, P. (2001). Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation*, **104**, 365-372.

Libby, P., Ridker, P.M. and Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation*, **105**, 1135-1143.

Loffredo, L., Marcoccia, A., Pignatelli, P., Andreozzi, P., Borgia, M.C., Cangemi, R., Chiarotti, F. and Violi, F. (2007). Oxidative-stress-mediated arterial dysfunction in patients with peripheral arterial disease. *European Heart Journal*, **28**, 608-612.

Loftin, M., Boileau, R.A., Massey, B.H. and Lohman, T.G. (1988). Effect of arm training on central and peripheral circulatory function. *Medicine and Science in Sports and Exercise*, **20**, 136-141.

Lucini, D., Milani, R.V., Costantino, G., Lavie, C.J., Porta, A. and Pagani, M. (2002). Effects of cardiac rehabilitation and exercise training on autonomic regulation in patients with coronary artery disease. *American Heart Journal*, **143**, 977-983.

Lundgren, F., Dahllof, A.G., Schersten, T. and Bylund-Fellenius, A.C. (1989). Muscle enzyme adaptation in patients with peripheral arterial insufficiency: Spontaneous adaptation, effect of different treatments and consequences on walking performance. *Clinical Science*, **77**, 485-493.

Lurie, F., Creton, D., Eklof, B., Kabnick, L.S., Kistner, R.L., Pichot, O., Schuller-Petrovic, S. and Sessa, C. (2003). Prospective randomized study of endovenous radiofrequency obliteration (closure procedure) versus ligation and stripping in a selected patient population (EVOLVeS study). *Journal of Vascular Surgery*, **38**, 207-214.

Lurie, F., Creton, D., Eklof, B., Kabnick, L.S., Kistner, R.L., Pichot, O., Sessa, C. and Schuller-Petrovic, S. (2005). Prospective randomised study of endovenous radiofrequency obliteration (closure) versus ligation and vein stripping (EVOLVeS): Two-year follow-up. *European Journal of Vascular and Endovascular Surgery*, **29**, 67-73.

Mack, C.A., Magovern, C.J., Budenbender, K.T., Patel, S.R., Schwarz, E.A., Zanzonico, P., Ferris, B., Sanborn, T., Isom, P., Ferris, B., Sanborn, T., Isom, O.W., Crystal, R.G. and Rosengart, T.K. (1998). Salvage angiogenesis induced by adenovirus-mediated gene transfer of vascular endothelial growth factor protects against ischemic vascular occlusion. *Journal of Vascular Surgery*, **27**, 699-709.

Mackenzie, R.K., (2004). The effect of long saphenous vein stripping on deep venous reflux. *European Journal of Vascular and Endovascular Surgery*, **28**, 104-107.

Malanin, K., Havu, V.K. and Kolari, P.J. (2004). Dynamics of cutaneous laser doppler flux with concentration of moving blood cells and blood cell velocity in legs with venous ulcers and in healthy legs. *Angiology*, **55**, 37-42.

Mallika, V., Goswami, B. and Rajappa, M. (2007). Atherosclerosis pathophysiology and the role of novel risk factors: A clinicobiochemical perspective. *Angiology*, **58**, 513-522.

Mancini, D.M., Bolinger, L., Li, H., Kendrick, K., Chance, B. and Wilson, J.R. (1994). Validation of near-infrared spectroscopy in humans. *Journal of Applied Physiology*, **77**, 2740-2747.

Mannarino, E., Pasqualini, L., Menna, M., Maragoni, G. and Orlandi, U. (1989). Effects of physical training on peripheral vascular disease: A controlled study. *Angiology*, **40**, 5-10.

Mavor, G.E. and Galloway, J.M. (1969). Iliofemoral venous thrombosis. pathological considerations and surgical management. *The British Journal of Surgery*, **56**, 45-59.

Mavor, G.E. and Galloway, J.M. (1967). Collaterals of the deep venous circulation of the lower limb. *Surgery, Gynecology & Obstetrics*, **125**, 561-571.

Mayberry, J.C., Moneta, G.L., Taylor, L.M. and Porter, J.M. (1991). Fifteen-year results of ambulatory compression therapy for chronic venous ulcers. *Surgery*, **109**, 575-581.

McDermott, M.M., Criqui, M.H., Liu, K., Guralnik, J.M., Greenland, P., Martin, G.J. and Pearce, W. (2000). Lower ankle/brachial index, as calculated by averaging the dorsalis pedis and posterior tibial arterial pressures, and association with leg functioning in peripheral arterial disease. *Journal of Vascular Surgery*, **32**, 1164-1171.

McDermott, M.M., Greenland, P., Ferrucci, L., Criqui, M.H., Liu, K., Sharma, L., Chan, C., Celic, L., Priyanath, A. and Guralnik, J.M. (2002a). Lower extremity performance is associated with daily life physical activity in individuals with and without peripheral arterial disease. *Journal of the American Geriatrics Society*, **50**, 247-255.

McDermott, M.M., Greenland, P., Liu, K., Guralnik, J.M., Celic, L., Criqui, M.H., Chan, C., Martin, G.J., Schneider, J., Pearce, W.H., Taylor, L.M. and Clark, E. (2002b). The ankle brachial index is associated with leg function and physical activity: The Walking and Leg Circulation Study. *Annals of Internal Medicine*, **136**, 873-883.

McDermott, M.M., Guralnik, J.M., Greenland, P., Pearce, W.H., Criqui, M.H., Liu, K., Taylor, L., Chan, C., Sharma, L., Schneider, J.R., Ridker, P.M., Green, D. and Quann, M. (2003). Statin use and leg functioning in patients with and without lower-extremity peripheral arterial disease. *Circulation*, **107**, 757-761.

McDermott, M.M., Liu, K., Guralnik, J.M., Mehta, S., Criqui, M.H., Martin, G.J. and Greenland, P. (1998). The ankle brachial index independently predicts walking velocity and walking endurance in peripheral arterial disease. *Journal of the American Geriatrics Society*, **46**, 1355-1362.

McDermott, M.M., Ohlmiller, S.M., Liu, K., Guralnik, J.M., Martin, G.J., Pearce, W.H. and Greenland, P. (2001). Gait alterations associated with walking impairment in people with peripheral arterial disease with and without intermittent claudication. *Journal of the American Geriatrics Society*, **49**, 747-754.

Meijer, W.T., Hoes, A.W., Rutgers, D., Bots, M.L., Hofman, A. and Grobbee, D.E. (1998). Peripheral arterial disease in the elderly: The Rotterdam Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **18**, 185-192.

Meissner, M.H., Eklof, B., Smith, P.C., Dalsing, M.C., DePalma, R.G., Gloviczki, P., Moneta, G., Neglén, P., O'Donnell, T., Partsch, H. and Raju, S. (2007). Secondary chronic venous disorders. *Journal of Vascular Surgery*, **46**, 68S-83S.

Merchant, R.F., DePalma, R.G. and Kabnick, L.S. (2002). Endovascular obliteration of saphenous reflux: A multicenter study. *Journal of Vascular Surgery*, **35**, 1190-1196.

Merchant, R.F., Pichot, O. and Closure Study Group (2005). Long-term outcomes of endovenous radiofrequency obliteration of saphenous reflux as a treatment for superficial venous insufficiency. *Journal of Vascular Surgery*, **42**, 502-9.

Meru, A.V., Mittra, S., Thyagarajan, B. and Chugh, A. (2006). Intermittent claudication: An overview. *Atherosclerosis*, **187**, 221-237.

Michaels, J.A., Brazier, J.E., Campbell, W.B., MacIntyre, J.B., Palfreyman, S.J. and Ratcliffe, J. (2006a). Randomized clinical trial comparing surgery with conservative treatment for uncomplicated varicose veins. *The British Journal of Surgery*, **93**, 175-181.

Michaels, J.A., Campbell, W.B., Brazier, J.E., Macintyre, J.B., Palfreyman, S.J., Ratcliffe, J. and Rigby, K. (2006b). Randomised clinical trial, observational study and assessment of cost-effectiveness of the treatment of varicose veins (REACTIV trial). *Health Technology Assessment*, **10**, 1-196.

Milani, R.V. and Lavie, C.J. (2007). The role of exercise training in peripheral arterial disease. *Vascular Medicine*, **12**, 351-358.

Milton, S. (1986). A sample size formula for multiple regression studies. *Public Opinion Quarterly*, **50**, 112-118.

Mlacak, B., Blinc, A., Gale, N. and Ivka, B. (2005). Microcirculation disturbances in patients with venous ulcer before and after healing as assessed by laser Doppler flux-metry. *Archives of Medical Research*, **36**, 480-484.

Mohler, E.R. (2007). Therapy insight: Peripheral arterial disease and diabetes--from pathogenesis to treatment guidelines. *Nature Clinical Practice*, **4**, 151-162.

Mohler, E.R. (2004). The effect of risk factor changes on peripheral arterial disease and cardiovascular risk. *Current Drug Targets*, **4**, 259-263.

Mohler, E.R., Hiatt, W.R. and Creager, M.A. (2003). Cholesterol reduction with atorvastatin improves walking distance in patients with peripheral arterial disease. *Circulation*, **108**, 1481-1486.

Mondillo, S., Ballo, P., Barbati, R., Guerrini, F., Ammaturo, T., Agricola, E., Pastore, M., Borrello, F., Belcastro, M., Picchi, A. and Nami, R. (2003). Effects of simvastatin on walking performance and symptoms of intermittent claudication in hypercholesterolemic patients with peripheral vascular disease. *The American Journal of Medicine*, **114**, 359-364.

Morales, F., Graaff, R., Smit, A.J., Bertuglia, S., Petoukhova, A.L., Steenbergen, W., Leger, P. and Rakhorst, G. (2005). How to assess post-occlusive reactive hyperaemia by means of laser Doppler perfusion monitoring: Application of a standardised protocol to patients with peripheral arterial obstructive disease. *Microvascular Research*, **69**, 17-23.

Morrison, N. (2005). Saphenous ablation: What are the choices, laser or RF energy. *Seminars in Vascular Surgery*, **18**, 15-18.

Motykie, G.D., Caprini, J.A., Arcelus, J.I., Reyna, J.J., Overom, E. and Mokhtee, D. (1999). Evaluation of therapeutic compression stockings in the treatment of chronic venous insufficiency. *Dermatologic Surgery*, **25**, 116-120.

Mullineaux, D.R., Bartlett, R.M. and Bennett, S. (2001). Research design and statistics in biomechanics and motor control. *Journal of Sports Sciences*, **19**, 739-760.

Mundy, L., Merlin, T.L., Fitridge, R.A. and Hiller, J.E. (2005). Systematic review of endovenous laser treatment for varicose veins. *The British Journal of Surgery*, **92**, 1189-1194.

Myers, R.H. (1990). *Classical and modern regression with applications,* 2nd ed. PWS and Kent Publishing, Boston.

Naoum, J.J., Hunter, G.C., Woodside, K.J. and Chen C. (2007). Current advances in the pathogenesis of varicose veins. *The Journal of Surgical Research*, **141**, 311-316.

Nawaz, S., Walker, R.D., Wilkinson, C.H., Saxton, J.M., Pockley, A.G. and Wood, R.F. (2001). The inflammatory response to upper and lower limb exercise and the effects of exercise training in patients with claudication. *Journal of Vascular Surgery*, **33**, 392-399.

Neglen, P. and Raju, S. (2004). In-stent recurrent stenosis in stents placed in the lower extremity venous outflow tract. *Journal of Vascular Surgery*, **39**, 181-187.

Neglen, P. and Raju, S. (2003). Venous reflux repair with cryopreserved vein valves. *Journal of Vascular Surgery*, **37**, 552-557.

Neglen, P., Thrasher, T.L. and Raju, S. (2003). Venous outflow obstruction: An underestimated contributor to chronic venous disease. *Journal of Vascular Surgery*, **38**, 879-885.

Nelson, E.A., Mani, R. and Vowden, K. (2008). Intermittent pneumatic compression for treating venous leg ulcers. *Cochrane Database of Systematic Reviews*, CD001899.

Nelzen, O. and Fransson, I. (2007). True long-term healing and recurrence of venous leg ulcers following SEPS combined with superficial venous surgery: A prospective study. *European Journal of Vascular and Endovascular Surgery*, **34**, 605-612.

Newman, A.B., Shemanski, L., Manolio, T.A., Cushman, M., Mittelmark, M., Polak, J.F., Powe, N.R. and Siscovick, D. (1999). Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. The Cardiovascular Health Study group. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **19**, 538-545.

Ng, M.Y., Andrew, T., Spector, T.D. and Jeffery, S. (2005). Linkage to the FOXC2 region of chromosome 16 for varicose veins in otherwise healthy, unselected sibling pairs. *Journal of Medical Genetics*, **42**, 235-239.

Nicholson, C.D., Angersbach, D. and Wilke, R. (1992). The effect of physical training on rat calf muscle, oxygen tension, blood flow, metabolism and function in an animal

model of chronic occlusive peripheral vascular disease. *International Journal of Sports Medicine*, **13**, 60-64.

Nicolaides, A.N. (2005). Chronic venous disease and the leukocyte-endothelium interaction: From symptoms to ulceration. *Angiology*, **56**, S11-9.

Nicolaides, A.N. (2003). From symptoms to leg edema: Efficacy of daflon 500 mg. *Angiology*, **54**, S33-44.

Nicolaides, A.N., Allegra, C., Bergan, J., Bradbury, A., Cairols, M., Carpentier, P., Comerota, A., Delis, C., Eklof, B., Fassiadis, N., Georgiou, N., Geroulakos, G., Hoffmann, U., Jantet, G., Jawien, A., Kakkos, S., Kalodiki, E., Labropoulos, N., Neglen, P., Pappas, P., Partsch, H., Perrin, M., Rabe, E., Ramelet, A.A., Vayssaira, M., Ioannidou, E. and Taft, A. (2008). Management of chronic venous disorders of the lower limbs: Guidelines according to scientific evidence. *International Angiology*, **27**, 1-59.

Nicolaides, A.N., Hussein, M.K., Szendro, G., Christopoulos, D., Vasdekis, S. and Clarke, H. (1993). The relation of venous ulceration with ambulatory venous pressure measurements. *Journal of Vascular Surgery*, **17**, 414-419.

Norgren, L., Hiatt, W.R., Dormandy, J.A., Nehler, M.R., Harris, K.A., Fowkes, F.G., Bell, K., Caporusso, J., Durand-Zaleski, I., Komori, K., Lammer, J., Liapis, C., Novo, S., Razavi, M., Robbs, J., Schaper, N., Shigematsu, H., Sapoval, M., White, C., White, J., Clement, D., Creager, M., Jaff, M., Mohler, E., Rutherford, R.B., Sheehan, P., Sillesen, H. and Rosenfield, K. (2007). Inter-society consensus for the management of peripheral arterial disease (TASC II). *European Journal of Vascular and Endovascular Surgery*, **33**, S1-75.

Oakley, C., Zwierska, I., Tew, G., Beard, J.D. and Saxton, J.M. (2008). Nordic poles immediately improve walking distance in patients with intermittent claudication. *European Journal of Vascular and Endovascular Surgery*, **36**, 689-94.

Obermayer, A., Gostl, K., Partsch, H. and Benesch, T. (2008). Venous reflux surgery promotes venous leg ulcer healing despite reduced ankle brachial pressure index. *International Angiology*, **27**, 239-246.

O'Hare, A.M., Glidden, D.V., Fox, C.S. and Hsu, C.Y. (2004). High prevalence of peripheral arterial disease in persons with renal insufficiency: Results from the National Health and Nutrition Examination Survey 1999-2000. *Circulation*, **109**, 320-323.

Ono, T., Bergan, J.J., Schmid-Schönbein, G.W. and Takase, S. (1998). Monocyte infiltration into venous valves. *Journal of Vascular Surgery*, **27**, 158-166.

Ouriel, K. (2001). Peripheral arterial disease. *Lancet*, **358**, 1257-1264.

Padberg, F.T., Johnston, M.V. and Sisto, S.A. (2004). Structured exercise improves calf muscle pump function in chronic venous insufficiency: A randomized trial. *Journal of Vascular Surgery*, **39**, 79-87.

Padberg, F.T., Pappas, P.J., Araki, C.T., Back, T.L. and Hobson, R.W. (1996). Hemodynamic and clinical improvement after superficial vein ablation in primary combined venous insufficiency with ulceration. *Journal of Vascular Surgery*, **24**, 711-718.

Palfreyman, S.J., Drewery-Carter, K., Rigby, K., Michaels, J.A. and Tod, A.M. (2004). Varicose veins: A qualitative study to explore expectations and reasons for seeking treatment. *Journal of Clinical Nursing*, **13**, 332-340.

Palma, E.C. and Esperon, R. (1960). Vein transplants and grafts in the surgical treatment of the postphlebitic syndrome. *The Journal of Cardiovascular Surgery*, **1**, 94-107.

Partsch, H. (1985). Pathogenesis of the venous leg ulcer. *Der Hautarzt*, **36**, 196-202.

Pedersen, T.R., Kjekshus, J., Pyörälä, K., Olsson, A.G., Cook, T.J., Musliner, T.A., Tobert, J.A. and Haghfelt, T. (1998). Effect of simvastatin on ischemic signs and symptoms in the Scandinavian simvastatin survival study (4S). *The American Journal of Cardiology*, **81**, 333-335.

Perala, J., Rautio, T., Biancari, F., Ohtonen, P., Wiik, H., Heikkinen, T. and Juvonen, T. (2005). Radiofrequency endovenous obliteration versus stripping of the long saphenous vein in the management of primary varicose veins: 3-year outcome of a randomized study. *Annals of Vascular Surgery*, **19**, 669-672.

Perkins, J.M., Collin, J., Creasy, T.S., Fletcher, E.W. and Morris, P.J. (1996). Exercise training versus angioplasty for stable claudication. Long and medium term results of a prospective, randomised trial. *European Journal of Vascular and Endovascular Surgery*, **11**, 409-413.

Perrin, M.R., Guex, J.J., Ruckley, C.V., dePalma, R.G., Royle, J.P., Eklof, B., Nicolini, P. and Jantet, G. (2000). Recurrent varices after surgery (REVAS), a consensus document. REVAS group. *Cardiovascular Surgery*, **8**, 233-245.

Pittler, M.H. and Ernst, E. (2004). Horse chestnut seed extract for chronic venous insufficiency. *Cochrane Database of Systematic Reviews*, CD003230.

Plate, G., Akesson, H., Einarsson, E., Ohlin, P. and Eklöf, B. (1990). Long-term results of venous thrombectomy combined with a temporary arterio-venous fistula. *European Journal of Vascular Surgery*, **4**, 483-489.

Pober, D.M., Braun, B. and Freedson, P.S. (2004). Effects of a single bout of exercise on resting heart rate variability. *Medicine and Science in Sports and Exercise*, **36**, 1140-1148.

Pogliaghi, S., Terziotti, P., Cevese, A., Balestreri, F. and Schena, F. (2006). Adaptations to endurance training in the healthy elderly: Arm cranking versus leg cycling. *European Journal of Applied Physiology*, **97**, 723-731.

Porter, R. (1997). *The greatest benefit to mankind*. Harper Collins, London, UK.

Pratt, C.M. (2001). Analysis of the cilostazol safety database. *The American Journal of Cardiology*, **87**, 28D-33D.

Proebstle, T.M., Lehr, H.A., Kargl, A., Espinola-Klein, C., Rother, W., Bethge, S. and Knop, J. (2002). Endovenous treatment of the greater saphenous vein with a 940-nm diode laser: Thrombotic occlusion after endoluminal thermal damage by laser-generated steam bubbles. *Journal of Vascular Surgery*, **35**, 729-736.

Rajendran, S., Rigby, A.J. and Anand, S.C. (2007). Venous leg ulcer treatment and practice--part 1: The causes and diagnosis of venous leg ulcers. *Journal of Wound Care*, **16**, 24-26.

Raju, S., Berry, M.A. and Neglen, P. (2000). Transcommissural valvuloplasty: Technique and results. *Journal of Vascular Surgery*, 32, 969-976.

Raju, S., Owen, S. and Neglen, P. (2002). The clinical impact of iliac venous stents in the management of chronic venous insufficiency. *Journal of Vascular Surgery*, **35**, 8-15.

Rasmussen, L.H., Bjoern, L., Lawaetz, M., Blemings, A., Lawaetz, B. and Eklof, B. (2007). Randomized trial comparing endovenous laser ablation of the great saphenous vein with high ligation and stripping in patients with varicose veins: Short-term results. *Journal of Vascular Surgery*, **46**, 308-315.

Rautio, T., Ohinmaa, A., Perälä, J., Ohtonen, P., Heikkinen, T., Wiik, H., Karjalainen, P., Haukipuro, K. and Juvonen, T. (2002). Endovenous obliteration versus conventional stripping operation in the treatment of primary varicose veins: A randomized controlled trial with comparison of the costs. *Journal of Vascular Surgery*, **35**, 958-965.

Regensteiner, J.G. (1997). Exercise in the treatment of claudication: Assessment and treatment of functional impairment. *Vascular Medicine*, **2**, 238-242.

Regensteiner, J.G. and Hiatt, W.R. (1995). Exercise rehabilitation for patients with peripheral arterial disease. *Exercise and Sport Sciences Reviews*, **23**, 1-24.

Regensteiner, J.G., Hiatt, W.R., Coll, J.R., Criqui, M.H., Treat-Jacobson, D., McDermott, M.M. and Hirsch, A.T. (2008). The impact of peripheral arterial disease on health-related quality of life in the peripheral arterial disease awareness, risk, and treatment: New resources for survival (PARTNERS) program. *Vascular Medicine*, **13**, 15-24.

Regensteiner, J.G., Steiner, J.F. and Hiatt, W.R. (1996). Exercise training improves functional status in patients with peripheral arterial disease. *Journal of Vascular Surgery*, **23**, 104-115.

Regensteiner, J.G., Ware, J.E., McCarthy, W.J., Zhang, P., Forbes, W.P., Heckman, J. and Hiatt, W.R. (2002). Effect of cilostazol on treadmill walking, community-based walking ability, and health-related quality of life in patients with intermittent claudication due to peripheral arterial disease: Meta-analysis of six randomized controlled trials. *Journal of the American Geriatrics Society*, **50**, 1939-1946.

Regensteiner, J.G., Wolfel, E.E., Brass, E.P., Carry, M.R., Ringel, S.P., Hargarten, M.E., Stamm, E.R. and Hiatt, W.R. (1993). Chronic changes in skeletal muscle histology and function in peripheral arterial disease. *Circulation*, **87**, 413-421.

Ridker, P.M., Stampfer, M.J. and Rifai, N. (2001). Novel risk factors for systemic atherosclerosis: A comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *The Journal of the American Medical Association*, **285**, 2481-2485.

Robeer, G.G., Brandsma, J.W., van den Heuvel, S.P., Smit, B., Oostendorp, R.A. and Wittens, C.H. (1998). Exercise therapy for intermittent claudication: A review of the quality of randomised clinical trials and evaluation of predictive factors. *European Journal of Vascular and Endovascular Surgery*, **15**, 36-43.

Rosfors, S., Arnetz, B.B., Bygdeman, S., Skoldo, L., Lahnborg, G. and Eneroth, P. (1990). Important predictors of the outcome of physical training in patients with intermittent claudication. *Scandinavian Journal of Rehabilitation Medicine*, **22**, 135-137.

Rossi, M. and Carpi, A. (2004). Skin microcirculation in peripheral arterial obliterative disease. *Biomedicine & Pharmacotherapy*, **58**, 427-431.

Rossi, M., Cupisti, A., Perrone, L., Mariani, S. and Santoro, G. (2002). Acute effect of exercise-induced leg ischemia on cutaneous vasoreactivity in patients with stage II peripheral artery disease. *Microvascular Research*, **64**, 14-20.

Rossiter, H.B., Ward, S.A., Doyle, V.L., Howe, F.A., Griffiths, J.R. and Whipp, B.J. (1999). Inferences from pulmonary O_2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. *The Journal of Physiology*, **518**, 921-932.

Rowlands, T.E. and Donnelly, R. (2007). Medical therapy for intermittent claudication. *European Journal of Vascular and Endovascular Surgery*, **34**, 314-321.

Rubanyi G.M. (1991). Endothelium-derived relaxing and contracting factors. *Journal of Cellular Biochemistry*, **46**, 27-36.

Sallis, J.F., Haskell, W.L., Wood, P.D., Fortmann, S.P., Rogers, T., Blair, S.N. and Paffenbarger, R.S. (1985). Physical activity assessment methodology in the Five-City Project. *American Journal of Epidemiology*, **121**, 91-106.

Saltin, B. (1977). The interplay between peripheral and central factors in the adaptive response to exercise and training. *Annals of the New York Academy of Sciences*, **301**, 224-231.

Saltin, B., Blomqvist, G., Mitchell, J.H., Johnson, R.L., Wildenthal, K. and Chapman, C.B. (1968). Response to exercise after bed rest and after training. *Circulation*, **38**, 1-78.

Samson, R.H. (2008). The role of statin drugs in the management of the peripheral vascular patient. *Vascular and Endovascular Surgery*, **42**, 352-366.

Sanada, H., Higashi, Y., Goto, C., Chayama, K., Yoshizumi, M. and Sueda, T. (2005). Vascular function in patients with lower extremity peripheral arterial disease: A comparison of functions in upper and lower extremities. *Atherosclerosis*, **178**, 179-185.

Sanderson, B., Askew, C., Stewart, I., Walker, P., Gibbs, H. and Green S. (2006). Short-term effects of cycle and treadmill training on exercise tolerance in peripheral arterial disease. *Journal of Vascular Surgery*, **44**, 119-127.

Savage, P., Ricci, M.A., Lynn, M., Gardner, A., Knight, S., Brochu, M. and Ades, P. (2001). Effects of home versus supervised exercise for patients with intermittent claudication. *Journal of Cardiopulmonary Rehabilitation*, **21**, 152-157.

Saxton, J.M., Zwierska, I., Hopkinson, K., Espigares, E., Choksy, S., Nawaz, S., Walker, R. and Pockley, A.G. (2008). Effect of upper- and lower-limb exercise training on circulating soluble adhesion molecules, hs-CRP and stress proteins in patients with intermittent claudication. *European Journal of Vascular and Endovascular Surgery*, **35**, 607-613.

Schillinger, M., Exner, M., Mlekusch, W., Haumer, M., Sabeti, S., Ahmadi, R., Wagner, O. and Minar, E. (2004). Effect of smoking on restenosis during the 1st year after lower-limb endovascular interventions. *Radiology*, **231**, 831-838.

Schmid-Schonbein, G.W., Takase, S. and Bergan, J.J. (2001). New advances in the understanding of the pathophysiology of chronic venous insufficiency. *Angiology*, **52**, S27-34.

Schubert, R. and Mulvany, M.J. (1999). The myogenic response: Established facts and attractive hypotheses. *Clinical Science*, **96**, 313-326.

Selvin, E. and Erlinger, T.P. (2004). Prevalence of and risk factors for peripheral arterial disease in the United States: Results from the National Health and Nutrition Examination Survey, 1999-2000. *Circulation*, **110**, 738-743.

Selvin, E. and Hirsch, A.T. (2008). Contemporary risk factor control and walking dysfunction in individuals with peripheral arterial disease: NHANES 1999-2004. *Atherosclerosis*, **201**, 425-433.

Sen, C.K. (1995). Oxidants and antioxidants in exercise. *Journal of Applied Physiology*, **79**, 675-686.

Sever, P. (2006). New hypertension guidelines from the National Institute for Health and Clinical Excellence and the British Hypertension Society. *Journal of the Renin-Angiotensin-Aldosterone System*, **7**, 61-63.

Shechter, M., Auslander, G., Weinmann, E.E. and Bass, A. (2003). Quality of life and social support following distal arterial bypass in elderly patients. *The Israel Medical Association Journal*, **5**, 322-325.

Shinoda, M., Toki, Y., Murase, K., Mokuno, S., Okumura, K. and Ito, T. (1997). Types of potassium channels involved in coronary reactive hyperemia depend on duration of preceding ischemia in rat hearts. *Life Sciences*, **61**, 997-1007.

Singer, D.R. and Kite, A. (2008). Management of hypertension in peripheral arterial disease: Does the choice of drugs matter? *European Journal of Vascular and Endovascular Surgery*, **35**, 701-708.

Siscovick, D.S., Weiss, N.S., Fletcher, R.H. and Lasky, T. (1984). The incidence of primary cardiac arrest during vigorous exercise. *The New England Journal of Medicine*, **311**, 874-877.

Skillman, J.J., Kent, K.C., Porter, D.H. and Kim, D. (1990). Simultaneous occurrence of superficial and deep thrombophlebitis in the lower extremity. *Journal of Vascular Surgery*, **11**, 818-23.

Smith, P.C. (2006). The causes of skin damage and leg ulceration in chronic venous disease. *The International Journal of Lower Extremity Wounds*, **5**, 160-168.

Sorlie, D. and Myhre, K. (1978). Effects of physical training in intermittent claudication. *Scandinavian Journal of Clinical and Laboratory Investigation*, **38**, 217-222.

Speakman, M.J. and Collin, J. (1986). Are swelling and aching of the legs reduced by operation on varicose veins? *British Medical Journal*, **293**, 105-106.

Spronk, S., Bosch, J.L., den Hoed, P.T., Veen, H.F., Pattynama, P.M. and Hunink, M.G. (2008). Cost-effectiveness of endovascular revascularization compared to supervised hospital-based exercise training in patients with intermittent claudication: A randomized controlled trial. *Journal of Vascular Surgery*, **48**, 1472-80.

Spronk, S., Bosch, J.L., Veen, H.F., den Hoed, P.T. and Hunink, M.G. (2005). Intermittent claudication: Functional capacity and quality of life after exercise training or percutaneous transluminal angioplasty--systematic review. *Radiology*, **235**, 833-842.

Stacey, M.C., Mata, S.D., Trengove, N.J. and Mather, C.A. (2000). Randomised double-blind placebo controlled trial of topical autologous platelet lysate in venous ulcer healing. *European Journal of Vascular and Endovascular Surgery*, **20**, 296-301.

Stankevicius, E., Kevelaitis, E., Vainorius, E. and Simonsen, U. (2003). Role of nitric oxide and other endothelium-derived factors. *Medicina*, **39**, 333-341.

Stein, R., Hriljac, I., Halperin, J.L., Gustavson, S.M., Teodorescu, V. and Olin, J.W. (2006). Limitation of the resting ankle-brachial index in symptomatic patients with peripheral arterial disease. *Vascular Medicine*, **11**, 29-33.

Stewart, A.H. and Lamont, P.M. (2001). Exercise for intermittent claudication. Supervised programmes should be universally available. *British Medical Journal*, **323**, 703-704.

Subramonia, S. and Lees, T.A. (2007). The treatment of varicose veins. *Annals of The Royal College of Surgeons of England*, **89**, 96-100.

Szuba, A., Oka, R.K., Harada, R. and Cooke, J.P. (2006). Limb hemodynamics are not predictive of functional capacity in patients with PAD. *Vascular Medicine*, **11**, 155-163.

Tan, K.H., Cotterrell, D., Sykes, K., Sissons, G.R., de Cossart, L. and Edwards, P.R. (2000). Exercise training for claudicants: Changes in blood flow, cardiorespiratory status, metabolic functions, blood rheology and lipid profile. *European Journal of Vascular and Endovascular Surgery*, **20**, 72-78.

Tan, K.H., De Cossart, L. and Edwards, P.R. (2000). Exercise training and peripheral vascular disease. *The British Journal of Surgery*, **87**, 553-562.

Tawes, R.L., Barron, M.L., Coello, A.A., Joyce, D.H. and Kolvenbach, R. (2003). Optimal therapy for advanced chronic venous insufficiency. *Journal of Vascular Surgery*, **37**, 545-551.

Taylor, D.J., Amato, A., Hands, L.J., Kemp, G.J., Ramaswami, G., Nicolaides, A. and Radda, G.K. (1996). Changes in energy metabolism of calf muscle in patients with intermittent claudication assessed by 31P magnetic resonance spectroscopy: A phase II open study. *Vascular Medicine*, **1**, 241-245.

Taylor, S.M., Kalbaugh, C.A., Healy, M.G., Cass, A.L., Gray, B.H., Langan, E.M., Cull, D.L., Carsten, C.G., York, J.W., Snyder, B.A. and Youkey, J.R. (2008). Do current outcomes justify more liberal use of revascularization for vasculogenic claudication? A single center experience of 1,000 consecutively treated limbs. *Journal of the American College of Surgeons*, **206**, 1053-62.

Tessari, L., Cavezzi, A. and Frullini, A. (2001). Preliminary experience with a new sclerosing foam in the treatment of varicose veins. *Dermatologic Surgery*, **27**, 58-60.

Thijssen, D.H., Bleeker, M.W., Smits, P. and Hopman, M.T. (2005). Reproducibility of blood flow and post-occlusive reactive hyperaemia as measured by venous occlusion plethysmography. *Clinical Science*, **108**, 151-157.

Thijssen, D.H., Rongen, G.A., Smits, P. and Hopman, M.T. (2008). Physical (in)activity and endothelium-derived constricting factors: Overlooked adaptations. *The Journal of Physiology*, **586**, 319-324.

Thompson, P.D., Buchner, D., Pina, I.L., Balady, G.J., Williams, M.A., Marcus, B.H., Berra, K., Blair, S.N., Costa, F., Franklin, B., Fletcher, G.F., Gordon, N.F., Pate, R.R., Rodriguez, B.L., Yancey, A.K. and Wenger, N.K. (2003). Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: A statement from the council on clinical cardiology (subcommittee on exercise, rehabilitation, and prevention) and the council on nutrition, physical activity, and metabolism (subcommittee on physical activity). *Circulation*, **107**, 3109-3116.

Thompson, P. D., Zimet, R., Forbes, W.P. and Zhang, P. (2002). Meta-analysis of results from eight randomized, placebo-controlled trials on the effect of cilostazol on

patients with intermittent claudication. *The American Journal of Cardiology*, **90**, 1314-1319.

Tinken, T.M., Thijssen, D.H., Black, M.A., Cable, N.T. and Green, D.J. (2008). Time course of change in vasodilator function and capacity in response to exercise training in humans. *The Journal of Physiology*, **586**, 5003-5012.

Tordi, N., Belli, A., Mougin, F., Rouillon, J.D. and Gimenez, M. (2001). Specific and transfer effects induced by arm or leg training. *International Journal of Sports Medicine*, **22**, 517-524.

Treesak, C., Kasemsup, V., Treat-Jacobson, D., Nyman, J.A. and Hirsch, A.T. (2004). Cost-effectiveness of exercise training to improve claudication symptoms in patients with peripheral arterial disease. *Vascular Medicine*, **9**, 279-285.

Turner, J., Belch, J.J. and Khan, F. (2008). Current concepts in assessment of microvascular endothelial function using laser Doppler imaging and iontophoresis. *Trends in Cardiovascular Medicine*, **18**, 109-116.

Vainas, T., Stassen, F.R., de Graaf, R., Twiss, E.L., Herngreen, S.B., Welten, R.J., van den Akker, L.H., van Dieijen-Visser, M.P., Bruggeman, C.A. and Kitslaar, P.J. (2005). C-reactive protein in peripheral arterial disease: Relation to severity of the disease and to future cardiovascular events. *Journal of Vascular Surgery*, **42**, 243-251.

Valencia, I.C., Falabella, A., Kirsner, R.S. and Eaglstein, W.H. (2001). Chronic venous insufficiency and venous leg ulceration. *Journal of the American Academy of Dermatology*, **44**, 401-21.

van der Zaag, E.S., Prins, M.H. and Jacobs, M.J. (1996). Treatment of intermittent claudication; prospective randomized study in the BAESIC-trial (bypass, angioplasty or endarterectomy patients with severe intermittent claudication). *Nederlands Tijdschrift voor Geneeskunde*, **140**, 787-788.

Vanscheidt, W., Rabe, E., Naser-Hijazi, B., Ramelet, A.A., Partsch, H., Diehm, C., Schultz-Ehrenburg, U., Spengel, F., Wirsching, M., Götz, V., Schnitker, J. and Henneicke-von Zepelin, H.H. (2002). The efficacy and safety of a coumarin-/troxerutin-combination (SB-LOT) in patients with chronic venous insufficiency: A double blind placebo-controlled randomised study. *Vasa*, **31**, 185-190.

Vickers, A.J. and Altman, D.G. (2001). Statistics notes: Analysing controlled trials with baseline and follow up measurements. *British Medical Journal*, **323**, 1123-1124.

Vidula, H., Tian, L., Liu, K., Criqui, M.H., Ferrucci, L., Pearce, W.H., Greenland, P., Green, D., Tan, J., Garside, D.B., Guralnik, J., Ridker, P.M., Rifai, N. and McDermott, M.M. (2008). Biomarkers of inflammation and thrombosis as predictors of near-term mortality in patients with peripheral arterial disease: A cohort study. *Annals of Internal Medicine*, **148**, 85-93.

Virgini-Magalhaes, C.E., Porto, C.L., Fernandes, F.F., Dorigo, D.M., Bottino, D.A. and Bouskela, E. (2006). Use of microcirculatory parameters to evaluate chronic venous insufficiency. *Journal of Vascular Surgery*, **43**, 1037-1044.

Vogel, T.R., Su, L.T., Symons, R.G. and Flum, D.R. (2007). Lower extremity angioplasty for claudication: A population-level analysis of 30-day outcomes. *Journal of Vascular Surgery*, **45**, 762-767.

Walker, R.D., Nawaz, S., Wilkinson, C.H., Saxton, J.M., Pockley, A.G. and Wood, R.F. (2000). Influence of upper- and lower-limb exercise training on cardiovascular function and walking distances in patients with intermittent claudication. *Journal of Vascular Surgery*, **31**, 662-669.

Wang, J., Zhou, S., Bronks, R., Graham, J. and Myers, S. (2008). Effects of supervised treadmill walking training on calf muscle capillarization in patients with intermittent claudication. *Angiology*, **60**, 36-41.

Wang, J., Zhou, S., Bronks, R., Graham, J. and Myers, S. (2006). Effects of supervised treadmill-walking training on strength and endurance of the calf muscles of individuals with peripheral arterial disease. *Clinical Journal of Sport Medicine*, **16**, 397-400.

Wang, J.S. (2005). Effects of exercise training and detraining on cutaneous microvascular function in man: The regulatory role of endothelium-dependent dilation in skin vasculature. *European Journal of Applied Physiology*, **93**, 429-434.

Wang, J.S., Yang, C.F., Wong, M.K., Chow, S.E. and Chen, J.K. (2000). Effect of strenuous arm exercise on oxidized-LDL-potentiated platelet activation in individuals with spinal cord injury. *Thrombosis and Haemostasis*, **84**, 118-123.

Watson, L., Ellis, B. and Leng, G.C. (2008). Exercise for intermittent claudication. *Cochrane Database of Systematic Reviews*, CD000990.

Weiss, R.A. and Weiss, M.A. (2002). Controlled radiofrequency endovenous occlusion using a unique radiofrequency catheter under duplex guidance to eliminate saphenous varicose vein reflux: A 2-year follow-up. *Dermatologic Surgery*, **28**, 38-42.

Weiss, T., Fujita, Y., Kreimeier, U. and Messmer, K. (1992). Effect of intensive walking exercise on skeletal muscle blood flow in intermittent claudication. *Angiology*, **43**, 63-71.

Weitz, J.I., Byrne, J., Clagett, G.P., Farkouh, M.E., Porter, J.M., Sackett, D.L., Strandness, D.E. and Taylor L.M. (1996). Diagnosis and treatment of chronic arterial insufficiency of the lower extremities: A critical review. *Circulation*, **94**, 3026-3049.

Welling, R.E., Cranley, J.J., Krause, R.J. and Hafner, C.D. (1981). Obliterative arterial disease of the upper extremity. *Archives of Surgery*, **116**, 1593-1596.

Whyman, M.R. and Ruckley, C.V. (1998). Should claudicants receive angioplasty or just exercise training? *Cardiovascular Surgery*, **6**, 226-231.

Willigendael, E.M., Teijink, J.A., Bartelink, M.L., Peters, R.J., Büller, H.R. and Prins, M.H. (2005). Smoking and the patency of lower extremity bypass grafts: A meta-analysis. *Journal of Vascular Surgery*, **42**, 67-74.

Wilmore, J.H. and Costill, D.L. (1999). *Physiology of sport and exercise*. Human Kinetics, Leeds, UK.

Wind, J. and Koelemay, M.J. (2007). Exercise therapy and the additional effect of supervision on exercise therapy in patients with intermittent claudication. Systematic review of randomised controlled trials. *European Journal of Vascular and Endovascular Surgery*, **34**, 1-9.

Winterborn, R.J., Foy, C. and Earnshaw, J.J. (2004). Causes of varicose vein recurrence: Late results of a randomized controlled trial of stripping the long saphenous vein. *Journal of Vascular Surgery*, **40**, 634-639.

Wollina, U., Abdel-Naser, M.B. and Mani, R. (2006). A review of the microcirculation in skin in patients with chronic venous insufficiency: The problem and the evidence available for therapeutic options. *The International Journal of Lower Extremity Wounds*, **5**, 169-180.

Womack, C.J., Sieminski, D.J., Katzel, L.I., Yataco, A. and Gardner, A.W. (1997). Improved walking economy in patients with peripheral arterial occlusive disease. *Medicine and Science in Sports and Exercise*, **29**, 1286-1290.

Yamaki, T., Nozaki, M. and Iwasaka, S. (2004). Comparative study of duplex-guided foam sclerotherapy and duplex-guided liquid sclerotherapy for the treatment of superficial venous insufficiency. *Dermatologic Surgery*, **30**, 718-22.

Yataco, A.R., Corretti, M.C., Gardner, A.W., Womack, C.J. and Katzel L.I. (1999). Endothelial reactivity and cardiac risk factors in older patients with peripheral arterial disease. *The American Journal of Cardiology*, **83**, 754-758.

Yvonne-Tee, G.B., Rasool, A.H., Halim, A.S. and Rahman, A.R. (2006). Noninvasive assessment of cutaneous vascular function in vivo using capillaroscopy, plethysmography and laser-doppler instruments: Its strengths and weaknesses. *Clinical Hemorheology and Microcirculation*, **34**, 457-473.

Zetterquist, S. (1970). The effect of active training on the nutritive blood flow in exercising ischemic legs. *Scandinavian Journal of Clinical and Laboratory Investigation*, **25**, 101-111.

Zwierska, I., Walker, R.D., Choksy, S.A., Male, J.S., Pockley, A.G. and Saxton, J.M. (2006). Relative tolerance to upper- and lower-limb aerobic exercise in patients with peripheral arterial disease. *European Journal of Vascular and Endovascular Surgery*, **31**, 157-163.

Zwierska, I., Walker, R.D., Choksy, S.A., Male, J.S., Pockley, A.G. and Saxton, J.M. (2005). Upper- vs lower-limb aerobic exercise rehabilitation in patients with

symptomatic peripheral arterial disease: A randomized controlled trial. *Journal of Vascular Surgery*, **42**, 1122-1130.

PAGE/PAGES EXCLUDED UNDER INSTRUCTION FROM THE UNIVERSITY