REFERENCE
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)
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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.

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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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABPI</td>
<td>ankle-brachial pressure index</td>
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<tr>
<td>ACE</td>
<td>angiotensin-converting enzyme</td>
</tr>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CD</td>
<td>claudication distance</td>
</tr>
<tr>
<td>CEAP</td>
<td>Clinical-Etiology-Anatomy-Pathophysiology</td>
</tr>
<tr>
<td>cGMP</td>
<td>cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>EDHF</td>
<td>endothelium-derived hyperpolarising factor</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>MET</td>
<td>metabolic equivalent</td>
</tr>
<tr>
<td>MRT</td>
<td>mean response time</td>
</tr>
<tr>
<td>MWD</td>
<td>maximum walking distance</td>
</tr>
<tr>
<td>NIRS</td>
<td>near-infrared spectroscopy</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>PAD</td>
<td>peripheral arterial disease</td>
</tr>
<tr>
<td>PTA</td>
<td>percutaneous transluminal angioplasty</td>
</tr>
<tr>
<td>PU</td>
<td>perfusion units</td>
</tr>
<tr>
<td>RPE</td>
<td>rating of perceived exertion</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SEPS</td>
<td>superficial endoscopic perforator surgery</td>
</tr>
<tr>
<td>SNP</td>
<td>sodium nitroprusside</td>
</tr>
<tr>
<td>SNS</td>
<td>sympathetic nervous system</td>
</tr>
<tr>
<td>StO₂</td>
<td>calf muscle oxygen saturation</td>
</tr>
<tr>
<td>( \tau )</td>
<td>time constant</td>
</tr>
<tr>
<td>TD</td>
<td>time delay</td>
</tr>
<tr>
<td>TEM</td>
<td>technical error of measurement</td>
</tr>
<tr>
<td>tHb</td>
<td>total haemoglobin/myoglobin</td>
</tr>
<tr>
<td>( \dot{V}O_{2} )</td>
<td>rate of oxygen consumption</td>
</tr>
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Publications and conference presentations arising from this research

Published articles


Published abstracts
*Oral communication at the Yorkshire Vascular Forum, Garforth, Leeds, Oct 2008*
*Awarded 'Registrar Research Prize'.

*Oral communication at the BASES Annual Conference, Brunel University, Sept 2008.*
Oral presentation at the European Society for Clinical Hemorheology and Microcirculation Annual Meeting, Switzerland, June 2009.

Other presentations

Oral communication at the BASES Student Conference, University of Bedfordshire, March 2008.
*Awarded 'Best Post-Graduate Oral Presentation Prize'.

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

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

Accepted for poster presentation at the BASES Annual Conference, Leeds Metropolitan University, September 2009.
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-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.
Intact
Initial lesion
Fatty streak
Atheroma
Fibroatheroma
Complicated lesion

Hypertension, Diabetes mellitus, Hypercholesterolemia, Heart failure, Obesity, Smoking, Aging, Menopause

Endothelial dysfunction

Occurrence of atherosclerosis

Development of atherosclerosis

Cardiovascular complications

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$\text{s}^{-1}$ 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$\text{s}^{-1}$. 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).
Table 2.1  Progression of intermittent claudication for a population >65 years
(Dormandy and Murray, 1991)

<table>
<thead>
<tr>
<th>Peripheral vascular outcomes</th>
<th></th>
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<tbody>
<tr>
<td>5 to 10% have critical limb ischaemia</td>
<td></td>
</tr>
<tr>
<td>Worsening claudication: 16%</td>
<td></td>
</tr>
<tr>
<td>Lower-limb bypass surgery: 7%</td>
<td></td>
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<tr>
<td>Major amputation: 5% within 5 years</td>
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</table>

<table>
<thead>
<tr>
<th>Other cardiovascular morbidity/total mortality</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Non-fatal cardiovascular event (myocardial infarction/stroke; 5-yr rate: 20%)</td>
<td></td>
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<tr>
<td>5-yr mortality: 30% (cardiovascular events 75%)</td>
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</tbody>
</table>

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 healthy 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).

Table 2.2  Classification of peripheral arterial disease: Fontaine’s stages and Rutherford’s categories (Dormandy and Rutherford, 2000)

<table>
<thead>
<tr>
<th>Fontaine</th>
<th>Rutherford</th>
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<tr>
<td>Stage</td>
<td>Clinical</td>
</tr>
<tr>
<td>I</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>IIa</td>
<td>Mild claudication</td>
</tr>
<tr>
<td>IIb Moderate-severe claudication</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>Minor tissue loss</td>
</tr>
<tr>
<td>IV</td>
<td>Ulceration or gangrene</td>
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<td></td>
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</tbody>
</table>

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 \( \leq 0.90 \) has a sensitivity of 95% and a specificity of 100%, relative to contrast angiography, for detecting a stenotic lesion in the limb of \( \geq 50\% \) (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 \( \leq 0.40 \) represent severe PAD (Hirsch et al., 2006). An abnormal ABPI (\( \leq 0.90 \)) is a potent predictor of cardiovascular events and premature mortality (Newman et al., 1999).
Right ABI = ratio of
Higher of the right ankle systolic pressures (posterior tibial or dorsalis pedis)
Higher arm systolic pressure (left or right arm)

Left ABI = ratio of
Higher of the left ankle systolic pressures (posterior tibial or dorsalis pedis)
Higher arm systolic pressure (left or right arm)

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·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 undertreated. 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).
Choosing drugs for patients newly diagnosed with hypertension

Abbreviations:
A = ACE inhibitor
C = calcium-channel blocker
D = thiazide-type diuretic

(consider angiotensin-II receptor antagonist if ACE intolerant)

Black patients are those of African or Caribbean descent, and not mixed-race, Asian or Chinese patients

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 μg·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 μg·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. Further 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.
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)

<table>
<thead>
<tr>
<th>Primary clinician role</th>
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<tbody>
<tr>
<td>Establish the PAD diagnosis using the ABPI measurement or other objective vascular laboratory evaluations</td>
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<tr>
<td>Determine that claudication is the major symptom limiting exercise</td>
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<tr>
<td>Discuss risk/benefit of claudication therapeutic alternatives, including pharmacological, percutaneous, and surgical interventions</td>
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<tr>
<td>Initiate systemic atherosclerosis risk modification</td>
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<tr>
<td>Perform treadmill stress testing</td>
</tr>
<tr>
<td>Provide formal referral to a claudication exercise rehabilitation program</td>
</tr>
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Exercise guidelines for claudication*

Warm-up and cool-down period of 5 to 10 minutes each

Types of exercise

Treadmill and track walking are the most effective exercise for claudication
Resistance training has conferred benefit to individuals with other forms of cardiovascular disease, and its use, as tolerated, for general fitness is complementary to but not a substitute for walking

Intensity

The speed and grade of the treadmill is set to that which elicits claudication symptoms within 3 to 5 minutes
Patients walk at this intensity until they achieve claudication of moderate severity, which is then followed by a brief period of standing or sitting rest to permit symptoms to resolve

Duration

The exercise-rest-exercise pattern should be repeated throughout the exercise session
The initial duration will usually include 35 minutes of intermittent walking and should be increased by 5 minutes each session until 50 minutes of intermittent walking can be accomplished

Frequency

Treadmill or track walking 3 to 5 times per week

Role of direct supervision

As patients improve their walking ability, the intensity of exercise should be increased by modifying the treadmill grade or speed (or both) to ensure that there is always the stimulus of claudication pain during the workout
As patients increase their walking ability, there is the possibility that cardiac signs and symptoms may appear (e.g., dysrhythmia, angina, or ST-segment depression). These events should prompt physician re-evaluation

* 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.

Table 2.4 Patient anxieties associated with walking exercise (Binnie et al., 1999)

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1</td>
<td>Fear of the pain (i.e. that walking with pain will do some damage)</td>
</tr>
<tr>
<td>2</td>
<td>A perception that going for a walk is not possible because of the pain</td>
</tr>
<tr>
<td>3</td>
<td>A fear of not being able to get home, or of legs ‘giving way’</td>
</tr>
<tr>
<td>4</td>
<td>Feeling foolish when having to stop</td>
</tr>
<tr>
<td>5</td>
<td>A perception of not having time to go for regular walks</td>
</tr>
<tr>
<td>6</td>
<td>A dislike of walking without a purpose</td>
</tr>
<tr>
<td>7</td>
<td>Fear or dislike of going out alone</td>
</tr>
<tr>
<td>8</td>
<td>Confusing ‘stop-start’ activities (e.g. ‘pottering about’; ‘I am on my feet all day’) with the sustained effort of a purposeful walk</td>
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</table>

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·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·kg⁻¹·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 Sortie 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; Sortie 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·kg⁻¹·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 (StO2) at 1 min and time-to-minimum StO2. 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 O₂ 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 O₂ 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 ≥12 months
- A resting ABPI ≤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 sphygmanometer) 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·h⁻¹ at 12% gradient; and, (ii) graded (incremental) treadmill protocol - starting horizontally at a constant speed of 3.2 km·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·h⁻¹) but the grade was varied, starting horizontally, and then increasing by 1%·min⁻¹. Grade increments of 1%·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 ≥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 (StO2) 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 StO2 percentage (Figure 3.1). Calf muscle StO2 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 StO2 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 separation 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 StO₂ at absolute (e.g. rest, 1 min, 2 min) and relative (e.g. MWD) time-points, and time-to-minimum StO₂. Time-to-minimum StO₂, 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-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-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·min⁻¹.
Figure 3.3  Electronically-braked cycle ergometer positioned for arm-crank exercise
3.3.3 Pulmonary oxygen uptake kinetics

Pulmonary \( \dot{V} \) \( \text{O}_2 \) 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 \( \dot{V} \) \( \text{O}_2 \), 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 \( \dot{V} \) \( \text{O}_2 \) can be well described by an exponential function, and can thus be described with an equation of the form:

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

[1]

where \( Y \) represents pulmonary \( \dot{V} \) \( \text{O}_2 \) at any time \( t \), \( b \) represents baseline, \( A \) is the amplitude of the increase in \( Y \) above the baseline value, \( \tau \) is the time constant defined as the time required for \( \dot{V} \) \( \text{O}_2 \) to reach 63% of its final amplitude (Jones and Poole, 2005), and \( TD \) is the time delay.
Square-wave increase in energetic (ATP) demand to sustain exercise

Oxygen uptake (VO$_2$) increases with finite kinetics

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, $\tau$, 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. $0.63 + 0.63 \times (1.0 - 0.63) = 0.86$); 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 $\tau$ therefore represent 'fast' $\dot{V} O_2$ response kinetics (e.g. a $\tau$ 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. $4 \times 20$ s), whereas high values of $\tau$ represent 'slow' $\dot{V} O_2$ kinetics (a $\tau$ of 50 s means that a steady-state $\dot{V} O_2$ is attained in approximately 200 s; $4 \times 50$ s).

The $\tau$ of the $\dot{V} O_2$ response after an increase in metabolic rate is thought to be an important determinant of 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 ($\tau$) 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 $\dot{V}O_2$ 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 $\tau$ describing the rate with which pulmonary $\dot{V}O_2$ rises in phase II reflects the $\tau$ 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-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 $\tau$ had a TEM of 18%. The mean response time (MRT) was also calculated ($MRT = TD + \tau$) 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 healthy 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·kg⁻¹·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 (PolarA1, 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, $\tau$, 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 StO₂, 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 coefficient ($r$). 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 (StO₂) 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 StO₂ 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 \( V_\text{O}_2 \)), ventilatory threshold, and \( V_\text{O}_2 \) kinetics, as such measures of cardiopulmonary fitness are correlated with exercise performance in other populations, including trained runners (Kilding \textit{et al.}, 2006), overweight adolescents (Drinkard \textit{et al.}, 2001), and patients with advanced heart failure (Cahalin \textit{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.

\textbf{4.2 METHODS}

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

\textit{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

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

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 (StCh) 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 $P < 0.05$.

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$_2$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Values, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWD (m)</td>
<td>523 ± 262$^a$</td>
</tr>
<tr>
<td>Resting ankle-brachial pressure index</td>
<td>0.71 ± 0.17$^a$</td>
</tr>
<tr>
<td>Resting calf blood flow (%·min$^{-1}$)</td>
<td>2.70 ± 0.97$^a$</td>
</tr>
<tr>
<td>Peak calf blood flow (%·min$^{-1}$)</td>
<td>10.05 ± 4.97$^a$</td>
</tr>
<tr>
<td>Peak $\dot{V}_O^2$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>17.8 ± 3.9$^a$</td>
</tr>
<tr>
<td>Ventilatory threshold (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>12.0 ± 2.7$^b$</td>
</tr>
<tr>
<td>$\tau$ (s)</td>
<td>45.9 ± 12.2$^c$</td>
</tr>
<tr>
<td>StO$_2$ at rest (%)</td>
<td>47 ± 8$^c$</td>
</tr>
<tr>
<td>StO$_2$ at 1 min (%)</td>
<td>41 ± 12$^c$</td>
</tr>
<tr>
<td>StO$_2$ at MWD (%)</td>
<td>38 ± 13$^c$</td>
</tr>
<tr>
<td>Time-to-minimum StO$_2$ (s)</td>
<td>345 ± 330$^c$</td>
</tr>
</tbody>
</table>

MWD, maximum walking distance; $\dot{V}_O^2$, pulmonary oxygen uptake; $\tau$, $\dot{V}_O^2$ time constant; StO$_2$, calf muscle oxygen saturation. $^a n = 45$, $^b n = 25$, $^c n = 43$.  

74
Figure 4.1 Representative near-infrared spectroscopy profile from a claudicant during graded treadmill exercise. A, calf muscle oxygenation (StO₂; 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 (StO₂) 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 StO₂ 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 StO₂ relative to baseline, and a low time-to-minimum StO₂ 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 ($\tau = 44$ 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 StO₂ variables are presented in Table 4.3. Time-to-minimum StO₂ was strongly and positively associated with MWD, whereas moderate positive correlations with MWD were observed for peak $\dot{V}O_2$ and StO₂ 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 $\tau$ and MWD. There were no relationships between MWD and resting calf blood flow, ventilatory threshold, or StO₂ at rest or at MWD.

Table 4.3 Univariate Pearson's correlation ($r$) between MWD and haemodynamic, cardiopulmonary fitness, and calf muscle StO₂ variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting ABPI</td>
<td>0.379</td>
<td>0.008</td>
</tr>
<tr>
<td>Resting calf blood flow</td>
<td>0.132</td>
<td>0.388</td>
</tr>
<tr>
<td>Peak calf blood flow</td>
<td>0.328</td>
<td>0.019</td>
</tr>
<tr>
<td>Peak $\dot{V}O_2$</td>
<td>0.553</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ventilatory threshold</td>
<td>0.141</td>
<td>0.192</td>
</tr>
<tr>
<td>$\tau$</td>
<td>-0.358</td>
<td>0.012</td>
</tr>
<tr>
<td>StO₂ at rest</td>
<td>0.075</td>
<td>0.622</td>
</tr>
<tr>
<td>StO₂ at 1 min</td>
<td>0.519</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>StO₂ at MWD</td>
<td>0.178</td>
<td>0.241</td>
</tr>
<tr>
<td>Time-to-minimum StO₂</td>
<td>0.752</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MWD, maximum walking distance; ABPI, ankle-brachial pressure index; $\dot{V}O_2$, pulmonary oxygen uptake; $\tau$, $\dot{V}O_2$ time constant; StO₂, calf muscle oxygen saturation
Figure 4.4 Scatter-plots: A, MWD and time-to-minimum StO₂; B, MWD and peak $\dot{V}_O_2$; and, C, MWD and StO₂ at 1 min. Linear regression lines are illustrated to highlight relationships. *$P < 0.001$.

MWD, maximum walking distance; StO₂, calf muscle oxygen saturation
Table 4.4 shows the results of the multiple regression analysis. The final regression model, which included time-to-minimum StO₂, peak $\dot{V}O_2$ and StO₂ at 1 min, explained 64% of the variation in MWD. As either StO₂ 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 StO₂) + (16.854 x peak $\dot{V}O_2$) + (5.841 x StO₂ 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

<table>
<thead>
<tr>
<th>Step</th>
<th>Unstandardised beta coefficient</th>
<th>Standard error</th>
<th>Standardised beta coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>322.52</td>
<td>39.54</td>
<td></td>
</tr>
<tr>
<td>Time-to-minimum</td>
<td>0.58</td>
<td>0.08</td>
<td>0.75*</td>
</tr>
<tr>
<td>StO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>127.48</td>
<td>91.56</td>
<td></td>
</tr>
<tr>
<td>Time-to-minimum</td>
<td>0.50</td>
<td>0.09</td>
<td>0.65*</td>
</tr>
<tr>
<td>StO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>StO₂ at 1 min</td>
<td>5.44</td>
<td>2.33</td>
<td>0.26*</td>
</tr>
<tr>
<td>Step 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-153.00</td>
<td>152.71</td>
<td></td>
</tr>
<tr>
<td>Time-to-minimum</td>
<td>0.40</td>
<td>0.09</td>
<td>0.51*</td>
</tr>
<tr>
<td>StO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>StO₂ at 1 min</td>
<td>5.84</td>
<td>2.22</td>
<td>0.28*</td>
</tr>
<tr>
<td>Peak $\dot{V}O_2$</td>
<td>16.85</td>
<td>7.54</td>
<td>0.25*</td>
</tr>
</tbody>
</table>

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

MWD, maximum walking distance; StO₂, 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 StO₂ was moderately
and positively associated with StO₂ at 1 min and peak $\tilde{V}O₂$, whereas StO₂ at 1 min was not correlated with peak $\tilde{V}O₂$. 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

<table>
<thead>
<tr>
<th></th>
<th>Time-to-minimum</th>
<th>StO₂ at 1 min</th>
<th>Peak $\tilde{V}O₂$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-to-minimum</td>
<td>1.000*</td>
<td>0.388*</td>
<td>0.502*</td>
</tr>
<tr>
<td>StO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>StO₂ at 1 min</td>
<td>0.388*</td>
<td>1.000*</td>
<td>0.081</td>
</tr>
<tr>
<td>Peak $\tilde{V}O₂$</td>
<td>0.502*</td>
<td>0.081</td>
<td>1.000*</td>
</tr>
</tbody>
</table>

*P < 0.05.

StO₂, calf muscle oxygen saturation; $\tilde{V}O₂$, 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 StO₂ 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 StO₂, likely due to the activation of the muscle pump (Bauer et al., 2004b). This was followed by a sharp decline in calf muscle StO₂ 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 $S_tC_2$. 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 $S_tO_2$, peak $\dot{V} O_2$ and $S_tO_2$ at 1 min, and together these variables explained 64% of the variation in MWD. Time-to-minimum $S_tO_2$ appeared to be the most important predictor variable, explaining over half of the variance (Table 4.4). The correlation coefficients for time-to-minimum $S_tO_2$ and $S_tO_2$ 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 $\tau$ 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.
StO₂) 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 \( \tau \) 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 \textit{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 \textit{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·kg⁻¹·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 \( \tau \), 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 \textit{et al.}, 2006).

Limitations

The main limitations of this study mostly relate to the NIRS and \( \dot{V}O_2 \) kinetics data. Although the StO₂ 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 StO₂ should be minimal beyond the initial phase of exercise (Mancini \textit{et al.}, 1994; Bauer \textit{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 $\tau$, and this might explain why the $\tau$ 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\textsubscript{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

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

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 $74 \pm 11\%$, $13.1 \pm 1.5$, and $3.3 \pm 1.4$, respectively (calculated using data from all of the completed training sessions). Training intensity increased from $39 \pm 8\ W$ (63 ± 7% peak W) at baseline to $55 \pm 11\ W$ (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

<table>
<thead>
<tr>
<th></th>
<th>Exercise group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 12 weeks</td>
<td>Baseline 12 weeks</td>
<td></td>
</tr>
<tr>
<td>Peak work rate (W)</td>
<td>62 ± 14</td>
<td>79 ± 16*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak ( \dot{V} O_2 ) (mL·kg(^{-1})·min(^{-1}))</td>
<td>13.5 ± 2.7</td>
<td>15.2 ± 2.7</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>2.7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilatory threshold (mL·kg(^{-1})·min(^{-1}))</td>
<td>8.4 ± 1.2</td>
<td>8.3 ± 1.4</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>8.8 ± 1.5</td>
<td>8.0 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Peak heart rate (beats·min(^{-1}))</td>
<td>121 ± 23</td>
<td>124 ± 21</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>116 ± 24</td>
<td>121 ± 21</td>
<td></td>
</tr>
<tr>
<td>Peak blood lactate (mM)</td>
<td>3.94 ± 4.34</td>
<td>3.63 ± 3.63</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>1.34</td>
<td>1.28</td>
<td>0.94</td>
</tr>
<tr>
<td>Peak exertion</td>
<td>15.7 ± 2.4</td>
<td>15.0 ± 2.6</td>
<td>0.614</td>
</tr>
<tr>
<td></td>
<td>15.6 ± 2.3</td>
<td>15.3 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Peak arm pain</td>
<td>4.1 ± 2.6</td>
<td>4.8 ± 2.6</td>
<td>0.972</td>
</tr>
<tr>
<td></td>
<td>3.8 ± 2.8</td>
<td>4.5 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Table 5.3 Incremental treadmill-walking test data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Exercise group</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Claudication distance (m)</td>
</tr>
<tr>
<td>Maximum walking distance (m)</td>
</tr>
<tr>
<td>Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$)</td>
</tr>
<tr>
<td>Ventilatory threshold (mL·kg$^{-1}$·min$^{-1}$)</td>
</tr>
<tr>
<td>Peak heart rate (beats·min$^{-1}$)</td>
</tr>
<tr>
<td>Peak lactate (mM)</td>
</tr>
<tr>
<td>Peak exertion</td>
</tr>
<tr>
<td>Peak leg pain</td>
</tr>
<tr>
<td>Time-to-minimum StO$_2$ (s)</td>
</tr>
<tr>
<td>End-exercise StO$_2$ (%)</td>
</tr>
</tbody>
</table>

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 (StO₂) data during the incremental treadmill walking test for A, the arm-crank exercise training group, and B, the control group. Min indicates minimum StO₂ 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. *$P$ \textless 0.05. The speed of the treadmill was fixed at 3.2 km\text{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>
<thead>
<tr>
<th>Table 5.4</th>
<th>Haemodynamic outcome measures assessed at rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exercise group</td>
</tr>
<tr>
<td></td>
<td>Baseline 12 weeks</td>
</tr>
<tr>
<td>Resting ABPI</td>
<td>0.67 ± 0.13</td>
</tr>
<tr>
<td>Resting calf blood flow (% min⁻¹)</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>Peak calf blood flow (% min⁻¹)</td>
<td>12.2 ± 7.0</td>
</tr>
<tr>
<td>Resting skin flux (PU)</td>
<td>5.1 ± 4.5</td>
</tr>
<tr>
<td>Peak skin flux (PU)</td>
<td>19.6 ± 11.6</td>
</tr>
<tr>
<td>Hyperaemic response (%)</td>
<td>502 ± 420</td>
</tr>
<tr>
<td>Skin temperature (°C)</td>
<td>28.4 ± 2.1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Exercise group</th>
<th>Control group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 weeks</td>
<td>Baseline</td>
</tr>
<tr>
<td>Resting $\dot{V}O_2$ (mL·min$^{-1}$)</td>
<td>$307 \pm 43$</td>
<td>$320 \pm 41$</td>
<td>$292 \pm 46$</td>
</tr>
<tr>
<td>End-exercise $\dot{V}O_2$ (mL·min$^{-1}$)</td>
<td>$915 \pm 138$</td>
<td>$923 \pm 179$</td>
<td>$855 \pm 168$</td>
</tr>
<tr>
<td>Time delay (s)</td>
<td>$13.1 \pm 5.9$</td>
<td>$12.4 \pm 6.6$</td>
<td>$12.9 \pm 4.9$</td>
</tr>
<tr>
<td>$\tau$ (s)</td>
<td>$44.7 \pm 10.4$</td>
<td>$41.3 \pm 14.4^*$</td>
<td>$44.2 \pm 11.1$</td>
</tr>
<tr>
<td>MRT (s)</td>
<td>$57.8 \pm 11.6$</td>
<td>$53.7 \pm 13.5^*$</td>
<td>$57.1 \pm 9.4$</td>
</tr>
<tr>
<td>Resting heart rate (beats·min$^{-1}$)</td>
<td>$75 \pm 13$</td>
<td>$73 \pm 14$</td>
<td>$71 \pm 14$</td>
</tr>
<tr>
<td>Steady state heart rate (beats·min$^{-1}$)</td>
<td>$95 \pm 20$</td>
<td>$91 \pm 17$</td>
<td>$87 \pm 15$</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Exercise group</th>
<th>Control group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 weeks</td>
<td>Baseline</td>
</tr>
<tr>
<td>Occupational activities</td>
<td>$25 \pm 51$</td>
<td>$25 \pm 44$</td>
<td>$3 \pm 16$</td>
</tr>
<tr>
<td>Household work</td>
<td>$71 \pm 58$</td>
<td>$60 \pm 37$</td>
<td>$55 \pm 35$</td>
</tr>
<tr>
<td>Leisure activities</td>
<td>$107 \pm 30$</td>
<td>$113 \pm 33$</td>
<td>$121 \pm 27$</td>
</tr>
<tr>
<td>Total activity</td>
<td>$202 \pm 56$</td>
<td>$197 \pm 32$</td>
<td>$180 \pm 30$</td>
</tr>
</tbody>
</table>

Values (in MET·hour·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 StO₂, peak \( \dot{V}O_2 \) and StO₂ 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·kg\(^{-1}\)·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 $\tau$ (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 $\tau$ 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 $\tau$ 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 \(\tau\), or the poor reproducibility of \(\tau\) \((\text{TEM} = 18\%)\) compared to maximum walking distance \((\text{TEM} = 6\%)\). Alternatively, \(\dot{V}O_2\) kinetics might not be an important determinant of exercise performance in claudicants. Indeed, in Study 1, \(\tau\) 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 \((\text{Gardner et al.}, 2008)\). 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 \((\text{cardiac output})\) 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 \((\text{Loftin et al.}, 1988)\), 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 \((\text{Walker et al.}, 2000)\). 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 $\tau$ 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 O₂ 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 StO₂, peak \( \dot{V}O_2 \) and StO₂ at 1 min. The results suggest that cardiopulmonary fitness and the ability to match O₂ delivery to metabolic demand are important determinants of MWD in this patient population, and that measures made during exercise (e.g. StO₂) 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 StO₂, peak \( \dot{V}O_2 \) and StO₂ at 1 min) were all improved after arm-crank exercise training, providing evidence of enhanced lower-limb O₂ delivery. Furthermore, the improvement in walking performance appears to be explained, at least in part, by enhanced lower-limb O₂ 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 (Nicolaiides 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 (Nicolaiides 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)

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

+, 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., 2003).
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 interendothelial 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 (Bumand 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)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description/Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C, Clinical</strong> (sub-divided into A for asymptomatic, S for symptomatic)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No venous disease</td>
</tr>
<tr>
<td>1</td>
<td>Telangiectases</td>
</tr>
<tr>
<td>2</td>
<td>Varicose veins</td>
</tr>
<tr>
<td>3</td>
<td>Oedema</td>
</tr>
<tr>
<td>4a</td>
<td>Pigmentation or eczema</td>
</tr>
<tr>
<td>4b</td>
<td>Lipodermatosclerosis or white atrophy</td>
</tr>
<tr>
<td>5</td>
<td>Healed venous ulcer</td>
</tr>
<tr>
<td>6</td>
<td>Active venous ulcer</td>
</tr>
<tr>
<td><strong>E, Etiologic classification</strong></td>
<td>Present since birth</td>
</tr>
<tr>
<td>Congenital</td>
<td>Present since birth</td>
</tr>
<tr>
<td>Primary</td>
<td>Undetermined etiology</td>
</tr>
<tr>
<td>Secondary</td>
<td>Post-thrombotic</td>
</tr>
<tr>
<td><strong>A, Anatomic distribution</strong></td>
<td>Great and short saphenous veins</td>
</tr>
<tr>
<td>Superficial</td>
<td>Great and short saphenous veins</td>
</tr>
<tr>
<td>Deep</td>
<td>Cava, iliac, gonadal, femoral, profunda, popliteal, tibial, and muscular veins</td>
</tr>
<tr>
<td><strong>P, Pathophysiology</strong></td>
<td>Axial and perforating veins</td>
</tr>
<tr>
<td>Reflux</td>
<td>Axial and perforating veins</td>
</tr>
<tr>
<td>Obstruction</td>
<td>Acute and chronic</td>
</tr>
<tr>
<td>Combination of both</td>
<td>Valvular dysfunction and thrombus</td>
</tr>
</tbody>
</table>
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 pathophysiologic features (Figure 8.2).
Signs and symptoms of CVI

Conservative management including compressive therapy

Satisfactory response

Continue treatment

Unsatisfactory response or advanced clinical disease

Non-Invasive Testing
- Duplex and/or APG

Non-acute Obstruction
- Venography
  - Consider Venous Stenting

Reflux
- Duplex and/or APG
  - Superficial Venography
  - Consider Ablation or Stripping
  - Deep Venography
  - Consider Valve Reconstruction
  - Perforator
  - Consider SEPS

Muscle Pump Dysfunction
- Consider Exercise Program

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 (Peralia 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 
oxidation 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 $\geq$ 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 (Labichella 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, Jarfalla, 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 (PeriOint 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-hr⁻¹; Controls: 5.0 ±0.1 km-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
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 \( \chi^2 \)-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 \( \chi^2 \)-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

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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<td>5 male, 7 female</td>
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</tr>
<tr>
<td>Age (years)</td>
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<td>50 ± 6</td>
<td>0.229b</td>
</tr>
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<td>Body mass (kg)</td>
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<td>78.9 ± 21.4</td>
<td>0.772b</td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>169.0 ± 9.6</td>
<td>169.0 ± 12.5</td>
<td>0.990b</td>
</tr>
</tbody>
</table>

Values are means ± SD. aChi-squared tests, bindependent $t$-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 $802 \pm 886$ at baseline to $1273 \pm 1078\%$ after acute exercise in the patients, with no such change observed for the controls ($1156 \pm 816$ to $1050 \pm 755\%$) (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 ≤ 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 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 subdermal 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:  **Study 4 - 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; Winterbom 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 of 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>
<thead>
<tr>
<th>Table 11.1 Demographic data</th>
<th>Exercise group</th>
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<td>Age (y)</td>
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<td>Body mass (kg)</td>
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<td>Stature (cm)</td>
<td>168.2 ± 5.0</td>
<td>166.2 ± 8.9</td>
<td>0.141&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SD. <sup>a</sup>Chi-squared tests, <sup>b</sup>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 ≤ 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 $6 \pm 3$ PU in the exercise group versus $7 \pm 5$ 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·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 $\dot{V}O_2$, StO2 at 1 min, and time-to-minimum StO2, 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., StO2) 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 O2 delivery in these patients. Arm-crank exercise training induced favourable changes in various NIRS (time-to-minimum StO2 and sub-maximal StO2) 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 activity 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


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


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


Dear ........

We are undertaking a study, to determine the effects of a 12-week training programme of arm exercise on the symptoms of claudication.

We have recently shown that both a six and 25-week programme of arm exercise can improve walking distances in claudicant patients, to a similar extent that was achieved through leg exercise. We now need to show how arm exercise results in the improvement in walking ability.

You may have been selected as being a suitable patient from attending the Vascular Clinic or because you have been involved in a previous study.

Please find enclosed a patient information sheet, which describes the study in detail and answers the most frequently asked questions.

One of my staff (Mr Garry Tew) will be phoning you shortly to see if you would be interested in participating in this study and will gladly answer any further questions you may have. It is important to note that there is no pressure to participate in this study and if you do not wish to be contacted please phone 0114 225 2358 before (insert date).

Yours sincerely,

Mr Shah Nawaz, M.D., F.R.C.S.
Consultant Vascular Surgeon
Sheffield Vascular Institute
Q: Why has my doctor asked me to take part in this study?

A: We are currently proposing to undertake a study, in order to compare the effects of a 12-week training programme of arm exercise on the symptoms of claudication. You may have been selected as being a suitable patient from your medical notes or because you have been involved in a previous study.

Exercising the legs can improve your walking distance, however this can be painful. We have recently shown that a 25-week programme of arm exercise improved walking distances in claudicant patients. The improvements were equivalent to those patients who undertook leg exercise. However, the main advantage of undertaking arm exercise is that patients are able to exercise without experiencing the pain associated with leg exercise.

Q: What will I have to do?

A: All patients entering the study will have a medical examination and will be randomly allocated to one of two groups. One group will be required to undertake a supervised 12-week arm exercise training programme at the Centre for Sport and Exercise Science, of Sheffield Hallam University, Collegiate Hall, Collegiate Crescent (off Ecclesall Road). The second group will not be required to undertake formal exercise training, but will be actively encouraged to exercise, and their level of physical activity will be monitored using an activity diary.
Q: How long will the study last?
A: The programme will last for a total of 12 weeks. We will monitor everyone's progress throughout with assessments at the start and at the end of the programme period.

Q: How long will I have to exercise for?
A: If allocated to the arm exercise training group you will be required to exercise twice per week, for a total of 20 minutes during a 40-minute training session – i.e. 2 minutes exercise, 2 minutes rest, 2 minutes exercise, 2 minutes rest etc. All exercise will be carefully supervised and you will be shown how to use the equipment.

Q: Are there side-effects?
A: No side effects have been observed in our previous study and therefore none are expected.

Q: What will the assessment visits entail?
A: During 3 assessment visits at the beginning and end of the study we will:

1. Collect a small 10-20 ml blood sample from an arm vein, then ask you to perform a treadmill walking test. During this session we will monitor your heart rate and take your blood pressure. We will also take a small finger-prick blood sample and measure your oxygen consumption whilst you are exercising. We will measure blood pressure in your arm and ankle and blood flow in your most painful calf. The blood flow measures will involve inflation of a blood pressure cuff placed around your thigh and measurement of the changes in the volume of your lower leg using sensitive non-invasive equipment. During this session, we will also ask you to fill in a questionnaire.

2. Ask you to perform arm exercise. During this session we will monitor your heart rate and take your blood pressure. We will also take a small finger-prick blood sample and measure your oxygen consumption whilst you are exercising.

3. Ask you to perform 3 short treadmill walking tests at moderate intensity. During these tests, we will measure your oxygen consumption whilst you are exercising. You will also have a small
piece of apparatus strapped to your calf during these assessments. This technique is completely painless and harmless.

These assessments will be performed over 3 separate visits to the Centre, with each visit lasting 45-90 minutes.

Q: What if I do not wish to take part?
A: This will in no way affect your treatment.

Q: What if I change my mind during the study?
A: You are free to withdraw from the study at any time without affecting your treatment.

Q: What will happen to the information from the study?
A: The overall conclusions of the study will be available to you, however it will not be possible to produce an individualised report of your performance.

Q: What if I have further questions?
A: If you have any further questions with regards to this study you may phone:-
### Table

<table>
<thead>
<tr>
<th>IF YOUR QUERY IS REGARDING ...</th>
<th>CONTACT</th>
<th>AT THE</th>
<th>TELEPHONE NUMBER</th>
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<tr>
<td>Participating in this study</td>
<td>MR GARRY TEW</td>
<td>CENTRE FOR SPORT &amp; EXERCISE SCIENCE</td>
<td>07773310360 (MOBILE LINE)</td>
</tr>
<tr>
<td>or</td>
<td></td>
<td>(SHEFFIELD HALLAM UNIVERSITY)</td>
<td>01142252358 (OFFICE LINE)</td>
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<td>Participating in this study</td>
<td>DR JOHN SAXTON</td>
<td></td>
<td>01142254414 (DIRECT LINE)</td>
</tr>
<tr>
<td>Your health (i.e. medically</td>
<td>MR SHAH NAWAZ</td>
<td>NORTHERN GENERAL HOSPITAL</td>
<td>01142714450 (DIRECT LINE)</td>
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<td>related)</td>
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### Q: What if I wish to complain about the way in which this study has been conducted

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study.

If you have any complaints or concerns please contact either the project co-ordinator:

Name: Dr John Saxton……………… Tel: 01142254414……………………...
Otherwise you can use the normal hospital complaints procedure and contact the following person:

Name: Chris Welch ...................... Tel: 0114 271 2178 .................

OR

Otherwise you can use the normal University complaints procedure and contact the following person:

Name: Office of the Registrar ........ Tel: 0114 225 5555 .................

Sheffield Hallam University has the following policies in place for the legal liability of the University; (a) Professional indemnity (£10 million); and (b) Public liability (£20 million).
Appendix 3: Ethics approval letter (Studies 1 & 2)

South Sheffield Research Ethics Committee
307 Western Bank
Sheffield
S10 2TJ

Telephone: 0114 226 1172
Facsimile: 0114 271 2394
Email: Louise.Horne@sth.nhs.uk

Chair: Dr. M Hatton
REC Coordinator: Ms. L Horne

02 November 2005

Dr J Saxton
Principal Research Fellow
Centre for Sport & Exercise Science
Sheffield Hallam University
Collegiate Crescent
Sheffield
S10 2BP

Dear Dr Saxton

Full title of study: The effects of upper-limb aerobic exercise training on walking performance and lower-limb circulatory function in patients with peripheral arterial disease.

REC reference number: 05/Q2305/128

Thank you for your letter of 18 October 2005, responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
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<tbody>
<tr>
<td>Application</td>
<td>4.1</td>
<td>26 August 2005</td>
</tr>
<tr>
<td>Application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Investigator CV Dr. M Saxton</td>
<td></td>
<td></td>
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<tr>
<td>Protocol</td>
<td>1</td>
<td>26 August 2005</td>
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</table>
Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/Q2305/128 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project

Yours sincerely

Dr. M Hatton
Chair

Enclosures: Standard approval conditions
           Site approval form

Copy to: STH Research Department
        305 Western Bank
        Sheffield
        S10 2TJ
South Sheffield Research Ethics Committee

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

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<tr>
<th>REC reference number:</th>
<th>05/Q2305/128</th>
<th>Issue number:</th>
<th>1</th>
<th>Date of issue:</th>
<th>02 November 2005</th>
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</thead>
</table>

Chief Investigator: Dr John Saxton

Full title of study: The effects of upper-limb aerobic exercise training on walking performance and lower-limb circulatory function in patients with peripheral arterial disease.

This study was given a favourable ethical opinion by South Sheffield Research Ethics Committee on 07 November 2005. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Post</th>
<th>Research site</th>
<th>Site assessor</th>
<th>Date of favourable opinion for this site</th>
<th>Notes (1)</th>
</tr>
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<tbody>
<tr>
<td>Dr John Saxton</td>
<td>Principal Research Fellow</td>
<td>STH Research Department305 Western BankSheffieldS10 2TJ</td>
<td>South Sheffield Research Ethics Committee</td>
<td>02/11/2005</td>
<td></td>
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</tbody>
</table>

Approved by the Chair on behalf of the REC:

.................................................. (Signature of Chair/Administrator)

.................................................. (Name)

(1) The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.
Appendix 4: Peripheral Arterial Disease Physical Activity Recall (PAD-PAR) questionnaire

1. How many hours do you sleep a night, on average?
   Hours x 7 =
   Sleep hours per week =

2. Explain to patient that you are going to ask about typical work activities performed during the past week (includes work for pay or regular volunteer activities).
   If patient is not employed, go to question 3.
   How many total hours did you work per week on average?
   Work hours per week?

   Here is a listing of typical work activities (show participant CARD A).
   Activities are classified as heavy, moderate, light and very light, depending on their average energy demands.
   With your job, time may be spent in more than one category of activity. Let’s start with heavy activities and then go on to moderate, light and then very light activities.

   a) Please tell me the average number of days during the last week you performed heavy activities at work
   b) Please tell me the average length of time you performed heavy activities in a day.

   Then, repeat above directions for all intensities of activity.

<table>
<thead>
<tr>
<th>Intensity of Activity</th>
<th>Days per Week (0.5 to 7.0)</th>
<th>Hours per Day (nearest 0.5 h)</th>
<th>Hours per Week</th>
<th>MET Hours per Week</th>
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<tbody>
<tr>
<td>Heavy (5.1 - 7.0 METs)</td>
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<tr>
<td>Moderate (3.1 - 5.0 METs)</td>
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</tr>
<tr>
<td>Light (2.1 - 3.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Light (0.9 - 2.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. Did you perform household chores or yard work around the home during the past week? (Follow instructions given above, except refer to CARD B). If yes, how many total hours did you spend in household chores?

Household or yard hours per week =

<table>
<thead>
<tr>
<th>Intensity of Activity</th>
<th>Days per Week (0.5 to 7.0)</th>
<th>Hours per Day (nearest 0.5 h)</th>
<th>Hours per Week</th>
<th>MET Hours per Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy (5.1 - 7.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate (3.1 - 5.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light (2.1 - 3.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Light (0.9 - 2.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Did you perform recreational or leisure-time activities during the past week (Refer to CARD C). If yes, how many total hours did you spend in leisure activities?

Recreation or leisure hours per week =

<table>
<thead>
<tr>
<th>Intensity of Activity</th>
<th>Days per Week (0.5 to 7.0)</th>
<th>Hours per Day (nearest 0.5 h)</th>
<th>Hours per Week</th>
<th>MET Hours per Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy (5.1 - 7.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate (3.1 - 5.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light (2.1 - 3.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Light (0.9 - 2.0 METs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Appendix 5: A range of physical activities

MET Values in Parentheses*

<table>
<thead>
<tr>
<th>Card A</th>
<th>Physical Activities at Work</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy</strong></td>
<td><strong>Moderate</strong></td>
</tr>
<tr>
<td>Heavy power tools</td>
<td>Locksmith</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(3.5)</td>
</tr>
<tr>
<td>Coal mining</td>
<td>Carrying &lt; 20 lbs</td>
</tr>
<tr>
<td>(7.0)</td>
<td>(5.0)</td>
</tr>
<tr>
<td>Loading truck</td>
<td>Farming</td>
</tr>
<tr>
<td>(6.5)</td>
<td>(4.5)</td>
</tr>
<tr>
<td>Shovelling</td>
<td>Machine tooling</td>
</tr>
<tr>
<td>(7.0)</td>
<td>(4.0)</td>
</tr>
<tr>
<td>Heavy carpentry</td>
<td>Forestry, chain saw</td>
</tr>
<tr>
<td>(7.0)</td>
<td>(4.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Card B</th>
<th>Household Chores and Yard Work</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy</strong></td>
<td><strong>Moderate</strong></td>
</tr>
<tr>
<td>Roofing</td>
<td>Food shopping</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(3.5)</td>
</tr>
<tr>
<td>Digging</td>
<td>Heavy cleaning</td>
</tr>
<tr>
<td>(5.0)</td>
<td>(4.5)</td>
</tr>
<tr>
<td>Chopping wood</td>
<td>Laying carpet</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(4.5)</td>
</tr>
<tr>
<td>Shovelling snow</td>
<td>Weeding</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(4.5)</td>
</tr>
<tr>
<td>Manual lawn mowing</td>
<td>Power lawn mowing</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(4.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Card C</th>
<th>Recreational Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy</strong></td>
<td><strong>Moderate</strong></td>
</tr>
<tr>
<td>Walking/hiking uphill</td>
<td>Walking, level</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(4.0)</td>
</tr>
<tr>
<td>Moderate canoeing</td>
<td>Easy bicycling</td>
</tr>
<tr>
<td>(7.0)</td>
<td>(5.0)</td>
</tr>
<tr>
<td>Bicycling 10-12 mph</td>
<td>Frisbee, ultimate</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(3.5)</td>
</tr>
<tr>
<td>Light stationary cycle</td>
<td>Ping pong</td>
</tr>
<tr>
<td>(5.5)</td>
<td>(4.0)</td>
</tr>
<tr>
<td>Aerobic dance</td>
<td>Weight lifting</td>
</tr>
<tr>
<td>(7.0)</td>
<td>(3.5)</td>
</tr>
<tr>
<td>Leisurely swimming</td>
<td>Light rowing</td>
</tr>
<tr>
<td>(6.0)</td>
<td>(3.5)</td>
</tr>
</tbody>
</table>

Appendix 6: Patient recruitment letter (Studies 3 & 4)

Sheffield Teaching Hospitals NHS Foundation Trust
Sheffield Hallam University

Dear

We are aware that you are soon to be having surgery for your varicose veins. Despite the considerable benefits that surgery offers, the recurrence rate remains high, therefore additional measures are required in order to reduce this rate and improve quality of life.

We are undertaking a pilot study, to assess whether an 8-week exercise training programme can improve circulatory function in your legs.

Please find enclosed a patient information sheet, which describes the study in detail and answers the most frequently asked questions.

If you are interested in participating in this study, please return the tear off slip below, and post it to "Centre for Sport and Exercise Science (Varicose Veins Study), Sheffield Hallam University, Collegiate Campus, Sheffield S10 2BP" as soon as possible. One of my staff (Dr Markos Klonizakis), will then contact you and will gladly answer any further questions you may have.

Yours sincerely,

Professor Jonathan Michaels
Professor of Vascular Surgery, Northern General Hospital

EFFECTS OF POST-OPERATIVE EXERCISE IN LOWER-LIMB VENOUS DISEASE

☐ Yes. I am interested in taking part in the above named study. I understand that a member of staff will be contacting me, regarding this study.

☐ No, I am not interested in taking part in the study

Name: ____________________________
Telephone Number: ____________________________
POST-OPERATIVE EXERCISE IN LOWER-LIMB VENOUS DISEASE

Introduction

Venous disease in general, and varicose veins in specific, are the most common factors in chronic leg ulceration. While surgery can improve ulcer healing rates and reduce ulcer reoccurrence, it does not completely rectify the situation and varicose veins can still reoccur in a high number of patients. This study aims to see whether exercise is beneficial for patients who have recently had varicose veins surgery, by improving leg circulation.

Patients will be randomly allocated to one of two groups, to an exercise rehabilitation group, or to a lifestyle advice group. At the beginning and throughout the study we will investigate the functioning of the small vessels just beneath the skin around the ankle area. This will be achieved using a non-invasive technique known as LDF. Your quality of life will also be assessed using questionnaires. The data from this pilot study will hopefully provide the basis for a further clinical trial, which will be aimed at developing more effective strategies for the treatment of patients with varicose veins.

Please find attached the patient information sheet, which answers the most frequently asked questions. Part 1 of the information sheet informs you about the study and what the study involves, whereas part 2 provides you with more information about how the study is organised, and what will happen to the data. This is your copy to retain.
PATIENT INFORMATION SHEET
PART 1

Q: What is the main purpose of this study?
A: The aim of this study is to see whether exercise therapy, in the form of treadmill-walking exercise, is beneficial for patients who have recently had varicose veins surgery, by improving leg circulation.

Q: Why has my doctor asked me to take part in this study?
A: You have been selected as being a suitable patient from your medical history, and because you have recently had surgery for your varicose veins.

Q: What will I have to do?
A: All patients entering the study will have a medical examination and will be randomly allocated to one of two groups. One group will undertake an 8-week programme of moderate-intensity treadmill-walking exercise and another group will act as the usual care (lifestyle advice only) control group.

All exercise training will be performed at the Centre for Sport and Exercise Science, Sheffield Hallam University, Collegiate Crescent Campus (off Ecclesall Road). If you are in the exercise training group, twice weekly attendance at the Centre will be required during the 8-week study period. You will be shown how to use the exercise equipment, and you will be carefully supervised at all times.

If you are in the lifestyle advice group, you will only be required to attend the Centre for Sport and Exercise Science for assessment visits, which will be at the beginning end of the 8-week study period. You will not be involved in any supervised exercise training.

Q: What will the assessment visits entail?
A: All assessments will be performed at the start of, and at 8-weeks of the study period. During the assessment visits we will:-

1. Assess the flow of blood in your legs. This is achieved by placing two sticky patches to the skin just above your ankle. Through these patches tiny quantities of two drugs will be administered, which will cause local relaxation of the small blood vessels in your leg. Special computer software will analyse the results. This procedure is completely painless, and will initially be performed when you are lying down, and then after a suitable rest period when you are standing. Each series of measurements will take approximately 25 minutes to perform. You will then be required to undertake a 25-min period of treadmill walking at a
comfortable, self-selected pace, after which we will repeat the above blood flow measurements. This session will last about 2 hours in total.

2. Ask you to complete a quality of life questionnaire.

**Q:** How long will the study last?
**A:** The programme will last for 8 weeks. We will monitor your progress throughout, with assessments at the start and 8-weeks of the study, as described above.

**Q:** How long will I have to exercise for?
**A:** Patients randomised to exercise training will be required to attend the Centre for Sport and Exercise Science twice per week for the 8-week study period. Each exercise session will last for approximately 40-50 minutes. You will undertake interval-type exercise during the session, based on two minutes of exercise, followed by two minutes of rest etc, so that in a 40-minute exercise session, you will undertake 20-minutes of exercise. All exercise will be carefully supervised and you will be shown how to use the equipment.

**Q:** Will my travel expenses be reimbursed?
**A:** Your travel expenses to the Centre for Sport and Exercise Science will be reimbursed at the end of the 8-week study period. A £2.50 reimbursement per visit will be made.

**Q:** What are the alternatives for diagnosis or treatment?
**A:** If you are diagnosed with varicose veins, surgery may be recommended to reduce ulcer re-occurrence. Following surgery your Consultant and GP typically recommends that you undertake exercise. We thus wish to assess whether a period of exercise rehabilitation after surgery can improve leg circulation, which might help to prevent these ulcers from re-occurring, and thus be of benefit to the patient.

**Q:** What are the possible benefits of taking part in this study?
**A:** We cannot promise that the study will help you, but the information which we obtain might help improve the treatment of patients with varicose veins.

**Q:** Are there side-effects of any treatment received when taking part?
**A:** Assessing the flow of blood in your legs is completely pain free, and is not associated with any side effects. We expect that exercise will make you feel tired, but as you do it more regularly you will feel increasingly better. Exercise might improve your fitness and quality of life.
Q: What are the possible disadvantages and risks of taking part?
A: The potential for risks to occur will be minimised, since all patients prior to study acceptance will have a full clinical assessment performed by a Vascular Surgeon. The likelihood of anything untoward happening during the exercise will be minimal.

Q: If I decide to participate, will my GP be notified?
A: With your consent, we will write and inform your GP about your decision to take part.

Q: What if I do not wish to take part?
A: Your participation is entirely voluntary. If you decide not to take part, your future treatment will not be affected.

Q: What if I change my mind during the study?
A: You are free to withdraw from the study at any time without it affecting your future treatment.

Q: What will happen to the information from the study?
A: The overall conclusions of the study will be available to you, however it will not be possible to produce an individualised report of your performance.

Q: What if I have further questions?
A: If you have any further questions with regards to this study you may phone:-
This is the end of part 1 of the information sheet.
If you are still interested in taking part in the study, please read part 2.

Q: Who is organising and funding the research?
A: The research is organised and sponsored by the Sheffield Teaching Hospital, in collaboration with the Centre for Sport and Exercise Science, Sheffield Hallam University.

Q: Who has reviewed this study?
A: The North Sheffield Research Ethics Committee has reviewed this study.

Q: Will my taking part in this study be kept confidential?
A: The confidentiality of our patients and the data, which this study will generate, is of utmost importance. All data from this study will be anonymised. In brief, you will be allocated a number during the study. We will need to obtain your permission to allow access for the research team to your medical records, and to information collected during the
study. This is one of the clauses, which you will sign in agreement on the official consent form.

Our procedures for handling, processing and storage of and destruction of data are compliant with the Data Protection Act 1998.

**Q: What will happen to the results of the study?**

**A:** The results of the study will be written up by the researchers. A paper will be published in a relevant journal, and also presented within the hospital research forum. An overall summary report will be sent to participants for their information, but it will not be possible to produce individualised reports for patients.

**Q: What will happen if I don't want to carry on with the study?**

**A:** You are free to withdraw at any stage of the study. If you decide to withdraw from the study, we might still utilise the data which we collected from you prior to your withdrawal.

**Q: What if I wish to complain about the way this study has been conducted?**

**A:** If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. The normal hospital complaints procedure does apply, and you should contact the following person:

Name: Professor Chris Welch (Medical Director) Tel: 0114 271 1900

You can also complain to any individual of the research team, contact details as above.

**Q: What if I am Harmed?**

**A:** In the event that something does go wrong and you are harmed during the research study, there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for legal action for compensation, but you may have to pay your legal costs.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will still be available to you.
14 December 2006

Professor Jonathan Michaels
Professor of Vascular Surgery
Sheffield Vascular Institute
Northern General Hospital

Dear Professor Michaels


REC reference number: 06/Q2308/142

Thank you for your letter of 30 November 2006, responding to the Committee’s request for further information on the above research [and submitting revised documentation].

The further information has been considered on behalf of the Committee by the Chairman.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation [as revised].

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application</td>
<td></td>
<td>13 September 2006</td>
</tr>
<tr>
<td>Investigator CV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol</td>
<td>2</td>
<td>29 November 2006</td>
</tr>
</tbody>
</table>
Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

06/Q2308/142 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project

Yours sincerely

Dr C A Moore
CHAIRMAN – North Sheffield Research Ethics Committee

Email: april.dagnall@sth.nhs.uk

Enclosures: Standard approval conditions [SL-AC1 for CTIMPs, SL-AC2 for other studies]
Site approval form

Copy to: Dr Simon Heller
Sheffield Teaching Hospitals
305 Western Bank
Royal Hallamshire Site
Sheffield
North Sheffield Local Research Ethics Committee

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

<table>
<thead>
<tr>
<th>REC reference number:</th>
<th>06/Q2308/142</th>
<th>Issue number:</th>
<th>1</th>
<th>Date of issue:</th>
<th>14 December 2006</th>
</tr>
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<tbody>
<tr>
<td>Chief Investigator:</td>
<td>Professor Jonathan Michaels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This study was given a favourable ethical opinion by North Sheffield Local Research Ethics Committee on 20 December 2006. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Post</th>
<th>Research site</th>
<th>Site assessor</th>
<th>Date of favourable opinion for this site</th>
<th>Notes (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Professor Jonathan Michaels</td>
<td>Professor of Vascular Surgery</td>
<td>Sheffield Teaching Hospitals NHS Foundation Trust</td>
<td>North Sheffield Local Research Ethics Committee</td>
<td>14/12/2006</td>
<td></td>
</tr>
<tr>
<td>Dr J.M. Saxton</td>
<td>Principal Research Fellow</td>
<td>Sheffield Hallam University, Collegiate Crescent, Sheffield</td>
<td>North Sheffield Local Research Ethics Committee</td>
<td>14/12/2006</td>
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</table>

Approved by the Chair on behalf of the REC:

[Signature of Chair/Co-ordinator]

(delete as applicable)

[Name]