



**INACTIVITY AND EXERCISE IN PERIPHERAL ARTERIAL DISEASE: EFFECT ON  
VASCULAR HEALTH AND FUNCTIONAL CAPACITY**

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VASCULAR HEALTH AND FUNCTIONAL CAPACITY**

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**Volume 1 of 1**

## Declaration

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## Abstract

### **Furlong, Bróna A. Inactivity and Exercise in Peripheral Arterial Disease: Effect on Vascular Health and Functional Capacity**

**Background:** Peripheral arterial disease (PAD) is a form of cardiovascular disease characterized by atherosclerotic occlusion of blood flow to peripheral tissue. Intermittent claudication, a symptom of PAD, can severely impair functional capacity and daily physical activity. Exercise is an established treatment for PAD and improvement in endothelial function is proposed as a mechanism through which exercise improves PAD symptoms.

**Study 1:** This study determined total daily sedentary time and the duration of sedentary bouts in 22 men and women,  $69.0 \pm 8.4$  yr, with PAD and examined the relation between these behaviours and disease severity, functional capacity, CV risk factors, endothelial function, and quality of life (QOL). Participants spent 68% of the waking day sedentary and 36% of this time was accumulated in sedentary bouts >60 min. Excessive sedentary time was related to PAD severity and QOL. Prolonged sedentary bouts were related to PAD severity and CV risk factors.

**Study 2:** This study compared the effect of acute intermittent walking to the onset of claudication (OC) and to maximal claudication (MC) on endothelial function and inflammatory markers in 10 men and women,  $70.4 \pm 7.9$  yr, with PAD. Acute intermittent exercise to both OC and MC had no effect on endothelial function or inflammatory markers.

**Study 3:** The effect of a 12-week community-based exercise programme on endothelial function, disease severity, functional capacity, daily activity and sedentary behaviour, and QOL was evaluated in 11 men and women,  $67.6 \pm 9.2$  yr, with PAD. There was a significant improvement in endothelial function, functional capacity, daily activity and sedentary behaviour, and QOL.

**Conclusion:** Reducing and breaking up sedentary time may have positive health implications in PAD. Intermittent exercise to MC may be a more effective and time efficient exercise prescription than submaximal exercise. Community-based exercise is an effective treatment option for PAD.

## Abbreviations

6MWD/T	Six minute walk distance/test
ABI	Ankle brachial index
ACC	American College of Cardiology
ACSM	American College of Sports Medicine
ActD	Actinomycin D
ADMA	Asymmetric dimethylarginine
ADP	Adenosine diphosphate
AHA	American Heart Association
ALI	Acute limb ischemia
ATP	Adenosine triphosphate
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CBVD	Cerebrovascular disease
cGMP	Cyclic guanosine monophosphate
CK	Creatine kinase
CLI	Critical limb ischemia
CRP	C-reactive protein
CS	Citrate synthase
CSA	Cross-sectional area
CVD	Cardiovascular disease
DLW	Doubly-labeled water
DM	Diabetes mellitus
EBCI	Event-based claudication index
EDD	Endothelial-dependent dilation
EEPA	Energy expenditure of physical activity
EID	Endothelial-independent dilation

EMG	Electromyography
eNOS	Endothelial nitric oxide synthase
ETC	Electron transport chain
FDA	Food and Drug Administration
FMD	Flow-mediated dilation
GPX	Glutathione peroxidase
GTN	Glyceryl trinitrate
HbA <sub>1c</sub>	Glycosylated hemoglobin
HDL-C	High-density lipoprotein cholesterol
HR	Heart rate
IC	Intermittent claudication
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-1 $\beta$	Interleukin-1 beta
IL-6	Interleukin-6
INF- $\gamma$	Interferon gamma
I-RI	Ischemia-reperfusion injury
ITW	Intermittent treadmill walking
LDL-C	Low-density lipoprotein cholesterol
LPL	Lipoprotein lipase
MC	Maximal claudication
MHC	Myosin heavy chain
MI	Myocardial infarction
MnSOD	Manganese superoxide dismutase
mtDNA	Mitochondrial deoxyribonucleic acid
MVPA	Moderate to vigorous physical activity
MWD/T	Maximal walking distance/time
NADPH	Nicotinamide adenine dinucleotide phosphate

NEAT	Non-exercise activity thermogenesis
NO	Nitric oxide
OC	Onset of claudication
PAD	Peripheral arterial disease
PAR-Q	Physical activity readiness questionnaire
PAQ	Peripheral Artery Questionnaire
PCr	Phosphocreatine
PFWD	Pain-free walking distance
PFWT	Pain-free walking time
PGE <sub>1</sub>	Prostaglandin E <sub>1</sub>
PTA	Percutaneous transluminal angioplasty
Q	Cardiac output
QOL	Quality of life
RBC	Red blood cell
RCT	Randomized controlled trial
ROS	Reactive oxygen species
RPP	Rate pressure product
RR	Relative risk
RT	Resistance training
SBP	Systolic blood pressure
SE	Supervised exercise
SF-36	Medical Outcomes Study Short Form
sICAM-1	Soluble intercellular adhesion molecule 1
SMC	Smooth muscle cell
SPPB	Short physical performance battery
StO <sub>2</sub>	Oxygen saturation
SV	Stroke volume
T2DM	Type 2 diabetes mellitus

TBARS	Thiobarbituric acid-reactive substances
TBI	Toe brachial index
TGs	Triglycerides
TNF- $\alpha$	Tumor necrosis factor alpha
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VLA4	Very late activation antigen-4
$\dot{V}O_2$	Oxygen uptake
WIQ	Walking Impairment Questionnaire
XD	Xanthine dehydrogenase
XO	Xanthine oxidase



## Chapter I

### INTRODUCTION

Peripheral arterial disease (PAD) is a form of cardiovascular disease (CVD) characterized by the obstruction of blood flow and consequently the delivery of oxygen and other nutrients to peripheral tissue. The arteries of the lower extremities are most commonly affected<sup>1</sup>. The prevalence of PAD increases sharply with age and it is a common disease among the elderly population, with a prevalence of >20% in those aged  $\geq 75$  yr<sup>2</sup>. It is associated with substantial morbidity and a 2-3 fold increased risk of all-cause and cardiovascular (CV) mortality<sup>3,4</sup>. Intermittent claudication, exercise-induced muscle pain that is relieved with rest, is the most common symptom of PAD and can severely impair functional capacity, therefore impeding daily physical activity and placing individuals with PAD at the extreme low end of the physical activity spectrum.

Emerging evidence indicates that excessive sedentary behaviour may have deleterious cardiometabolic health effects, independent of physical activity<sup>5,6</sup>. In addition, the pattern in which sedentary time is accumulated is clinically significant. Breaking up sedentary time can have a beneficial effect on metabolic biomarkers, independent of total sedentary time<sup>7</sup>. To date, no published study has directly and objectively measured total daily sedentary time and the duration of sedentary bouts in individuals with PAD and determined the relation between these behaviours and CV risk, disease severity, and functional capacity.

Exercise rehabilitation is an effective treatment for PAD and is associated with substantial improvements in symptom severity, functional capacity and quality of life (QOL) <sup>8,9</sup>. Although the effectiveness of exercise rehabilitation is well established, the mechanisms through which these improvements occur are not fully understood. Proposed mechanisms include the formation of collateral circulation and improvements in hemorheological properties, muscle metabolism, walking economy, muscular strength and endothelial function <sup>10</sup>.

Previous research has found significant improvements in endothelial function with exercise rehabilitation in individuals with PAD <sup>11-13</sup>. However, acute exercise is associated with a transient impairment in endothelial function in individuals with PAD <sup>12,14-17</sup>. This is in contrast to no change or increases in endothelial function following acute exercise in healthy individuals <sup>18</sup> and individuals with coronary artery disease (CAD) <sup>19</sup>. The impairment in endothelial function in individuals with PAD is dependent upon the degree of ischemia produced in the diseased limb during exercise and occurs following exercise to maximal claudication but not exercise to the onset of claudication <sup>14</sup>. The acute exercise protocols in previous studies have consisted of a single short continuous bout of exercise, whereas exercise rehabilitation programs designed for individuals with PAD and research studies investigating the effects of chronic exercise in individuals with PAD often employ intermittent exercise protocols to allow participants to accumulate minutes of exercise.

Previous research investigating the effect of chronic exercise on endothelial function in individuals with PAD has involved submaximal intermittent exercise programmes. To date, no published study has determined the effect of exercise rehabilitation involving intermittent exercise to maximal claudication on endothelial function in individuals with PAD. In addition, the previous studies have employed hospital- or laboratory-based exercise rehabilitation programmes. The growing epidemic of CVD and its escalating economic burden requires the employment of viable secondary prevention strategies. Home- and community-based exercise programmes provide an alternative to hospital-based programmes, but it is first necessary to determine the efficacy of these alternatives if their use is to become more widespread in the treatment of PAD.

### **Purpose**

The purpose of the following series of studies is to determine i) sedentary behaviour and its effects, and ii) the effects of acute and chronic exercise on endothelial function in men and women with PAD.

### **Objectives**

1. To determine total daily sedentary time and the duration of sedentary bouts in men and women with PAD and the relation between these behaviours and CV risk factors, disease severity, functional capacity, endothelial function, and QOL

2. To compare the effects of an acute bout of intermittent walking to the onset of claudication and maximal claudication on endothelial-dependent and endothelial-independent dilation in men and women with PAD
3. To determine the effect of a 12-week community-based exercise rehabilitation programme on endothelial function, disease severity, functional capacity, daily physical activity, and QOL in men and women with PAD

### **Hypotheses**

1. Total daily sedentary time and the duration of sedentary bouts will be associated with CV risk factors, disease severity, functional capacity, and QOL in men and women with PAD
2. Acute intermittent exercise to maximal claudication, but not to the onset of claudication, will impair endothelial-dependent dilation in men and women with PAD
3. Twelve weeks of community based-exercise rehabilitation will be associated with significant improvements in endothelial function, functional capacity, daily physical activity, and QOL in men and women with PAD

## **Chapter II**

### **REVIEW OF LITERATURE**

#### **Cardiovascular Disease**

Cardiovascular disease is a class of disorders affecting the heart and circulatory system and includes coronary artery disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, heart failure, deep vein thrombosis and pulmonary embolism. Cardiovascular disease is the leading cause of mortality worldwide, accounting for approximately 30% of global death annually<sup>20</sup>. In Ireland, approximately 10,000 people die each year from CVD, representing 35% of all deaths. Cardiovascular disease is also a leading cause of morbidity, 1,391 per 100,000 disability-adjusted life years lost per annum in Ireland are a consequence of CVD<sup>21</sup>. The economic burden of CVD is substantial, costing the EU economy €192 billion in 2006<sup>21</sup>. Cardiovascular disease is a growing epidemic and the annual mortality rate is expected to rise by 6.3 million by 2030 as a consequence of population growth and aging<sup>20</sup>.

#### **Peripheral Arterial Disease**

Peripheral arterial disease is a term that incorporates a collection of disorders characterized by altered structure and function of non-coronary arteries<sup>22</sup>. Arteries commonly affected include the aorta and its visceral branches, such as the

renal and mesenteric arteries, the carotid and arteries of the upper and lower extremities<sup>23</sup> (figure 2.1). The site of disease is important in determining symptoms.

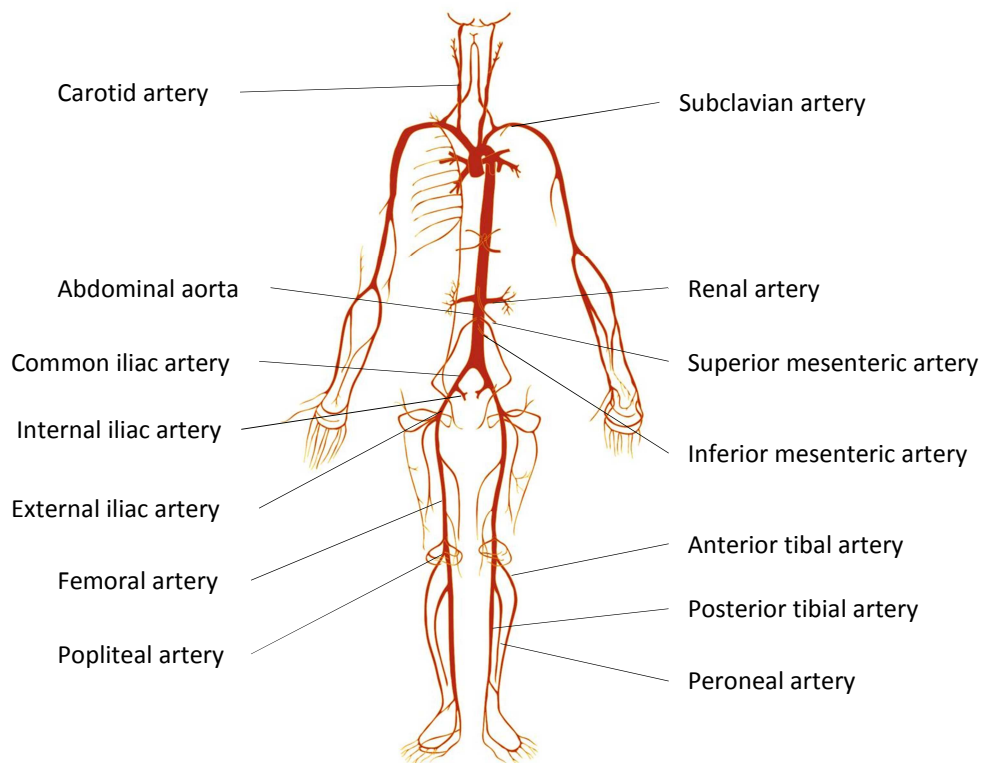


Figure 2.1: The arteries commonly affected by peripheral arterial disease

PAD is characterized by the stenotic occlusion or aneurysmal dilation of the artery, which results in compromised blood flow. Several pathophysiological processes may contribute to the development of PAD including atherosclerosis, degenerative diseases, dysplastic disorders, vasculitis, and thrombosis and thromboembolism<sup>22</sup>. Atherosclerosis is the most common cause of PAD and the focus of the following series of studies is atherosclerotic PAD of the lower extremities.

PAD is a progressive arterial occlusive disease and hence a spectrum of symptoms is observed ranging from asymptomatic PAD to symptomatic PAD or intermittent claudication to critical limb ischemia (figure 2.2).

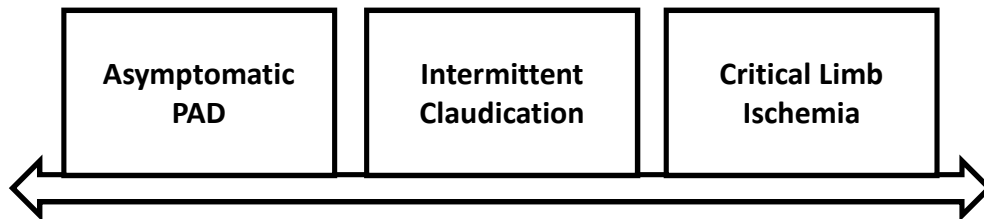


Figure 2.2: The peripheral arterial disease spectrum

### **Intermittent Claudication**

Intermittent claudication (IC) is defined as exercise-induced muscle ischemia that is accompanied with pain, discomfort, cramping or fatigue in specific muscle groups, and is relieved by rest. It is the most common symptom of PAD and occurs following shorter periods of exercise as the disease progresses<sup>24</sup>. Exercise increases the local muscle demand for oxygen to support metabolism. Stenosis and impaired compensatory mechanisms, such as impaired endothelial dilation, restrict blood flow to the peripheral tissue leading to an oxygen supply-demand mismatch, producing ischemia in the affected region. The severity of exercise-induced muscle ischemia in individuals with IC is commonly classified using either the Fontaine or Rutherford scale<sup>25,26</sup> (table 2.1).

Table 2.1: The Fontaine and Rutherford classification scales<sup>25,26</sup>

Fontaine		Rutherford		
Stage	Clinical	Grade	Category	Clinical
I	Asymptomatic	0	0	Asymptomatic
IIa	Mild claudication	0	1	Mild claudication
IIb	Moderate to severe claudication	I	2	Moderate claudication
III	Ischemic rest pain	I	3	Severe claudication
IV	Ulceration or gangrene	II	4	Ischemic rest pain
		III	5	Minor tissue loss
		III	6	Major tissue loss

The site of the claudication pain often corresponds to the site of the stenotic artery. Occlusion of the iliac artery tends to produce hip, buttock, and thigh pain, whereas femoral and popliteal artery occlusion typically causes calf pain. Tibial artery stenosis can produce calf pain or, in some cases, foot pain and numbness. The superficial femoral and popliteal arteries are the most commonly affected by atherosclerosis, and hence IC is most frequently experienced in the calf muscles<sup>1</sup>.

Classic claudication is defined by the World Health Organization (WHO) as leg pain that occurs in one or both calves while walking that does not begin at rest, does not disappear with walking, is provoked by hurrying or walking uphill, makes the individual stop or decrease walking speed, but disappears after stopping for  $\leq 10$  min<sup>27</sup>. Many individuals do not meet all the criteria of classic claudication and are classified as having atypical claudication. Atypical leg pain may arise because of the strict limitations of the WHO definition<sup>28</sup> and/or the presence of comorbidities that alter the symptoms, such as arthritis, neuropathy and spinal stenosis, or prevent



sufficient activity to produce limb symptoms, such as heart failure, severe pulmonary disease and musculoskeletal disease <sup>29</sup>.

### **Critical Limb Ischemia**

Critical limb ischemia (CLI) is defined as ischemic limb pain at rest or impending limb loss due to severely impaired blood flow to the affected limb. It is distinct from acute limb ischemia by the presence of symptoms for >2 weeks and if untreated the natural history would lead to major limb amputation within 6 months <sup>22</sup>. In CLI, resting perfusion is inadequate to meet the metabolic demand of distal tissues or to sustain viability of distal vessels. It is characterized by chronic ischemic related pain at rest, ulcers, or gangrene attributable to atherosclerotic occlusion of the arteries <sup>30</sup> and is associated with a reduction in ankle brachial index (ABI), the ratio of systolic blood pressure (SBP) at the ankle and the brachial artery, to  $\leq 0.4$ , an ankle SBP <50 mmHg, or a toe pressure <40 mmHg <sup>26,31</sup>. The atherosclerosis that gives rise to CLI is often diffuse, multisegmental and multilevel and its systemic nature means the contralateral limb may also be affected by ischemic symptoms.

### **Acute Limb Ischemia**

Acute limb ischemia (ALI) is a sudden reduction in limb perfusion that threatens tissue viability <sup>30</sup>. Presentation is generally within 2 weeks of the acute event. An acute ischemic event may be the first presentation of PAD in previously asymptomatic individuals or it may progress claudication symptoms. Progression along the PAD spectrum may be the result of multiple acute ischemic events. The

severity of an acute event depends on the location and extent of the arterial occlusion and the extent to which collateral blood vessels have perfused the area <sup>22</sup>.

The two most common causes of ALI are embolic and *in situ* thrombotic occlusion. Thrombosis due to atherosclerotic plaque rupture or thrombosis of a lower limb bypass graft account for 85% of ALI cases <sup>32</sup>. Embolic occlusion originating from the heart or an arterial aneurysm account for the remaining 15%. Approximately 90% of the emboli causing ALI are of cardiac origin, generally as a result of atrial fibrillation or an acute myocardial infarction (MI).

## **Epidemiology of Peripheral Arterial Disease**

### **Prevalence**

The prevalence of PAD varies across epidemiological studies depending on the diagnostic method used and the study population. The use of questionnaires determines the prevalence of claudication symptoms and therefore measures only one aspect of the PAD spectrum and underestimates the prevalence of PAD. In the Framingham Offspring Study of 3313 men and women aged  $\geq 40$  yr, IC, defined by questionnaire, was found in 1.9% of men and 0.8% of women <sup>33</sup>. Using the objective measure of ABI  $< 0.90$ , PAD was evident in 3.6% of the population. Similarly, in the Edinburgh Artery Study of 1592 men and women aged 55 to 74 yr, an ABI  $< 0.90$  was found in 9% and questionnaire-determined IC was found in 4.5% of the population

<sup>34</sup>.

The use of ABI <0.90 to diagnose PAD has been given an American Heart Association (AHA) Class I recommendation <sup>35</sup> and table 2.2 summarizes the prevalence of PAD in epidemiological studies employing this criteria. The prevalence ranges from 3.6% to 19%.

Table 2.2: The prevalence of peripheral arterial disease, defined as ABI <0.90, in epidemiological studies

<b>Author</b>	<b>n</b>	<b>Age (yr)</b>	<b>Cohort</b>	<b>Prevalence of PAD (%)</b>
Murabito <i>et al.</i> , 2002 <sup>33</sup>	3313	≥40	Framingham Offspring Study	3.6
McDermott <i>et al.</i> , 2005 <sup>36</sup>	6570	45-84	Multi-Ethnic Study of Atherosclerosis	3.7
Selvin & Erlinger 2004 <sup>37</sup>	2174	≥40	NHANES	4.3
Kröger <i>et al.</i> , 2006 <sup>38</sup>	4735	45-75	Heinz Nixdorf Recall Study	6.4 M, 5.1 F
Stoffers <i>et al.</i> , 1996 <sup>39</sup>	3171	40-75	Limburg PAOD Study	6.9
Fowkes <i>et al.</i> , 1991 <sup>34</sup>	1592	55-74	Edinburgh Artery Study	9
Criqui <i>et al.</i> , 1985 <sup>2</sup>	624	38-82	Californian community	11.7
Newman <i>et al.</i> , 1993 <sup>40</sup>	5084	≥65	Cardiovascular Health Study	12.4
Sigvant <i>et al.</i> , 2007 <sup>41</sup>	5080	60-90	Swedish population	18
Meijer <i>et al.</i> , 1998 <sup>42</sup>	7715	≥55	The Rotterdam Study	19

F, female; M, male; n, number of study participants; NHANES, National Health and Nutrition Examination Survey; PAD, peripheral arterial disease; PAOD, peripheral arterial occlusive disease

The prevalence of PAD increases sharply with age. In a population of 624 men and women aged 38 to 82 yr, the prevalence of PAD, defined by 4 non-invasive measures of limb perfusion, increased from <3% at <60 yr to >20% at ≥75 yr <sup>2</sup> (figure

2.3). In the National Health and Nutrition Examination Survey (NHANES) the prevalence of PAD was 4.3% in 2174 US adults aged  $\geq 40$  yr and 14.5% in the men and women aged  $\geq 70$  yr<sup>37</sup>. In the Framingham Offspring Study, the risk of developing PAD increased 2.6-fold for each decade of life<sup>33</sup>.

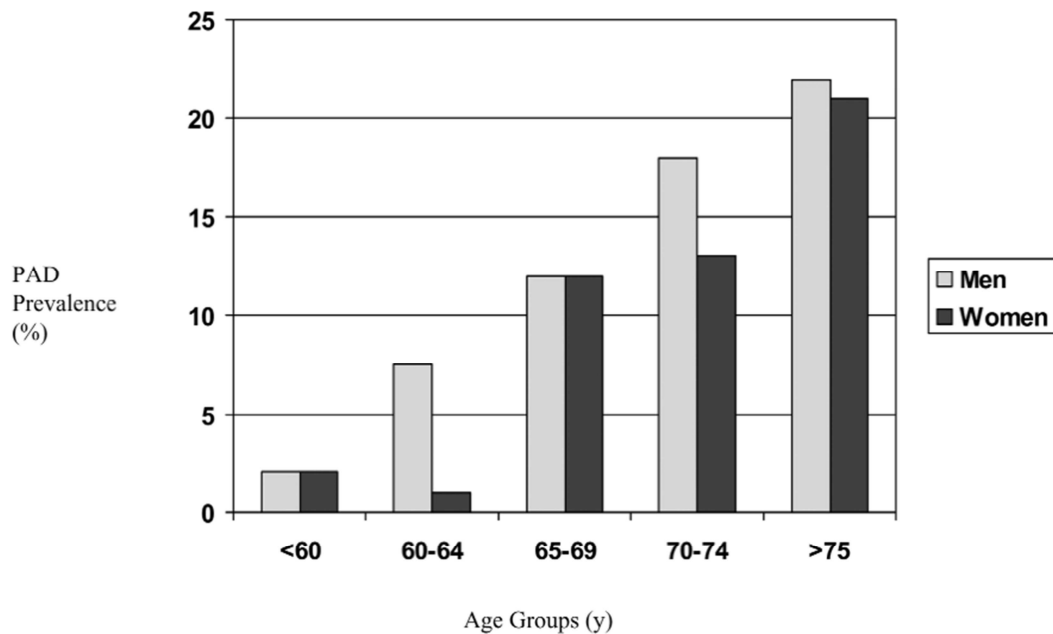


Figure 2.3: Prevalence of peripheral arterial disease by age and gender<sup>2</sup>  
 The prevalence of PAD, defined by segmental blood pressure, flow velocity by Doppler ultrasound, post-occlusive reactive hyperemia, and pulse reappearance half-time, increased with age in 624 men and women with PAD from <3% at <60 yr to >20% at  $\geq 75$  yr. At most ages PAD was slightly more common in men than women.

### Risk Factors

In addition to age, cigarette smoking and diabetes mellitus (DM) are the most powerful risk factors for PAD (figure 2.4). Other modifiable risk factors include dyslipidemia, hypertension, hyperhomocysteinemia, and elevated C-reactive protein (CRP) levels.

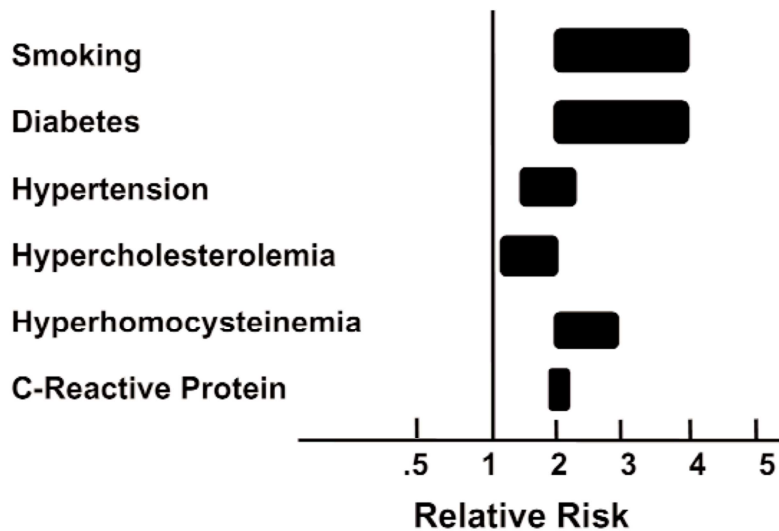


Figure 2.4: Modifiable risk factors for peripheral arterial disease<sup>22</sup>

The range of relative risk (RR) for the modifiable risk factors of PAD estimated from epidemiological studies. The RR for smoking is ~2-4-fold compared with former smokers and non-smokers. The presence of DM has a RR of ~2-4-fold compared with the absence of DM. The presence of hypertension has a RR of ~1.5-2.5-fold compared with the absence of hypertension. The RR of ~1-2-fold for hypercholesterolemia is based on a 10% risk for each 10 mg·L<sup>-1</sup> increase in total cholesterol. The highest quartiles of homocysteinemia and CRP have a ~2-3 fold and ~2-fold RR for PAD, respectively, compared with the lowest quartile.

**Cigarette smoking** is a 2-3 fold more powerful risk factor for PAD than for CAD<sup>43</sup>. Most epidemiological studies have found a 2-5 fold increase risk of developing PAD among smokers compared with former smokers and non-smokers<sup>33,37,40</sup>. Smokers develop PAD 10 years earlier than non-smokers<sup>44</sup>. The association is dose-dependent. The risk of PAD increases with increasing years smoked and packs per year smoked. Price *et al.*, (1999) found that the prevalence of PAD increased from 2.6% in never smokers to 4.5% in moderate smokers (0-25 packyears) to 9.8% in heavy smokers (>25 packyears)<sup>43</sup>. At least 80% of individuals with PAD are current or former smokers<sup>4,42</sup>. Smoking is also associated with the progression of PAD and with an enhanced risk of developing CLI<sup>45</sup>.

**Diabetes mellitus** increases the risk of PAD by 2-4 fold and is present in 10-27% of PAD patients <sup>36,37,40,42</sup>. The duration and severity of DM affects the associated risk for PAD. For every 1% increase in glycosylated hemoglobin (HbA<sub>1c</sub>) there is an associated increased relative risk (RR) of developing PAD of 1.28 and 1.77 fold in diabetic and non-diabetic individuals, respectively <sup>46</sup>. The presence of DM may accelerate the atherosclerotic development and increase the risk of progressing to CLI <sup>47</sup>. The risk of lower limb amputation is approximately 5 times greater in diabetic PAD patients compared with non-diabetic PAD patients <sup>48</sup>.

**Dyslipidemia** is a risk factor for PAD. In the Framingham Study, a fasting cholesterol level  $>7 \text{ mmol}\cdot\text{L}^{-1}$  was associated with a doubling of IC incidence <sup>49</sup>. In the Cardiovascular Health Study, each  $10 \text{ ml}\cdot\text{dL}^{-1}$  increase in total cholesterol increased the risk of developing PAD by approximately 10% <sup>40</sup>. Some studies have failed to find an association between PAD and hypercholesterolemia <sup>50,51</sup>, but there is evidence that treatment of dyslipidemia decreases the progression of atherosclerosis in PAD and the incidence of IC <sup>52</sup>. The ratio of total cholesterol to high-density lipoprotein cholesterol (HDL-C) is a strong risk factor for CAD and the ratio has been found to be higher in PAD patients than controls <sup>51</sup>. In a prospective study, Ridker *et al.*, (2001) found this ratio to be the strongest lipid predictor of risk for developing PAD <sup>53</sup>.

**Hypertension** is the most prevalent CVD risk factor worldwide <sup>54</sup>. The Framingham Study reported a strong association between hypertension and the risk of developing IC and there was a gender difference in this association <sup>55</sup>. The odds

ratio was 2.5 and 4.0 for males and females, respectively. Other epidemiological studies have reported age and gender adjusted risk ratios of 1.4-2.6<sup>33,37,40,56</sup>, while some have failed to find an association between PAD and hypertension<sup>4,57</sup>.

**Hyperhomocysteinemia**, defined as plasma homocysteine  $>15 \mu\text{mol}\cdot\text{L}^{-1}$ , has been identified as an independent risk factor for PAD<sup>58</sup>. Graham *et al.*, (1997) described a 1.35 and 1.42 fold increased risk of PAD for males and females, respectively, with each  $5 \mu\text{mol}\cdot\text{L}^{-1}$  increase in fasting homocysteine level<sup>59</sup>. Higher levels of **CRP**, a marker of systemic inflammation, have been found in PAD patients than healthy controls<sup>53,60</sup>. In a prospective study, Ridker *et al.*, (1998) reported that individuals in the highest quartile of baseline CRP levels were twice as likely to develop PAD than the lowest quartile (RR, 2.1)<sup>61</sup>. Vainas *et al.*, (2005) found a significant inverse relation between CRP levels and ABI in 387 men and women with PAD, suggesting that CRP levels may be related to the severity of PAD<sup>62</sup>. Other CVD risk factors such as obesity and alcohol consumption have shown inconsistent associations with PAD.

### **Natural History of Peripheral Arterial Disease**

The clinical course of PAD is relatively benign. Within 5 years, 7-15% of asymptomatic PAD patients will develop IC<sup>3</sup>, 25% of claudicants will experience a deterioration in symptoms<sup>30</sup>, <5% will develop CLI<sup>63</sup>, <10% will require revascularization<sup>1</sup> and only 1-3.3% of claudicants will require amputation<sup>64</sup>. Therefore, approximately 70% of PAD patients will experience symptom stabilization or improvement<sup>65</sup>. This may be due in part to physiological adaptations resulting

from chronic exposure to ischemia such as the development of collateral vessels or metabolic adaptations, or to an alteration in gait in favour of non-ischemic muscle groups to minimize claudication pain<sup>30</sup>. In 25% of claudicants, deterioration occurs most frequently during the first year, before physiological adaptations have time to occur, with 7-9% deterioration compared with 2-3% per year thereafter<sup>66</sup>. In addition, the systemic nature of atherosclerosis means stenosis typically develop at >1 site, limiting local progression<sup>67</sup>. The risk of deterioration in individuals with PAD is influenced by risk factors such as smoking, DM, and dyslipidemia<sup>30</sup>. The progression of PAD may be gradual or rapid, if ALI occurs suddenly decreasing limb perfusion.

Although PAD represents a spectrum of symptoms, progression along the spectrum is the exception rather than the rule. In a systematic review of 13 population studies, Hooi *et al.*, (1999) reported that only a small percentage of asymptomatic patients developed IC and similarly only a small percentage of patients with IC advanced to CLI<sup>68</sup>. In 100 patients with CLI, Matzke and Lepantalo (2001) found that CLI was the first indication of PAD in 37% of patients<sup>69</sup>. In some cases, IC may not precede CLI due to extreme sedentary behaviour, which does not allow IC to manifest.

The best predictor of deterioration in individuals with PAD is lower limb hemodynamics. Ankle SBP <70 mmHg, toe SBP <40 mmHg, and ABI <0.50 have been found to be independent predictors of the progression of atherosclerotic disease, defined as the development of rest pain or gangrene<sup>66</sup>. Low ABI is also significantly



associated with functional decline. In a study by McDermott *et al.*, (2004), a baseline ABI <0.50 was associated with a 13 fold increased risk of being unable to walk continuously for 6 min 2 yr later compared with an ABI of 1.10-1.50<sup>70</sup>.

Despite the relatively stable prognosis of the diseased limb, atherosclerosis is systemic in nature and individuals with PAD have significant concomitant CAD and cerebrovascular disease (CBVD). In fact, in the prognosis of individuals with PAD, CV events and mortality are a greater concern than lower limb ischemia.

### **Concomitant Coronary and Cerebrovascular Disease**

Coronary artery and/or cerebrovascular disease is present in 40% to 60% of individuals with PAD<sup>30,71</sup>. Cardiovascular causes are by far the leading cause of mortality in individuals with PAD, with approximately 50% of deaths attributed to CAD, 15% to CBVD, and 10% to other vascular events, such as a ruptured aortic aneurysm. Individuals with PAD have a 2-3 fold increased risk of fatal or non-fatal MI, stroke, and all-cause mortality<sup>3,4,40,72,73</sup>. The annual major CV event rate in individuals with PAD is approximately 5-7%<sup>30</sup>. The 5-, 10-, and 15-yr morbidity and all-cause mortality rates in individuals with PAD are approximately 30%, 50% and 70%, respectively.

ABI is a robust and independent predictor of fatal and non-fatal CV events and all cause mortality<sup>40,74</sup>. Research has found a significant association between ABI and the degree of coronary involvement (number of vessels with >75% stenosis)<sup>75</sup> and coronary artery calcium scores<sup>36</sup>. A U-shaped association between ABI and

mortality has been reported <sup>76</sup> (figure 2.5). In a historical cohort study, Sikkink *et al.*, (1997) reported a RR of mortality of 3.1 per 0.50 decrease in resting ABI and 2.4 per 0.50 decrease in post-exercise ABI <sup>77</sup>. The cumulative survival after 5 years was 63% for resting ABI <0.50, 71% for ABI 0.50-0.69 and 91% for ABI of 0.70-0.89. ABI >1.40 predicts mortality to a similar strength as ABI <0.90. An ABI >1.40 is indicative of non-compressible vessels, common in individuals with DM.

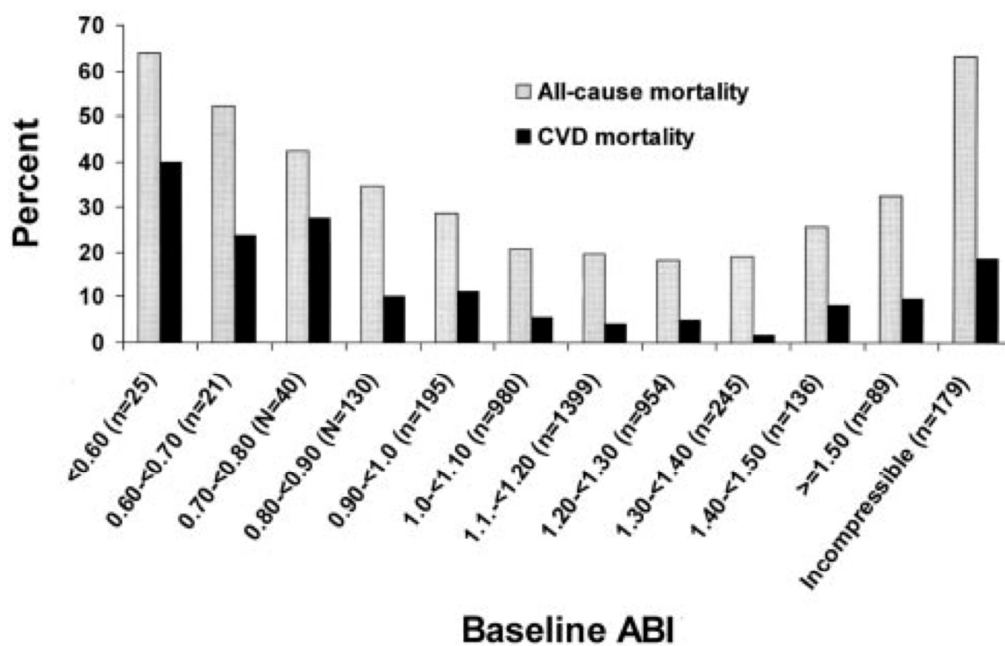


Figure 2.5: Ankle brachial index and mortality <sup>76</sup>  
 The association between low and high ABI and all-cause and cardiovascular mortality in 4393 men and women, mean age 57.5 yr, was U-shaped. Mortality risk increased at ABI <1.0 and progressively increased with decreasing ABI. ABI >1.40 predicts mortality with similar strength as ABI <0.90.

### Diagnosis of Peripheral Arterial Disease

Establishment of an accurate diagnosis can facilitate the formation of a therapeutic plan and diminish the morbidity and mortality associated with PAD. The diagnosis of PAD begins with an accurate history and physical examination. Vascular tests are required to confirm the diagnosis. Intra-arterial angiography is considered

the gold standard test for the diagnosis of PAD <sup>78</sup>. However, this technique is invasive, expensive, and time-consuming. There are a number of non-invasive hemodynamic measurements that have been validated against angiography and can be used to detect the presence, location, and severity of PAD and identify patients for further investigations.

### **Ankle Brachial Index**

Ankle brachial index is the ratio of SBP at the ankle and the brachial artery. It is the standard test used for the diagnosis of PAD in field epidemiological studies, vascular laboratories and office practice <sup>22</sup>. It is determined by measuring SBP in both brachial arteries and the dorsalis pedis and posterior tibial arteries of both ankles (figure 2.6). A number of techniques may be used to determine the ankle SBP, including Doppler ultrasound, oscillometric methods, and plethysmography. The Doppler method has received an AHA Class I recommendation for the use in determining ankle SBP <sup>35</sup>.

The most commonly used method to calculate ABI is to divide the highest of the posterior tibialis and dorsalis pedis arteries pressures by the higher of the two brachial pressures. However, it has been suggested that this method may underestimate the prevalence and/or severity of PAD and using the lowest ankle artery pressure or the average of the 2 pressures may be more suitable <sup>79</sup>. Using the highest pressure has less sensitivity but greater specificity than using the lowest pressure <sup>80</sup> and the AHA has given a Class I recommendation to using the highest ankle pressure <sup>35</sup>.

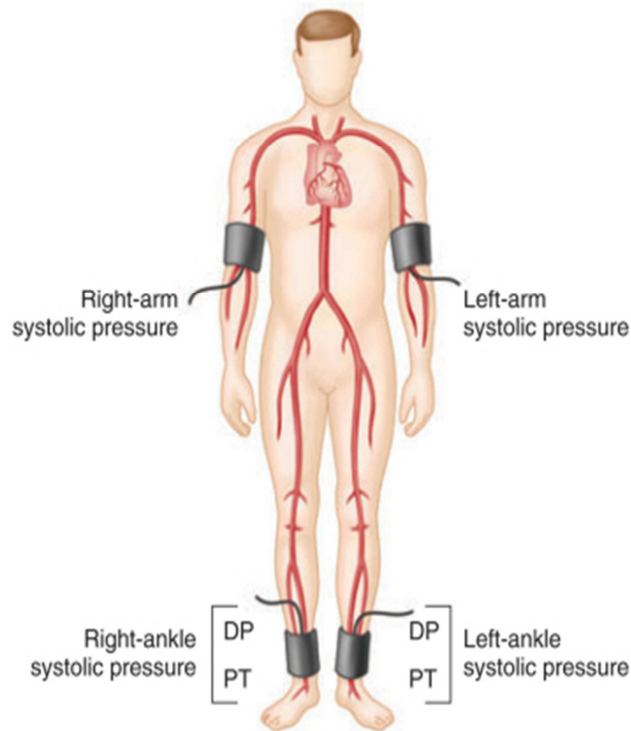


Figure 2.6: Ankle brachial index measurement sites <sup>51</sup>  
 DP, dorsalis pedis artery; PT, posterior tibial artery

Table 2.3 outlines the American College of Cardiology (ACC)/AHA guidelines for the interpretation of ABI <sup>22</sup>. Pulse wave reflection in healthy individuals results in an ankle SBP 10-15 mm Hg higher than brachial SBP and therefore, a healthy ABI is generally >1.00. An abnormally high ABI, >1.40, is a false positive result. Calcification of arterial walls, prevalent in individuals with DM and renal insufficiency, inhibits the abolishment of SBP by cuff inflation resulting in an artificially high ABI. An alternative diagnostic test such as toe brachial index (TBI) is required to detect PAD in these situations.

Table 2.3: Interpretation of ankle brachial index results <sup>22</sup>

<b>ABI</b>	<b>Classification</b>
>1.40	Noncompressible arteries
0.90-1.29	Healthy
0.81-0.89	Mild disease
0.51-0.80	Moderate disease
0.30-0.50	Severe disease
<0.30	Critical disease

The diagnostic performance of ABI varies depending on the cut-off threshold, the technique used to measure flow in the ankle arteries, and the population studied. In a review of the accuracy of an ABI  $\leq 0.90$  to detect a  $\geq 50\%$  stenosis, specificity and accuracy were high at 83.3-99.0% and 72.1-89.2%, respectively, however sensitivity varied from 15% to 79% <sup>82</sup>. The sensitivity was lowest in the elderly and in those with DM. The optimal ABI threshold for detecting PAD has not been clearly defined. The lower limit of the 95% CI ranges from 0.85 to 0.97 in older studies <sup>83,84</sup>, whereas more recent studies using the ROC curve proposed an optimal threshold of 0.92 to 0.97 <sup>78,85</sup>. The AHA gave a Class I recommendation for the use of an ABI threshold  $\leq 0.90$  for the diagnosis of PAD. However, when ABI is  $>0.90$  but there is clinical suspicion of PAD based on symptoms and clinical findings, post-exercise ABI or other non-invasive tests should be used. Intraobserver variability in the measurement of ABI ranges from 4.7% to 13.0%, with an average of 10% <sup>35</sup>. Interobserver variability ranges from 5.4% to 24%, with an average of 13%.

In addition to diagnosing PAD, ABI can be used to quantitatively assess the severity of PAD, determine the temporal progression of PAD and the response to

treatment, and provide prognostic information to predict limb survival and patient survival. There is also evidence that ABI may enhance the predictive accuracy of the Framingham risk score <sup>86</sup>. Overall, ABI is a valid biological parameter, non-invasive, cost-effective, and easy to perform and has been given an AHA Class I recommendation as the tool that should be used to establish lower-extremity PAD diagnosis in patients suspected of PAD <sup>35</sup>.

### **Toe Brachial Index**

In individuals with DM and renal insufficiency, calcification of arterial walls renders lower limb arteries incompressible and an accurate ABI difficult to obtain. An abnormally high ABI (>1.40) or elevated ankle pressure (>250 mmHg) may indicate incompressible arteries <sup>30</sup>. The prevalence of PAD in individuals with DM is approximately 20-fold higher than in age- and gender-matched controls <sup>87</sup> and in a situation where incompressible arteries are suspected, the measurement of toe SBP is recommended to obtain an accurate assessment of distal limb perfusion <sup>22</sup>. Calcification does not tend to extend to digital arteries and the calculation of TBI can be used to diagnose PAD.

Toe SBP is measured by placing a small occlusive cuff on the proximal portion of the first or second toe and detecting the pressure using a plethysmographic device. Toe SBP is generally 30 mmHg less than ankle pressure and a TBI <0.70 is considered diagnostic of lower extremity PAD <sup>88</sup>. The sensitivity and specificity of TBI for the detection of vessel stenosis are 90-100% and 65-100%, respectively <sup>88</sup>.

## **Exercise Testing**

A normal ABI may be obtained despite clinical suspicion of PAD based on symptoms and clinical findings. This may occur in individuals with mild arterial narrowing or isolated iliac stenosis, whereby the stenosis is not hemodynamically significant at rest. In this situation the measurement of post-exercise ABI is recommended<sup>22</sup>. Lower extremity exercise produces peripheral vasodilation and a decrease in ankle SBP. In healthy individuals, a mild decrease in ABI is evident immediately post exercise, which rapidly returns to baseline within 1-2 min. In individuals with PAD, the post-exercise decrease in ABI is of a greater magnitude and persists for a longer duration. A decrease of 30 mm Hg in ankle SBP or a 15-20% decrease in ABI post-exercise are proposed as diagnostic of PAD<sup>30,89</sup>.

Post-exercise ABI provides information on the dynamic functional significance of a stenosis and can also be used to differentiate arterial claudication from non-arterial or pseudoclaudication in those with exertional leg symptoms.

## **Segmental Pressure Examination**

Segmental pressure examination can be used to diagnose PAD and determine the anatomic location of the lower extremity stenosis. Specially designed plethysmographic cuffs, with bladders that encircle the entire limb, are placed sequentially along the limb at various locations, most commonly the upper thigh, lower thigh, upper calf, and lower calf<sup>22</sup>. A Doppler probe is used to determine the SBP in the major arteries under the cuff and the identification of a significant

pressure gradient can determine the location of an arterial stenosis. In general, a gradient of >20 mmHg between adjacent segments is considered a physiologically important focal stenosis<sup>90</sup>.

### **Pulse Volume Recording**

The location of the stenotic occlusion can also be determined by pulse volume recording. Similar to segmental pressure examination, blood pressure cuffs are placed sequentially along the limb, generally at the thigh, calf and ankle<sup>30</sup>. The cuffs are connected to a plethysmograph, an instrument that detects and graphs alterations in limb volume. Arterial blood flow to the lower limbs is pulsatile and results in changes in limb volume with each cardiac cycle. Detection of these cyclical volume changes by the plethysmograph are translated into pulsatile pressure and an arterial pressure waveform profile is produced<sup>90</sup>. The magnitude and amplitude of the waveform shape are used to measure vessel patency and correlate with blood flow. Changes in waveform shape or amplitude signify the presence of a flow-limiting stenosis<sup>22</sup>.

### **Continuous-Wave Doppler Ultrasound**

Continuous-wave Doppler ultrasound is used to obtain arterial flow velocity waveforms and to measure SBP at sequential segments of the lower limb and can provide an estimate of disease location, severity, progression, and response to treatment<sup>22,30</sup>. PAD not only changes lower extremity pressure but also the pattern of blood flow velocity distal to the stenosis. Flow-limiting lesions can result in an



increase in peak systolic velocity at the site of narrowing, a decrease in peak systolic velocity distal to the site of narrowing, turbulence distal to the lesion, and loss of the reverse flow component <sup>1</sup>. The pulsatility index is a commonly used quantitative measure of limb perfusion and is defined as peak systolic velocity minus minimum diastolic velocity divided by mean blood flow velocity. A decrease in pulsatility index between adjacent proximal and distal limb segments indicates the presence of an occlusive lesion between the 2 segments. The magnitude of the decrease is generally proportional to the severity of disease <sup>22</sup>.

### **Duplex Ultrasound**

Duplex ultrasound is an imaging technique that is useful to diagnose the anatomic location and degree of blood vessel stenosis and can be used to identify lesions suitable for revascularization and monitor patients following revascularization <sup>22</sup>. Quantitative data on the degree of stenosis is provided by the analysis of blood flow velocities by Doppler ultrasound.

### **Pathophysiology of Peripheral Arterial Disease**

#### **Atherosclerosis**

Inflammation, the movement of cellular elements from the blood to tissues in response to an injury on the vascular wall or of surrounding tissues, is the basis of all pathogenetic aspects of atherosclerosis <sup>91</sup>. Many of the molecular aspects of vascular inflammation present in atherosclerosis have as their basis a condition of endothelial dysfunction (endothelial “activation”) and subsequent early selective

expression by the endothelium of an adhesive phenotype towards monocytes from peripheral blood. Specifically, the expression of immunoglobulin vascular cell adhesion molecule-1 (VCAM-1) and subsequent interaction with the integrin very late activation antigen-4 (VLA4) results in monocyte recruitment. In addition to the adhesion and migration of monocytes (inception), inflammation is also involved in the growth (accumulation of cells and matrix) and clinical emergence (fissuring) of atheroma. Endothelial dysfunction occurs when established CVD risk factors conjure with altered physical forces to increase adhesion of circulating monocytes that subsequently infiltrate the arterial intima resulting in a proatherogenic phenotype<sup>92</sup>.

### **Ischemia-Reperfusion Injury**

Ischemia-reperfusion injury (I-RI), tissue damage caused by the restoration of blood-flow following an ischemic episode, may contribute to disease progression in PAD. Exercise to claudication followed by rest may be considered a low-grade I-RI<sup>93</sup> (figure 2.7). Ischemia results in a decrease in cellular oxidative phosphorylation and consequently a failure to resynthesize adenosine triphosphate (ATP) levels. Adenosine triphosphate is degraded to hypoxanthine by the purine metabolic pathway. Under normoxic conditions endothelial cell xanthine dehydrogenase (XD) subsequently converts hypoxanthine to xanthine. Under hypoxic conditions, cellular ionic gradients are disrupted leading to a calcium-induced protease attack on XD, forming xanthine oxidase (XO). Xanthine oxidase requires oxygen as a co-factor and consequently, ischemia results in the accumulation of hypoxanthine. Reperfusion with oxygenated blood allows XO to convert hypoxanthine to xanthine with the

production of large quantities of oxygen free radicals, superoxide and hydrogen peroxide.

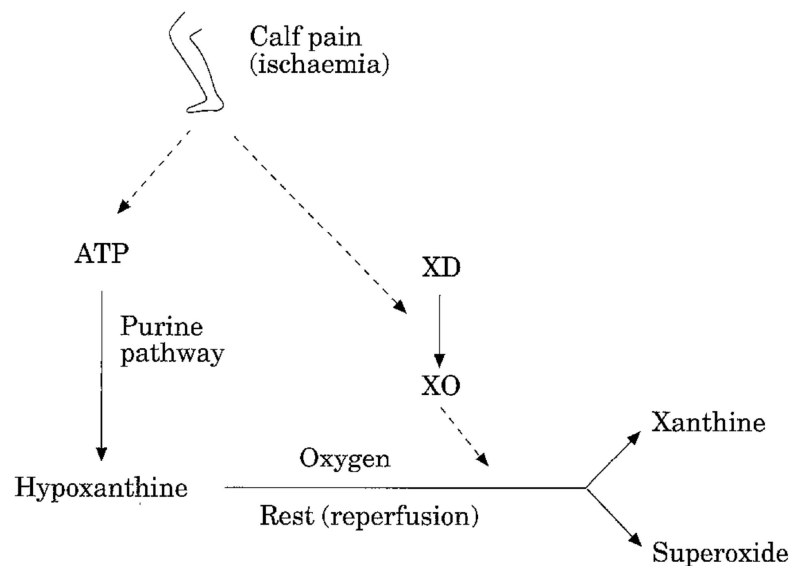


Figure 2.7: Generation of reactive oxygen species during ischemia-reperfusion injury in claudication<sup>93</sup>

ATP, adenosine triphosphate; XD, xanthine dehydrogenase; XO, xanthine oxidase

Ischemia is induced in the skeletal muscle of the lower limb in individuals with PAD during exercise to maximal claudication pain. Ischemia results in a decrease in cellular oxidative phosphorylation and consequently failure to resynthesize ATP. ATP is degraded to hypoxanthine by the purine pathway. Under normoxic conditions endothelial cell XD converts hypoxanthine to xanthine. Under hypoxic conditions, cellular ionic gradients are disrupted leading to a calcium-induced protease attack on XD, forming XO. XO requires oxygen as a co-factor and reperfusion with oxygenated blood allows XO to convert hypoxanthine to xanthine with the production of superoxide.

Reperfusion is followed by an acute inflammatory response triggered by tissue damage incurred during ischemia. Restoration of blood flow promotes the recruitment of leukocytes including neutrophils into the post-ischemic tissue. Activated neutrophils contain the membrane-bound enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that oxidizes cytoplasmic NADPH to  $\text{NADP}^+$  and reduces molecular oxygen to superoxide, which can dismutate to form hydrogen peroxide<sup>94</sup>.

Plasma markers of oxidative stress <sup>14</sup> and oxidative damage <sup>95</sup> are significantly increased in PAD patients compared with healthy controls following acute exercise. Exercise to maximal claudication appears necessary to produce I-RI, as plasma levels of thiobarbituric acid reactive substances (markers of oxidative stress) were unchanged following exercise to the onset of claudication <sup>14</sup>. In addition, there is evidence of impaired antioxidant defences in individuals with PAD. Compared with healthy controls, there is a significant reduction in the activity of manganese superoxide dismutase (MnSOD) <sup>96</sup> and glutathione peroxidase (GPX) and in plasma levels of selenium <sup>97</sup>, an essential cofactor of GPX, in the skeletal muscle of individuals with PAD. Exposure to I-RI, in combination with reduced antioxidant defences, may result in damage to endothelial cells contributing to the progression of atherosclerosis in individuals with PAD. Ischemia-reperfusion injury may also result in damage to PAD skeletal muscle.

### **Skeletal Muscle Myopathy**

Although hemodynamic restriction to peripheral tissue caused by atherosclerotic stenosis is the primary pathophysiology of PAD, chronic restriction of blood flow may lead to secondary complications distal to the stenosis. This is indicated by a number of findings in individuals with PAD, including 1) a weak or absent relation between limb hemodynamics and functional capacity <sup>98</sup>, 2) the absence of changes in functional capacity despite improvement in limb hemodynamics with extremity revascularization <sup>99</sup>, 3) the absence of changes in limb hemodynamics despite improvements in functional capacity with exercise training

<sup>100</sup> or no relation between changes in limb hemodynamics and improvements in functional capacity with exercise training <sup>101</sup>, 4) impairment in oxygen-independent attributes such as anaerobic power <sup>102</sup>, and 5) no relation between limb hemodynamics and impaired exercise oxygen uptake ( $\dot{V}O_2$ ) kinetics <sup>103</sup>. These findings imply the presence of a skeletal muscle myopathy in PAD, which is likely to contribute to functional impairment in individuals with PAD.

### **Alterations in Skeletal Muscle Metabolism**

One of the earliest indications of a metabolic abnormality in PAD was the unexpected finding of increased mitochondrial content, as assessed by mitochondrial enzyme activity <sup>104–107</sup>. The increased mitochondrial content was significantly related with the severity of PAD, determined by ABI ( $r=-0.54$ ) <sup>105</sup>, suggesting that it may represent a compensatory mechanism for impaired oxygen delivery. In contrast, more recent research has reported a significant decrease in mitochondrial enzyme activity <sup>96,108</sup>. Whether the mitochondrial content is increased or not, mitochondrial function appears to be compromised and PAD is associated with impaired mitochondrial respiration. Using gastrocnemius muscle, Pipinos *et al.*, (2003) measured respiratory rate in vitro under normoxic conditions (to remove the influence from potential blood flow limitations) in 9 men and women with PAD and 9 healthy controls <sup>109</sup>. Mitochondrial respiratory rate after maximal stimulation of the electron transport chain (ETC) with Krebs cycle substrates malate and glutamate in the presence of adenosine diphosphate (ADP) ( $V_{ADP}$ ) was significantly lower in PAD muscle than control, whereas respiratory rate after maximal inhibition of ETC with

atractyloside, which inhibits ATP-ADP translocase, depriving mitochondria of ADP ( $V_{AT}$ ) was similar between groups. The ratio of  $V_{ADP}$  to  $V_{AT}$ , an indicator of mitochondrial function, was significantly lower in PAD muscle than control.

Elevated levels of ROS resulting from I-RI in combination with impaired antioxidant defences can lead to significant oxidative damage to the ETC in skeletal muscle distal to the stenosis. Complexes I and III of the ETC are particularly susceptible to oxidative damage and the enzyme activity of complexes I, III and IV have been found to be significantly diminished in PAD gastrocnemius muscle compared with healthy controls<sup>96</sup>. The decline in enzyme activity in complexes I and III was significantly related with an impairment in oxidative phosphorylation. In addition, complexes I and III are the main sites of mitochondrial ROS production required at low levels for cellular signal transduction<sup>110</sup>. Damage to these sites can dramatically increase ROS production, which in turn can act in an autocrine fashion, damaging the ETC further and also resulting in significant oxidative damage to cellular proteins and lipids<sup>96</sup>.

Mitochondrial DNA (mtDNA) may be susceptible to oxidative damage in men and women with PAD. Complex II, the only ETC complex entirely encoded by nuclear DNA, is the only ETC complex unaffected in PAD skeletal muscle<sup>96</sup>. All other complexes are partly encoded by mtDNA, suggesting that close proximity to the ETC exposes mtDNA to the elevated levels of ROS. Mitochondrial DNA mutations have been found in PAD skeletal muscle<sup>111</sup>. The mutations may result in the transcription of defective ETC complexes and consequently further increases in ROS and impaired

oxidative phosphorylation <sup>112</sup>, thus initiating and perpetuating a destructive cycle of oxidative damage and mitochondrial dysfunction (figure 2.8).

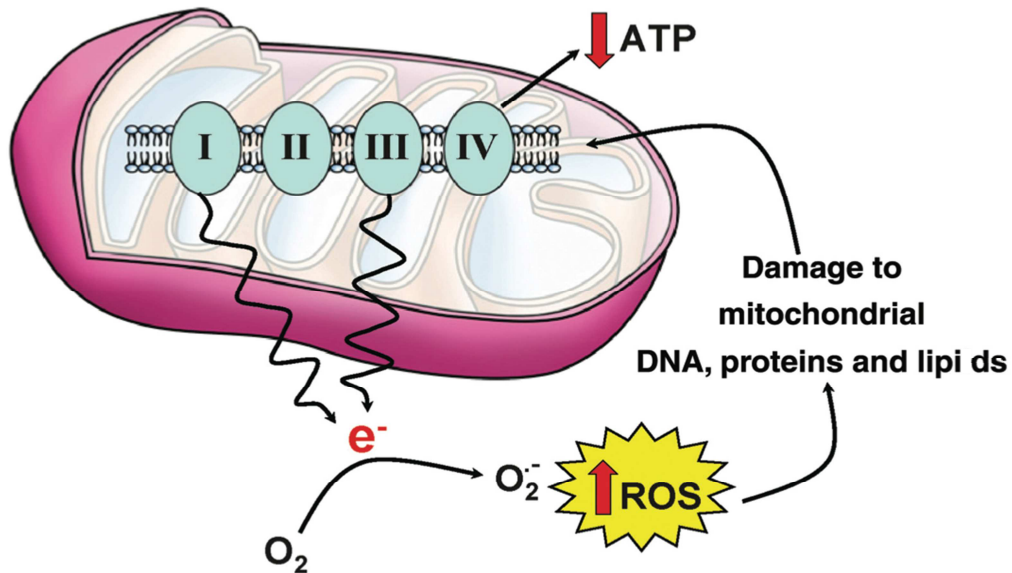


Figure 2.8: The cycle of mitochondrial dysfunction

ATP, adenosine triphosphate; DNA, deoxyribonucleic acid;  $e^-$ , electrons;  $O_2$ , oxygen;  $O_2^-$ , oxide ion; ROS, reactive oxygen species.

Damage to the electron transport chain leads to leakage of electrons and increased production of reactive oxygen species. The ROS cause damage to mitochondrial DNA, proteins, and lipids, perpetuating a destructive cycle of oxidative damage and mitochondrial dysfunction.

Indicators of impaired mitochondrial respiration in PAD include prolonged post-exercise phosphocreatine (PCr) and ADP recovery rates and acyl-carnitine accumulation. PCr and ADP are restored to pre-exercise levels using ATP generated mainly by oxidative phosphorylation, which occurs exclusively in the mitochondria <sup>113</sup>. Using 31 magnetic resonance spectroscopy, it was found that post-exercise PCr and ADP recovery rates were prolonged in PAD skeletal muscle <sup>114,115</sup>.

### Acylcarnitine Accumulation

Carnitine serves as a buffer for the acyl-CoA pool through the reversible transfer of acyl groups (figure 2.9). Changes in the distribution of total carnitine

between free carnitine and acylcarnitine reflects changes in the acyl-CoA pool and thus is an index of metabolic function <sup>116</sup>. Under conditions of metabolic stress, acetyl-CoA production may exceed utilization in the Krebs cycle leading to acetyl-CoA accumulation <sup>117</sup>, which can inhibit pyruvate dehydrogenase leading to the production of lactate. Transfer of the acetyl group from acetyl-CoA to carnitine to form acetyl-carnitine and CoA, decreases acetyl-CoA concentration alleviating the inhibition of pyruvate dehydrogenase. During exercise the increase in lactate and acylcarnitine are closely related (Hiatt, 1992) and acylcarnitine accumulation occurs during high-intensity exercise exceeding the lactate threshold but not during low-intensity exercise.

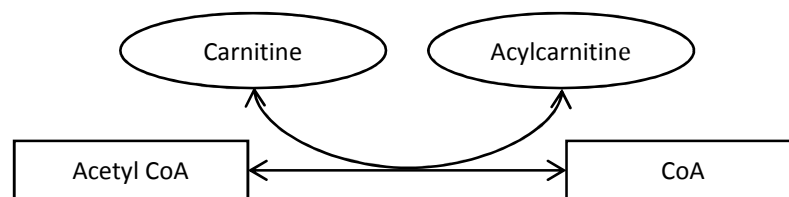


Figure 2.9: The role of carnitine as a buffer for the acyl-CoA pool

Carnitine buffers acetyl-CoA through the reversible transfer of acyl groups to produce acylcarnitine and CoA

In PAD, the increase in muscle short-chain acylcarnitine concentration in the symptomatic limb during exercise was not related with the systemic lactate threshold or the change in muscle lactate content but was instead significantly related with exercise duration ( $r=0.82$ ) <sup>118</sup>. In contrast, in the asymptomatic limb muscle short-chain acylcarnitine concentration significantly increased in participants who exceeded their lactate threshold only and the change in muscle short-chain acylcarnitine content was significantly related with peak blood lactate concentration ( $r=0.67$ ,  $p<0.05$ ), a response similar to healthy controls. These findings indicate a



disruption in skeletal muscle intermediary metabolism during exercise in PAD. In addition, there was an inverse relation between resting muscle short-chain acylcarnitine concentration and maximal walking time (MWT) ( $r=0.70$ ,  $p<0.05$ ) and  $\dot{V}O_2$ peak ( $r=0.75$ ,  $p<0.05$ )<sup>118</sup> and between plasma short-chain acylcarnitine concentration and MWT ( $r=0.51$ ,  $p<0.05$ )<sup>119</sup>. These associations were not observed in the asymptomatic limb or in healthy controls and indicate that the greater the accumulation of short-chain acylcarnitine at rest, the greater the functional impairment in individuals with PAD.

The association between functional capacity and acylcarnitine concentration also indicates that individuals with PAD with more severe functional impairment have a greater accumulation of acylcarnitine. In fact, individuals with severe PAD, Fontaine Stage III and IV, display carnitine deficiency<sup>120</sup>, indicating that the more severe the ischemic disease, the greater the amount of carnitine required to buffer the excess acylCoA, which can ultimately lead to carnitine deficiency. Carnitine deficiency has also been found in other ischemic conditions such as following an MI<sup>121</sup>. Supplementation with L-carnitine and L-propionylcarnitine can improve walking performance in individuals with PAD compared with placebo<sup>122,123</sup>. L-carnitine therapy was associated with an increase in post-exercise lactate removal and an increase in lactate/pyruvate ratio during recovery, suggesting improved pyruvate utilization and oxidative phosphorylation efficiency<sup>124</sup>. This is further supported by the finding of increased short-chain acylcarnitine, implying that part of the supplemented carnitine was taken up by the muscle to buffer acyl-CoA.

Improvements in walking performance with L-carnitine were only evident in those with an abnormal plasma acylcarnitine responses to exercise <sup>124</sup> or carnitine deficiency <sup>120</sup>.

The functional impairment associated with PAD is not related to peripheral hemodynamics but is significantly related with acylcarnitine accumulation, which highlights the importance of the skeletal muscle myopathy in the pathophysiology of PAD. Individuals with PAD are in double jeopardy; the blood and oxygen supply to the working muscles distal to the stenosis is compromised and the oxygen that is received by these muscles cannot be utilized to its maximum potential.

### **Alterations in Skeletal Muscle Histology**

In addition to the metabolic myopathy evident in skeletal muscle, PAD is also associated with alterations in skeletal muscle morphology and function. Ischemia-reperfusion injury can result in denervation, fiber and muscle atrophy, and changes in muscle fiber type distribution that may contribute to the functional impairment associated with PAD <sup>125</sup>.

### **Neuropathy**

Chronic ischemia and I-RI can result in tissue damage including damage to nerve tissue. Nerve conduction and EMG studies have reported evidence of neurological abnormalities in individuals with PAD. The incidence of neurological abnormalities is significantly related to the severity of ischemia. Findings of prolonged motor and sensory nerve conduction velocities, prolonged distal motor

latencies, and small amplitude of the compound muscle action potential suggest axonal degeneration and demyelination in PAD <sup>126</sup>. Histological analysis of sciatic and sural nerves from amputated PAD limbs demonstrate both axonal degeneration-regeneration and segmental demyelination-remyelination <sup>127</sup>.

Denervation may result due to distal motor axon and nerve twig injury from repeated exposure to ischemia-reperfusion. Evidence of denervation in individuals with PAD includes the presence of angular or target fibers, muscle fiber atrophy, muscle fiber grouping, and longer duration motor unit action potentials <sup>126,128</sup>. Denervation can result in a decrease in excitatory and trophic effects of motor neurons, rendering them unable to maintain normal muscle fiber structure. The muscle fibers are paralyzed in a flaccid state and begin to lose their contractile elements leading to muscle fiber atrophy unless reinnervated by a neighbouring motor neuron.

Impaired lower extremity motor nerve innervation to skeletal muscles can result in muscle weakness, either directly or through muscle atrophy, and consequently contribute to functional impairment. In men with unilateral IC, a significant relation has been found between walking performance and the number of angular fibers in the anterior tibial muscle of the symptomatic limb <sup>129</sup>. Peroneal nerve function, measured by electroneurography, was significantly related with calf muscle cross-sectional area (CSA), 6 minute walk test distance (6MWD), usual- and fast-paced 4 m walking velocity, and short physical performance battery (SPPB) score (a functional performance measure of leg strength and balance, that combines data

from usual paced 4 m walking velocity, time to rise and stand from a seated position 5 times, and standing balance) in 413 men and women with PAD, but not in 271 age- and gender-matched controls <sup>130</sup>. The relation between nerve function and functional performance was attenuated after adjustment for calf muscle CSA.

### **Muscle Atrophy**

Denervation and I-RF associated with PAD can lead to fiber and muscle atrophy. Clyne *et al.*, (1985) found that the CSA of both type I and II muscle fibers are significantly smaller in men and women with PAD than in healthy controls <sup>131</sup>. It was speculated that the smaller CSA was due to reduced mobility in patients with PAD. However, when physical activity levels were matched among 14 men and women with PAD and 8 age-matched controls, both type I and type II muscle fiber area were still significantly smaller in PAD patients than controls, suggesting that the muscle atrophy is caused by PAD rather than disuse <sup>132</sup>. Type II fiber CSA has also been found to be smaller in symptomatic limb of men with unilateral PAD compared with the asymptomatic and activity-matched control limbs <sup>128</sup>.

Ischemia-reperfusion is associated with an inflammatory response and markers of inflammation are significantly related to calf muscle characteristics in PAD. McDermott *et al.*, (2007) found a significant inverse relation between calf muscle area and blood levels of CRP, interleukin-6 (IL-6), sVCAM-1, and D-dimer and between calf muscle density and blood levels of D-dimer, sVCAM-1, and homocysteine among 423 men and women with PAD <sup>133</sup>. These associations were generally unchanged after adjustment for physical activity levels. There was a

significant inverse relation between sVCAM-1 and plantar flexion strength, which was attenuated after additional adjustment for calf muscle area, calf muscle percentage fat, and calf muscle density, indicating that calf muscle characteristics are involved in the association between inflammation and muscle function.

Inflammation can directly alter muscle homeostasis by inhibiting muscle repair and promoting muscle proteolysis <sup>134</sup>. In men with unilateral IC substantial muscle fiber damage has been found in the anterior tibial muscle of the symptomatic limb compared with the asymptomatic limb and healthy controls <sup>129</sup>. Morphological abnormalities included centrally localized nuclei, single fibers undergoing necrosis with and without phagocytosis, infiltrates of inflammatory cells between fibers, increased connective tissue, and focal and extensive Z-line streaming.

Muscle atrophy in PAD may be related to the degree of ischemia. A lower ABI has been associated with smaller calf muscle CSA, independent of physical activity <sup>130</sup>. A recent study by Parmenter *et al.*, (2013) found a significant relation between ABI and bilateral hip extensor strength ( $r=0.54$ ) and whole body strength ( $r=0.44$ ) among 22 men and women with IC <sup>135</sup>. Bilateral hip extensor strength was also significantly related to 6MWD ( $r=0.75$ ) and SPPB score ( $r=0.75$ ), suggesting the presence of a pathway from a decrease in ABI to muscle atrophy and weakness, functional impairment and reduced ambulatory capacity.

## Muscle Fiber Type Shift

Type II muscle fibers may be more susceptible to ischemic damage than type I fibers. In addition, denervation-reinnervation and physical inactivity may lead to changes in myosin isoforms resulting in fiber type transitions. PAD appears to be associated with a muscle fiber type shift towards type I fibers, however the research is inconsistent with the shift to type I fibers reported in most<sup>128,136</sup> but not all studies<sup>132</sup>. Controversial findings are likely due to variability in populations studied, in terms of disease severity, comorbidities, and physical activity level, the methods used for fiber type determination, and the fiber type composition of the control population.

Fiber type alteration in PAD appears to be related to disease severity, with progressive increases of the relative amount of myosin heavy chain 1 (MHC I) with increasing ischemia. Steinacker *et al.*, (2000) determined the proportion of MHC isoforms in 13 PAD patients with varying degrees of PAD severity and 5 age-matched controls<sup>136</sup>. There was no difference in the proportion of MHC isoforms between participants with Fontaine stage II PAD and controls. Individuals with Fontaine stage III PAD had a significantly reduced proportion of MHC IIb isoforms and a corresponding significant increase in the percentage of MHC I compared with controls. In Fontaine stage IV, there was a significant decrease in MHC IIa compared with controls, resulting in a remarkably high MHC I content and the ratio of MHC I/II demonstrated a shift towards the more oxidative type I fiber.

## Daily Physical Activity in Peripheral Arterial Disease

Intermittent claudication severely limits mobility and functional capacity, with impairments in walking speed and distance<sup>29,137</sup>. This ambulatory limitation significantly reduces daily physical activity levels and in a destructive cycle, low daily physical activity leads to further impairments in functional capacity<sup>138</sup>. Low daily physical activity is a risk factor for the development of PAD. A sedentary lifestyle was associated with a 1.46-fold increased risk of developing PAD<sup>139</sup>. Daily physical activity has been found to be significantly lower in individuals with PAD than healthy controls. In a study by Sieminski *et al.*, (1997), energy expenditure and step count, measured by accelerometer and pedometer, were significantly lower in 85 participants with IC compared with 59 healthy controls<sup>140</sup>. Women with IC have been shown to be less physically active than their male counterparts<sup>141,142</sup> and individual with IC who smoke are less physically active than non-smokers<sup>143</sup>.

The lower daily physical activity levels observed in individuals with PAD are due to both less time spent walking and fewer strides taken, especially at medium and high cadences. The pattern of ambulatory activity was compared in 98 men and women with PAD and 129 age- and gender-matched controls, using a step activity monitor, worn for 7 d, by Gardner *et al.*, (2007)<sup>144</sup>. Participants with PAD took fewer total strides·d<sup>-1</sup> and fewer strides at medium, 15-30 strides·min<sup>-1</sup> and high, >30 strides·min<sup>-1</sup>, cadence than controls. A higher percentage of the PAD patient's strides were at a low cadence, <15 strides·min<sup>-1</sup>. The average daily cadence and the peak activity index (average stride rate for the highest 30 min of the day) were

significantly lower in the PAD group than controls and participants with IC had a significantly slower ambulation at paces characteristics of bursts of activity and ambulation at sustained endurance activities than controls. The total time spent ambulating per day was significantly lower in the PAD group than controls, but there was no difference in sedentary time between groups.

The fragmented nature of ambulation in individuals with IC was objectively quantified using an ActivPAL™ physical activity monitor by Clarke *et al.*, (2013)<sup>145</sup>. Thirty men and women with IC and 30 age- and gender-matched controls wore the monitor for 7 d and the ratio of the number of walking events to upright events was taken as an event-based claudication index (EBCI). There was no difference in the number of upright events between groups, whereas the number of walking events and therefore the EBCI was significantly higher in participants with IC than controls, reflecting the need to stop and start more frequently due to claudication pain. To achieve the same step count, participants with PAD would require more walking events per upright event.

### **Daily Physical Activity and Disease Severity**

Daily physical activity has been found to be significantly related with PAD severity. Sieminski *et al.*, (1997) found a significant relation between ABI and physical activity determined by both accelerometer ( $r=0.41$ ) and pedometer ( $r=0.41$ )<sup>140</sup>. For every 0.10 decrease in ABI, daily activity was  $42 \text{ kcal}\cdot\text{d}^{-1}$  or  $612 \text{ steps}\cdot\text{d}^{-1}$  lower. Gardner *et al.*, (1999) found no relation between energy expenditure of physical activity (EEPA), determined using both doubly-labelled water (DLW) and



indirect calorimetry, and markers of macrocirculation, ABI and calf blood flow <sup>146</sup>. There was, however, a significant relation between EEPA and markers of microcirculation, calf transcutaneous heating power at rest ( $r=-0.41$ ), after post-occlusion reactive hyperemia ( $r=-0.38$ ), and after maximal exercise ( $r=-0.46$ ).

### **Daily Physical Activity and Functional Capacity**

Daily physical activity is significantly related with functional capacity in individuals with PAD. Gardner *et al.*, (1998) reported a significant association between EEPA, measured using DLW and indirect calorimetry, and maximal walking distance (MWD) ( $r=0.47$ ) and 6MWD ( $r=0.63$ ) <sup>147</sup>. Functional capacity is associated with both the duration and intensity of physical activity. Gardner *et al.*, (2008) found a significant relation between MWD and total daily strides ( $r=0.31$ ), total activity time ( $r=0.21$ ), strides at medium ( $r=0.26$ ) and high cadence ( $r=0.34$ ), time at medium ( $r=0.28$ ) and high cadence ( $r=0.31$ ), average cadence ( $r=0.31$ ), and peak activity index ( $r=0.47$ ) <sup>148</sup>. Pain-free walking distance (PFWD) was associated with physical activity intensity rather than duration. There was a significant relation between PFWD and average cadence ( $r=0.19$ ) and peak activity index ( $r=0.31$ ), indicating the onset of claudication limits the intensity of physical activity.

Daily physical activity is also associated with functional decline in PAD. In a cohort of 417 men and women with PAD followed for a median of 36 months, those who reported walking for exercise  $\geq 3$  d·wk<sup>-1</sup> had a significantly smaller average annual decline in 6MWD, usual-paced 4 m walking velocity and fast-paced 4 m walking velocity than those who exercised less frequently <sup>149</sup>. Similar findings were

observed with accelerometer-measured physical activity. Higher baseline accelerometer-measured physical activity was significantly associated with less average annual decline in 6 MWD, 4 m walking velocity, and SPPB score in 203 men and women with PAD followed for an average of 33.6 months <sup>138</sup>.

Greater sedentary time is associated with accelerated functional decline. Greater self-reported hours spent sitting per day and greater self-reported total sedentary time were associated with faster average annual decline in 6MWD, and usual-paced and fast-paced 4 m walking velocity in 384 men and women with PAD followed for a median of 47 months <sup>150</sup>.

### **Daily Physical Activity and CV Risk Factors**

Gardner *et al.*, (2013) reported a significant association between hs-CRP and the total number of daily strides ( $r=0.30$ ), total daily ambulatory time ( $r=0.28$ ), daily average cadence ( $r=0.19$ ), and peak activity index ( $r=0.24$ ) in 134 men and women with IC <sup>151</sup>. An impaired endogenous fibrinolytic profile has been shown in sedentary individuals with PAD compared with their physically active counterparts. Gardner *et al.*, (2002) classified 106 men and women with IC into low, moderate, and high tertiles of physical activity based on data from Caltrac accelerometers worn over 2 weekdays <sup>141</sup>. Tissue plasminogen activator activity was significantly lower and plasminogen activator inhibitor activity was significant higher in the low physically active group than the moderate and high groups. There was no difference in fibrinolytic profile between the moderate and high activity groups. Expending <175

kcal·d<sup>-1</sup> in physical activities is associated with impaired endogenous fibrinolysis in individuals with PAD and increases the risk of having a prothrombotic state.

### **Daily Physical Activity and Mortality**

Low physical activity is associated with increased risk of CV morbidity and mortality in healthy individuals<sup>152,153</sup>. Individuals with PAD are already at an increased risk of mortality<sup>3,40</sup> and a sedentary lifestyle further compounds this risk. Higher physical activity, determined using an accelerometer worn over 7 d, was associated with lower all-cause mortality after adjustment for confounders in 460 men and women, mean age 71.9 ± 8.4 yr, with PAD at 57 month follow-up<sup>154</sup>. Those in the lowest activity quartile had a 3.48 fold higher mortality rate than those in the highest quartile. In a retrospective, natural history follow-up study, men and women with IC were classified as physically active (n=135) or sedentary (n=135) using the Johnson Space Centre physical activity scale<sup>155</sup>. At follow-up, median 5.0 yr, the risk of mortality was significantly lower in the physically active than sedentary (hazard ratio = 0.51). Physical activity status was an independent predictor of mortality. Participants who engaged in any amount of physical activity exceeding light intensity had a lower risk of mortality than the sedentary individuals who performed either no or only light-intensity physical activity.

### **Sedentary Behaviour**

Moderate to vigorous physical activity (MVPA) has established health benefits and low levels of MVPA are associated with CVD, type 2 diabetes mellitus

(T2DM), obesity, and some site specific cancers <sup>152,153</sup>. Over the last decade, evidence has emerged suggesting that excessive sedentary behaviour has deleterious CV and metabolic effects, independent of physical activity levels. The term inactivity physiology was proposed in 2004 to describe the research on the potential causal role of sedentary behaviour in the development of CV and metabolic diseases and to distinguish it from exercise physiology <sup>156</sup>. The effects of inactivity may not simply be a mirror image of the effects of activity or reflect the bottom end of the physical activity continuum. There may be potentially unique molecular, physiologic, and clinical effects of too much sitting compared with too little exercise <sup>157</sup>.

### **Epidemiological Evidence**

Epidemiological studies have provided a wealth of information on the association between sedentary behaviour and cardiometabolic risk. In the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) of 11 247 men and women aged  $\geq 25$  yr, self-reported TV viewing time and overall sitting time and/or accelerometer-derived sedentary time were significantly related with body mass index (BMI), blood pressure (BP), total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C), triglycerides (TGs), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), 2-h plasma glucose, fasting insulin, insulin resistance, clustered metabolic risk score, and the metabolic syndrome, independent of physical activity <sup>158-163</sup>. In NHANES, self-reported total TV and computer time was significantly associated with the metabolic syndrome <sup>164,165</sup> and accelerometer-

derived sedentary time demonstrated a detrimental linear association with waist circumference, HDL-C, TGs, CRP, insulin, B-cell function, and insulin sensitivity <sup>6</sup>. Similarly, Henson *et al.*, (2013) reported detrimental linear associations of accelerometer-derived sedentary time with 2-h plasma glucose, TGs, and HDL-C, among 878 men and women with a mean age of  $58.4 \pm 13.8$  yr <sup>166</sup>.

In the EPIC-Norfolk study of 14189 men and women, mean age  $60.5 \pm 9.0$  yr, TV viewing time was significantly associated with BMI, waist to hip ratio, percentage body fat, BP, total cholesterol, LDL, HDL, TGs, and HbA<sub>1c</sub> <sup>167</sup>. In the ProActive UK trial involving 258 overweight men and women, mean age  $40.8 \pm 6.4$  yr, with a family history of T2DM, accelerometer-measured sedentary time was significantly associated with insulin resistance <sup>168</sup>. There was a significant association between TV viewing time and the risk of T2DM among 37918 men and 50277 women in the Health Professionals Follow-up Study <sup>169</sup> and the Nurses' Health Study <sup>170</sup>, respectively.

High levels of TV viewing time can have detrimental effects on cardiometabolic health even in individuals meeting the current physical activity recommendations. Among 4064 participants in the AusDiab study who reported  $\geq 2.5$  h·wk<sup>-1</sup> of MVPA, there was a significant dose-response association between TV viewing time and waist circumference, SBP, and 2-h plasma glucose in men and women, and FPG, TG, and HDL in women <sup>171</sup>.

In a prospective study, the change in TV viewing from baseline to 5 yr follow-up was significantly associated with changes in waist circumference, insulin

resistance, fasting serum insulin, and  $\beta$ -cell function in men and women, 2-h plasma glucose in men, and FPG and DBP and clustered metabolic risk score in women, independent of baseline and change in physical activity <sup>172</sup>

Sedentary behaviour has also been associated with CV events and mortality. In the EPIC-Norfolk study, there was a significant association between TV viewing time and incident total CVD, non-fatal CVD and CHD at  $6.9 \pm 1.9$  yr follow-up <sup>173</sup>. In 123216 US men and women, mean age  $63.6 \pm 6.0$  yr, self-reported sitting time was associated with all-cause and CVD mortality at 14 yr follow-up <sup>174</sup>. In 4,512 Scottish men and women, mean age  $57.8 \pm 14.2$  yr, screen-based entertainment time was significantly associated with all-cause mortality and CVD events at  $4.3 \pm 0.5$  yr follow-up <sup>175</sup>. Time spent riding in a car and a combination of TV and car time were significantly associated with CVD mortality in 7744 men aged 20-89 years at 21 yr follow-up <sup>176</sup>.

In the Canada Fitness Survey, there was a dose-response association between levels of sitting time and risk of all-cause and CV mortality in 17013 men and women aged 18-90 yr at mean follow-up of 12.9 yr <sup>177</sup>. In 83034 Japanese men and women aged 45-76 yr, there was significantly greater all-cause mortality at mean follow-up of 8.7 yr in men, but not women, who reported sitting for  $\geq 8$  h·d<sup>-1</sup> compared with  $< 3$  h·d<sup>-1</sup> <sup>178</sup>. In the AusDiab study, each 1 h·d<sup>-1</sup> increase in TV viewing time was associated with hazard ratios of 1.11 and 1.18 for all-cause and CVD mortality, respectively at a median follow-up of 6.6 yr <sup>179</sup>. All these associations were independent of physical activity.

## Breaks in Sedentary Time

In addition to the detrimental effects of total sedentary time, the manner in which it is accumulated is of clinical relevance. Breaking up sedentary time may have a beneficial effect on cardiometabolic risk, independent of total sedentary time. In the AusDiab Study, breaks in sedentary time, defined as interruptions in sedentary time of  $>100$  accelerometer counts $\cdot$ min<sup>-1</sup>, were inversely associated with waist circumference, BMI, TGs, and 2-h plasma glucose, independent of total sedentary time, MVPA, and the mean intensity of breaks<sup>7</sup>. Similarly, in the NHANES study there was a significant beneficial association between breaks in sedentary time and waist circumference, CRP, and FPG, independent of total sedentary time<sup>6</sup>. Henson *et al.*, (2013) found inverse associations between breaks in sedentary time and measures of adiposity, BMI and waist circumference, among 878 men and women, mean age  $58.4 \pm 13.8$  yr, independent of total sedentary time<sup>166</sup>.

## Mechanisms

The mechanisms through which sedentary behaviour is associated with cardiometabolic risk are yet to be determined. Both physiological and behavioural mechanisms have been proposed. Behaviours associated with TV viewing time such as increased snacking have been suggested. It is worth noting that the association between TV viewing time and cardiometabolic risk markers remains significant after adjustment for dietary intake. Physiologically, inactivity may have unique negative effects on specific cellular and molecular processes important for disease-related proteins<sup>156</sup>. Findings from bed rest studies may provide insight but need to be

inferred with caution because these studies usually involve lying down for several days. In an animal study, just 12 h of immobilization was associated with changes in expression of 63 genes and 58 expressed sequence tags compared with controls performing normal spontaneous standing and light ambulation<sup>180</sup>. There is limited evidence that inactivity activates specific molecular responses leading to impaired lipid metabolism by suppression of skeletal muscle lipoprotein lipase (LPL) activity<sup>181,182</sup>. Lipoprotein lipase is among the first proteins directly involved in lipid metabolism to be studied at the cellular level during physical inactivity and has served as the prototype for understanding the impact of inactivity on metabolic health and highlighting the distinct cellular responses of activity and inactivity.

### **Lipoprotein Lipase**

Lipoprotein lipase is a plasma enzyme synthesized primarily by muscle cells and adipocytes<sup>183</sup>. When present on the vascular endothelium, LPL binds to circulating lipoproteins and mediates TG lipolysis. A reduction in LPL activity impairs tissue-specific uptake of lipoprotein-derived fatty acids and may contribute to the impaired lipid metabolism evident in obesity, T2DM, and CVD. Even a small reduction in LPL activity with specific polymorphisms is associated with a significant increase in the risk of CVD and mortality compared with healthy individuals<sup>184</sup>.

The amount of LPL activity in skeletal muscles is extremely sensitive to physical inactivity and activity. Bey and Hamilton (2003) reported a significant reduction in heparin-releasable LPL activity in rodent skeletal muscle after both acute, 12 h, and chronic, 10 h·d<sup>-1</sup> for 11 d, hindlimb unloading compared with



ambulating controls<sup>181</sup>. Lipoprotein lipase activity began to decline after just 4 h of inactivity. Following acute unloading, a recovery period of 4 h of normal cage activity and low-intensity treadmill walking was sufficient to reverse the LPL activity reduction. The inactivity-induced decrease in LPL activity was localized to the immobilized skeletal muscle. There was no decrease in LPL activity in the diaphragm or cardiac muscle, which continued contractile activity, or in the contralateral limb during unilateral limb unloading. This suggests the decrease in skeletal muscle LPL activity is associated with the loss in local contractile activity and not a generalized response to a decrease in systemic energy demands.

The decrease in LPL activity was in association with a significant decrease in [<sup>3</sup>H]TG-derived fatty acid uptake locally in hindlimb muscles and a significant decrease in HDL-C concentration after both acute and chronic unloading. The decrease in LPL activity occurred in the absence of a change in LPL mRNA concentration. Instead there was a significant decrease in heparin-releasable and total LPL protein mass. The decrease in heparin-releasable LPL protein mass was significant after just 6 h compared with 12 h for total LPL protein mass, indicating that the LPL protein at the endothelium and other heparin binding sites is depleted before the larger intracellular pool. This suggests a possible posttranslational regulation of the distribution of LPL. Actinomycin D (ActD) is a potent global inhibitor of transcription and has been shown to raise LPL activity without raising LPL mRNA concentration. Intraperitoneal injection of ActD immediately prior to unloading prevented the decrease in heparin releasable LPL activity. Actinomycin D

infusion did not change LPL activity in ambulatory controls or during the reloading phase, suggesting the presence of a short-lived and potent inhibitor protein for posttranslational regulation of LPL induced by physical inactivity.

In a subsequent rodent study, intraperitoneal administration of nicotinic acid completely prevented the inactivity-induced decrease in heparin-releasable LPL activity<sup>182</sup>. Nicotinic acid was administered at a dose known to acutely impede the appearance of plasma TG from the liver and FFA from adipose tissue. In contrast to inactivity, nicotinic acid had no effect on heparin-releasable LPL activity in control rats with normal standing and ambulatory activity, indicating that the sensitivity of LPL activity to circulating plasma lipids is heightened by low contractile activity.

Research on the effects of LPL activity during run training in rodents<sup>183</sup> permits the comparison of the effects of activity and inactivity on LPL activity and highlights that the effects of inactivity are not simply the opposite of activity. Firstly, the magnitude of the LPL activity response to activity and inactivity differs greatly. After just 10 h of inactivity, LPL activity was 10 fold lower compared with a  $\geq 2.5$  fold increase after 14-20 d of run training. Secondly, the muscle fiber types affected differ. Running was associated with a significant increase in LPL activity in glycolytic fibers only, whereas inactivity was associated with a greater decrease in LPL activity in oxidative fibers. The higher sensitivity of oxidative fibers to inactivity compared with glycolytic fibers may be because oxidative fibers are generally recruited during standing, for example in postural muscle. Lastly, run training is associated with a

significant increase in LPL mRNA compared with no change in LPL mRNA during inactivity.

### **Insulin Action**

Epidemiological studies have reported a positive association between sedentary time and insulin resistance and the risk of T2DM. Prolonged muscular unloading by hind limb suspension in rodents and bed rest in humans can decrease insulin-mediated glucose uptake<sup>185,186</sup>. Brooke *et al.*, (2011) investigated the effect of 1 day of sitting on insulin action in 14 healthy men and women, mean age  $26.1 \pm 4.5$  yr<sup>187</sup>. Insulin action was measured during a continuous infusion of [6,6-2H]-glucose the morning after three 24 h conditions: 1) an active, no sitting condition with a high energy expenditure of  $2944 \pm 124$  kcal and a matched energy intake (NO-SIT), 2) a sitting condition with low energy expenditure of  $2195 \pm 121$  kcal and no reduction in energy intake (SIT), and 3) a sitting condition with low energy expenditure of  $2139 \pm 118$  kcal and a reduction in energy intake to match expenditure (SIT-BAL). Insulin action, defined as whole-body rate of glucose disappearance normalized to mean plasma insulin, was decreased by 39% in the SIT ( $p < 0.001$ ) and by 18% in the SIT-BAL group ( $p = 0.07$ ) compared with the NO-SIT group. Insulin action was significantly lower in the SIT group than the SIT-BAL group. One day of sitting substantially reduced insulin action; this effect was attenuated, but not prevented, when energy intake was decreased to match expenditure. These findings suggest that energy surplus is central to the acute metabolic response to sitting but other factors are also involved. There was no significant difference in

fasting glucose, hepatic insulin action, or fatty acid oxidation between groups, suggesting the effect of prolonged sitting may be mediated in skeletal muscle and may be related to reduced skeletal muscle insulin sensitivity. The factors responsible for this response are unknown but potential factors include greater circulating levels of counterregulatory hormones (e.g., glucagon, epinephrine, cortisol) or hemodynamic changes, including decreased insulin-mediated muscle blood flow and capillary recruitment.

Breaking up sedentary time has a beneficial effect on insulin. In a study by Dunstan *et al.*, (2012), 19 sedentary overweight men and women, mean age  $53.8 \pm 4.9$  yr, performed either 5 h uninterrupted sitting, or 5 h sitting with 2 min bouts of light ( $3.2 \text{ km}\cdot\text{h}^{-1}$ ) or moderate-intensity ( $5.8\text{-}6.4 \text{ km}\cdot\text{h}^{-1}$ ) treadmill walking every 20 min. There was a significant reduction in both the glucose and insulin positive incremental area under the curve following the interrupted conditions compared with the uninterrupted condition. Interrupting with light or moderate-intensity exercise lowered glucose and insulin by a similar extent.

### **Low Energy Expenditure**

The associations between sedentary behaviour and cardiometabolic risk are independent of MVPA. There is little or no relation between sedentary time and MVPA time<sup>158</sup>. On average only ~4% of waking hours are spent in MVPA, with the majority spent in either sedentary or light-intensity activity<sup>162</sup>. The deleterious health effects of sedentary behaviour are therefore not the result of sedentary behaviour replacing MVPA but may arise from sedentary behaviour replacing light-

intensity activity. Sedentary time and light-intensity time are strongly inversely related<sup>162,163</sup>.

Light-intensity activity consists of unintentional or non-exercise behaviours. This “active living” increases skeletal muscle energy demand and effects whole body metabolic rate. Non-exercise activity thermogenesis (NEAT) typically accounts for a much greater portion of total energy expenditure than structured exercise<sup>157</sup>, can be sufficient to prevent diet-induced obesity<sup>188</sup> and may prevent the negative molecular impact of inactivity. Contractile activity through non-exercise activity may signal biochemical and molecular processes to prevent the effects of inactivity. For example, rats performing normal cage activity and low-intensity ambulation did not experience the decrease in LPL activity of sedentary counterparts<sup>181</sup>. Maintaining a high level of daily low-intensity activity may be important for metabolic risk, independent of MVPA.

### **Management of Peripheral Arterial Disease**

The primary goals of treatment for PAD are to prevent the progression of the disease, prevent a coronary or cerebrovascular event, and for individuals with symptomatic PAD to relieve symptoms, and improve functional capacity and quality of life (QOL)<sup>81</sup>. A number of treatment strategies are utilized in PAD, including risk factor modification, pharmacotherapy, revascularization, and exercise rehabilitation.

## **Exercise Rehabilitation**

Exercise was originally recommended by Erb in 1898 as a treatment modality for patients with PAD<sup>189</sup>. The concept was reintroduced by Foley in 1957<sup>190</sup> and in 1988, Housley<sup>191</sup> summarized the treatment of PAD in the statement “stop smoking and keep walking”. Exercise is a non-invasive, inexpensive, relatively safe, and effective treatment for PAD and the ACC/AHA recommend exercise as the first-line of treatment for the majority of patients<sup>22</sup>. The relatively benign natural history of PAD makes non-operative therapy such as exercise a viable and preferred approach to treating PAD. Exercise training has been given a Class I recommendation by the ACC/AHA for the treatment of PAD, indicating the general agreement of its effectiveness, supported by Level of Evidence A arising from multiple RCTs and meta-analyses<sup>22</sup>.

## **Exercise Rehabilitation and Walking Performance**

The improvement of walking performance is a primary objective for the treatment of individuals with PAD. The effect of exercise rehabilitation on walking performance in patients with PAD has been extensively studied. In 1998, Brandsma *et al.*, conducted a systematic review of 10 randomized controlled trials (RCTs) investigating the effect of exercise on walking performance in individuals with IC<sup>192</sup>. The methodological quality of the studies were scored and ranged from 47 to 75 points out of a maximum 100, with a mean of  $62.5 \pm 8.5$ <sup>193</sup>. All studies reported that exercise had a positive effect on walking distance or walking time, despite the differences in demographic data, baseline characteristics, and exercise protocols

across the studies. Improvements ranged from 28% to 210%, with a mean of 105% ± 55.8%. Gardner *et al.*, (1995) included RCTs and non-randomized and uncontrolled trials in a meta-analysis of exercise trials in individuals with PAD <sup>8</sup>. In the 18 non-randomized and uncontrolled trials, PFWD and MWD increased from baseline by 179% and 122%, respectively. In the RCTs, the exercise group had a 190% and 39% increase in PFWD and MWD, respectively, which were significantly greater than changes in the control group.

A recent Cochrane review included 22 RCTs investigating the effect of exercise rehabilitation on relieving symptoms and improving walking ability in individuals with IC <sup>9</sup>. Exercise was concluded to be an effective therapeutic modality for the treatment of IC. Fourteen of the twenty-two trials compared exercise with placebo or usual care and exercise significantly improved PFWD and MWD by 82.19 m and 113.20 m, respectively. The overall improvement in walking ability was between 50% and 200%. In the majority of trials the data were not normally distributed demonstrating the individual variance in response to exercise. Differences in participant demographic and clinical characteristics, exercise programme components, training adherence, and outcome measures may account for the large range in the degree of improvement.

The most recent meta-analysis by Fakhry *et al.*, in 2012 included 25 RCTs examining the effect of supervised walking therapy as a treatment in individuals with IC <sup>194</sup>. There was a weighted mean difference of 128 m and 180 m in PFWD and MWD, respectively between exercise and control, in favour of exercise.

## **Exercise rehabilitation compared with usual care/placebo**

The first RCT to investigate the effect of an exercise intervention in PAD was performed in 1966 by Larsen and Lassen<sup>195</sup>. Fourteen men with IC were randomized to an exercise or placebo-controlled group for 26 weeks. The exercise group was instructed to walk intermittently to near maximal claudication pain for 60 min daily. Pain free and maximal walking distance increased significantly from baseline by 155% and 176%, respectively, whereas there was no change in walking performance in the control group. The effect of supervised exercise therapy was first investigated in a RCT in 1974 by Dahllöf *et al.*,<sup>196</sup>. Eighteen men and women with IC were randomly assigned to receive either supervised exercise training (dynamic leg exercises for 30 min to the onset of claudication pain 3 d·wk<sup>-1</sup> for 26 weeks) or a placebo drug treatment. Pain free and maximal walking distance increased by 191% and 120%, respectively in the exercise group. Subsequently, numerous RCTs have confirmed the beneficial effect of exercise training compared with usual care on walking performance in individuals with PAD. Table 2.4 provides a summary overview of these studies.



Table 2.4: RCTs examining the effect of exercise training on pain free walking time and maximum walking time in individuals with peripheral arterial disease

Author	n	Mean age (yr)	Duration (wks)	Frequency (.wk <sup>-1</sup> )	Time (min)	Mode	Intensity	Claudication pain end-point	Results	
									PFWT/PFWD	MWT/MWD
Allen <i>et al.</i> , (2010) <sup>11</sup>	35	67 ± 2	12	3	30-40	ITW	Workload to induce IC at baseline, progressed	Moderately severe	↑ by 138s	↑ by 260s
Cheetham <i>et al.</i> , (2004) <sup>197</sup>	59	67	24	1	45	W + circuits	Self-selected pace + 7 x 2 min circuits	Moderate to maximal		↑ by 129%
Crowther <i>et al.</i> , (2008) <sup>198</sup>	21	69.2 ± 7.7	52	3	25→40	ITW	3.2 km.h <sup>-1</sup> , progressed	Near maximal	↑ by 171%	↑ by 120%
Dahllof <i>et al.</i> , (1974) <sup>196</sup>	18	61.4 ± 1.7	26	3	30	DLE		Onset	↑ by 191%	↑ by 120%
Dahllöf <i>et al.</i> , (1976) <sup>199</sup>	34		26	3	30	DLE		Onset	↑ by 172%	↑ by 128%
Gardner <i>et al.</i> , (2001) <sup>200</sup>	52	70.5 ± 1	24	3	15→40	ITW	~2mph, grade at 50-80% max workload	Near maximal	↑ by 134%	↑ by 77%
Helgerud <i>et al.</i> , (2009) <sup>201</sup>	21	67.5 ± 6.5	16	3	4 x 4 min	ITW + CE	3.2 km.h <sup>-1</sup> , grade at 90-95%HRpeak + CE at 55-65 rpm and 80% max workload			↑ by 35%
Hiatt <i>et al.</i> , (1990) <sup>101</sup>	19	60 ± 12.5	12	3	60	ITW	2mph, progressed	Moderately severe		↑ by 117%
Langbein <i>et al.</i> , (2002) <sup>202</sup>	52	67.1 ± 7.8	24	3-5	30→40-60	PS	60-80% $\dot{V}O_2$ peak			↑ 51%
Larsen & Lassen (1966) <sup>195</sup>	14	57.8	26	7	60	IW		Near maximal	↑ by 155%	↑ by 176%

Table 2.4 continued

Author	n	Mean age (yr)	Duration (wks)	Frequency (.wk <sup>-1</sup> )	Time (min)	Mode	Intensity	Claudication pain end-point	Results	
									PFWT/PFWD	MWT/MWD
McGuigan <i>et al.</i> , (2001) <sup>203</sup>	20	69.5 ± 6	24	3		RT	2 sets of 8-15 reps of 8 exercises		↑ by 158%	
Mika <i>et al.</i> , (2005) <sup>204</sup>	80	60.2 ± 6	12	3		ITW	3 x 85% PFWD at 3.2 km.h <sup>-1</sup> and 12° grade	Pain-free	↑ by 119%	
Nicolai <i>et al.</i> , (2010) <sup>205</sup>	304	66.2 ± 9.4	52	2-3	30	ITW		Near maximal		↑ by 131%
Patterson <i>et al.</i> , (1997) <sup>206</sup>	55	69.1 ± 8.1	12	3	40	ITW + AE + LE	To induce IC within 3-5 min	Onset	↑ by 315%	↑ by 190%
Stewart <i>et al.</i> , (2008) <sup>207</sup>	60	68 ± 8.3	12	2	60	Circuits	8 min x 5 stations	Maximal	↑ by 89%	↑ by 93%
Tew <i>et al.</i> , (2009) <sup>208</sup>	51	69.5±8.5	12	2	10 x 2 min	AE	50 rpm at 60-70% max workload		↑ by 53%	↑ by 33%
Tsai <i>et al.</i> , (2002) <sup>209</sup>	53	76.2 ± 3.8	12	3	30	ITW	2 mph and 0% grade, with 1% grade increase every 10 min		↑ by 88%	↑ by 70%
Wang <i>et al.</i> , (2008) <sup>210</sup>	25	66.5±6.5	8	3	4 x 4 min	CE	80% max workload			↑ by 20%
Womack <i>et al.</i> , (1997) <sup>211</sup>	26	65.3 ± 7.9	16	3	15-30	ITW	2 mph, grade at 60-70% $\dot{V}O_2$ peak	Near maximal	↑ by 108%	

AE, arm ergometry; CE, calf ergometry; DLE, dynamic leg exercise; HR, heart rate; IC, intermittent claudication; ITW, intermittent treadmill walking; IW, intermittent walking; LE, leg ergometry; MWD, maximal walking distance; PFWT, pain-free walking time; PWD, pain-free walking distance; MWT, maximal walking time; PS, polestriding; RT, resistance training; W, walking

## Exercise rehabilitation compared with surgery

Surgery is an accepted treatment method for PAD<sup>22</sup>, although it is not widely applied, perhaps because of the associated risks. In contrast, exercise is a relatively safe therapeutic modality. Research directly comparing exercise and surgery in PAD is limited. In a large scale RCT, 264 men and women with IC were assigned to an exercise training, surgery, or control group<sup>212</sup>. Exercise training involved 30 min of supervised walking training 3 d·wk<sup>-1</sup> for 6 months, and 2 d·wk<sup>-1</sup> for a further 6 months. At 1 yr follow-up, MWD improved significantly in the surgery group only. The lack of improvement in walking performance in the exercise group is atypical and may be the result of poor training compliance as only 49% of the exercise group completed the training program. Using the same training protocol, Lundgren *et al.*, (1998) did find significant improvements in walking performance in the exercise group among 75 men and women with IC, randomized to receive surgery, surgery and exercise training, or exercise training only<sup>213</sup>. The improvement in the two surgery groups was significantly greater than in the exercise only group, although surgery was associated with more complications than exercise. The combination of surgery and exercise had significantly greater improvements than both single treatment groups. These findings were supported by a RCT by Badger *et al.*, (2007), in men and women undergoing infrainguinal reconstructive surgery<sup>214</sup>. Patients randomized to receive exercise following surgery had a significant increase in MWD compared with those randomized to control following surgery.

## Exercise rehabilitation compared with angioplasty

The less invasive nature of angioplasty in comparison to surgery has made it an attractive and widely used treatment for PAD. Low complication rates have been reported for percutaneous transluminal angioplasty (PTA) in PAD <sup>215</sup>. Research comparing exercise and PTA in the treatment of PAD is equivocal. A recent systematic review <sup>216</sup> of all RCTs comparing PTA with exercise therapy in individuals with PAD could not conclude the superiority of one treatment option over the other. The heterogeneity of interventions, in particular the exercise therapy, baseline walking performance, lesion location and the assessment of outcomes made direct comparisons between studies impossible and a definitive conclusion could not be drawn. In addition, the methodological quality of the studies was average, sample size was small, studies were underpowered and some were terminated prematurely.

The first RCT to compare PTA and exercise therapy in PAD found an improvement in MWD in the exercise group only <sup>217</sup>. ABI improved in the PTA group only. Revascularization was associated with a significant improvement in walking performance in a RCT by Murphy *et al.*, (2012) <sup>218</sup>. However, improvements in MWT were still not to the same extent as with exercise. In contrast, Hobbs *et al.*, (2006) reported a superiority of PTA over exercise therapy in improving walking performance <sup>219</sup>. There was no significant improvement in walking performance with exercise therapy, which involved circuit training 2 d·wk<sup>-1</sup> for 12 weeks. This is an atypical finding and may be due to follow-up measures not being assessed until 3 months after the cessation of the exercise program.

Revascularization may achieve more rapid improvements in walking performance in individuals with PAD, however the invasive nature and associated risks of revascularization must be considered and weighed-up against the non-invasive and relatively safe treatment with exercise. Furthermore, exercise is associated with additional benefits such as improved CV risk factors and CV fitness. Improvements with exercise take longer to achieve but may be more durable than with PTA. Indeed, a 2000 Cochrane review of the effect of PTA on IC questioned the sustainability of the immediate improvements achieved in walking performance <sup>220</sup>. In a RCT by Spronk *et al.*, (2009), the initial advantage of PTA over exercise training in claudication symptoms was lost <sup>221</sup>. One week after the start of the exercise programme and after revascularization, there was a significantly greater improvement in clinical success rate, defined as an improvement by at least one category on the Rutherford scale, in the PTA group than the exercise group. After 6 months, there was no longer a significant difference between the groups.

The combination of exercise and PTA may have additional benefits on walking performance in PAD. Kruidenier *et al.*, (2011) randomized 70 men and women with IC to receive PTA only or PTA plus supervised exercise (SE) <sup>222</sup>. There was a similar significant increase in MWD in both groups immediately following revascularization. At 6 months, MWD remained the same as the postoperative level in the PTA only group, whereas the addition of SE increased MWD significantly above the postoperative level in the PTA + SE group. Maximal walking distance was significantly greater in the PTA + SE group than the PTA only group. Greenhalgh *et*

*al.*, (2008) compared PTA + SE to SE only in men and women with PAD in a multicenter RCT <sup>223</sup>. After 6 months, MWD increased significantly from baseline in both groups, but to a significantly greater extent in the PTA + SE group.

Mazari *et al.*, (2010) compared the combination of PTA and exercise with each treatment alone in men and women with PAD <sup>224</sup>. Pain free and maximal walking distances were significantly increased above baseline in all 3 groups at 1, 3, 6, and 12 month follow-up. Analysis of clinical outcomes according to the International Society for Cardiovascular Surgery found that at 3 months follow-up 62.7%, 66.6%, and 81.6% of patients improved following SE, PTA, and PTA + SE, respectively. The improvements in the PTA + SE group were significantly greater than the PTA alone and SE alone, although this was not sustained at 12 months.

### **Exercise rehabilitation compared with pharmacotherapy**

Pentoxifylline and cilostazol are the two drugs that have been approved by the US Food and Drug Administration (FDA) for the treatment of IC. Pentoxifylline was FDA-approved in 1984 and believed to reduce blood viscosity. Recent evidence suggests pentoxifylline is not significantly different from placebo in the treatment of IC <sup>225</sup>. The effect of pentoxifylline in comparison to exercise therapy for the treatment of IC has not been extensively studied. A meta-analysis of controlled clinical trials investigating treatment strategies for IC included pentoxifylline trials and exercise trials <sup>226</sup>. The trials were classified based on the quality of design; level 1, RCT with either double-blind or specified blind assessment; level 2, other RCTs; levels 3, nonrandomized controlled studies. Compared with the placebo condition,

pentoxifylline increased PFWD by 21.0 m and MWD by 43.8 m in 6 level 1 studies. In 5 level 2 studies, exercise rehabilitation resulted in a significant increase in PFWD by 139.0 m and MWD by 179.1 m compared with controls.

The effect of pentoxifylline therapy and exercise rehabilitation in the treatment of IC was directly compared in a 3 month RCT by Ciuffetti *et al.*, (1994)<sup>227</sup>. Participants with IC were randomly assigned to receive pentoxifylline, 800 mg three times daily, or exercise therapy involving walking 3 d·wk<sup>-1</sup> with an initial goal of 500 m in 20 min, which doubled each week. After 13 weeks, MWD increased significantly in both groups, with significantly greater improvements in the pentoxifylline group. The administration of pentoxifylline in conjunction with exercise rehabilitation did not infer additional benefits in walking performance compared with exercise rehabilitation alone in a study by Scheffler *et al.*, (1994)<sup>228</sup>. Men and women with IC were randomized to receive 4 weeks of daily exercise therapy alone, or in combination with either pentoxifylline or prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), administered as 200 mg and 40 µg, respectively, twice daily. Pain free and maximal walking distance increased significantly in all 3 groups. There was no difference in improvements between the exercise only and exercise and pentoxifylline groups. However, the improvement in the exercise and PGE<sub>1</sub> group was significantly greater than the other groups. In contrast, iloprost, an analogue of PGE, has been shown to be less effective than exercise rehabilitation in improving walking performance in individuals with PAD<sup>229</sup>.

Cilostazol, a phosphodiesterase 3 inhibitor, was FDA-approved in 1999 and is associated with significantly greater improvements in walking performance in individuals with IC than pentoxifylline or placebo <sup>225</sup>. The effect of cilostazol and supervised exercise on walking performance was directly compared in men and women with IC randomized in a 2 x 2 factorial design to receive best medical therapy (BMT) only, BMT + SE, BMT + cilostazol, or BMT + SE + cilostazol <sup>230</sup>. Cilostazol was administered at a dose of 100 mg twice daily and the SE involved circuit training 2 d·wk<sup>-1</sup> for 12 weeks. Pain free and maximal walking distance significantly improved in the exercise and cilostazol groups and the improvement was similar between groups.

The effect of antiplatelet therapy compared with exercise rehabilitation has also been investigated in men and women with PAD by Mannarino *et al.*, (1991) <sup>231</sup>. Thirty PAD patients were randomized to one of 3 groups. Group 1 was administered dipyridamole 75 mg three times daily and aspirin 330 mg once daily. Group 2 performed physical exercise in the form of walking and dynamic leg exercise to the onset of pain daily and group 3 received both the antiplatelet and exercise therapy. After 6 months, PFWT and MWT improved significantly from baseline in all 3 groups. The improvements in both exercise groups were significantly greater than antiplatelet therapy alone.

The effectiveness of exercise therapy in comparison to pharmacotherapy is relatively unknown due to the small number of trials conducted and the small number of participants within these trials. Side effects of pentoxifylline include



abdominal bloating, diarrhea, flushing, and palpitations. Side effects of cilostazol include diarrhea, headache, and palpitations<sup>232</sup>. Exercise is a safer and cheaper alternative that can provide additional health benefits. However, exercise rehabilitation requires commitment and adherence can significantly impact upon the improvements obtained. Exercise rehabilitation may not be suitable for all patients and individual patient characteristics and likelihood to adhere to exercise should be considered when deciding the course of treatment.

There is evidence for the effectiveness of exercise, revascularization, and pharmacotherapy compared with usual care or placebo in the treatment of IC and current guidelines include all 3 as treatment options. However, the relative benefits of these therapies are unknown, as no multicenter clinical trials have directly compared the 3 treatments. The study of the comparative effectiveness of claudication treatments is ranked in the top 50 of all American health challenges by the Institute of Medicine<sup>233</sup>.

### **Exercise Rehabilitation and Quality of Life**

Intermittent claudication impairs functional capacity, which can have a detrimental effect on personal, social, and occupational aspects of daily living and severely diminish QOL<sup>234-236</sup>. Treadmill performance and ABI relate poorly to perceived functional capacity and health status measures in individuals with PAD<sup>237,238</sup>. These commonly used objective outcome measures do not reflect a patient's perceived QOL and do not directly define the degree of functional disability of a patient in the community setting. In addition, QOL predicts long-term survival in

patients with PAD, independent of established prognostic risk factors <sup>239</sup>. The evaluation of the efficiency of treatment strategies now extends beyond objective measures of clinical symptoms to include the broader impact of the disease and treatment on the patient's QOL and several studies have even proposed the use of QOL as a primary endpoint when determining treatment effects <sup>235,238,240</sup>.

Exercise rehabilitation can have a beneficial effect on QOL in individuals with PAD. A recent systematic review identified 23 exercise trials, including 5 RCTs, measuring the impact on QOL in patients with IC <sup>241</sup>. Eleven studies used the Medical Outcomes Study Short Form (SF-36) and all found improvements in the physical functioning domain, with some also reporting improvements in the bodily pain, role functioning, vitality, general health and health transition domains. Improvements across a range of QOL domains also occurred in studies using a disease-specific questionnaire. Using the Walking Impairment Questionnaire (WIQ), a number of RCTs have found significant improvements in walking distances and speeds, and ability to climb stairs following exercise rehabilitation <sup>101,209</sup>. In contrast, Gardner *et al.*, (2001) failed to find an improvement in WIQ or SF-36 scores in 61 men and women, mean age  $70.5 \pm 1$  yr, with IC randomized to 24 weeks of exercise or control <sup>200</sup>. The training protocol used was similar to a study by Hiatt *et al.*, (1990), which did find improvements in WIQ with exercise in 19 men, mean age  $61 \pm 13$  yr, with IC compared to age-matched controls <sup>101</sup>. Both studies reported improvements in treadmill walking performance, however  $\dot{V}O_2$ peak improved in the study by Hiatt *et al.*, only, perhaps suggesting a link between improvements in

aerobic fitness and QOL. Participants in the study by Gardner *et al.*, were also older and had a greater number of comorbidities. These findings indicate that in elderly or chronically ill individuals, ambulatory function may be only one of many factors influencing QOL.

Taft *et al.*, (2001) compared the effects of exercise rehabilitation and surgery on QOL in 264 men and women with IC<sup>242</sup>. Compared with exercise, surgery was associated with significantly greater improvements in disease-specific symptoms and complaints during walking and Sickness Impact Profile – Intermittent Claudication scores. Spronk *et al.*, (2005) pooled data from 8 trials using a random-effects model and weighted mean to compare QOL results between exercise and angioplasty treatment<sup>243</sup>. At 3 months there were significant improvements in SF-36 physical function and bodily pain domain scores in the exercise group and in the physical role functioning and general health in the PTA group. There was no significant difference in the change in QOL between groups. Although a combination of exercise and revascularization has been suggested to have added benefit in improving walking performance over either treatment alone, this may not apply for QOL<sup>222,224,244</sup>.

### **Exercise Rehabilitation and Daily Physical Activity**

The ambulatory limitation associated with IC places individuals with PAD at the extreme low end of the physical activity spectrum. Exercise rehabilitation is effective at improving claudication symptoms and ambulatory capacity in individuals with PAD and therefore, may remove the limitations preventing individuals with PAD from leading physically active lives. Studies investigating the effects of exercise

rehabilitation on free-living daily physical activity have produced inconsistent findings. Following 6 months of intermittent treadmill walking (ITW) training to near-maximal claudication 3 d·wk<sup>-1</sup>, Gardner *et al.*, (2000) reported a significant improvement in daily physical activity, determined by both a Caltrac accelerometer worn over 2 d and the Minnesota LTPA questionnaire, in individuals with PAD <sup>245</sup>. The change in accelerometer-derived physical activity, but not self-reported physical activity, was significantly related with the change in PFWD and MWD. In a subsequent RCT using the same exercise protocol, Gardner *et al.*, (2001) found a significant increase in accelerometer-derived physical activity, but no change in self-reported physical activity in individuals with PAD compared with controls <sup>200</sup>. These findings suggest the increase in physical activity was primarily unstructured activity, such as walking around the home.

In contrast, a study including both symptomatic and asymptomatic PAD patients found no significant improvement in accelerometer-derived daily physical activity, following either ITW or lower extremity resistance training (RT) for 6 months <sup>246</sup>. It is possible that the lack of impairment in functional capacity experienced by asymptomatic participants at baseline may explain the findings. Despite significant improvements in PWFT and MWT, Crowther *et al.*, (2008) found no improvement in daily step count, determined by pedometer over 7 d, in men and women with IC following supervised treadmill walking training to near-maximal or maximal pain 3 d·wk<sup>-1</sup> for 12 months <sup>198</sup>. Similarly, improvements in treadmill walking performance

following both SE and stent revascularization did not translate to improvements in community-based step activity in a study by Murphy *et al.*, (2012)<sup>218</sup>.

### **Exercise Rehabilitation and CV Risk Factors**

Risk factor modification is central to the treatment of PAD. It can prevent the progression of atherosclerotic disease and prevent CV events. In healthy individuals, exercise is an established therapy for risk factor reduction<sup>153</sup>. The functional limitations imposed by IC may restrict individuals with PAD from achieving sufficient exercise volume or intensity to induce risk factor modification. The findings are inconsistent. In a nonrandomized-controlled trial specifically addressing the effect of exercise rehabilitation on CV risk factors, Izquierdo-Porrera *et al.*, (2000) found a significant reduction in total cholesterol, LDL-C and SBP following 6 months of supervised ITW 3 d-wk<sup>-1</sup> in 34 men and women with IC<sup>247</sup>. There was no change in lipids or BP in 14 age-matched controls with IC. However, between group differences were not significant and lipid profile and BP remained abnormal in both groups. The findings from several additional studies investigating the effect of exercise training on lipid profiles, WBCs, haematocrit, fasting glucose, fibrinogen, hs-CRP, plasminogen, platelet count, prothrombin time, and activated partial thromboplastin time in individuals with PAD have been equivocal<sup>248-251</sup>.

### **Exercise Rehabilitation and Cardiovascular Events**

Cardiovascular causes are the primary cause of mortality in individuals with PAD. Individuals with PAD are at a 2-3 fold increased risk of fatal or non-fatal MI or

stroke<sup>3,40,72</sup>. Sakamoto *et al.*, (2009) found that completing an exercise programme may reduce mortality rates in individuals with PAD<sup>252</sup>. In a retrospective cohort study, 118 men and women with PAD were assigned to a 12-week supervised exercise programme involving ITW 3 d·wk<sup>-1</sup> and the long-term outcomes of 64 participants who completed the training programme were compared with 54 participants who did not. At mean follow-up of 5.7 ± 3.9 yr, CV death-free and event-free rates were significantly higher in completers than non-completers. Completing the programme reduced CV mortality and morbidity by 52% and 30%, respectively. Completion of the training programme was an independent predictor of CV mortality.

### **Exercise Prescription**

A meta-analysis of 21 exercise trials in individuals with PAD investigated the components of exercise programmes that were most effective in alleviating symptoms and improving walking ability<sup>8</sup>. There were significantly greater improvements when walking was the sole mode of exercise compared with a combination of exercises and when exercise was performed to near-maximal IC compared with the onset of IC, for >30 min compared with ≤30 min, for ≥3 sessions·wk<sup>-1</sup> compared with <3 sessions·wk<sup>-1</sup>, and for ≥6 months compared with <6 months. Increases in PFWD and MWD were independently related to claudication pain endpoint used during training, duration of the training programme, and mode of exercise. Collectively these 3 exercise components explained 89% and 87% of the variance in the increases in PFWD and MWD, respectively.

## Intensity

Claudication pain endpoint, the pain threshold to which participants exercise explained 55% and 40% of the variance in the increases in PFWD and MWD, respectively, making it the most important programme component for determining improvements in walking performance<sup>8</sup>. The greatest improvement in walking performance occurred when exercise was performed to near-maximal IC compared with the onset of IC. This may be a consequence of greater local ischemia, which may lead to greater hemodynamic and metabolic adaptations. The TASC-II working group currently recommends walking to a moderate-high level of claudication pain as a treatment for IC<sup>30</sup>.

To date only one study has directly compared training protocols to varying claudication pain intensities<sup>250</sup>. Fifty-two men and women with IC were randomized to either an onset of IC or moderate IC group. Both groups performed ITW to their designated IC end-point at 3.2 km·h<sup>-1</sup> and a grade to induce IC within 3-5 min, 3 d·wk<sup>-1</sup> for 12 weeks. Pain free and maximal walking time improved significantly and similarly in both groups.

Exercise intensities based on maximal exercise capacity rather than claudication pain have been compared in other studies. Gardner *et al.*, (2005) randomized 64 men and women with IC to walk at 40% or 80% of the maximal grade achieved during a baseline test<sup>253</sup>. Both groups performed ITW to near-maximal IC at ~3.2 km·h<sup>-1</sup>, 3 d·wk<sup>-1</sup> for 24 weeks. The low-intensity training (LIT) group accumulated 15 min of walking initially and progressed to 40 min, whereas the

walking duration of the high-intensity training (HIT) group was shorter to match the caloric expenditure of the LIT group. Pain free and maximal walking distance, 6MWD and  $\dot{V}O_2$ peak increased significantly in both groups. Walking economy, measured during a submaximal constant load treadmill test, and fractional utilization of oxygen also increased significantly in both groups. The changes in each measure were similar between groups.

In contrast, Slørdahl *et al.*, (2005) reported a difference in outcomes between LIT and HIT in men and women with IC<sup>100</sup>. Participants randomized to both groups performed treadmill walking 3 d·wk<sup>-1</sup> for 8 weeks. The LIT group exercised at 60% $\dot{V}O_2$ peak and walked continuously for 30 min or if IC became intolerable, they walked intermittently and accumulated the 30 min of exercise. The HIT group exercise at 80% $\dot{V}O_2$ peak and performed 8 x 2 min bouts of exercise, interspersed with 3 min of passive recovery. Maximal walking distance was increased significantly in both groups. The improvements were significantly greater in the HIT than LIT group.  $\dot{V}O_2$ peak was significantly increased in the HIT group only.

Recently, a pain-free model of exercise training for individuals with IC has been proposed. Some studies have shown that exercise to maximal claudication pain can induce an inflammatory response and may be associated with potentially harmful systemic effects associated with I-RI. Exercise to maximal claudication pain can lead to elevated plasma markers of oxidative stress<sup>14</sup>, oxidative damage<sup>95</sup>, and inflammation<sup>17,254,255</sup>. Improvements in walking performance in the absence of an inflammatory response have been reported using a pain-free training model in



individuals with PAD <sup>204</sup>. In a RCT, the training group performed 3 bouts of treadmill walking to 85% of their PFWD, 3 d·wk<sup>-1</sup> for 12 weeks. Pain free walking distance increased significantly in the training group compared with controls. There was no significant increase in neutrophil count, WBC count, or microalbuminuria with exercise.

Barak *et al.*, (2009) <sup>256</sup> employed a different pain-free training protocol involving continuous treadmill walking at  $\leq 2.4$  km·h<sup>-1</sup> for 2-3 min, with subsequent increases in velocity every 1-2 min. If IC symptoms occurred, velocity was decreased until symptoms subsided, before being increased again gradually for a total duration of 25 min. Participants trained 2 d·wk<sup>-1</sup> for 6 weeks. The walking distance, walking time, and walking rate during a training session increased significantly from the first to the last session. There were also significant increases in the estimated relative  $\dot{V}O_2$  consumption, mean METs, estimated total energy expenditure, and estimated rate of energy expenditure from the first to the last training session.

### **Duration**

Exercise programme duration explained 22% and 28% of the variance in the increases in PFWD and MWD, respectively in individuals with PAD <sup>8</sup>. Improvement in walking performance was significantly greater when participants exercised for  $\geq 6$  months compared with  $< 6$  months. In contrast, a recent RCT investigating the optimal exercise programme duration for individuals with PAD, found that improvements in PFWD and MWD are achieved rapidly within the first 2-4 months, with no further improvement from 4-6 months <sup>257</sup>. Similar findings have been

reported in other studies, whereby improvements in walking performance are achieved rapidly and further exercise maintains but does not enhance these improvements<sup>258,259</sup>. Improvements in walking performance in individuals with PAD have been found after as little as 2 weeks of exercise training<sup>229,260</sup>. However the exercise training protocols in these studies were intensive, involving several hours of exercise daily. Other studies involving less intense protocols have found improvements in walking performance in individuals with PAD after 3-4 weeks<sup>202,261,262</sup>.

A number of studies have shown continuous improvements in walking performance in individuals with PAD with prolonged exercise training<sup>263–265</sup>. However, if the continuous improvements are expressed relative to the volume and intensity of exercise completed during training, a trend of diminishing returns may be evident. More prolonged exercise training programmes may be required to achieve additional benefits, such as improvements in CV fitness, and to maintain improvements in walking performance. Improvements can be maintained even when the frequency of training sessions is reduced<sup>266</sup> or supervised training is followed by an unsupervised training period<sup>207</sup>.

## **Mode**

Walking is the most commonly used mode of exercise prescribed in PAD rehabilitation and is central to the ACC/AHA guidelines for the management of PAD<sup>22</sup>. Numerous RCTs have confirmed the effectiveness of walking exercise to improve walking performance in individuals with PAD<sup>101,200 198</sup>. A recent systemic review of

RCTs concluded that aerobic modalities other than walking appear equally beneficial for improving walking performance in individuals with PAD, although the data is limited <sup>267</sup>. The efficiency of alternative modes of exercise to improve walking performance may require a cross-transfer effect, possibly through central and/or peripheral adaptations. Additional aims of PAD treatment are to prevent the progression of the disease, prevent CV events and improve QOL. Alternative modes of exercise may be effective in achieving these aims.

## **Ergometry**

### **Lower-limb Ergometry**

Leg ergometry induces similar acute physiological responses to walking in individuals with PAD <sup>268</sup>. However, the primary exercise-limiting symptom and the post-exercise hemodynamic response in the lower extremity differ between the 2 modes. The effects of chronic leg ergometry and walking training on walking performance also differ. In a RCT, men and women with IC performed 6 weeks of either treadmill training (TM) or lower-limb ergometry (LLE), matched for volume and intensity <sup>269</sup>. Pain free and maximal walking time significantly increased in the TM group only and this was significantly greater than the change in the LLE and control groups. Maximal cycle time significantly increased in the LLE group only and the change was significantly greater than the change in the control group, but not the change in the TM group. Claudication pain in the calf and quadriceps were the most commonly cited symptoms during TM and LLE training, respectively, and the

difference in location of the ischemic pain between the 2 modes may explain the absence of a cross-transfer effect.

Calf ergometry utilizes the muscle groups most commonly affected by claudication pain during walking and exercise-induced muscle ischemia may stimulate local skeletal muscle adaptations and hence may improve walking performance. In addition, decreasing the muscle mass used in exercise increases the rate of muscle blood flow and metabolic extraction <sup>270</sup>. Wang *et al.*, (2008) found significant improvements in MWT and  $\dot{V}O_{2peak}$  following 8 weeks of plantar flexion training compared with controls. The fact that there was no change in cardiac output (Q) or stroke volume (SV) measured at  $\dot{V}O_{2peak}$ , would indicate that the improvements in MWT and  $\dot{V}O_{2peak}$  were attributable to peripheral adaptations. Plantar flexion power output increased significantly in the exercise group, suggesting enhanced metabolic capacity.

Improvements in Q and SV were found when calf training was combined with walking training in individuals with IC <sup>201</sup>. A combination of lower-extremity ergometry and walking training may be required to attain both central and peripheral adaptations. Concurrent calf ergometry and walking training has been found to improve PFWT, MWT, and  $\dot{V}O_{2peak}$  <sup>271,272</sup>.

### **Upper-limb Ergometry**

Upper-limb ergometry has been proposed as an alternative mode of exercise training to allow individuals with PAD to exercise without inducing lower limb

claudication pain, which may increase exercise adherence. It may also potentially increase the intensity of training an individual can achieve, thereby resulting in greater potential for improvements in cardiorespiratory fitness. The cross-transfer effect of aerobic exercise training represents a potential mechanism by which upper-limb ergometry may lead to improvements in walking performance. Tew *et al.*, (2009) found significant improvements in PFWT, MWT and  $\dot{V}O_{2peak}$  following 12 weeks of upper-limb ergometry training in men and women with IC compared with controls<sup>208</sup>.

In a RCT, Walker *et al.*, (2000) compared upper- and lower-limb ergometry training in men and women with IC<sup>273</sup>. Both groups performed 10 x 2 min bouts at 50 rpm and ~85-95% of limb-specific  $\dot{V}O_{2peak}$ , interspersed with 2 min passive recovery. After 6 weeks, PFWT, MWD, and QOL increased significantly in both exercise groups. The change did not differ between groups. There was no change in the control group. Using the same training protocol, Zwierska *et al.*, (2009) compared the effects of 24 weeks of upper- and lower-limb ergometry training in men and women with PAD<sup>264</sup>. There was a significant improvement in PFWT and MWD in both groups, with no difference between groups and no change in controls. Quality of life also improved in both exercise groups<sup>274</sup>.

Upper-limb ergometry may result in improvements in walking performance similar to walking training. Treat-Jacobson *et al.*, (2009) randomized 41 men and women with IC to either a treadmill, arm-ergometry, combination, or control group<sup>275</sup>. Participants in all 3 exercise groups performed 60 min of intermittent exercise 3

d·wk<sup>-1</sup> for 12 weeks. The combination group performed 20 min of arm-ergometry exercise, followed by 40 min of ITW. MET-minutes of exercise training per session were similar between all exercise groups. Pain free and maximal walking distance increased significantly in all exercise groups. There was no change in controls. The change in MWD did not differ between exercise groups. The change in PFWD was significantly different from controls in the arm-ergometry group only.

Upper-limb ergometry training also improved cardiorespiratory function to a similar extent as treadmill training in individuals with PAD. Submaximal rate of  $\dot{V}O_2$ , rate pressure product (RPP),  $O_2$  uptake kinetics, minute ventilation,  $\dot{V}O_2$  at the anaerobic threshold, and  $\dot{V}O_{2peak}$  significantly improved in those randomized to the arm-ergometry group and treadmill training group compared with the control group<sup>276</sup>. There was no significant difference between the exercise groups.

### **Polestriding**

Polestriding is a modified form of walking that incorporates the use of handheld walking sticks. The additional muscle recruitment associated with the upper-body work during polestriding has been found to significantly increase heart rate (HR),  $\dot{V}O_2$ , RER and caloric expenditure compared with walking without poles in healthy individuals<sup>277</sup> and in individuals with PAD<sup>278</sup>, in the absence of increased RPE. Walking with poles can acutely improve PFWT, MWT, and perceived leg pain in individuals with IC<sup>278</sup>. The use of poles may also increase walking speed, stride length, and stance time, and reduce vertical ground and knee joint reaction forces<sup>279</sup>. Greater stance time provides longer relaxation time between “toe off” and “heel

strike” in the non-weight bearing leg, which theoretically, may allow greater limb perfusion. In addition, reduced ground reaction forces lead to less tension in antigravity muscles contracting to balance forces, thus potentially slowing the accumulation of metabolic by-products and extending exercise duration.

Langbein *et al.*, (2002) investigated the potential of polestriding training to improve functional capacity in individuals with PAD<sup>202</sup>. In a RCT, the polestriding group performed interval training to maximal or near-maximal IC at 60-80%  $\dot{V}O_{2peak}$  to accumulate 30 min initially and eventually 40-60 min of exercise. After 24 weeks, there was a significant improvement in MWT,  $\dot{V}O_{2peak}$  and QOL in the polestriding group compared with controls. Polestriding was also associated with an improvement in clinical indicators of CV fitness. The change in the slope of the relation between SBP, HR, RPP,  $\dot{V}O_2$ , and leg pain measured over time was significantly greater in the polestriding group than controls<sup>280</sup>.

The effect of walking with and without poles in individuals with PAD was examined by Collins *et al.*, (2012)<sup>281</sup>. Randomized participants followed identical training programs, which involved interval training with combinations of light-, moderate-, and high-intensity exercise, 3 d·wk<sup>-1</sup> for 12 weeks. Maximal walking time increased in both groups, but to a greater extent in the traditional walking group. Pain free walking time increased significantly in the traditional walking group only, but there was no difference between groups. The greater improvements in the traditional walking group may be due to greater peripheral adaptations in the skeletal muscle of the lower limb. Time to nadir StO<sub>2</sub> was significantly increased in

the calf muscle of the traditional walking group only. Exercise training were matched for intensity based on HR and the addition of upper-body work in polestriding contributes to exercise intensity, resulting in less work being performed by the lower limbs.

### **Stair-climbing**

Stair-climbing exercise evokes similar acute responses to walking in individuals with PAD. Oxygen consumption, time to the onset of IC and to maximal IC, foot transcutaneous oxygen tension, ankle SBP, and ABI were similar between acute walking and stair-climbing over a range of intensities<sup>282</sup>. However, responses to chronic exercise differ between these modalities. Jones *et al.*, (1996) randomized 12 men and women with IC to either stairmaster (SM) or treadmill (TM) training<sup>283</sup>. Both groups performed intermittent exercise to severe IC to accumulate 60 min of exercise, 2 d·wk<sup>-1</sup> for 12 weeks. Pain free walking time significantly improved with training in both groups on both a treadmill test and stair-climbing test. However, greater improvement occurred with the apparatus on which they trained. Improvements in MWT were training-mode specific. The TM group increased MWT on the treadmill test only and the SM group improved on the stair-climbing test only.

### **Resistance Training**

Muscle strength and endurance is decreased in individuals with PAD<sup>128</sup>. Histological changes in PAD skeletal muscle include denervation, muscle fiber atrophy, and alterations in myosin heavy chain isoforms<sup>128,136</sup>. The reduced muscle



strength is related to disease severity<sup>102</sup> and functional performance<sup>128</sup>. There is therefore a strong rationale for RT as a successful training modality for individuals with PAD.

McGuigan *et al.*, (2001) found significant improvements in walking performance and lower-extremity strength following 24 weeks of RT in men and women with IC compared with controls<sup>203</sup>. The RT programme involved 2 sets of 8-15 repetitions of 8 exercises for upper- and lower-body strength, 3 d·wk<sup>-1</sup>. There were significant increases in PFWT, MWT, 6MWD, 10 RM leg press strength, and 10 RM calf press strength in the RT group only. Biopsies of the medial head of the gastrocnemius muscle showed a significant increase in the percentage of type IIA fibers and the CSA of all 3 fiber types and a significant decrease in the percentage of MHC IIB and the percentage of type IIB/AB fibers in the RT group. Capillary density increased significantly in the RT group only. There was no change in fiber type distribution, CSA, or capillary density in controls. Walking performance and leg strength were also significantly improved in individuals with PAD following 8 weeks of maximal leg press RT<sup>284</sup>. Participants performed 4 sets of 5 repetitions of leg press at 85-90%1RM, with 4 min rest between sets. There was a significant improvement in MWT, walking economy, 1 RM leg press and the dynamic rate of force development.

The effect of RT in comparison to walking training in individuals with PAD has been examined in a number of RCTs. McDermott *et al.*, (2009) randomized 156 men and women with asymptomatic or symptomatic PAD to a treadmill exercise, lower

extremity RT, or control group <sup>246</sup>. The two exercise groups trained 3 d·wk<sup>-1</sup> for 6 months. The TM group performed ITW to near maximal IC or to a level between 12 and 14 on the Borg RPE scale (asymptomatic participants) to accumulate 15 min initially and gradually increasing to 40 min. The RT group performed 3 sets of 8 repetitions of 3 leg exercises at 50%1RM initially, increasing gradually to 80%1RM and 3 sets of 8 reps of body-weight squat and toe rise exercises. Maximal walking time increased significantly in both exercise groups compared with controls. Pain free walking time and 6MWD improved in the TM group only compared with controls. The improvements in MWT and 6MWD were significantly greater in the TM group than the RT group. Maximum isometric knee extension strength improved in the RT group compared with the TM and control groups. Quality of life improved significantly in both exercise groups compared with controls.

Similar improvements in walking performance between modalities was found when the exercise training was matched for intensity <sup>285</sup>. Thirty men and women with IC were randomized to perform 30 min of either ITW or RT at an RPE of 11-13, with 30 min passive recovery, 2 d·wk<sup>-1</sup> for 12 weeks. The ITW group performed 15 x 2 min bouts at an intensity to induce IC in the last 30 s of each bout, with 2 min passive recovery. The RT group performed 3 sets of 10 repetitions of 8 exercises, with 2 min recovery between sets. There was a significant improvement in PFWD and MWD in both exercise groups, with no between group differences. Resting and exercise CV responses also improved significantly and similarly following training in

both groups. There was a significant reduction in resting SBP, HR, and RPP, and submaximal SBP and RPP <sup>286</sup>.

Hiatt *et al.*, (1994) compared the effect of walking and RT in individuals with PAD and then investigated the effect of a combined programme <sup>263</sup>. Twenty-nine men with IC were randomized to a TM, RT, or non-exercising control group for 12 weeks. In the second phase of the study (i) the TM group continued training for an additional 12 weeks, (ii) the RT group crossed-over to 12 weeks of treadmill training to determine if an initial period of muscle strengthening would augment the training benefits of walking and (iii) participants originally assigned to the control group undertook 12 weeks of a combined programme of treadmill walking and RT. All exercise groups trained 3 d·wk<sup>-1</sup>. The TM group performed 50 min of ITW to moderate IC at an intensity that induced IC pain within 3-5 min. The RT group performed 3 sets of 6 repetitions of 5 leg exercises at 6 RM. The combined programme involved 50 min of ITW and 3 RT exercises.

There was a significant improvement in MWT after 12 weeks in the TM group (74%) and RT group (36%). The difference in the improvement between groups did not reach statistical significance. Pain free walking time and  $\dot{V}O_2$ peak were improved in the TM group only. There were no changes in any measure in the control group after 12 weeks. After an additional 12 weeks of TM training, the original TM group had further significant increases in MWT and PFWT compared with baseline and week 12. Both the original RT group who undertook 12 weeks of TM training and the combination group significantly increased MWT compared with

baseline and week 12, but did not improve PFWT or  $\dot{V}O_2$ peak. Gastrocnemius muscle strength increased in the RT group only after week 12, but these improvements were lost when the group crossed to TM training. The combination group had no improvement in calf muscle strength.

Quality of life improved significantly in the TM and RT groups at week 12 and week 24 with no improvements in the control or combination group<sup>287</sup>. There was a significant increase in daily physical activity levels, measured using the PAR questionnaire and a Vitalog activity monitor, in the TM group at week 12 and week 24. There was no change in daily physical activity in the control or combination group or the RT group until they crossed to TM training. The finding that a combination of modalities do not appear to be associated with additive benefits, suggests that the total volume of exercise is more important than the mode in determining functional improvements.

### **Supervised versus Non-supervised Exercise Rehabilitation**

In 2006, a Cochrane review evaluated the effects of supervised versus non-supervised exercise therapy on walking performance in individuals with IC<sup>265</sup>. Eight randomized and controlled trials were included in the analysis. Although, heterogeneity existed among the studies with regard to exercise training protocols, there was a significantly greater improvement in PFWD and MWD with supervised exercise compared with non-supervised, with overall effect sizes of 0.61 and 0.58, respectively, at 3 month follow-up. Only 2 of the trials monitored compliance with non-supervised exercise training<sup>197,288</sup> and the results were inconsistent. Quality of

life was significantly different between groups in only 3 of the trials, bringing into focus the clinical, as opposed to the statistical, relevance of the results.

Wind *et al.*, (2007) conducted a systematic review of 5 RCTs comparing supervised to non-supervised exercise in individuals with IC <sup>289</sup>. The weighted difference in PFWD and MWD was 143.81 m and 250.40 m, respectively, in favour of supervised exercise.

Structuring unsupervised exercise programs can result in greater improvements in walking performance than traditional home-based exercise or “go home and walk” advice. In a non-randomized study, 143 men and women with IC undertook 6 months of traditional home-based free walking exercise (Tr-E) or test in-train out (Ti-To) exercise <sup>290</sup>. The Tr-E group was advised to perform intermittent walking at a self-selected pace to IC tolerance for 20-30 min  $\geq 6$  d·wk<sup>-1</sup>. The Ti-To group performed 2 daily 10 min interval walking sessions, involving 1 min at maximal asymptomatic velocity with 1 min passive recovery, 6 d·wk<sup>-1</sup>. Maximal asymptomatic velocity was determined monthly during a hospital visit and converted into a walking cadence and followed at home using a metronome. The Tr-E group also attended monthly assessments of functional capacity to ensure similar motivation between groups. Pain free and maximal walking distance, and self-reported claudication symptoms improved significantly in both groups, with significantly greater improvement with Ti-To.

Using a monitoring device during home-based exercise can achieve improvements in walking performance similar to supervised exercise. Gardner *et al.*,

(2011) randomized 119 men and women with IC to a home-based exercise program, supervised exercise program, or usual-care control <sup>291</sup>. Both exercise groups performed intermittent walking to near-maximal IC 3 d·wk<sup>-1</sup> for 12 weeks and wore a step activity monitor during each session. The supervised group walked at 2.0 mph and 40% of maximal baseline grade and accumulated 15 min for the first two weeks, progressing by 5 min biweekly thereafter up to 40 min duration. The home-based group was instructed to walk 5 min extra in an attempt to match the programs on total volume of exercise. The home-based group reported to the laboratory biweekly to receive feedback from their step activity monitor. The adherence to the exercise programs was similar between groups and was >80%. The home-based group exercised for a longer duration but at a lower cadence, resulting in similar total exercise volume. After 12 weeks, PFWT and MWT increased significantly in both groups and there was no significant difference between groups. Daily average cadence increased in the home-based group only and this was significantly different from the supervised group. There were no changes in walking performance or daily physical activity in the control group.

### **Mechanisms of Exercise Rehabilitation**

Although the beneficial effect of exercise rehabilitation on functional capacity in individuals with PAD is well established, the mechanisms underlying these improvements are not fully understood. It is likely that the physiological, metabolic, and mechanical alterations that occur with exercise training induce an adaptive response that decreases IC symptoms, improving ambulatory capacity. Several

potential mechanisms for such benefits have been proposed, including the formation of collateral vessels and improvements in endothelial function, hemorheological properties, muscle metabolism, walking economy, and muscle strength.

### **Improvement in Endothelial Function**

Endothelial dysfunction is a primary event in the pathogenesis of atherosclerosis. A feature of endothelial dysfunction is an imbalance in the regulation of vascular tone, which is due, at least in part to a reduction in level of endothelial-derived nitric oxide (NO). In PAD, the delivery of blood flow to the lower limbs depends not only on the severity of the stenosis but also on the regulatory mechanisms that control blood flow, specifically vascular tone <sup>292</sup>. Vascular reactivity, assessed non-invasively by brachial artery flow-mediated dilation (FMD), is impaired in individuals with PAD compared with healthy controls <sup>293–297</sup>. There is no impairment in glyceryl trinitrate (GTN)-mediated dilation, indicating that the impairment in FMD is due to endothelial dysfunction and not smooth muscle cell (SMC) dysfunction. Flow-mediated dilation is an independent predictor of short- and long-term CV events in individuals with PAD <sup>298,299</sup> and can add to the prognostic value of ABI, the most consistent and powerful prognostic indicator in PAD <sup>300,301</sup>. It may also serve as an indicator of subclinical coronary artery abnormalities <sup>302</sup>.

An association between FMD and the severity of PAD has been found in some <sup>294,303,304</sup> but not all studies <sup>296,297</sup>. The studies reporting a relation between FMD and the severity of PAD have involved asymptomatic or symptomatic participants. In

studies involving participants at more advanced stages of PAD, such as CLI, FMD is associated with the presence but not the severity of PAD, suggesting that endothelial dysfunction is not linearly related to the severity of the disease.

Impaired FMD in PAD has been attributed to various mechanisms. A decrease in NO bioavailability may be caused by a decrease in endothelial nitric oxide synthase (eNOS) expression, insufficient L-arginine or cofactor tetrahydrobiopterin for eNOS, presence of eNOS inhibitors (e.g. asymmetric dimethylarginine (ADMA)), impaired eNOS activation, or increased NO degradation. Plasma concentrations of ADMA have been shown to be elevated above the normal range and urinary excretion rates of nitrate and cyclic guanosine monophosphate (cGMP), indices of systemic NO production, below the normal range in individuals with IC<sup>305</sup>. Treatment with L-arginine improves FMD in individuals with PAD and is associated with a significant increase in the L-arginine/ADMA ratio, which suggests improved eNOS substrate availability. L-arginine treatment was also associated with a significant increase in urinary nitrate and cGMP excretion rates. The change in the L-arginine/ADMA ratio and urinary excretion rates were related to improvements walking performance.

Oxidative stress, which impairs eNOS activation and increases NO degradation, is elevated in patients with PAD<sup>306</sup> and is significantly inversely related with FMD, plasma levels of nitrites and nitrates and walking performance in individuals with PAD<sup>306,307</sup>. Antioxidant supplementation reduces the level of oxidative stress and is associated with increased NO bioavailability, FMD, and MWD.



Inflammation is a major determinant of endothelial dysfunction<sup>308</sup>. Significant inverse relations have been reported between FMD and plasma levels of CRP, fibrinogen, and soluble intercellular adhesion molecule-1 (sICAM-1) in individuals with PAD<sup>294,303,309,310</sup>. Finally, CV risk factors, the prevalence of which is high in PAD, may also contribute to impairment in endothelial function, although the findings of an association with FMD are inconsistent<sup>60,296</sup>.

## **Flow-mediated Dilation and Exercise in Peripheral Arterial Disease**

### **Cross-Sectional Studies**

In a cross-sectional study, Payvandi *et al.*, (2009) investigated the association between FMD and daily physical activity levels, measured over a 7 d period using both an accelerometer and pedometer, in 111 men and women with PAD<sup>311</sup>. After adjustment for confounding factors, there was a significant positive relation between daily physical activity, measured by both the accelerometer and step counter, and both absolute and percent change in brachial artery diameter.

### **Acute Exercise**

Acute exercise has been found to transiently impair FMD in individuals with PAD. Andreozzi *et al.*, (2007) found a significant reduction in FMD following an acute bout of treadmill walking to maximal IC in 22 men with IC<sup>12</sup>. The impairment in FMD with acute exercise in PAD is dependent upon the degree of ischemia produced in the diseased limb during exercise. In a RCT, Silvestro *et al.*, (2002) found a significant decrease in FMD following acute treadmill exercise to maximal IC, but no change

following exercise to the onset of IC and exercise in healthy age- and gender-matched controls <sup>14</sup>. Exercise-induced changes in FMD differed significantly between treatment conditions. Endothelial-independent dilation was unaffected by acute exercise. There was a significant difference in post-exercise plasma levels of thiobarbituric acid-reactive substances (TBARS), an index of oxidative stress, and sICAM-1 between groups. Plasma levels TBARS and sICAM-1 increased significantly following exercise to maximal IC, and remained unchanged following exercise to the onset of IC and in controls. There was no significant relation between increases in TBARS and changes in FMD and sICAM-1. However, the impairment in FMD and elevation of sICAM-1 were eliminated by administration of an antioxidant, vitamin C.

The acute exercise-induced impairment in FMD is also eliminated by PLC administration, which exerts both antioxidant and anti-inflammatory effects <sup>15</sup>. Flow-mediated dilation was significantly reduced following acute treadmill exercise to maximal claudication in 40 men and women with IC. Participants were subsequently randomized in a double-blind fashion to receive either placebo or PLC, administered as 600 mg in a single bolus followed by an infusion of  $1.0 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  for 60 min. Following treatment, FMD continued to decrease significantly post-exercise in the placebo group, but not in the PLC group. However, the difference between the 2 treatments did not reach statistical significance. There was a significant inverse relation between the post-exercise decrease in FMD before treatment ( $\Delta\% \text{ FMD1}$ ) and the difference between post-exercise change in FMD after PLC ( $\Delta\% \text{ FMD2}$ ) and  $\Delta\% \text{ FMD1}$ , indicating the greater the post-exercise decrease in

FMD at baseline, the greater the benefit attained from PLC treatment. Subsequently, the data of participants with a post-exercise decrease in FMD  $\geq 45\%$  (the median FMD decrease) at baseline were analyzed. In this cohort, there was a significant protective effect on post-exercise FMD with PLC compared with placebo.

The time course of the changes in FMD following acute exercise in men and women with IC was examined by Joras and Poredos (2008)<sup>16</sup>. Flow-mediated dilation was determined before and 30 min, 2 h and 4 h after a constant load treadmill test to maximal claudication for PAD participants and for 10 min for controls. Following exercise, FMD decreased significantly from baseline at 30 min and 2 h post-exercise in participants with IC, before returning to baseline values at 4 h post-exercise. Controls experienced a slight non-significant increase in FMD post-exercise. In comparison with controls, FMD was significantly lower in participants with IC at 30 min, 2 h and 4 h post-exercise. The difference between groups in the change in FMD over time was significant.

### **Chronic Exercise**

In contrast to the detrimental effect of acute exercise on FMD in PAD, chronic exercise has been found to have a positive effect. The majority of studies investigating the effects of acute exercise on FMD in PAD have involved exercise to ischemia-inducing maximal IC, whereas the majority of exercise training studies have involved submaximal exercise protocols. Acute exercise to submaximal claudication, unlike exercise to maximal claudication, does not lead to transient impairment of endothelial function<sup>14</sup>. The moderate hemodynamic stress associated with

submaximal exercise may in fact lead to improvements in endothelial function, through ischemic preconditioning.

In a study by Andreozzi *et al.*, (2007)<sup>12</sup> 22 participants with IC performed ITW to 60-70% of maximal walking ability to accumulate 1-2 km, 3 d·wk<sup>-1</sup>. After 6 weeks, there was a significant improvement in pre-exercise FMD. Post-exercise FMD significantly increased from pre-training levels, but was still significantly reduced compared with pre-exercise. Exercise training was also associated with significant improvements in MWD and ABI.

The effect of a longer duration exercise programme on FMD in PAD was considered by Brendle *et al.*, (2001)<sup>13</sup>. Nineteen overweight men and women with IC performed a 6 month aerobic exercise programme involving 3 d·wk<sup>-1</sup> of ITW to near-maximal IC at 2.0 mph and a grade equivalent to 50-80%HRpeak. Participants accumulated 15 min of walking initially and gradually progressed to ≥30 min. Following the program, there was a significant improvement in FMD, PFWT, MWT and maximal calf blood flow. There was a significant relation between change in FMD and reactive hyperemic blood before and after the exercise programme and maximal blood flow at baseline, indicating those with the lowest calf blood flow experienced the least improvement in FMD.

Improvements in FMD are likely due to an increase in NO bioavailability. Exercise training is associated with exposure to repeated episodes of increased shear stress, which upregulates the mRNA expression of eNOS, consequently increasing NO production and improving endothelial function<sup>312</sup>. Allen *et al.*, (2010)

investigated the response of NO bioavailability and FMD to exercise training in 35 men and women with IC<sup>11</sup>. Participants were randomized to either a supervised or home exercise group for 3 months. The supervised exercise involved ITW to moderately severe IC, accumulating 30-40 min, 3 d·wk<sup>-1</sup>. The home exercise group was instructed to walk for 30 min 3 d·wk<sup>-1</sup>. At baseline, plasma nitrite concentration (a surrogate measure of NO bioavailability) was significantly lower in PAD participants than 41 healthy controls following an acute bout of maximal exercise. Following the supervised exercise program, plasma nitrite concentration after maximal exercise no longer differed from controls and plasma nitrite flux became positive rather than negative. Flow-mediated dilation improved significantly in the supervised group only. There was also a significant improvement in PFWT, MWT and  $\dot{V}O_{2peak}$  in the supervised exercise group only. The change in PFWT was significantly related to the change in plasma nitrite flux for both the supervised exercise group and the home exercise group, individually and combined, suggesting that the degree of change in the onset of ischemia is related to the change in NO bioavailability.

The effect of the intensity of exercise training on FMD in PAD was investigated by Mika *et al.*, (2012)<sup>313</sup>. Fifty-two men and women with symptomatic PAD were randomized into either a pain-free training (PFT) or moderate pain training (MT) group. Both groups performed ITW at 3.2 km·h<sup>-1</sup> and a grade to induce IC within 3-5 min to accumulate 35-60 min, 3 d·wk<sup>-1</sup> for 12 weeks. The PFT group exercised to the onset of IC and the MT group exercised to moderate IC. Following

training, FMD increased by 36% and 56% in the PFT and MT groups, respectively. There was no difference between groups. There was no change in plasma levels of hs-CRP and fibrinogen in either group following the training programme. There were also significant and similar improvements in PFWT and MWT in both groups.

The effect of different training modalities on FMD was evaluated by McDermott *et al.*, (2009) in 156 men and women with asymptomatic or symptomatic PAD<sup>246</sup>. Participants were randomized to a treadmill exercise, lower extremity RT, or control group. The two exercise groups trained 3 d·wk<sup>1</sup> for 6 months. Compared with controls, FMD improved significantly in the treadmill exercise group, but not the RT group. There were also significant improvements in MWT in both exercise groups compared with controls. However, PFWT and 6MWD improved in the treadmill exercise group only compared with controls.

### **Formation of Collateral Vessels**

Arteriogenesis refers to the increase in diameter of pre-existing arterioles<sup>314</sup>. It can result in the formation of mature arteries and the development of collateral circulation to supply the tissues distal to a stenotic occlusion. This process is not a passive dilation but involves active proliferation and remodelling. The primary stimulus for arteriogenesis is likely increased shear stress resulting from increased blood flow<sup>315</sup>. Shear stress triggers a complex cascade of molecular and cellular events resulting in the enlargement of the vessel lumen and wall thickness (figure 2.10).

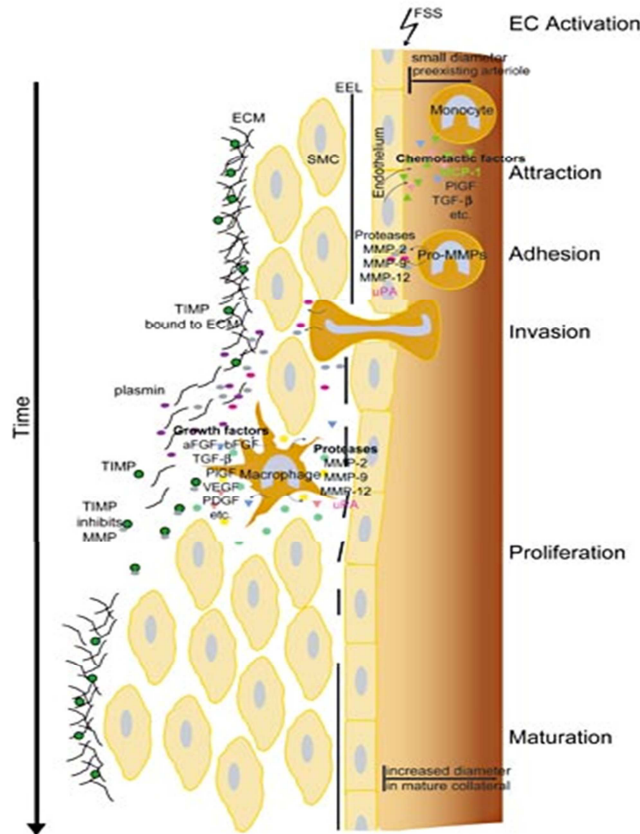


Figure 2.10: The process of arteriogenesis<sup>316</sup>

EC, endothelial cell; ECM, extracellular matrix; EEL, external elastic lamina; FGF, fibroblast growth factor; FSS, fluid shear stress; MCP-1, monocyte chemoattractant protein; MMP, matrix-metalloproteinase; PDGF, platelet-derived growth factor, PIGF, placenta growth factor; SMC, smooth muscle cells; TGF- $\beta$ , transforming growth factor beta; TIMP, tissue inhibitor of metalloproteinase; VEGF, vascular endothelial growth factor

FSS triggers EC activation, which is associated with the upregulation of chemotactic factors, in particular, MCP-1. This results in the recruitment of monocytes, which release proteases such as MMP. These proteases degrade surrounding structures such as the internal elastic lamina and extracellular matrix, creating space for the expanding vessel and remodelling the structure of the vessel itself. Monocytes also release growth factors essential for vascular remodelling. The vascular growth begins with endothelial proliferation, followed by that of SMCs to the subendothelial space, where they exhibit a “synthetic” phenotype, characterized by production of ECM, collagen and elastin to form a new internal elastic lamina, ultimately producing a mature artery.

In PAD, a pressure gradient develops between the regions proximal and distal to the stenotic occlusion, resulting in increased blood flow and thus shear stress<sup>317</sup>.

Arteriogenesis may be a compensatory adaptation in PAD. Exercise results in increased blood flow to the active muscles, rendering it a source of shear stress,

which may accelerate compensatory arteriogenesis in PAD leading to increased collateral blood flow and ultimately improved walking economy. Exercise training is associated with collateral vessel development in animal models of PAD. Following femoral artery ligation in rodents, collateral vessel wall CSA, maximal luminal diameter, and total hindlimb blood flow increased in both sedentary and exercise trained animals, but to a significantly greater extent in the trained animals<sup>318</sup>. The improvements in total hindlimb blood flow are associated with improved exercise tolerance<sup>319,320</sup>. Exercise accelerated the time course of the developments in collateral circulation. Maximal luminal diameter increased significantly in exercised rodents 1 week in advance of significant improvements in sedentary rodents<sup>318</sup>. Restoration of blood flow by collaterals was incomplete as calf muscle blood flow in the exercise animals still remained at ~60% that of the nonoccluded control group. Arteriogenesis is a self-limiting process. Since shear stress is inversely proportional to the cube of the vessel radius there will be a decrease in shear stress and consequently a decrease in arteriogenesis following the initial vessel enlargement.

The effect of exercise training on collateralization in human studies of PAD is equivocal. Collateralization in human studies is not measured directly, but inferred from lower limb hemodynamic measures including calf blood flow and ABI. The majority of studies have found no improvement in calf blood flow following exercise training, despite significant improvements in walking capacity. A 2010 systematic review of RCTs using an exercise intervention for the treatment of IC, reported small and insignificant effect sizes for resting and reactive hyperemic blood flow<sup>321</sup>. Hiatt



*et al.*, (1990) did find moderate increases in maximal calf blood flow, measured using venous occlusion plethysmography, in 19 men with PAD following 12 weeks of ITW 3 d·wk<sup>-1</sup> compared with age-matched PAD controls. The changes in maximal calf blood flow were not related to changes in MWT. In contrast, Gardner *et al.*, (2001)<sup>200</sup> found a significant relation between the change in maximal calf blood flow, measured using venous occlusion plethysmography, and the change in both PFWD and MWD in men and women with IC following 24 weeks of ITW 3 d·wk<sup>-1</sup> <sup>200</sup>. However, in a 12 month continuation of this study, in which training frequency was reduced to 2 d·wk<sup>-1</sup>, the improvements in maximal calf blood flow decreased, despite continued improvement in PFWD and MWD.

The disconnect between improvements in walking performance and improvements in blood flow is also evident in revascularization studies. Improvements in ABI following PTA did not translate to improvements in walking performance <sup>217</sup> or were not related to improvements in walking performance <sup>213</sup>. The apparent contradiction between findings of collateral development in animals but not humans may be explained by a number of factors <sup>322</sup>. Firstly, animal models may not reflect the disease process in humans with regard to the location of the occlusion, level of ischemia, and rate of onset. In humans, the ischemia associated with PAD is intermittent, the onset of the occlusion is gradual, and the disease is often multilevel, which impairs the development of an effective collateral circuit because of the increasingly lower perfusion pressure at each level <sup>323</sup>. Secondly, shear stress is the primary stimulus of arteriogenesis and the endothelial dysfunction

evident in individuals with PAD may inhibit vascular remodelling. Finally, more sophisticated measures of collateralization such as magnetic resonance imaging or computed tomography may be necessary to detect changes in humans.

### **Improvement in Hemorheological Properties**

Hemorheology refers to the study of the deformation and flow of blood and its formed elements and their interaction with other tissues <sup>324</sup>. Hemorheological properties are a determinant of blood flow in the macro- and microcirculation, especially in low flow states such as PAD <sup>325</sup>, and hence significantly affect tissue perfusion. Abnormal hemorheology has been reported in individuals with PAD, specifically increased blood and plasma viscosity, increased RBC aggregation and decreased RBC deformability <sup>326–328</sup>. Blood viscosity refers to the resistance of blood to flow. Under low-shear conditions such as in PAD the tendency of RBCs to aggregate increases <sup>329</sup> and RBC aggregation is a main cause of increased blood viscosity <sup>330</sup>. RBC aggregation is influenced by plasmatic factors, including fibrinogen concentration, and cellular factors such as I-RI <sup>331</sup>. Red blood cell deformability refers to ease of deformation of blood cells by external force. Deformability allows RBC to change shape and traverse microcirculation <sup>332</sup> and the loss of deformability decreases microcirculation blood flow and consequently tissue perfusion <sup>333</sup>.

The degree of hemorheological abnormality in PAD may be related to disease severity. Blood viscosity, hematocrit, hematocrit-corrected blood viscosity, plasma viscosity, fibrinogen, leukocyte elastase, and uric acid have been found to be significantly related to ABI and the presence of IC <sup>328</sup>. Blood viscosity and fibrinogen

were identified as independent predictors of ABI. In addition, plasma viscosity<sup>334</sup> and RBC deformability<sup>335</sup> are significantly lower in patients with CLI compared with those with IC. Abnormal hemorheology may have prognostic value for PAD. Dormandy *et al.*, (1973) found a significant relation between progressive deterioration of peripheral blood flow and initial blood viscosity and plasma fibrinogen levels<sup>336</sup>. Abnormal hemorheology may also be regarded as a risk factor for PAD. Impaired blood viscosity in individuals aged 45-65 years doubled the risk of developing arterial occlusive disease in the following 2 years, independent of classic CV risk factors<sup>337</sup>.

Exercise training can potentially normalize hemorheology in individuals with PAD. In a study by Ernst and Matrai (1987), 42 men and women with IC were assigned to an exercise or control group<sup>327</sup>. The exercise group performed supervised continuous treadmill walking to maximal IC twice per day, 5 d·wk<sup>-1</sup> for 8 weeks. At baseline, there was a significant difference in blood and plasma viscosity, blood cell filterability, and cell aggregation between all PAD patients and 40 healthy matched controls. After 8 weeks, there was a significant difference for blood and plasma viscosity, blood cell filterability, red cell aggregation, PFWD, and MWD between the exercise and PAD control group and the exercise group no longer differed from healthy controls. Similarly, Mika *et al.*, (2006) found significant improvements in RBC deformability following 12 weeks of ITW 3 d·wk<sup>-1</sup> in individuals with IC compared with usual care controls<sup>338</sup>. These improvements were accompanied by significant improvements in PFWD and MWT in the training group.

Häfner *et al.*, (2009) found a significant inverse relation between the change in PFWD and the change in plasma viscosity following 12 months of pedal dynamometry in men and women with IC<sup>272</sup>. In contrast, Tan *et al.*, (2000) reported no change in plasma viscosity following 12 weeks of walking for 60 min to maximal IC 5 d<sup>wk</sup><sup>-1</sup> in men and women with IC, despite a significant improvement in MWD<sup>339</sup>.

The mechanisms through which exercise leads to the normalization of hemorheology are not fully understood. In healthy individuals exercise produces an increase in plasma volume<sup>340</sup>, which may explain the decrease in blood and plasma viscosity. However, hematocrit was unchanged, although exercise may have stimulated erythropoiesis. A decrease in plasma fibrinogen is also a potential contributor to the improvement in blood and plasma viscosity. Plasma fibrinogen is elevated above the normal range in individuals with PAD and is significantly related to blood viscosity<sup>326</sup>.

An improvement in the resistance of blood to flow would theoretically improve macro- and microcirculation and thus tissue perfusion, especially in low flow states such as PAD. Blood viscosity is related to exercise performance<sup>341</sup> and improvements in viscosity are associated with improvements in walking performance in individuals with PAD. However, the association is weak and improvements in walking performance can occur in the absence of improvements in hemorheological properties, indicating that hemorheological adaptations are not the primary mechanisms responsible for improvements in walking performance with exercise rehabilitation in PAD.

## Improvement in Muscle Metabolism

Chronic exposure to I-RI leads to significant oxidative damage to the mitochondria of skeletal muscle distal to the stenosis in PAD. This results in diminished activity of oxidative enzymes and consequently impaired mitochondrial respiration<sup>96</sup>. Improved muscle metabolism is a common adaptive response to exercise training in healthy individuals and may, at least in part, explain the beneficial response to exercise rehabilitation in individuals with PAD. An improvement in mitochondrial respiration in PAD skeletal muscle would yield greater oxygen extraction per unit of blood delivered, leading to improvements in exercise performance without the need for improvements in limb hemodynamics.

In PAD, skeletal muscle acylcarnitine accumulation is inversely related with exercise performance and it is believed to be an indicator of impaired mitochondrial respiration. Hiatt *et al.*, (1990) found a significant decrease in plasma concentration of short-chain acylcarnitines following 12 weeks of ITW training in men with PAD compared with PAD controls<sup>101</sup>. There was also a significant decrease in the ratio of plasma short-chain acylcarnitine concentration to the total acid-soluble carnitine concentration, an index of the distribution of total carnitine between free and acyl carnitines. This ratio was strongly inversely related with the increase in MWT. In a subsequent study Hiatt *et al.*, (1996) randomized 26 men with PAD to a TM, RT or control group<sup>105</sup>. There was no significant change in plasma or muscle concentrations of carnitine or acylcarnitines in any group. However, the change in

the ratio of plasma short-chain acylcarnitines to total acid-soluble carnitine was significantly related with the increase in MWT.

Improvement in muscle metabolism with exercise rehabilitation may be attributable to a number of factors including an increase in the activity of oxidative enzymes, increased capillary density and consequently O<sub>2</sub> extraction, and alterations in muscle fiber properties.

### **Increased Oxidative Enzyme Activity**

The effect of exercise rehabilitation on oxidative enzyme activity in PAD skeletal muscle has not been extensively investigated and the findings are inconclusive. Duscha *et al.*, (2011)<sup>261</sup> found no change in citrate synthase (CS) activity in men and women with PAD randomized to 12 weeks of supervised or unsupervised exercise training. An increase in CS activity is a normal training response in healthy individuals. The absence of an increase in CS activity in PAD patients may be due to concomitant denervation with exercise, which has been shown to decrease the activities of a variety of oxidative enzymes<sup>342</sup>, or due to a number of other insults to oxidative metabolism. Hiatt *et al.*, (1996) also failed to find a significant change in CS activity in men with PAD randomized to a TM, RT or control group for 12 weeks<sup>105</sup>. However, there was a significant increase in phosphofructokinase activity in the TM group, which may suggest a larger dependence on glucose than fatty acids under conditions of limited oxygen delivery. In a RCT involving 26 weeks of supervised exercise training in men and women with IC Dahllöf *et al.*, (1974) found a significant increase in the muscle contents of

cholesterol and phospholipids and the incorporation rate of glucose-carbon in glycogen, lipids, and CO<sub>2</sub> and a significant decrease in the incorporation of glucose-carbon into lactate<sup>196</sup>. Succinic oxidase activity was significantly increased and the change in the pattern of metabolic activity was significantly related with the improvement in walking performance.

In a study by Lundgren *et al.*, (1989), 33 participants with IC were randomized to receive surgery, combined surgery and exercise, or exercise only<sup>107</sup>. Cytochrome-c oxidase activity increased significantly in the exercise only group. The increase was significantly related with the improvement in PFWD. The absence of an improvement in oxidative enzyme activity in the combined surgery and exercise group, in which blood flow was restored, suggests that both exercise and a reduced calf blood flow are necessary for positive enzymatic adaptation.

### **Increased Capillary Density**

Increased capillary density enhances diffusion capacity through increased diffusion surface area and decreased diffusion distance. This leads to increased O<sub>2</sub> extraction and consequently increased mitochondrial respiration, despite no change in limb blood flow<sup>343</sup>. It is a common adaptive response to exercise training in healthy individuals and represents a potential mechanism through which exercise training increases functional capacity in individuals with PAD. It occurs through angiogenesis, the formation of new blood vessels from pre-existing vessels. Angiogenesis is a complex process depending on the balance of multiple stimulating and inhibiting factors (figure 2.11).

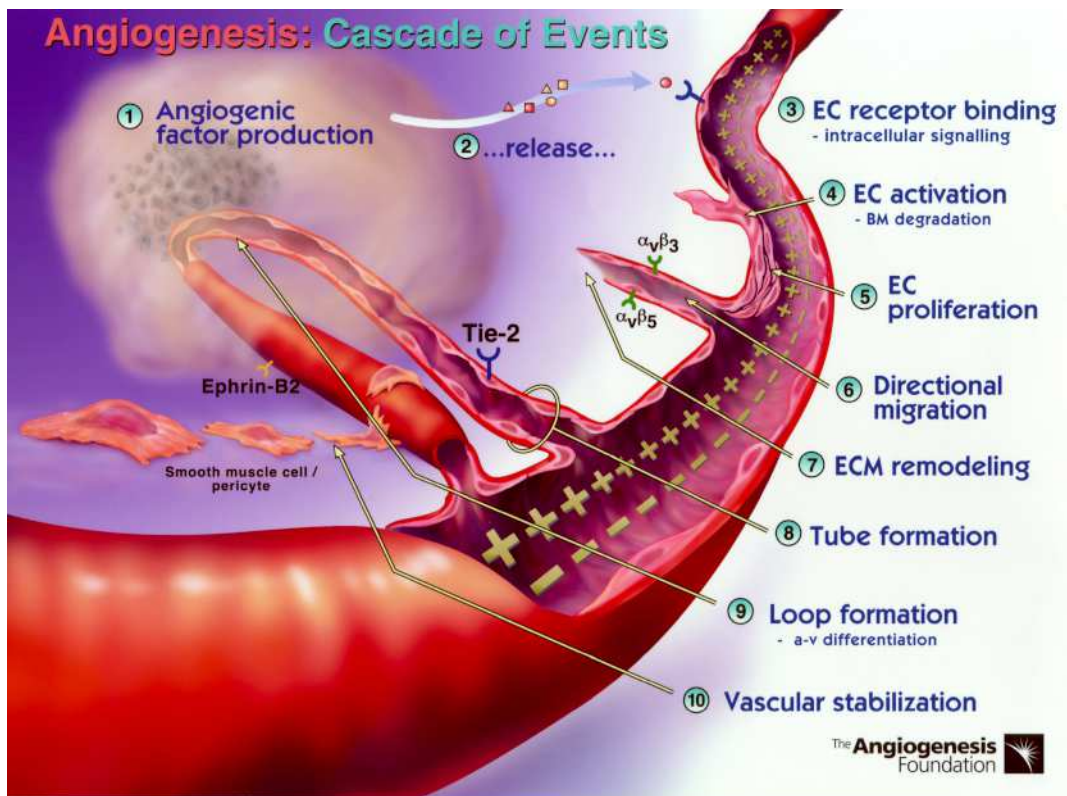


Figure 2.11: The process of angiogenesis<sup>344</sup>

EC, endothelial cell; ECM, extracellular matrix

Angiogenesis begins with the production of angiogenic factors. Vascular endothelial growth factor (VEGF) is the primary angiogenic factor and the most powerful stimulus for VEGF expression is hypoxia, which upregulates VEGF primarily through increased hypoxia-inducible factor 1 (HIF-1)-mediated transcription. VEGF-A exerts its biological effects via interaction with receptors located on EC membranes. Selected ECs, known as “tip-cells”, react to the VEGF gradient that specifies the direction of their migration and hence the direction of capillary growth. “Tip-cells” undergo phenotypic change acquiring invasive and migratory properties and activate secreted or cell surface proteases for partial degradation of the adjacent basement membrane. Proliferation and migration of ECs localized in the growing capillary branch progress the formation of the new capillary. Vessel maturation begins with the fusion of the newly formed capillary with other capillaries and the formation of the vessel lumen. The emerging blood flow removes the hypoxia stimulus for VEGF expression, which ceases EC proliferation. Recruitment of mural cells, pericytes and SMCs, form the walls of capillaries and immature blood vessels, and mature blood vessels, respectively. Pericytes, in turn, exert a stabilizing effect on the newly formed vessels, halting their growth and aiding the transition to a quiescent, mature state.

Vascular endothelial growth factor (VEGF) is the primary angiogenic factor and the most powerful stimulus for VEGF expression is hypoxia. In healthy individuals, exercise under conditions of restricted blood flow result in a significantly greater increase in VEGF mRNA expression than exercise under normoxic conditions



<sup>345</sup>. Therefore, PAD represents a prime condition for increasing VEGF expression with exercise. In a RCT, Sandri *et al.*, (2005) found a significant increase in investigated VEGF expression in individuals with PAD following ischemic but not non-ischemic exercise training <sup>346</sup>. Ischemic training involved treadmill walking to maximal IC. The non-ischemic group walked to 75% of MWD. Both exercise groups performed 2 bouts of treadmill walking at 3.5 km·h<sup>-1</sup> and 12% grade, 6 times daily, 5 d·wk<sup>-1</sup> for 4 weeks. Ischemia appears to be a prerequisite to enhance VEGF expression. Additional studies employing submaximal exercise programmes have reported no change in VEGF expression <sup>347,348</sup>.

Increased capillary density has been found after both endurance and resistance training in individuals with PAD <sup>203,261</sup>. Wang *et al.*, (2009) found a significant relation between the change in the number of capillaries in contact with type IIx and IIa muscle fibers and the increase in PFWT following 12 weeks of ITW training in individuals with IC <sup>349</sup>. Duscha *et al.*, (2011) found that improvements in capillary density preceded improvements in  $\dot{V}O_{2peak}$  with supervised exercise training in men and women with PAD <sup>261</sup>. There was a significant increase in gastrocnemius muscle capillary density after 3 weeks of training, although there was no significant improvement in  $\dot{V}O_{2peak}$  until week 12. Capillary density was related to  $\dot{V}O_{2peak}$  at baseline and week 12. Enhanced perfusion may be first required before improvements in mitochondrial function become evident.

Increased capillary density enhances diffusion capacity leading to increased O<sub>2</sub> extraction. Numerous studies have investigated the effects of exercise training

on calf muscle StO<sub>2</sub> in individuals with PAD. Exercise training has been associated with significant increases in calf muscle oxygenation at rest, dynamic muscle perfusion during exercise <sup>350</sup>, time to nadir StO<sub>2</sub> <sup>208,281</sup> and significant decreases in popliteal <sup>351</sup> and femoral venous StO<sub>2</sub> during exercise <sup>352</sup> in individuals with PAD. Transcutaneous oxygen tension appears unchanged with exercise training in individuals with PAD <sup>231,272,283</sup>.

Manfredini *et al.*, (1991) found that calf O<sub>2</sub> consumption at rest and dynamic muscle perfusion during exercise in men and women with PAD approached the stable values of untrained healthy participants following exercise training <sup>350</sup>. In a study by Sørliie *et al.*, (1978) the decrease in popliteal StO<sub>2</sub> was accompanied by a decrease in lactate release, indicating less reliance on anaerobic glycolysis and improved oxidative capacity, despite no change in limb blood flow <sup>351</sup>. The improvements in O<sub>2</sub> extraction are associated with improvements in walking performance. Collins *et al.*, (2012) found a significant relation between the reduction in time to nadir StO<sub>2</sub> and improvements in PFWT and MWT <sup>281</sup>.

### **Alterations in Muscle Fiber Properties**

A recent non-randomized controlled trial found that exercise rehabilitation may result in a shift in skeletal muscle fiber properties towards a more oxidative fibre type <sup>353</sup>. Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a significant increase in MHC I expression was found in the symptomatic gastrocnemius muscle of 27 men and women with IC following 12 weeks of circuit

training compared with 11 IC control patients. There was a significant relation between the percentage increase in MWD and the increase in MHC I expression.

Myosin heavy chain is a key determinant of skeletal muscle metabolism. An increase in the proportion MHC I increases oxidative capacity and reduces the reliance of claudicants on anaerobic glycolysis, thereby decreasing the painful accumulation of lactate. Previous research using immunohistochemistry has measured the effect of exercise rehabilitation on fiber type composition and fiber type area and found no significant change<sup>105,261,349</sup>.

### **Improvement in Walking Economy**

Walking economy is impaired in individuals with PAD. During constant-load exercise at high-intensity,  $\dot{V}O_2$  continues to rise with exercise duration in individuals with PAD rather than remaining steady-state as in healthy controls<sup>354</sup>. Impaired walking economy in combination with the low  $\dot{V}O_{2peak}$  evident in individuals with PAD results in ambulation at a given pace being performed at a higher relative metabolic cost than in healthy individuals.

Exercise rehabilitation is associated with an improvement in walking economy in individuals with PAD<sup>101,200,253,263,276,280,285,355</sup>. Studies have shown significant decreases in  $\dot{V}O_2$ , HR, RER, and ventilation during submaximal constant-load treadmill tests. Improvement in walking economy decreases the metabolic cost at a given workload, which would allow the activity to be maintained for a longer

duration. In claudication, this would allow individuals to walk for longer before developing an oxygen supply-demand mismatch and consequently claudication pain.

Although improvements in walking economy with exercise training are evident in the vast majority of trials, a significant relation between the change in walking economy and the change in PFWT and MWT has not always been reported. The absence of a significant relation between the change in walking economy and the change in PFWT and MWT tends to occur in studies measuring walking economy as  $\dot{V}O_2$  during the first stage of treadmill testing<sup>101,263,285</sup>. When walking economy is measured as  $\dot{V}O_2$  at the beginning of the final min of treadmill walking, a significant relation between the change in walking economy and a change PFWT, MWT, and 6MWD has been reported<sup>200,245,253</sup>. Fractional utilization (walking economy/ $\dot{V}O_{2peak}$ ) has been identified as an independent predictor of PFWT and MWT, suggesting that improvements in walking performance is due, at least in part, to combined changes in  $\dot{V}O_{2peak}$  and walking economy<sup>253</sup>.

Measuring changes in  $\dot{V}O_2$  at one time-point during exercise does not highlight whether improvements in walking economy are due to decreased steady-state  $\dot{V}O_2$  or a decrease in the slow component of  $\dot{V}O_2$  or both. Womack *et al.*, (1997) measured  $\dot{V}O_2$  at the third min and final min of a submaximal exercise test in 26 men and women with IC before and after a 4 month supervised exercise programme<sup>211</sup>. There was a significant increase in exercise time, a significant decrease in end-exercise  $\dot{V}O_2$ , and no change in  $\dot{V}O_2$  during the third min of submaximal exercise. The increase in  $\dot{V}O_2$  from the third min to the end of

submaximal exercise bout (the slow component of  $\dot{V}O_2$ ) present at baseline was eliminated with training.

The mechanisms through which exercise rehabilitation improves walking economy in individuals with PAD remain unclear, although a number of potential mechanisms have been proposed. Increased lower limb perfusion would reduce reliance on anaerobic metabolism, however change in limb perfusion is an inconsistent finding in PAD. Increased local skeletal muscle metabolism through increased mitochondrial content, oxidative enzyme activity, and capillary density would reduce the  $\dot{V}O_2$  at a given workload. Improvements in lower extremity strength have also been associated with improved walking economy. Following 12 weeks of RT, Ritti-Dias *et al.*, (2010) found a significant improvement in walking economy in 15 men and women with IC<sup>285</sup>. There was a significant inverse relation between the change in  $\dot{V}O_2$  at the first stage of the treadmill test and the change in strength in the leg with lower ABI and the change in the sum of strength of both legs.

Another potential mechanism through which exercise rehabilitation improves walking economy is improvements in biomechanical efficiency. Individuals with claudication may respond to exercise-induced pain in the lower extremities by altering their gait. Using this compensatory or protective mechanism, individuals with IC favor stability at the expense of speed by reducing both stride length and stride frequency and by spending a lower percentage of the gait cycle in the swing phase and more in the stance phase<sup>356,357</sup>. Within the stance phase, claudicants spend less time in single stance and more in double stance. Self-selected walking

pace is typically the most energy efficient, yielding the lowest O<sub>2</sub> consumption per meter travelled, and velocities above or below self-regulated pace are performed at a higher energy cost<sup>358</sup>. Individuals with IC may alter their gait in response to lower extremity pain by transferring the work to unaffected muscles, which may result in a less energy efficient gait. In fact, walking economy is further impaired after the onset of claudication pain<sup>359</sup>.

Exercise rehabilitation may improve the biomechanical efficiency of ambulation, thereby improving walking economy. Following 6 weeks of supervised exercise training, Hiatt *et al.*, (1990) found a significant decrease in  $\dot{V}O_2$ , minute ventilation, and HR during a submaximal exercise test in 10 men with IC compared with 9 age-matched controls<sup>101</sup>. This improvement occurred in the absence of a change in  $\dot{V}O_{2peak}$  but fewer participants experienced claudication pain during the submaximal test following training. A reduction in claudication pain may allow participants to select a more natural, energy-efficient gait.

Crowther *et al.*, (2008) directly assessed the effects of exercise rehabilitation on gait parameters in individuals with IC and found no change in any of the temporal-spatial gait parameters measured, despite significant improvements in PFWT and MWT<sup>198</sup>. However, gait parameters were measured during pain-free walking only.

## Improvement in Muscular Strength

Histological changes in PAD skeletal muscle including denervation, muscle fiber atrophy, and alterations in myosin heavy chain isoforms contribute to reduced muscle strength in individuals with PAD, which is related to functional performance<sup>128</sup>. Ritti-Dias *et al.*, (2010) found a significant relation between improvements in lower extremity strength and improvements in walking performance following 12 weeks of RT in 15 men and women with IC<sup>285</sup>. There was a significant relation between the change in knee extension strength in the higher ABI limb and PFWT and between both the change in the knee extension strength in the lower ABI limb and the change in the sum of strength in both limbs and the change in  $\dot{V}O_2$  during the first stage of the treadmill test. Similarly, Wang *et al.*, (2010) found a significant relation between the increase in 1 RM leg press and the improvement in walking economy in IC patients following 8 weeks of supervised maximal strength training<sup>284</sup>. The improvement in walking economy was also significantly related with the improvement in MWT.

The mechanisms through which improvements in lower extremity strength may potentially improve walking performance in individuals with PAD have not been fully investigated. An increase in muscle strength would decrease the relative load placed on the muscle during submaximal efforts. Individuals with PAD may alter their gait in favour of stability<sup>357</sup> and a reduced relative load could improve stability and decrease the  $O_2$  cost of walking. The improvements in strength occurred in the absence of increases in body weight, which may suggest the role of neural

adaptations, for example increased neural drive leading to increased frequency of motor unit stimulation and increased number of motor units recruited. An increase in the rate of force development would result in longer relaxation periods between contractions and potentially increased muscle perfusion<sup>360</sup>.



## Chapter III

### STUDY 1

#### SEDENTARY PATTERNS AND THEIR IMPACT IN PERIPHERAL ARTERIAL DISEASE

##### Rationale

The ambulatory limitation caused by PAD-associated intermittent claudication significantly reduces daily physical activity levels, placing these individuals at the extreme low end of the physical activity spectrum. A low level of physical activity is a risk factor for the development of PAD<sup>139,361</sup>. Recent epidemiological studies in the general population have found significant associations between sedentary time and cardiometabolic biomarkers, independent of physical activity<sup>6,162</sup>. Even in adults meeting the current physical activity recommendations, high levels of sedentary behaviour can have a detrimental effect on cardiometabolic health<sup>171</sup>. In addition to the detrimental effects of total sedentary time, the pattern of its accumulation may also be of clinical relevance. Prolonged sedentary bouts appear particularly detrimental to health and breaking up sedentary time can have a beneficial effect on metabolic biomarkers, independent of total sedentary time<sup>7</sup>.

The mechanisms responsible for the adverse cardiometabolic effects associated with prolonged sedentary behaviour are not fully understood. Chronic unbroken periods of muscular unloading may have deleterious biological consequences. The loss of contractile stimulation induced through sitting may lead

to the suppression of LPL activity leading to impairment in lipid metabolism<sup>181</sup>. In contrast, standing involves isometric contraction of the antigravity muscles and produces changes in skeletal muscle LPL activity. Other potential mechanisms include the reduction of insulin action<sup>187</sup> and the replacement of light-intensity physical activity<sup>157</sup>. Interrupting sedentary time with light or moderate-intensity exercise has been shown to lower glucose and insulin<sup>362</sup>.

Several studies have measured the physical activity levels of individuals with PAD<sup>140,144</sup>. However, to our knowledge no study has directly and objectively measured total daily sedentary time and the duration of sedentary bouts in individuals with PAD. Thresholding of accelerometer data classifies low counts as sedentary behaviour, which misleadingly classifies standing as a sedentary behaviour. ActivPAL<sup>3™</sup> triaxial physical activity logger is an inclinometer-based motion sensor that measures sedentary behaviour using postural orientation. It can differentiate between standing and sitting/lying<sup>363</sup> and is a valid measure of sedentary behaviour in adults<sup>364</sup>.

Previous research has reported a significant inverse relation between daily physical activity levels and mortality<sup>154</sup>, disease severity<sup>140</sup>, CV risk factors<sup>151</sup>, functional capacity<sup>148</sup>, and functional decline<sup>138</sup> among individuals with PAD. The purpose of this study was to measure total sedentary time and the number and duration of sedentary bouts in men and women with PAD and to determine the relation between sedentary time and patterns and CV risk factors, disease severity, functional capacity, and endothelial function.

## **Study Aims**

1. To determine total daily sedentary, standing, and ambulating time in men and women with PAD
2. To determine the number and duration of sedentary bouts in men and women with PAD
3. To examine the relation between activity and sedentary time and patterns and disease severity, functional capacity, CV risk factors, endothelial function and QOL in men and women with PAD

## **Study Hypotheses**

1. The majority of the day will be spent sedentary in men and women with PAD
2. The majority of daily sedentary time will be spent in bouts >60 min in men and women with PAD
3. There will be a significant relation between sedentary time and the duration of sedentary bouts and CV risk factors, disease severity, functional capacity, endothelial function, and QOL in men and women with PAD

## **Methodology**

### **Participants**

Eighteen men and five women with documented PAD were recruited. Peripheral arterial disease was confirmed by an ABI <0.90 at rest or a decrease in ankle systolic pressure  $\geq 30$  mmHg after exercise. Participants were excluded if they had ischemic rest pain, ulceration or gangrene, unstable angina, uncontrolled hypertension (systolic blood pressure >180 mmHg and/or diastolic blood pressure >100 mmHg), resting tachycardia, or unstable or acute heart failure. Participants were also excluded if their exercise tolerance was limited by factors other than intermittent claudication or if they were unable to walk on a treadmill at a velocity  $\geq 3.2$  km·h<sup>-1</sup>. Participants were fully informed of the experimental procedures and provided with a plain language statement before giving written informed consent in accordance with the Research Ethics Committee at Dublin City University (appendix A).

### **Overview of Study Design**

#### **Research Design**

Participants visited the Vascular Research Unit in DCU on 2 separate occasions and then wore an ActivPAL<sup>3</sup>™ triaxial physical activity logger for 7 d. The first laboratory visit was used to screen potential participants and during the second visit endothelial vasomotor function, walking performance, ABI, and QOL were assessed and a blood sample was taken.

## **Visit 1**

Participants were fully informed of the experimental procedures and provided with a plain language statement before giving written informed consent. Participants completed a general health questionnaire (appendix B), a physical activity readiness questionnaire (PAR-Q) (appendix C), and had their blood pressure, height, and body mass measured.

## **Visit 2**

An overview of visit 2 is outlined in figure 3.1. Participants arrived at the Vascular Research Unit following an 8 h overnight fast. Water consumption was permitted during the fasting period and where possible, all vasoactive medications were withheld for at least 4 half-lives. Participants refrained from strenuous physical activity for 24 h and from substances that may affect FMD such as caffeine, vitamin C, and tobacco for 6 h prior to the visit. Since the female participants were post-menopausal there was no need to control for menstrual cycle phase.

Following a 10 min rest period in a quiet, temperature-controlled room, a blood sample was drawn, and endothelial function was assessed. The participants then rested for a further 10 min in a supine position after which resting ABI was measured. Post-exercise ankle pressure was measured immediately after a treadmill walking test. Participants then completed the Peripheral Artery Questionnaire (PAQ) and finally performed a 6MWT.

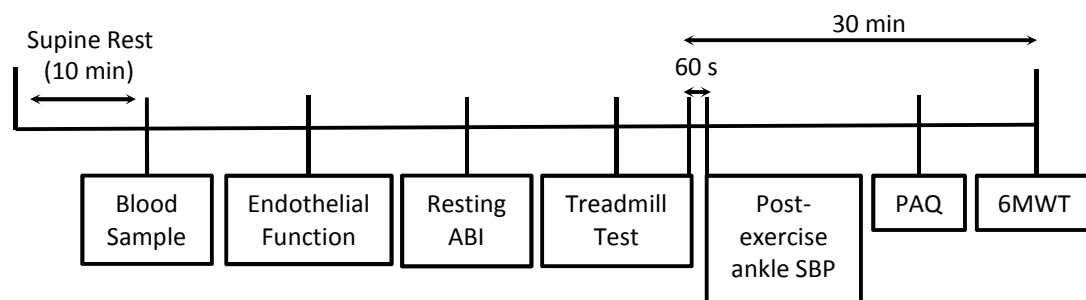


Figure 3.1: An overview of visit 2

### Blood Pressure

Resting blood pressure was taken from an upright sitting position using a mercury sphygmomanometer (Dekamet Model Accoson Sphygmomanometers, Harlow Essex) and stethoscope (Classic II 3M Littmann, St. Paul, MN).

### Anthropometric Characteristics

Height and body mass were measured using a wall stadiometer and electronic balance, respectively (Seca 797, USA). Footwear was removed prior to measurement. Height was measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg.

### Blood Sampling and Storage

Participants rested in a seated position for 5 min with legs uncrossed in order to minimize plasma volume shifts. Blood samples were drawn from the antecubital vein. Serum vacutainers were allowed to stand for 30 min before centrifugation at 3000 rpm (1600 g) for 15 min at 4°C.

## **Biochemical Analysis**

Serum triglycerides, total cholesterol, HDL-C, LDL-C, CRP, and creatine kinase (CK) were determined using spectrophotometric assays, performed on an automated bench-top clinical chemistry system (ACE®, Alfa Wassermann B.V., Netherlands) using the appropriate reagents, calibrators and controls (Randox Laboratories, UK). Concentration of haematocrit, haemoglobin and circulating leukocytes were determined from an EDTA whole blood sample using an automated haematology analyzer (AcTdiff2, Beckman Coulter, USA).

## **Endothelial Function**

### **Overview of Endothelial Function Assessment**

Brachial artery FMD was determined using high-resolution ultrasonography<sup>365</sup>. Measurements were performed by the same investigator using a SonoSite, MicroMaxx® (Sonosite Inc., Bothell, Washington, US) ultrasound system with a linear array transducer, operating at a frequency of 12.0 MHz (figure 3.2). The investigator displayed reliability in the use of ultrasonography to assess brachial arterial diameter ( $r=0.81$ ,  $p<0.1$ ).



Figure 3.2: Sonosite MicroMaxx<sup>®</sup> ultrasound system and 12.0 MHz linear array transducer

All brachial artery images were acquired with the participants in a supine position in a quiet, temperature-controlled room with their right arm rested on an examination table perpendicular to the bed and extended and externally rotated to permit imaging of the right brachial artery. An automated pneumatic cuff was placed on the right forearm, distal to the brachial artery (figure 3.3) and electrodes for a 3-lead ECG were placed on the chest. The ECG tracing was activated on the ultrasound system and settings adjusted to ensure clear identification of the R wave, which corresponds to the end of diastole in the cardiac cycle.



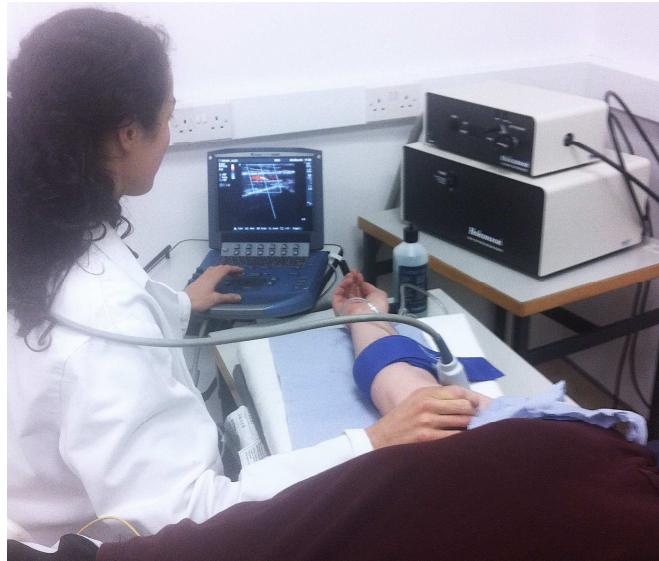


Figure 3.3: Arm position and cuff placement

### **Ultrasound Technique and Image Acquisition**

Anatomic landmarks such as veins and fascial planes were noted and used to ensure that all M-mode images and Doppler measurements were recorded at the same site. A longitudinal image of the brachial artery was obtained using B mode ultrasound. The brachial artery was insonated 3-7 cm above the antecubital crease. Great care was taken to ensure that the anterior and posterior intimal interfaces between the lumen and the arterial wall were clearly visible. Depth and gain settings were optimized to delineate the lumen-arterial interface optimally on both the near (anterior) and far (posterior) wall. The boundaries were clearly visualized with the angle of insonation perpendicular to the vessel. It is recommended that the imaging plane should bisect the vessel in the longitudinal direction to ensure that diameter measurements obtained from the images reflect the true diameter of the vessel<sup>366</sup>. Images were magnified using a “zoom” function.

### **M Mode Imaging**

The brachial artery was imaged using M mode function to facilitate arterial diameter measurements at appropriate time points (figure 3.4). Brachial artery images were named and saved for subsequent off-line analysis of arterial diameter. Each image acquired incorporated a minimum of 2 and a maximum of 3 consecutive ECG R waves, which represent the end of diastole in the cardiac cycle. The brachial artery diameter measurements were obtained at cross sections corresponding to the R waves, a process referred to as gating. The mean of the 2-3 measurements was taken as the brachial artery diameter.

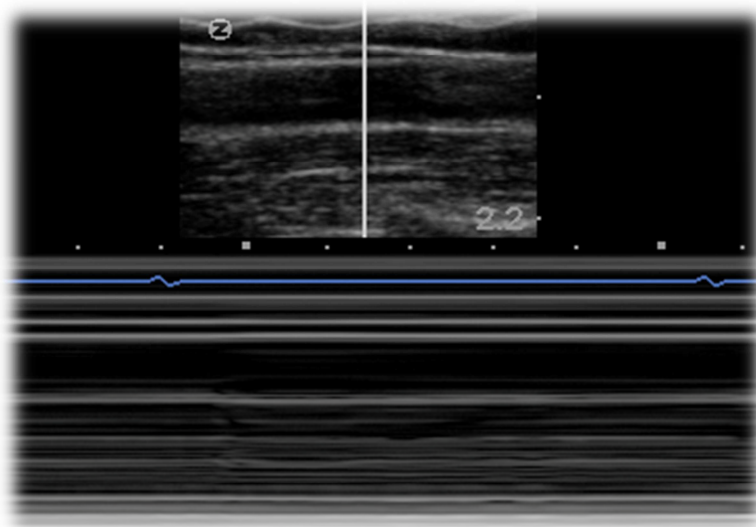


Figure 3.4: M mode ultrasound image of the brachial artery

### **Doppler Imaging**

Doppler imaging was used to measure blood flow velocity ( $\text{cm}\cdot\text{s}^{-1}$ ) in the brachial artery. The Doppler scale was adjusted to accommodate the spectral signal and the expected increase in blood flow following cuff release. The scale was

maintained at the minimum range to decrease measurement error. The Doppler gate was set to minimum (1.5 mm) and was positioned in the center of the artery lumen. The Doppler gate was aligned with the direction of flow and the transducer was adjusted to achieve an angle of insonation of  $60^\circ$ <sup>367</sup>. The insonation angle between the pulsed-wave Doppler beam and the vessel walls was adjusted by manipulation of the transducer, to allow the beam to be steered and the angle corrected in alignment with the vessel orientation/parallel, and blood flow axis at a discrete segment of vessel  $60^\circ$ <sup>367</sup>. The Doppler function traced the spectral waveform (figure 3.5). The image was frozen and peak systolic velocity was manually measured using the in-built ultrasound calipers (SonoSite MicroMaxx®).

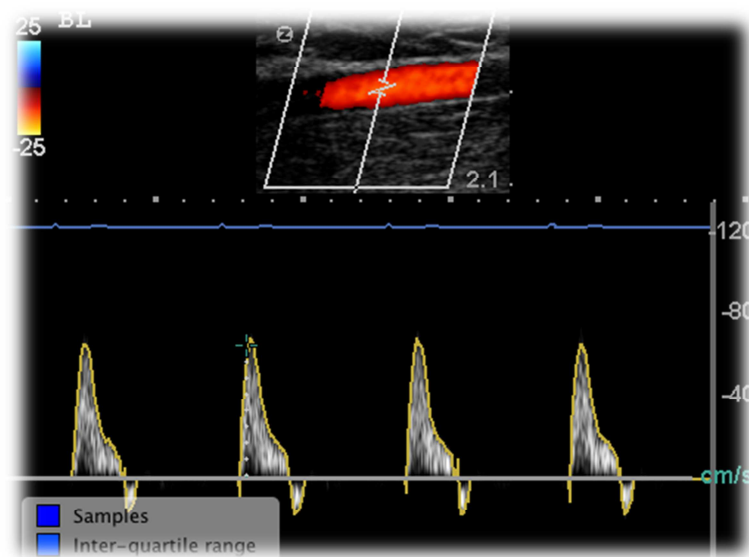


Figure 3.5: Frozen screen shot of a Doppler image

### Endothelial-Dependent Dilation

Figure 3.6 illustrates the endothelial-dependent dilation assessment procedure. Following a 10 min rest period, the brachial artery was identified. M

mode images were recorded for the measurement of baseline artery diameter and baseline systolic velocity was measured using Doppler. The pneumatic cuff was inflated to 250 mmHg for 5 min<sup>366</sup>. Following 5 min of occlusion, the cuff was rapidly deflated resulting in reactive hyperemia and a subsequent increase in brachial artery blood flow. Post-deflation peak systolic velocity was measured using Doppler within 15 s of cuff release. M-mode images of the brachial artery were named and recorded every 30 s post-deflation for 5 min. Brachial artery diameter was analyzed off-line.

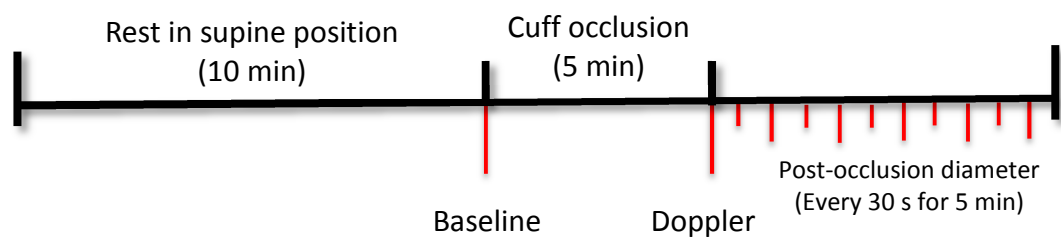


Figure 3.6: Overview of endothelial-dependent dilation assessment

### Endothelial-Independent Dilation

Figure 3.7 illustrates the endothelial-independent dilation assessment procedure. Participants rested for 15 min to eliminate endothelium-dependent effects on the brachial artery diameter. Following this period, a new baseline brachial artery image was recorded. Glyceryl trinitrate (GTN; 400  $\mu$ g) was then administered sublingually. M-mode images were named and recorded every 30 s for 5 min following GTN administration, for the off-line analysis of brachial artery diameter.

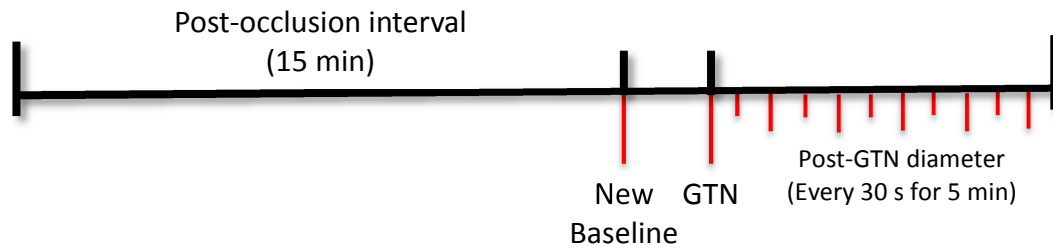


Figure 3.7: Overview of endothelial-independent dilation assessment

### Off-line Arterial Measurement Software

Brachial artery diameter was determined by the off-line analysis of ultrasound images using a custom-designed, semi-automated ultrasound arterial measurement software <sup>368</sup>. Measurements were performed in a blinded fashion. Images were selected for analysis using a standard dialog box (figure 3.8). For each image, the artery was located and the area between the anterior and posterior arterial walls was manually selected. The software used this point to segment the arterial boundary using a constrained region-growing algorithm, and the result was depicted visually in that the segmented arterial lumen was highlighted using grey shading. The segmentation of the artery was updated in real-time. The automated values could be overridden by selecting a new seed point or by using a slider to adjust the threshold intensity values of the segmentation until a satisfactory diameter estimate was obtained. Gated measurements of the brachial artery diameter were recorded using a minimum of 2 and maximum of 3 consecutive R waves on the ECG tracing. The mean of the 2-3 measurements was taken as the brachial artery diameter.

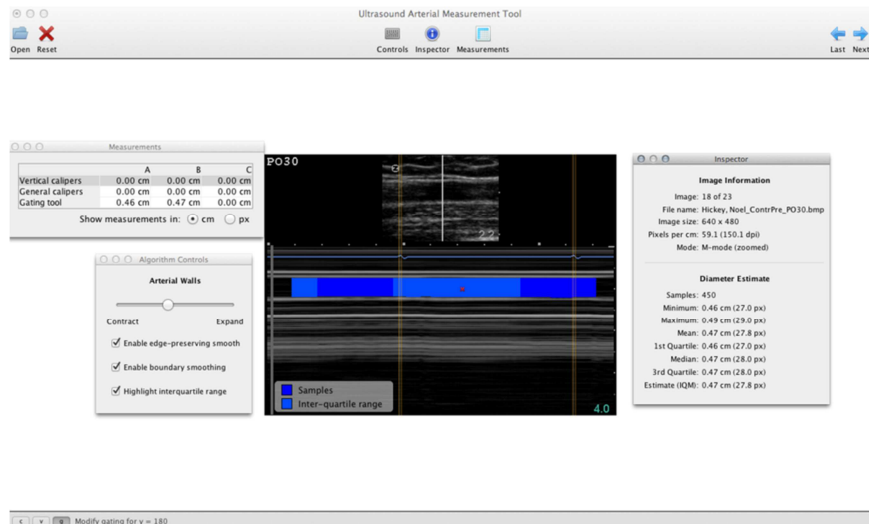


Figure 3.8: Standard dialog box

## Resting Ankle Brachial Index

Ankle brachial index was measured in accordance with recommendations from the AHA<sup>35</sup> (figure 3.9).

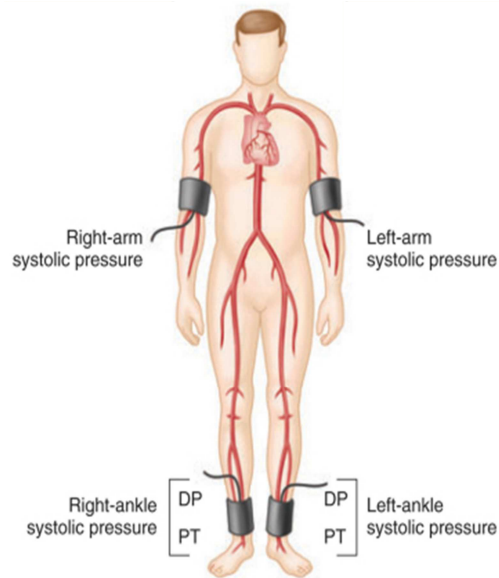


Figure 3.9: Overview of the ankle brachial index procedure

Following 10 min of supine rest, blood pressure cuffs were placed on both arms, just above the antecubital fossa, and both ankles, just above the malleolus.

Ankle and brachial systolic blood pressures were measured using a hand-held Doppler device (Elite 100™, Nicolet) with an 8 MHz pencil probe (figure 3.10). The Doppler device was used to detect the pulse. A sphygmomanometer was used to inflate the cuff to 20 mmHg above SBP. The cuff was then slowly deflated and the first reappearance of the pulse was recorded as the systolic pressure.



Figure 3.10: Nicolet Elite 100™ hand-held Doppler device with an 8 MHz pencil probe

Systolic blood pressures were measured in the right brachial artery, and the posterior tibial artery and dorsalis pedis artery of the right ankle followed by the left brachial artery and the posterior tibial artery and dorsalis pedis artery of the left ankle. This process was repeated and the average of the two pressures for each artery was taken. If any pair of values differed by more than 6 mmHg, repeat pressures were taken. Ankle brachial index was calculated by dividing the highest ankle artery pressure in the more severely diseased lower limb by the highest brachial artery pressure.

### **Post-Exercise Ankle Pressure**

Immediately following the treadmill exercise, participants returned to a supine position and SBP was measured within 60-150 s using a hand-held Doppler device (Elite 100™, Nicolet) in the artery yielding the highest resting pressure, in the more severely diseased lower limb.

### **Treadmill Walking Capacity**

Walking capacity was assessed on a treadmill (Marquette 2000, General Electric, USA) using an incremental protocol of 3.2 km·h<sup>-1</sup> and 0% grade, with a subsequent 2% increase in grade every 2 min to maximal claudication<sup>369</sup>. Pain free walking time and maximal walking time were recorded. Respiratory metabolic measures were monitored continuously throughout the test and  $\dot{V}O_{2peak}$  was determined by averaging the 3 highest consecutive 20 s values. Heart rate was recorded continuously throughout the test using telemetry (Polar Vantage NV™ Polar, Port Washington, NY).

### **Cardiorespiratory and Metabolic Measures**

Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (Sensormedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analyzers were calibrated with standard gases of known concentration. A mass flow sensor (Sensormedics, Loma Linda, CA, USA) was used to collect breath-by-breath measurement of ventilation. A 3 L volume syringe



(SensorMedics, Loma Linda, CA, USA) was used to calibrate the mass flow sensor prior to each test.

### **Mass Flow Sensor Heated Wire Anemometer – Mode of Operation**

The mass flow sensor is a low resistance tube with a tapered internal diameter extending from both ends of a laminar flow throat. A cold and hot stainless steel wire electrically heated to  $-180^{\circ}\text{C}$  and  $-240^{\circ}\text{C}$  respectively, are centered in the flow stream. These wires are elements in a servo-controller bridge circuit that maintain the resistance ratio of the two wires at a constant value. If only the temperature of the inspired gases changes, then both wires lose heat at the same rate and no current change is required to keep the bridge balanced. As air flows across the wires, the hot air loses heat more rapidly than the cold air and current must be added to keep the bridges balanced at a 3:4 ratio. The amount of current required is proportional to the mass flow of the gas. This method ensures that the sensor measures only the heat loss from the molecular convection of the moving gas stream, and not the artifact due to cooling of the gas as it passes through a breathing assembly. The mass flow meter responds to instantaneous flow rates between  $0\text{-}16\text{ L}\cdot\text{s}^{-1}$  and integrated flow between  $0\text{-}350\text{ L}\cdot\text{min}^{-1}$  with flow resistance  $<1.5\text{ cmH}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$ . The mass flow sensor was outputted to the analyzer module of the Vmax 229 and was sampled at a rate of 125 Hz.

## **Mass Flow Sensor Calibration**

A 3 L volume syringe (Sensormedics, Loma Linda, CA, USA) was connected to the mass flow sensor and stroked four times in order to measure inspired and expired volumes. The volumes were calculated by expressing 3 L as a fraction of each measured inspired and expired volume achieved during calibration. An average correction factor was calculated for inspired and expired volumes, and used to fine-tune the volume measurement.

A verification procedure was performed. This involved stroking the 3 L volume syringe four times. Inspired and expired volumes were measured using the newly calculated correction factors. In order to pass the calibration procedure, one of the four strokes had to have an average flow rate  $<0.5 \text{ L}\cdot\text{s}^{-1}$  and at least one of the four strokes had to have an average flow  $>3.0 \text{ L}\cdot\text{s}^{-1}$ .

## **Gas Analyzers**

The Vmax 229 utilizes a rapid response infrared measurement technique. An  $\text{O}_2$  and  $\text{CO}_2$  analyser is integrated with the Vmax 229. A small sample of inspired air is drawn through a sample cell and exposed to an infrared light through an optical that is passed through a band pass filter and the sample cell. An infrared detector responds to the amount of infrared light that passes through the sample cell. The amount of light that passes through the sample cell varies according to the concentration of  $\text{CO}_2$  in the sample cell. Based on measured levels of infrared light intensity, the analyzer computes the  $\text{PCO}_2$  in the gas sample. The  $\text{CO}_2$  analyzer is

linearly scaled across the 0-100% range with a resolution of 0.01% CO<sub>2</sub> and a response time of <130 ms (10-90%) at 500 ml·min<sup>-1</sup> flow. The O<sub>2</sub> analyzer is based on the high paramagnetic susceptibility of O<sub>2</sub>. A diamagnetic glass dumbbell suspended in a magnetic field rotates in proportion to the PO<sub>2</sub>. The analyzer is linearly scaled across the 0-100% range with a resolution of 0.01% O<sub>2</sub> and a response time of <130 ms (10-90%) at 500ml·min<sup>-1</sup> flow.

### **Calibration of CO<sub>2</sub> and O<sub>2</sub> Analyzers**

The gas analyzers were calibrated with standard gases of known concentration (BOC gases, Dublin, Ireland). The first calibration gas contained 26.00 ± 0.02% O<sub>2</sub> and the balance nitrogen (N<sub>2</sub>). The second calibration gas contained 4.00 ± 0.02% CO<sub>2</sub>, 16.00 ± 0.02% O<sub>2</sub>, and the balance N<sub>2</sub>. A small bore drying tube connected to the CO<sub>2</sub> and O<sub>2</sub> analyzers sampled the calibration gases. The absorption and evaporative properties of the drying tube ensured that the relative humidity of the calibration gas was equilibrated to ambient conditions prior to sampling by the O<sub>2</sub> and CO<sub>2</sub> analyzers. The calibration gas was sampled at a rate of 125 Hz. The response time was similar between O<sub>2</sub> and CO<sub>2</sub> analyzer.

### **The Peripheral Artery Questionnaire**

The PAQ is a self-administered, 20-item questionnaire, encompassing 6 domains concerning; physical function, symptom stability, symptom frequency/burden, treatment satisfaction, quality of life, and social function (appendix D)<sup>370,371</sup>. The PAQ is scored by allocating 1 point to the response

signifying the most limited function and an additional point for each higher response. Domain scores were calculated by summing the individual items within the domain and subtracting the lowest possible score for that domain, dividing by the range of that domain and multiplying by 100. Domain scores ranged from 0 to 100, and a lower score indicated greater limited function. A summary score was generated by averaging the scores from the physical function, symptom frequency/burden, social function, and quality of life domains.

### **Six Minute Walk Test**

Prior to the test, participants rested for 10 min in a seated position. The test was performed along a flat, enclosed 30 m corridor with a hard surface, according to ATS Guidelines<sup>372</sup>. No warm up was permitted and participants were instructed to walk as far as possible, turning 180° every 20 m in the allotted time of 6 min. The total distance walked was measured to the nearest metre, and recorded. Heart rate was recorded throughout the test using telemetry (Polar Vantage NV™ Polar, Port Washington, NY).

### **Activity and Sedentary Behaviour**

The ActivPAL<sup>3™</sup> triaxial physical activity logger (PAL Technologies Ltd., Glasgow, UK) (figure 3.11) is a single unit triaxial accelerometer measuring 53 x 35 x 7 mm and weighing approximately 15 g. The device samples at 10 Hz and measures bodily accelerations using a triaxial accelerometer. An inbuilt inclinometer is used to identify the posture of the wearer. The data was allocated into 15 s epochs of

sitting/lying, standing, and stepping using the on-board microprocessor. Proprietary algorithms provided outputs including time spent sitting/lying, standing, stepping, step counts, cadence and activity counts.



Figure 3.11: ActivPAL<sup>3</sup>™ triaxial physical activity logger

Each participant was provided with an ActivPAL<sup>3</sup>™ and given a verbal description and written demonstration of its use. Participants were instructed to wear the device continuously for 7 d, except during water activity periods. The ActivPal<sup>3</sup>™ was worn on the midpoint of the anterior aspect of the right thigh and attached using a Tegaderm™ film adhesive frame dressing (3M Health Care, Neuss, Germany) (figure 3.12). If required, an elastic tube bandage was worn over the device for further stability. After 7 d, the ActivPAL<sup>3</sup>™ was retrieved and the data was downloaded to a PC via a USB interface.



Figure 3.12: ActivPAL<sup>3</sup>™ placement

### Data Processing

For data to be included in the analysis, participants were required to provide  $\geq 4$  valid days, including one weekend day, where a valid day was defined as  $\geq 600$  min of recording during daytime hours, i.e., 7 am to 11 pm<sup>373</sup>. Non-wear time was defined as  $\geq 60$  min of continuous unbroken zero counts from the output data file<sup>363</sup>. Recorded data was accessed using the ActivPAL™ proprietary software (ActivPAL™ Professional VX) and exported to a Microsoft Excel format file (Microsoft Excel 2010, Microsoft Corporation, WA, USA). Data was displayed as the number of seconds that the participants engaged in sitting/lying, standing, and stepping for each 15 s epoch. Values over 24 h were summed to provide the total time spent sitting/lying, standing and stepping and the average daily time spent in these behaviour categories during the recording period were calculated.

A technical issue occurred during the analysis of accelerometer counts to identify MVPA. Step cadence was subsequently used to identify MVPA, which is robustly defined as  $>25 \text{ steps}\cdot\text{epoch}^{-1}$ . Moderate-to-vigorous physical activity was also classified using the definition of  $>16 \text{ steps}\cdot\text{epoch}^{-1}$  to account for the ambulatory limitation associated with PAD. The treadmill velocity used by Gardner *et al.*, (1991) to test functional capacity in individuals with PAD is equivalent to a cadence of 16  $\text{steps}\cdot\text{epoch}^{-1}$  <sup>369</sup>.

Sedentary behaviour characteristics were examined by processing the ActivPAL™ data output files using a customized MATLAB® (version 7.0.1, Mathworks Inc, Natick, MA, USA) computer software programme <sup>363</sup>.

### **Number and Duration of Sedentary Bouts**

The customized MATLAB® programme examined the ActivPAL™ output file epoch-by-epoch and binary coded each epoch. A sedentary epoch was categorized as an epoch spent entirely sitting/lying, i.e., a full 15 s (code = 1) and a non-sedentary epoch was categorized as an epoch containing  $>0$  s of standing or stepping or  $<15$  s of sitting/lying (code = 0) <sup>374</sup>. A sedentary epoch identified the start of a sedentary bout and the last consecutive sedentary epoch marked the end of the sedentary bout. The number and mean duration of sedentary bouts per day were calculated. Sedentary bouts were categorized by specific durations, namely  $<5$  min, 5-10 min, 11-20 min, 21-30 min, 31-40 min, 41-60 min,  $>60$  min,  $>90$  min,  $>2$  h,  $>3$  h, and  $>4$  h. The number of sedentary bouts and total duration spent in sedentary bouts in each category were calculated.

### **Breaks in Sedentary Behaviour**

Breaks in sedentary behaviour were defined as time spent standing or stepping that lasted  $\geq 15$  s<sup>363</sup>. The total number of breaks in sedentary behaviour, and the number of these breaks that involved ambulation was calculated.

### **Statistical Analysis**

Prior to statistical analysis the data were checked for normality using the Shapiro-Wilks test. Pain-free and maximal walking time data was log transformed to  $\log_{10}$  to normalize data. Univariate analysis was undertaken using both the Pearson product-moment correlation ( $r$ ) (parametric) and the Spearman correlation ( $r_s$ ) (non-parametric). Statistical significance was accepted at the  $p < 0.05$  level of confidence. SPSS for Windows statistical software (V21.0, SPSS Inc, IL) was used to perform the statistical analysis.



## Results

### Participant Characteristics

Participant's physical and biological characteristics are summarized in table 3.1. One participant provided <4 valid days of ActivPAL data and was excluded from the analysis. Based on BMI and blood pressure data the participants were on average overweight and stage I hypertensive<sup>375</sup>. Total cholesterol, LDL-C, and HDL-C were within the normal range and triglyceride levels were above the normal range

<sup>376</sup>

Table 3.1: Physical and biological characteristics of participants (n=22)

Age (y)	68.95 ± 8.43
Height (cm)	167.83 ± 8.18
Body mass (kg)	80.11 ± 17.07
BMI (kg·m <sup>-2</sup> )	28.28 ± 4.97
Systolic blood pressure (mmHg)	156.52 ± 19.35
Diastolic blood pressure (mmHg)	83.94 ± 14.83
Ankle brachial index	0.73 ± 0.18
Post-exercise change in ankle SBP (mmHg)	-33.71 ± 23.74
Total cholesterol (mmol·L <sup>-1</sup> )	4.83 ± 1.16
HDL-cholesterol (mmol·L <sup>-1</sup> )	1.17 ± 0.25
LDL-cholesterol (mmol·L <sup>-1</sup> )	1.76 ± 0.65
Triglycerides (mmol·L <sup>-1</sup> )	2.04 ± 2.14

Values are means ± SD

Table 3.2 summarizes the medications taken by the study participants.

Table 3.2: Medications taken by study participants

Drug class	N	%
Statins	18	82
Anti-platelet	16	73
Beta blocker	7	32
Angiotensin-converting-enzyme inhibitor	7	32
Proton pump inhibitors	7	32
Nonsteroidal anti-inflammatory drugs	7	32
Calcium channel blocker	6	27
Diuretic	6	27
Angiotensin II receptor blockers	6	27
Hypoglycemic	6	27
Antispasmodic	2	9

### Activity and Sedentary Behaviour

The daily activity and sedentary behaviour of the participants is outlined in figure 3.13. Participants spent a mean of  $18.9 \pm 2.1$  h (79.0%) of the total day sedentary,  $3.9 \pm 1.8$  h (16.1%) standing, and  $1.2 \pm 0.6$  h (4.9%) ambulating.

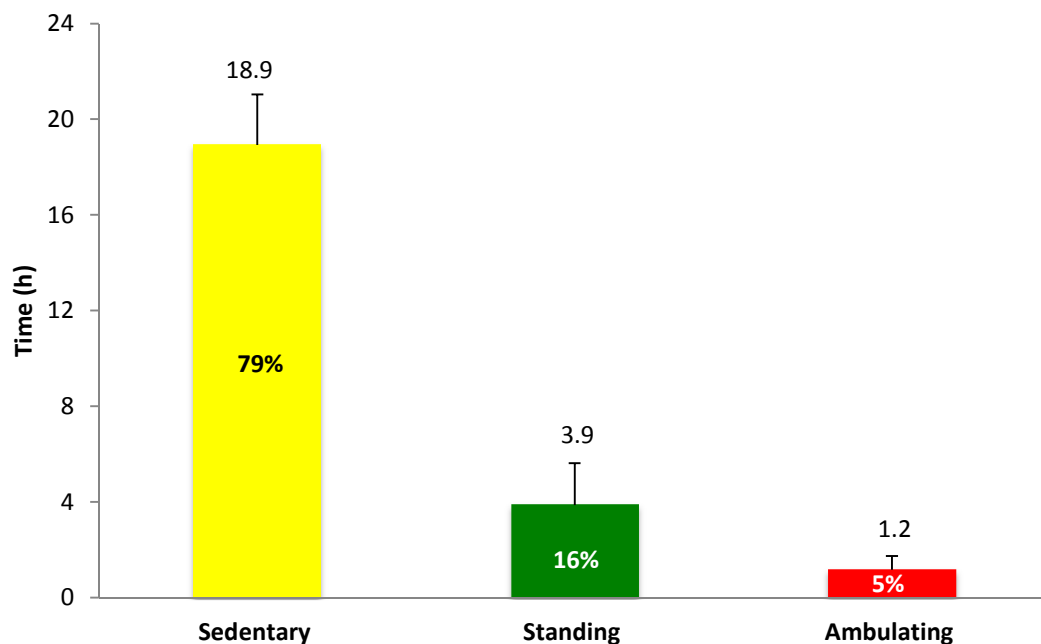


Figure 3.13: Total daily time (h) spent sedentary, standing, and ambulating

Figure 3.14 outlines the sedentary, standing and ambulating behaviours during waking hours. Participants spent  $67.6 \pm 13.3\%$ ,  $24.9 \pm 11.0\%$ , and  $7.6 \pm 3.5\%$  of the waking day sedentary, standing, and ambulating, respectively. The mean step count was  $5575.0 \pm 3197.3$  steps·d<sup>-1</sup>. A total of  $0.24 \pm 0.30$  h·d<sup>-1</sup> was spent in MVPA, defined as  $>25$  steps·epoch<sup>-1</sup>. When the definition of MVPA was altered to  $>16$  steps·epoch<sup>-1</sup> to account for the ambulatory limitation associated with PAD, the value was  $0.50 \pm 0.38$  h·d<sup>-1</sup>.

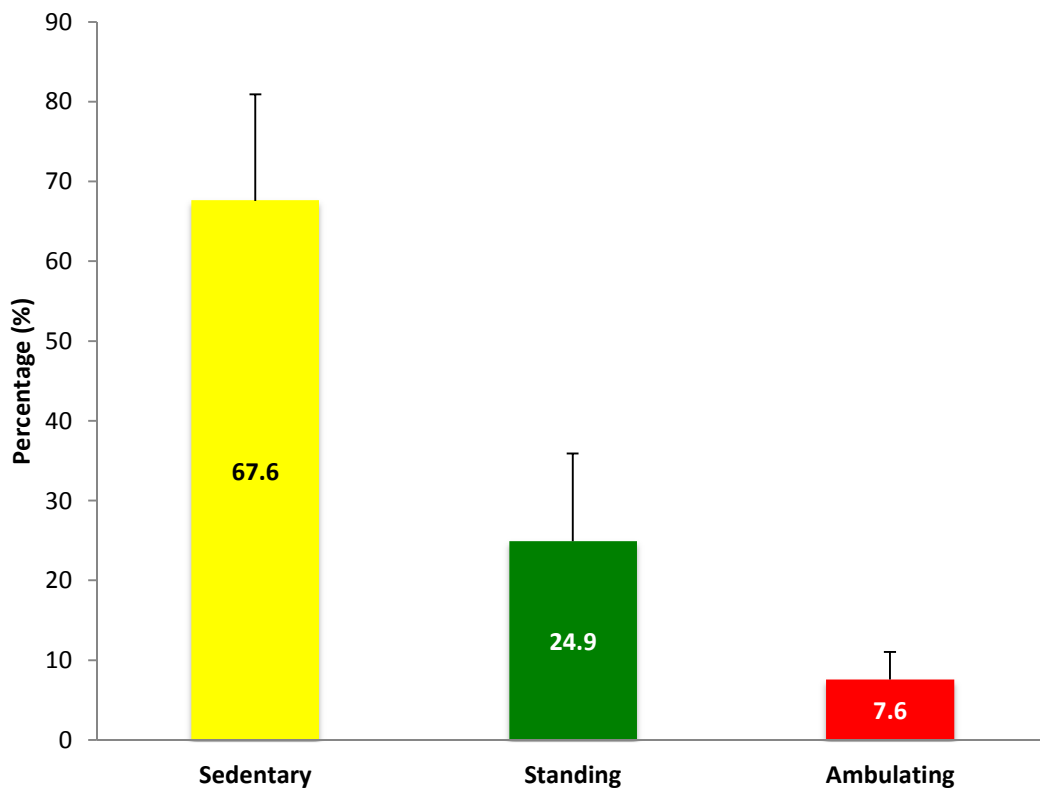


Figure 3.14: Percentage of the waking day spent sedentary, standing, and ambulating

### Sedentary Behaviour Characteristics

The characteristics of the participant's sedentary behaviour are outlined in figure 3.15. The majority of sedentary bouts were <5 min, however the greatest duration of sedentary time was spent in bouts >60 min. A mean of  $4.0 \pm 2.3 \text{ h}\cdot\text{d}^{-1}$  was spent in sedentary bouts >60 min. This represented 35.6% of the total waking sedentary time.

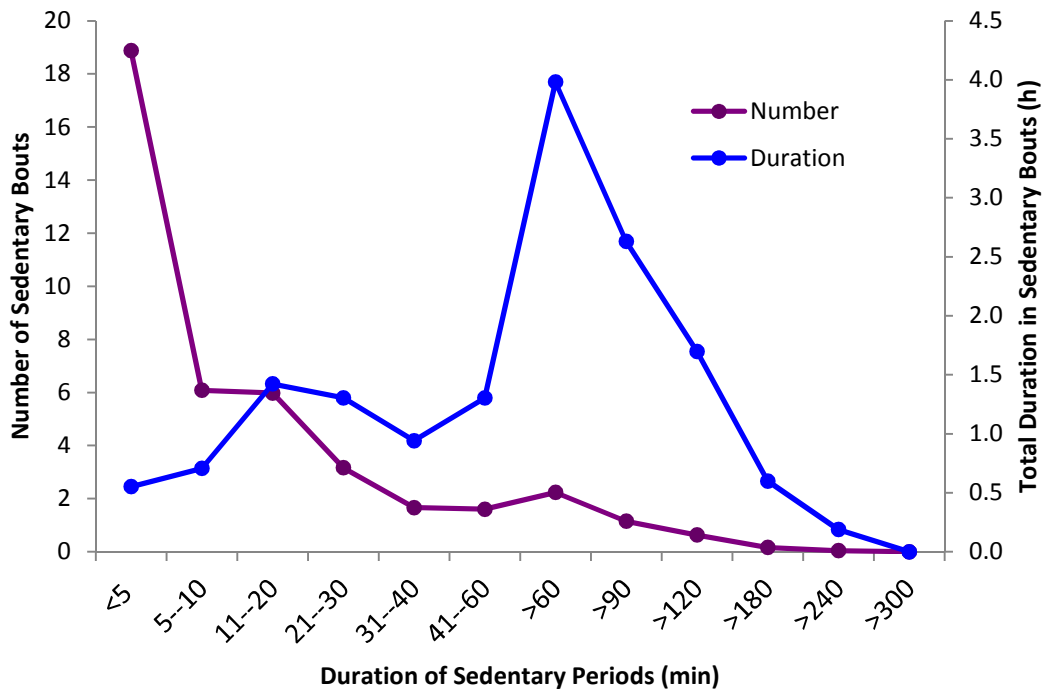


Figure 3.15: Characteristics of sedentary behaviour

## Breaks in Sedentary Time

Figure 3.16 outlines the breaks in sedentary time. Participants broke sedentary time on a mean of  $39.1 \pm 11.0$  occasions daily. The majority of these breaks,  $91.1 \pm 4.5\%$ , involved ambulation.

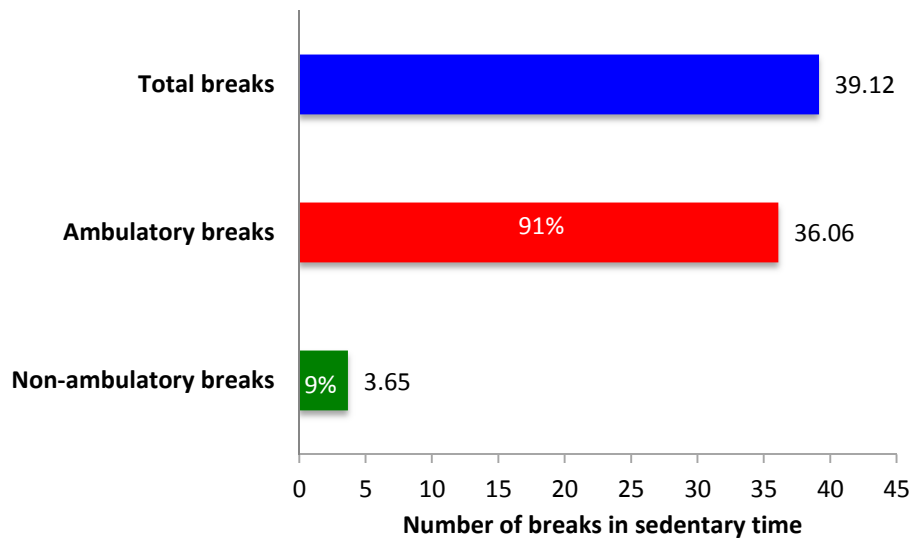


Figure 3.16: Total number of breaks in sedentary time and the number of ambulatory and non-ambulatory breaks

## Relation between activity and sedentary behaviour and cardiovascular risk factors

Sedentary time characteristics were associated with CV risk factors. Prolonged sedentary bouts were significantly related to cardiometabolic risk factors. There was a relation between DBP and the number of sedentary bouts >60 min ( $r_s=0.57$ ,  $p<0.05$ ) and the total duration spent in sedentary bouts >60 min ( $r_s=0.60$ ,  $p<0.01$ ) (figure 3.17). Total serum cholesterol was significantly related with the number of sedentary bouts >3 h and >4 h and the total duration spent in sedentary bouts >3 h and >4 h (table 3.3). The mean circulating level of CRP was  $3.75 \pm 3.16$   $\text{mg}\cdot\text{L}^{-1}$  and CRP levels were related to the number of sedentary bouts >4 h and the total duration spent in sedentary bouts >4 h (table 3.3).

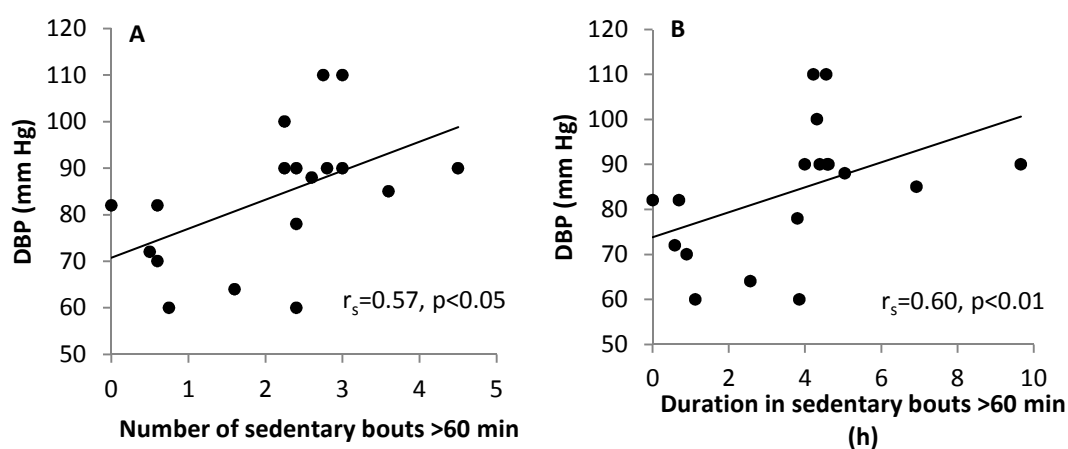


Figure 3.17: Relation between diastolic blood pressure and (A) the number of sedentary bouts >60 min and (B) total duration spent in sedentary bouts >60 min

Table 3.3: Relation between CV risk factors and sedentary bouts >3 h

Sedentary bouts	CRP	Total Cholesterol
Total number of bouts >3 h	ns	$r_s=0.45$ , $p<0.05$
Total duration in bouts >3 h	ns	$r_s=0.45$ , $p<0.05$
Total number of bouts >4 h	$r_s=0.44$ , $p<0.05$	$r_s=0.48$ , $p<0.01$
Total duration in bouts >4 h	$r_s=0.44$ , $p<0.05$	$r_s=0.48$ , $p<0.01$

ns, non-significant

There was a positive relation between BMI and the number of non-ambulatory breaks ( $r=0.53$ ,  $p<0.05$ ) and the percentage of non-ambulatory breaks ( $r=0.57$ ,  $p<0.01$ ) and an inverse relation between BMI and the percentage of ambulatory breaks ( $r=0.57$ ,  $p<0.01$ ) (figure 3.18). Body mass was also positively related with the percentage of non-ambulatory breaks ( $r=0.44$ ,  $p<0.05$ ) and inversely associated with the percentage of ambulatory breaks ( $r=0.44$ ,  $p<0.05$ ).

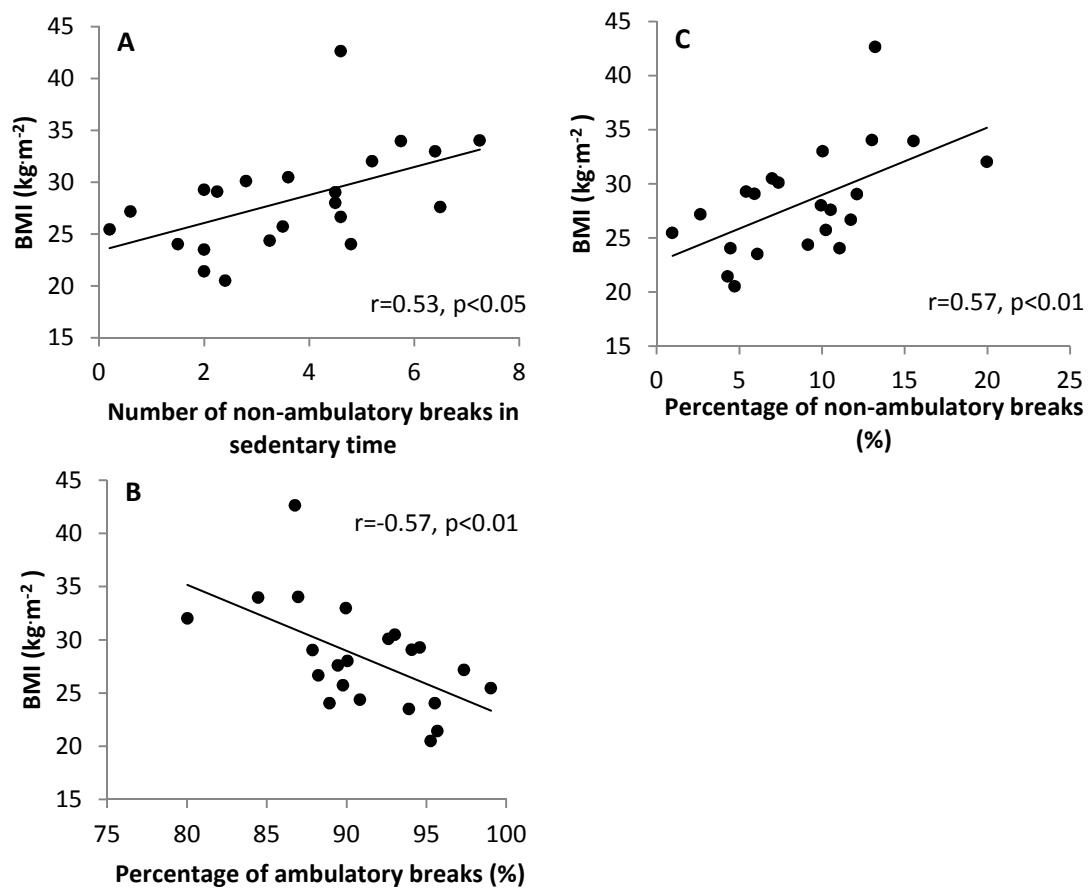


Figure 3.18: Relation between BMI and (A) the number of non-ambulatory breaks, (B) the percentage of non-ambulatory breaks, and (C) the percentage of ambulatory breaks

### Relation between activity and sedentary behaviour and disease severity

Resting ABI was related with MVPA defined as  $>25$  steps-epoch<sup>-1</sup> ( $r_s=0.44$ ,  $p<0.05$ ) (figure 3.19). The post-exercise change in ankle SBP was inversely related with total sedentary time ( $r=0.49$ ,  $p<0.05$ ) and positively related with total standing time ( $r=0.46$ ,  $p<0.05$ ) (figure 3.20). The post-exercise change in ankle SBP was also associated with the characteristics of sedentary time (figure 3.21). It was positively related with the total number of sedentary bouts ( $r=0.49$ ,  $p<0.05$ ) and the number of sedentary bouts  $<5$  min ( $r_s=0.60$ ,  $p<0.01$ ) and inversely related to the number of sedentary bouts  $>60$  min ( $r_s=0.48$ ,  $p<0.05$ ).

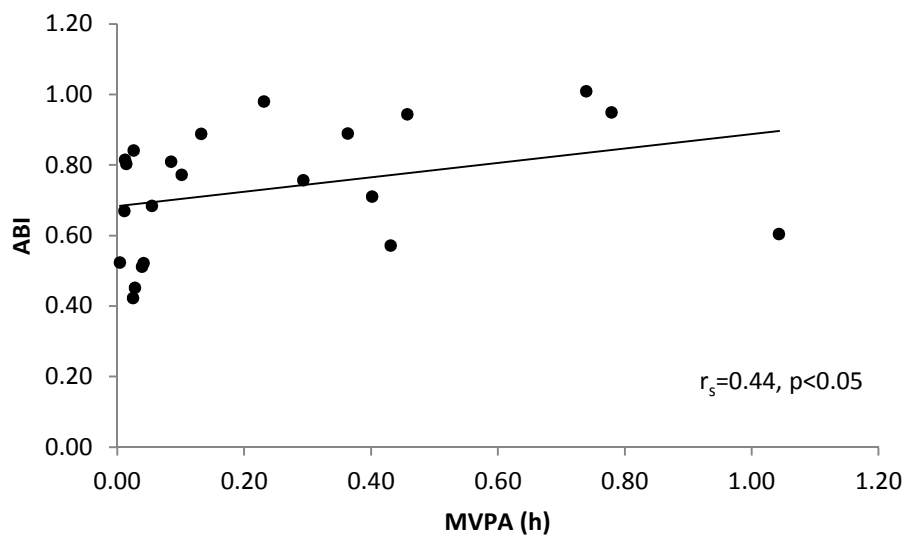


Figure 3.19: Relation between resting ABI and MVPA ( $>25$  steps-epoch<sup>-1</sup>)



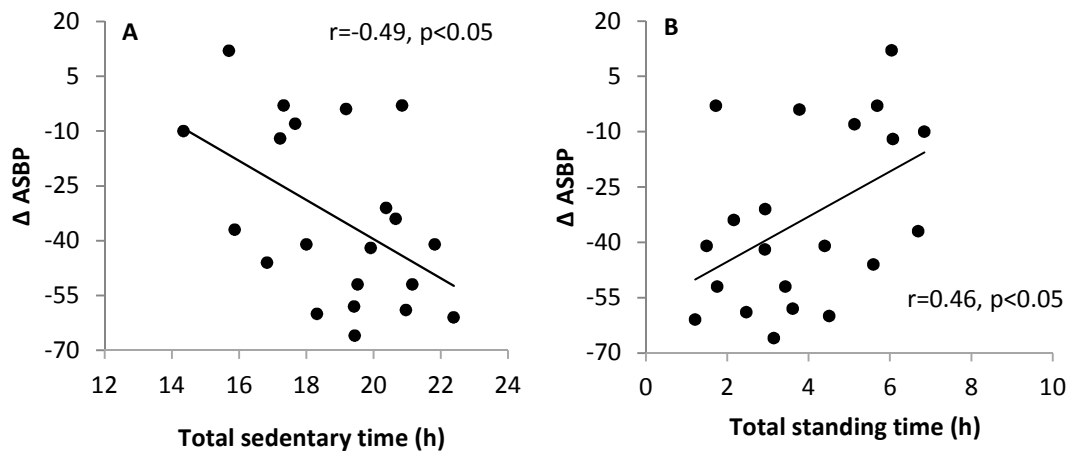


Figure 3.20: The relation between the post-exercise change in ankle systolic blood pressure and (A) total sedentary time and (B) total standing time

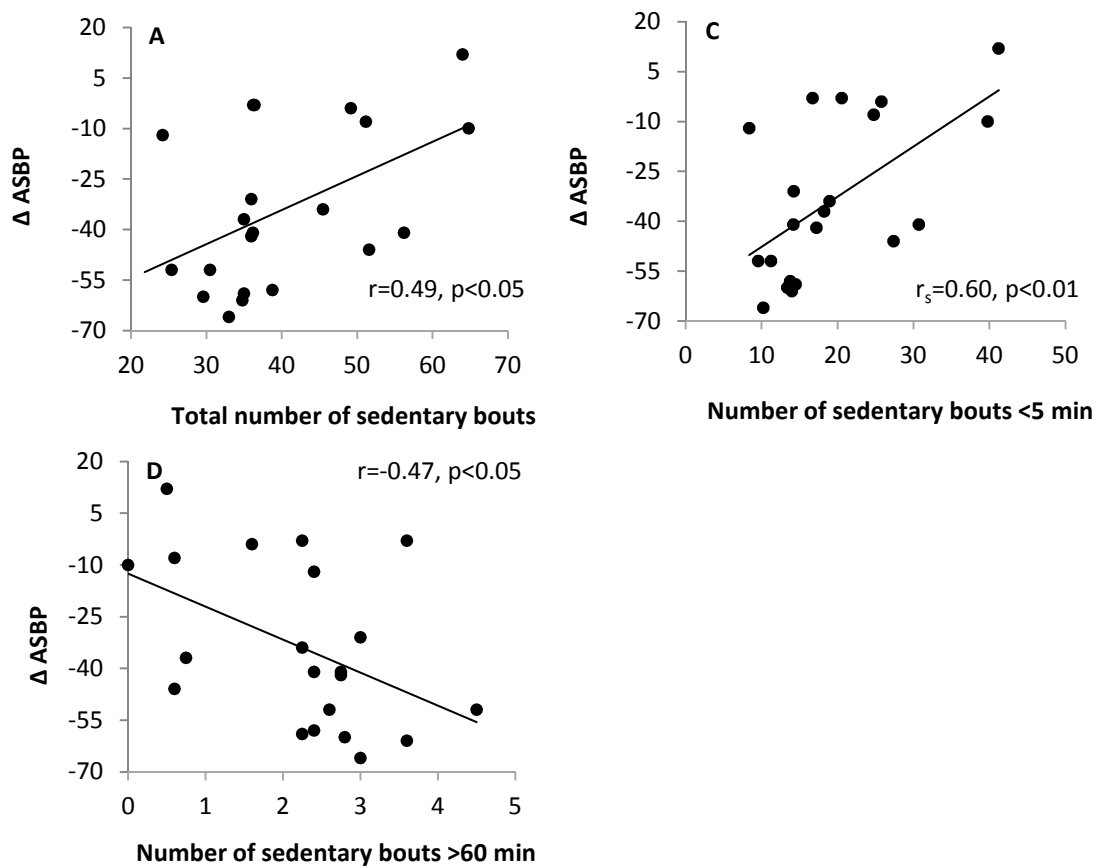


Figure 3.21: The relation between the post-exercise change in ankle systolic blood pressure and (A) the total number of sedentary bouts, and the number of sedentary bouts (B) < 5 min and (C) > 60 min

### Relation between activity and sedentary behaviour and walking performance

The mean PFWT and MWT were 4 min 18 s and 7 min 7 s, respectively. The mean 6MWD was  $377.95 \pm 77.57$  m. There was no significant relation between walking performance and any measure of activity or sedentary behaviour.

### Relation between activity and sedentary behaviour and endothelial function

Endothelial-dependent dilation in response to reactive hyperemia was  $4.54 \pm 4.62\%$ . Endothelial-independent dilation in response to GTN administration was  $16.96 \pm 7.35\%$ . There was a relation between endothelial-dependent dilation and MVPA defined as  $>25$  steps·epoch<sup>-1</sup> ( $r=0.43$ ,  $p<0.05$ ) (figure 3.22).

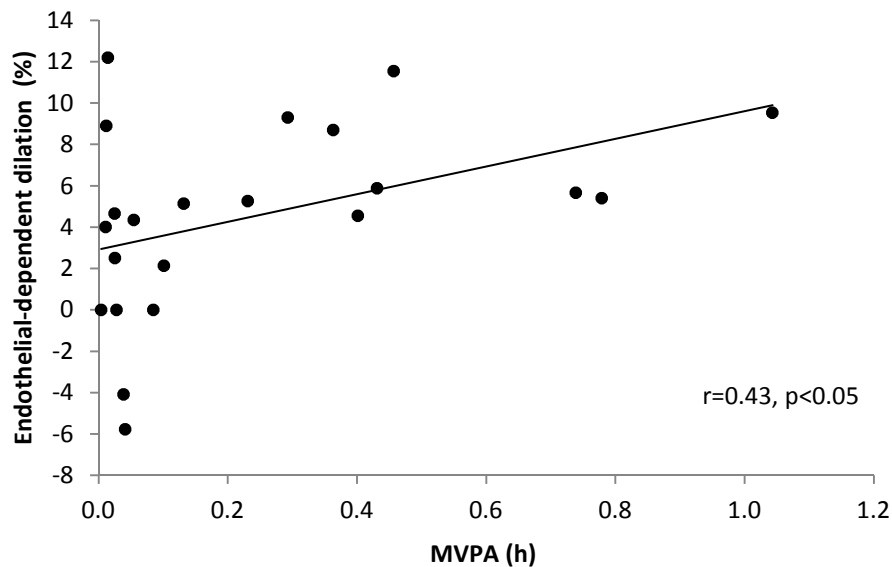


Figure 3.22: The relation between endothelial-dependent dilation and moderate-to-vigorous physical activity defined as  $>25$  steps·epoch<sup>-1</sup>

## **Relation between activity and sedentary behaviour and quality of life**

Table 3.4 summarizes the association between PAQ scores and activity and sedentary behaviour. The PAQ summary score was significantly related with total sedentary, standing, and ambulating time; step count, and MVPA (figure 3.23). The social function domain was significantly related with total sedentary, standing, and ambulating time; step count, and MVPA (table 3.4). The quality of life domain was significantly related with total sedentary and standing time. There was a significant relation between the physical function domain and measures of activity, i.e. total stepping time, step count, and MVPA. Treatment satisfaction was significantly related with MVPA, defined as  $>25 \text{ steps-epoch}^{-1}$ . There was no relation between activity and sedentary behaviour and the symptom stability or symptom frequency/burden domains of the PAQ.

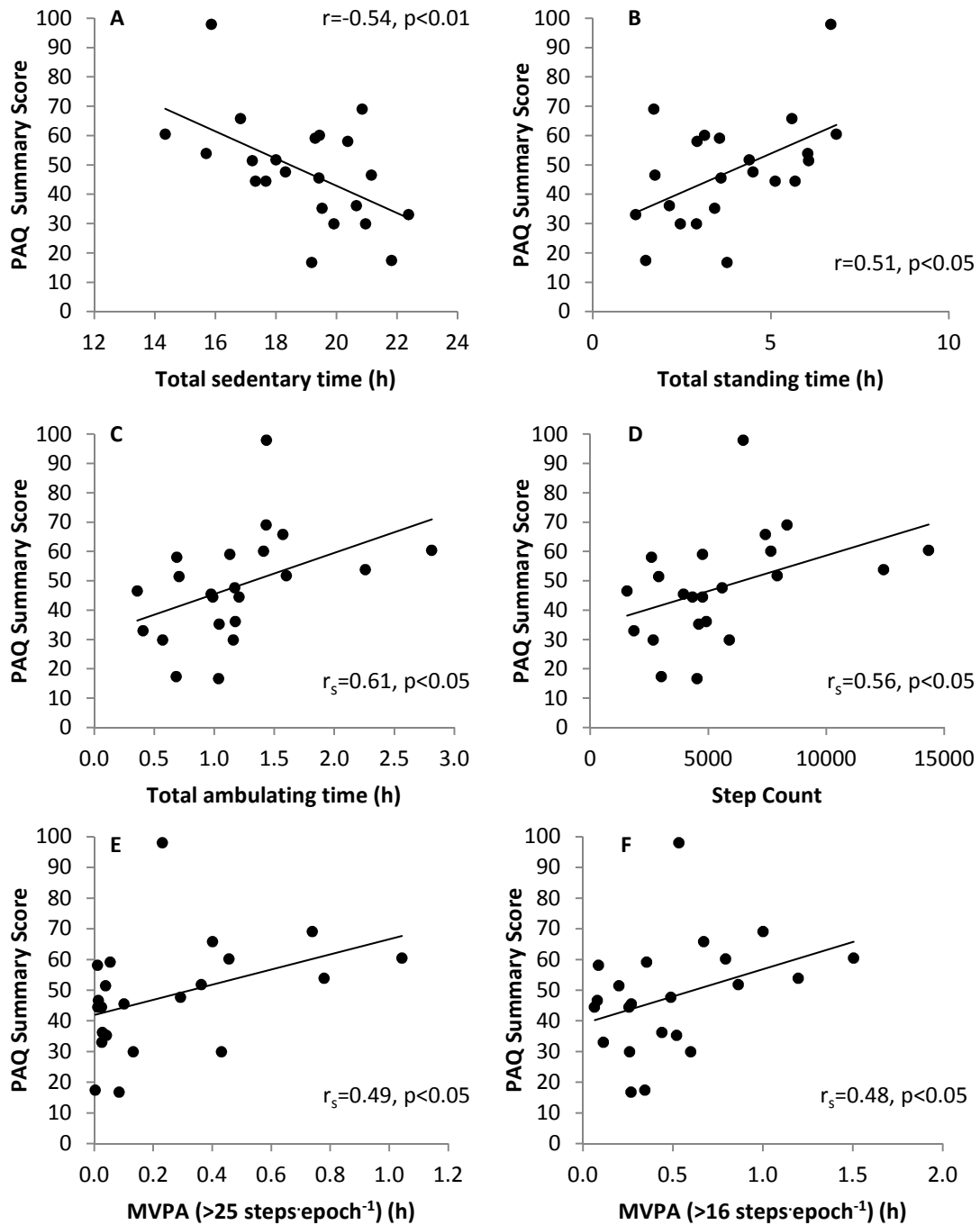


Figure 3.23: The relation between the Peripheral Artery Questionnaire summary score and (A) total sedentary time, (B) total standing time, (C) total ambulating time, (D) step count, and moderate-to-vigorous physical activity defined as both (E) >25 and (F) >16 steps·epoch<sup>-1</sup>

Table 3.4: The relation between PAQ scores and activity and sedentary behaviour

PAQ Domain	Sedentary time	Standing time	Ambulating time	Step count	MVPA (>25 steps·epoch <sup>-1</sup> )	PAD MVPA (>16 steps·epoch <sup>-1</sup> )
Physical function	ns	ns	$r_s=0.58, p<0.01$	$r_s=0.52, p<0.01$	$r_s=0.43, p<0.05$	$r_s=0.48, p<0.01$
Treatment satisfaction	ns	ns	ns	ns	$r_s=0.50, p<0.05$	ns
Quality of life	$r_s=-0.44, p<0.01$	$r_s=0.44, p<0.05$	ns	ns	ns	ns
Social function	$r=-0.74, p<0.01$	$r=0.71, p<0.01$	$r_s=0.70, p<0.01$	$r_s=0.65, p<0.01$	$r_s=0.53, p<0.05$	$r_s=0.49, p<0.05$
Summary score	$r=-0.54, p<0.01$	$r=0.51, p<0.05$	$r_s=0.61, p<0.01$	$r_s=0.56, p<0.01$	$r_s=0.49, p<0.05$	$r_s=0.48, p<0.05$

ns, non-significant

## **Summary of Results**

Participants spent 79.0%, 16.1%, and 4.9% of the total day sedentary, standing, and ambulating, respectively. The greatest duration of sedentary time was spent in bouts >60 min; 36% of the total daily waking sedentary time was spent in sedentary bouts >60 min. Excessive sedentary time and prolonged sedentary bouts were related to PAD severity. Prolonged sedentary bouts were also related to CV risk factors. Breaking up sedentary time and having numerous and short sedentary bouts was found to have a beneficial effect on PAD severity and CV risk factors. Quality of life was associated with overall daily activity and sedentary behaviours in PAD.

## Chapter IV

### STUDY 2

#### EFFECT OF ACUTE INTERMITTENT EXERCISE ON ENDOTHELIAL FUNCTION IN PERIPHERAL ARTERIAL DISEASE

##### Rationale

In the pathophysiology of PAD the delivery of blood flow to the peripheral tissues depends not only on the severity of the stenotic occlusion but also on the regulatory mechanisms that control blood flow, including vascular tone<sup>292</sup>. Vascular tone is regulated by the endothelium and endothelial dysfunction represents one of the earliest events in the pathogenesis of atherosclerosis<sup>377</sup>. The ability of large conduit vessels to dilate in response to a brief period of occlusion is termed endothelial-dependent dilation (EDD) and is commonly used to non-invasively assess endothelial function<sup>365</sup>. Impairment in EDD is well established in individuals with PAD, whereas there is no change in endothelial-independent dilation (EID)<sup>294,296,297,303</sup>, signifying that the impairment in vasodilation is due to endothelial dysfunction. In addition to being an early event in the development of PAD, endothelial dysfunction can be exacerbated by chronic exposure to I-RI in PAD.

Improvement in endothelial function is proposed as a mechanism through which exercise rehabilitation relieves symptoms and enhances functional capacity in individuals with PAD<sup>10</sup>. Previous research has found significant improvements in

EDD with exercise rehabilitation in individuals with PAD <sup>11-13</sup>. However, acute exercise is associated with a transient impairment in EDD in individuals with PAD <sup>12,14-17</sup>. This is in contrast to significant increases in EDD following acute exercise in healthy individuals <sup>18</sup> and individuals with CAD <sup>19</sup>. The impairment in EDD in individuals with PAD is dependent upon the degree of ischemia produced in the diseased limb during exercise and occurs following exercise to maximal claudication but not exercise to the onset of claudication <sup>14</sup>. Exercise to maximal claudication is also associated with an acute inflammatory response in individuals with PAD <sup>14,17</sup>.

Previous studies have employed acute exercise protocols consisting of a single short continuous bout of exercise, ranging in duration from approximately 2 min to 10 min. Current American College of Sports Medicine (ACSM) physical activity guidelines for adults, older adults, and adults with a clinically significant chronic condition or functional limitation recommend a minimum of 30 min moderate-intensity aerobic physical activity on 5 d·wk<sup>-1</sup> or a minimum of 20 min vigorous-intensity aerobic physical activity on 3 d·wk<sup>-1</sup> <sup>378,379</sup>. Activity can be accumulated from bouts of  $\geq 10$  min. The functional limitation associated with intermittent claudication means that exercise rehabilitation programs designed for individuals with PAD often employ intermittent walking consisting of multiple exercise bouts to allow participants to accumulate minutes of exercise. A similar approach has been adopted in research studies investigating the effects of chronic exercise in individuals with PAD.



In addition, all but one of the previous studies investigating the effects of acute exercise in individuals with PAD have failed to measure EID, which prohibits conclusions being drawn that the observed impairment in vasodilation is endothelial-dependent. The purpose of this study was to determine and compare the effect of acute intermittent exercise to the onset of claudication (OC) and maximal claudication (MC) on endothelial function and inflammatory markers in men and women with PAD.

### **Study Aims**

1. To determine the effect of an acute bout of intermittent walking to the onset of claudication on endothelial-dependent and endothelial-independent dilation and inflammation in men and women with PAD
2. To determine the effect of an acute bout of intermittent walking to maximal claudication on endothelial-dependent and endothelial-independent dilation and inflammation in men and women with PAD
3. To compare the effects of an acute bout of intermittent walking to the onset of claudication and maximal claudication on endothelial-dependent and endothelial-independent dilation and inflammation in men and women with PAD

### **Study Hypotheses**

1. There will be no significant change in endothelial-dependent or endothelial-independent dilation, or inflammation following an acute bout of

intermittent walking to the onset of claudication in men and women with PAD

2. There will be a significant decrease in endothelial-dependent dilation and increase in inflammation following an acute bout of intermittent walking to maximal claudication in men and women with PAD
3. There will be a significant decrease in endothelial-dependent dilation and increase in inflammation following an acute bout of intermittent walking to maximal claudication compared with an acute bout of intermittent walking to the onset of claudication in men and women with PAD

## **Methodology**

### **Participants**

Ten men and five women with documented PAD were recruited. Two participants from Study 1 participated in Study 2. Peripheral arterial disease was confirmed by an ABI <0.90 at rest or a decrease in ankle systolic pressure  $\geq 30$  mmHg after exercise. Participants were excluded if they had ischemic rest pain, ulceration or gangrene, unstable angina, uncontrolled hypertension (systolic blood pressure >180 mmHg and/or diastolic blood pressure >100 mmHg), resting tachycardia, or unstable or acute heart failure. Participants were also excluded if their exercise tolerance was limited by factors other than intermittent claudication or if they were unable to walk on a treadmill at a velocity of  $3.2 \text{ km}\cdot\text{h}^{-1}$ . Participants were fully informed of the experimental procedures and provided with a plain language statement before giving written informed consent in accordance with the Research Ethics Committee at Dublin City University (appendix E).

### **Overview of Study Design**

#### **Research Design**

An overview of the study design is presented in figure 4.1. Participants visited the Vascular Research Unit in DCU on 3 separate occasions. The first visit was used to screen potential participants and measure treadmill-walking performance. During the second and third visit endothelial vasomotor function was assessed

before, and immediately and 1 h after an acute bout of intermittent treadmill walking to OC or MC.

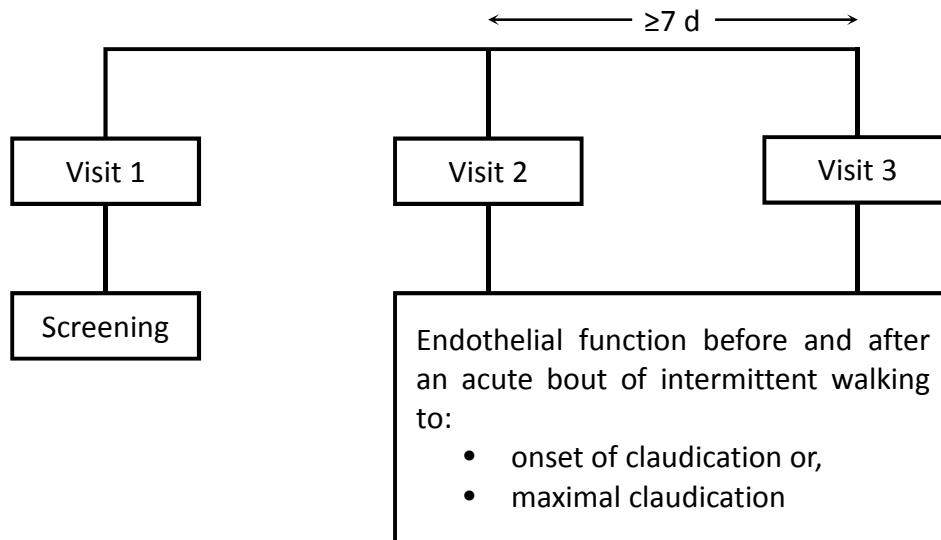


Figure 4.1: Overview of research design

### Visit 1

Participants fasted for 4 h and refrained from strenuous physical activity for 24 h prior to the visit. They were fully informed of the experimental procedures and provided with a plain language statement before giving written informed consent. Participants completed a general health questionnaire (appendix B), a PAR-Q (appendix C), and had their blood pressure, height, and body mass measured. Following a 10 min rest period in a supine position, resting ABI was measured before and post-exercise ankle pressure was measured immediately after a treadmill walking test.

## Visit 2 and Visit 3

An overview of visits 2 and 3 is outlined in figure 4.2. Both visits were identical except for the exercise endpoint – OC or MC. The order of exercise endpoints was randomized. Participants arrived at the Vascular Research Unit following an 8 h overnight fast. Water consumption was permitted during the fasting period and where possible, all vasoactive medications were withheld for at least 4 half-lives. Participants refrained from strenuous physical activity for 24 h and substances that may affect FMD such as caffeine, vitamin C, and tobacco for 6 h prior to the visit. Since the female participants were post-menopausal there was no need to control for menstrual cycle phase.

Following a 10 min rest period in a quiet, temperature-controlled room, a blood sample was drawn, and endothelial function was assessed. Participants then undertook an acute bout of intermittent treadmill walking to either OC or MC to accumulate 30 min of exercise. Within 5 min of exercise cessation a blood sample was taken and endothelial function was again assessed. One hour after exercise cessation a blood sample was taken and endothelial function was assessed.

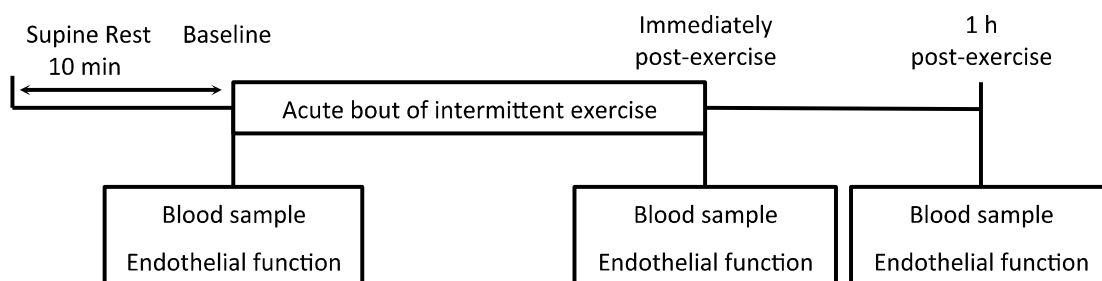


Figure 4.2: Overview of visit 2 and visit 3

### **Blood Pressure**

As described in the methodology section of Chapter III

### **Anthropometrics**

As described in the methodology section of Chapter III

### **Resting Ankle Brachial Index**

As described in the methodology section of Chapter III

### **Post-exercise Ankle Pressure**

As described in the methodology section of Chapter III

### **Treadmill Walking Capacity**

Walking capacity was assessed on a treadmill (Marquette 2000, General Electric, USA) using an incremental protocol of 3.2 km·h<sup>-1</sup> and 0% grade, with a subsequent 2% increase in grade every 2 min to maximal claudication (Gardner, 1991). Pain free walking time and maximal walking time were recorded. Heart rate was recorded continuously throughout the test using telemetry (Polar Vantage NV™ Polar, Port Washington, NY).

### **Blood Sampling and Storage**

Participants rested in a seated position for 5 min with legs uncrossed in order to minimize plasma volume shifts. Blood samples were drawn from the antecubital

vein. Serum vacutainers were allowed to stand for 30 min before centrifugation at 3000 rpm (1600 g) for 15 min at 4°C.

### **Biochemical Analysis**

Circulating leukocytes, haematocrit, RBC and haemoglobin concentrations were determined from an EDTA whole blood sample using an automated haematology analyzer (AcTdiff2, Beckman Coulter, USA). Serum concentration of interferon gamma (INF- $\gamma$ ), interleukin-1 beta (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- $\alpha$ ) were determined in duplicate using a electrochemiluminescent multi-spot quantitative sandwich immunoassay system (MSD, Maryland, USA). Appropriate reagents and calibrators were used (MSD, Maryland, USA).

### **Endothelial Function Assessment**

As described in the methodology section of Chapter III

### **Acute Intermittent Exercise**

Participants performed intermittent treadmill walking to accumulate 30 min of exercise. Treadmill intensity was self-selected by participants under instructions to select an intensity that induced the onset of claudication within 3-5 min (Norgren, 2005). Participants walked to either OC or MC. The severity of claudication pain experienced by the patient was determined using an adapted version of the perceived claudication pain scale, where 0 = no symptoms, 0.5 = tiredness, heaviness, or tightness in legs without pain; 1 = onset of pain; 2 = mild pain; 3 =

moderate pain; 4 = maximal pain (Barak, 2009) (appendix F). When participants reached the required point on the pain scale they rested seated until the pain subsided before continuing. Participants were allowed to alter the treadmill velocity and grade during the first minute of each exercise bout. The velocity control arrows were kept visible to allow participants alter the treadmill grade and velocity when signalled by the research assistant. Participants continued in this fashion until 30 min of walking had been accumulated. Heart rate was recorded continuously throughout the test using telemetry (Polar Vantage NV™ Polar, Port Washington, NY).

### **Statistical Analysis**

Prior to statistical analysis the data was checked for normality using the Shapiro-Wilks test. A dependent t-test (parametric) and the Wilcoxon signed-rank test (non-parametric) were used to compare mean differences in exercise characteristics and physiological responses during exercise to OC and MC. A condition (OC and MC) x time (baseline, immediately post-exercise, and 1 h post-exercise) repeated measures ANOVA was used to compare mean differences in endothelial-dependent and –independent dilation and inflammatory markers within and between exercise conditions. Significant main effects were probed using a Bonferroni post hoc test (parametric). Statistical significance was accepted at the  $p < 0.05$  level of confidence. SPSS for Windows statistical software (V19.0, SPSS Inc, IL) was used to perform the statistical analysis.



## Results

### Participant Characteristics

Four patients did not complete all 3 laboratory visits and were excluded. Two completed the screening visit only, 1 was lost due to ill health and 1 due to a family bereavement. Two did not complete the second exercise session, 1 due to vacation and 1 underwent revascularization. One participant could not complete the 30 min of exercise during the exercise to OC session due to fatigue and was excluded. Participant's physical characteristics are summarized in table 4.1. Based on BMI and blood pressure data the participants were on average overweight and stage I hypertensive<sup>375</sup>.

Table 4.1: Physical characteristics of participants (n=10)

Age (y)	70.40 ± 7.91
Height (cm)	169.58 ± 8.04
Body mass (kg)	72.75 ± 14.90
BMI (kg·m <sup>-2</sup> )	25.24 ± 4.39
Systolic blood pressure (mmHg)	141.29 ± 17.65
Diastolic blood pressure (mmHg)	79.86 ± 11.88
Ankle brachial index	0.71 ± 0.15
Pain-free walking time (min:s)	7:36 ± 4:20
Maximal walking time (min:s)	13:19 ± 3:41

Values are means ± SD

### Acute Exercise Session

The mean exercise characteristics and physiological responses during the exercise sessions to OC and MC are summarized in table 4.2. The treadmill grade was significantly higher during the exercise session to MC than OC. The bout duration was significantly longer and the number of exercise bouts was significantly lower in the MC condition than OC condition. The recovery duration did not differ

between the two conditions. The total recovery time and total session time was significantly longer in the OC condition than the MC condition. The average heart rate and the percentage of HR<sub>peak</sub> were significantly higher in the MC condition than OC.

Table 4.2: Average exercise characteristics and physiological responses during exercise to onset of claudication and maximal claudication

	Exercise Condition	
	Onset of Claudication	Maximal Claudication
Treadmill velocity (km·h <sup>-1</sup> )	3.85 ± 0.67	4.05 ± 0.56
Treadmill grade (%)	1.10 ± 1.64	1.99 ± 1.66*
Number of bouts	6.10 ± 1.85	3.60 ± 1.78*
Bout duration (min:s)	5:51 ± 3:23	13:03 ± 9:12†
Recovery duration (min:s)	3:58 ± 1:18	4:07 ± 2:53
Total exercise time (min:s)	30:26 ± 1:13	29:58 ± 0:50
Total recovery time (min:s)	19:23 ± 9:03	10:59 ± 10:05*
Total session time (min:s)	49:49 ± 9:20	40:57 ± 10:17*
Heart rate (beats·min <sup>-1</sup> )	85.22 ± 14.67	98.32 ± 18.24†
Percentage of treadmill test HR <sub>peak</sub> (%)	74.62 ± 12.76	85.69 ± 12.79†
Peak heart rate (beats·min <sup>-1</sup> )	105.90 ± 22.90	115.9 ± 22.37

Values are means ± SD, \*p<0.05 vs. OC; †p<0.01 vs. OC

### Endothelial Function

There was no significant main effect for time or condition and no significant time x condition interaction effect on endothelial-dependent (figure 4.3) or independent dilation. There was no significant main effect for time or condition and no significant time x condition interaction effect on baseline or peak blood flow velocity or the change in blood flow velocity.

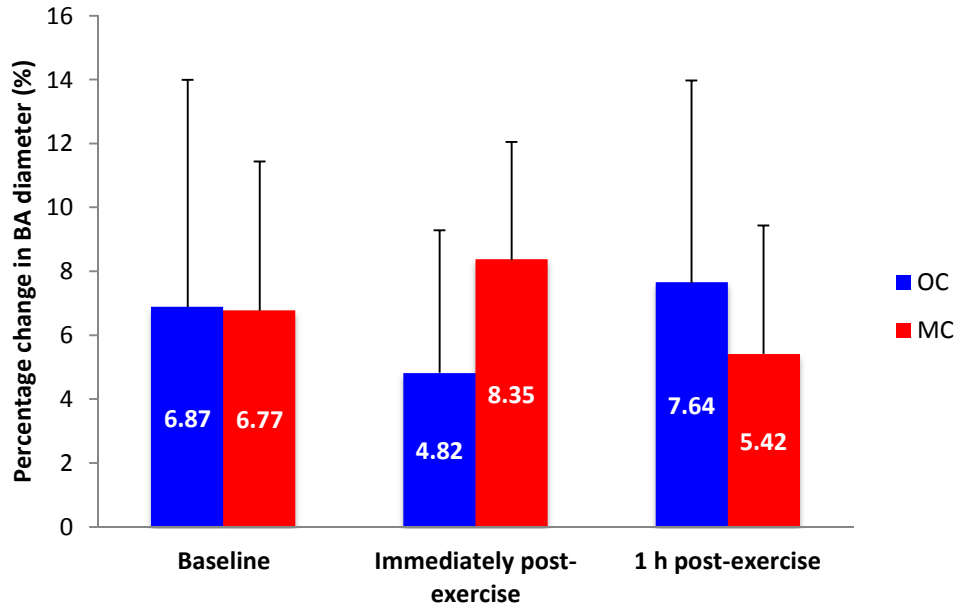


Figure 4.3: The effect of acute intermittent exercise to the onset of claudication (OC) and maximal claudication (MC) on FMD

### Inflammatory Markers

Compared with baseline there was a significant decrease in plasma concentration of  $\text{INF-}\gamma$  1 h after acute exercise to both OC and MC (figure 4.12). There was no significant difference between conditions. There was no significant change in  $\text{TNF-}\alpha$ , IL-6, IL-1 $\beta$ , or lymphocytes following either condition (table 4.3).

Table 4.3: Inflammatory markers during exercise to the onset of claudication and maximal claudication

	Onset of claudication			Maximal claudication		
	Baseline	Immediately post exercise	1 h post exercise	Baseline	Immediately post exercise	1 h post exercise
INF- $\gamma$ (pg·mL <sup>-1</sup> )	20.94 ± 16.73	19.58 ± 15.12	17.85 ± 14.78 <sup>†</sup>	21.41 ± 18.38	19.18 ± 16.49	18.15 ± 14.49 <sup>†</sup>
TNF- $\alpha$ (pg·mL <sup>-1</sup> )	24.75 ± 8.87	26.40 ± 7.50	24.04 ± 7.44	26.81 ± 7.71	25.30 ± 8.06	25.16 ± 8.86
IL-6 (pg·mL <sup>-1</sup> )	4.14 ± 2.39	4.82 ± 2.81	4.46 ± 2.51	3.98 ± 1.62	4.06 ± 1.82	4.01 ± 1.36
IL-1- $\beta$ (pg·mL <sup>-1</sup> )	0.27 ± 0.58	0.15 ± 0.31	0.13 ± 0.30	0.13 ± 0.27	0.32 ± 0.54	0.25 ± 0.52
WBCs (x10 <sup>3</sup> ·uL <sup>-1</sup> )	7.63 ± 2.17	8.05 ± 2.86	7.18 ± 1.48	7.79 ± 2.44	8.45 ± 3.00	7.69 ± 2.64
Lymphocytes (%)	27.43 ± 7.40	26.14 ± 8.36	25.40 ± 8.36	26.39 ± 9.21	25.36 ± 8.52	24.73 ± 8.55
Lymphocytes (x10 <sup>3</sup> ·uL <sup>-1</sup> )	2.06 ± 0.74	2.13 ± 1.00	1.91 ± 0.81	2.05 ± 1.01	2.22 ± 1.13	1.89 ± 0.94
Monocytes (%)	6.58 ± 3.57	5.28 ± 1.70	5.23 ± 2.07	5.70 ± 3.06	5.56 ± 1.30	5.86 ± 2.31
Monocytes (x10 <sup>3</sup> ·uL <sup>-1</sup> )	0.49 ± 0.27	0.45 ± 0.25	0.37 ± 0.14	0.46 ± 0.30	0.45 ± 0.22	0.56 ± 0.34
Granulocytes (%)	66.15 ± 5.83	68.50 ± 7.97	69.27 ± 8.28	74.49 ± 23.03	68.23 ± 8.74	70.48 ± 9.00
Granulocytes (x10 <sup>3</sup> ·uL <sup>-1</sup> )	7.77 ± 8.51	5.48 ± 2.02	9.62 ± 16.40	5.29 ± 1.86	5.80 ± 2.23	5.35 ± 1.97

Values are means ± SD, <sup>†</sup>p<0.01 vs. baseline

## **Summary of Results**

Acute intermittent exercise to both OC and MC had no effect on either endothelial-dependent or endothelial-independent dilation. There was no inflammatory response following either exercise condition. Mean recovery time during the acute exercise bouts did not differ whether participants walked to OC or MC. Fewer exercise bouts were required to accumulate 30 min and the overall session time was shorter when exercising to MC than OC.

## **Chapter V**

### **STUDY 3**

#### **EFFECT OF A 12-WEEK COMMUNITY-BASED EXERCISE REHABILITATION PROGRAMME ON ENDOTHELIAL FUNCTION IN PERIPHERAL ARTERIAL DISEASE**

##### **Rationale**

Exercise rehabilitation is a well-established treatment for PAD and is recommended by the ACC/AHA as the first-line of treatment for the majority of individuals with PAD <sup>22</sup>. The mechanism(s) through which exercise rehabilitation improves symptom severity and functional capacity in PAD is not fully understood. A number of potential mechanisms have been proposed including the formation of collateral vessels and improvements in hemorheological properties, muscle metabolism, walking economy, muscular strength, and endothelial function. Endothelial function can be assessed non-invasively by measuring the ability of large conduit vessels to dilate in response to a brief period of occlusion <sup>365</sup>. Previous research has reported significant improvements in EDD following exercise rehabilitation in individuals with PAD <sup>11-13,246,313</sup>. The training protocols employed in previous studies have consisted of submaximal intermittent exercise. To date, no published studies have evaluated the effect of exercise rehabilitation involving intermittent exercise to maximal claudication on endothelial function in individuals with PAD. Findings from Study 2 found no adverse effect of acute intermittent

exercise to maximal claudication on endothelial function or inflammatory markers in PAD.

Previous studies that have examined the effect of exercise training on endothelial function in PAD have employed hospital- or laboratory-based exercise rehabilitation programmes. The growing epidemic of CVD and its escalating economic burden requires the employment of viable secondary prevention strategies. Home- and community-based exercise rehabilitation programmes provide an alternative to hospital-based programmes for PAD. It is however, first necessary to determine the efficacy of home- and community-based exercise rehabilitation programmes if their use is to become more widespread in the treatment of PAD. The purpose of this study was to determine the effect of a 12-week community-based exercise rehabilitation programme on endothelial function, disease severity, walking performance, daily physical activity and sedentary levels, and QOL in men and women with PAD.

### **Study Aims**

1. To evaluate the effect of a 12-week community-based exercise rehabilitation programme on endothelial-dependent dilation in men and women with PAD
2. To evaluate the effect of a 12-week community-based exercise rehabilitation programme on endothelial-independent dilation in men and women with PAD

3. To evaluate the effect of a 12-week community-based exercise rehabilitation programme on ABI, walking performance, daily physical activity and sedentary levels, and QOL in men and women with PAD

### **Study Hypotheses**

1. There will be a significant increase in endothelial-dependent dilation after a 12-week community-based exercise rehabilitation programme in men and women with PAD
2. There will be no change in endothelial-independent dilation after a 12-week community-based exercise rehabilitation programme in men and women with PAD
3. There will be a significant increase in walking performance, daily physical activity levels, and QOL, and a significant decrease in daily sedentary behaviour after a 12-week community-based exercise rehabilitation programme in men and women with PAD



## **Methodology**

### **Participants**

Eleven men and four women with documented PAD, who had been referred for exercise rehabilitation by vascular surgeons in Beaumont Hospital and the Mater Misericordiae Hospital, were recruited. All Study 3 participants participated in Study 1. Two participants participated in all 3 studies. Peripheral arterial disease was confirmed by an ABI  $<0.90$  at rest or a decrease in ankle systolic pressure  $\geq 30$  mmHg after exercise. Participants were excluded if they had ischemic rest pain, ulceration or gangrene, unstable angina, uncontrolled hypertension (systolic blood pressure  $>180$  mmHg and/or diastolic blood pressure  $>100$  mmHg), resting tachycardia, or unstable or acute heart failure. Participants were also excluded if their exercise tolerance was limited by factors other than intermittent claudication or if they were unable to walk on a treadmill at a velocity  $\geq 3.2$  km·h<sup>-1</sup>. Participants were fully informed of the experimental procedures and provided with a plain language statement before giving written informed consent in accordance with the Research Ethics Committee at Dublin City University (appendix A).

### **Overview of Study Design**

#### **Research Design**

An overview of the study design is presented in figure 5.1. Participants visited the Vascular Research Unit in DCU on 3 separate occasions. The first visit was used to screen potential participants. The second and third visits took place

before and after completing a 12 week community-based exercise rehabilitation programme, respectively. Both visits involved taking blood samples and the assessment of endothelial function, walking performance, ABI, and QOL. Following the second and third laboratory visits, participants wore an ActivPAL<sup>3</sup>™ triaxial physical activity logger for 7 d. Participants did not begin the community-based exercise rehabilitation programme until after the 7 d physical activity recording period following the second laboratory visit.

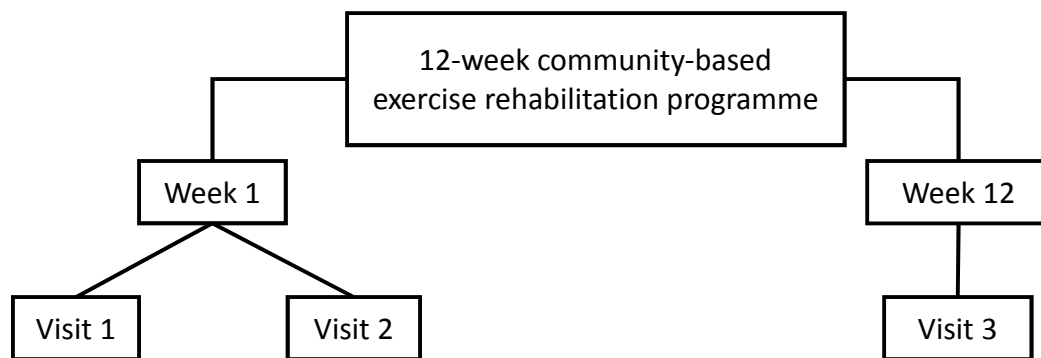


Figure 5.1: Overview of research design

### Visit 1

Participants were fully informed of the experimental procedures and provided with a plain language statement before giving written informed consent. They completed a general health questionnaire (appendix B), PAR-Q (appendix C), and had their blood pressure, height, and body mass measured.

### Visit 2 and Visit 3

An overview of visit 2 and visit 3 is outlined in figure 5.2. Participants arrived at the Vascular Research Unit following an 8 h overnight fast. Water consumption

was permitted during the fasting period and where possible, all vasoactive medications were withheld for at least 4 half-lives. Participants refrained from strenuous physical activity for 24 h and substances that may affect FMD such as caffeine, vitamin C, and tobacco for 6 h prior to the visit. Since the female participants were post-menopausal there was no need to control for menstrual cycle phase.

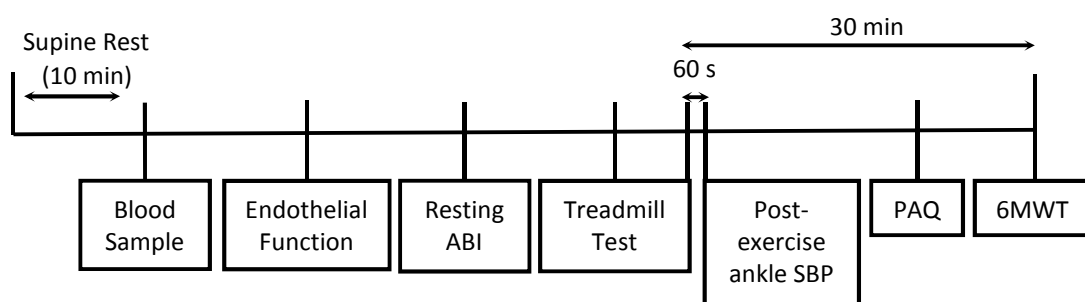


Figure 5.2: Overview of visit 2 and visit 3

Following a 10 min rest period in a quiet, temperature-controlled room, a blood sample was drawn, and endothelial function was assessed. The participants then rested for a further 10 min in a supine position after which resting ABI was measured before and post-exercise ankle pressure was measured immediately after a treadmill walking test. Participants then completed the PAQ and finally performed a 6MWT.

### Blood Pressure

As described in the methodology section of Chapter III

**Anthropometrics**

As described in the methodology section of Chapter III

**Blood Sampling and Storage**

As described in the methodology section of Chapter III

**Biochemical Analysis**

As described in the methodology section of Chapter III

**Endothelial Function Assessment**

As described in the methodology section of Chapter III

**Resting Ankle Brachial Index**

As described in the methodology section of Chapter III

**Post-exercise Ankle Pressure**

As described in the methodology section of Chapter III

**Treadmill Walking Capacity**

As described in the methodology section of Chapter III

**The Peripheral Artery Questionnaire**

As described in the methodology section of Chapter III

### **Six Minute Walk Test**

As described in the methodology section of Chapter III

### **Activity and Sedentary Behaviour**

As described in the methodology section of Chapter III

Participants continued to attend the exercise programme after the initial 12 weeks. Analysis of ActivPAL data was performed including and excluding the days participants attended the exercise programme.

### **Exercise Programme**

A community-based exercise rehabilitation programme for individuals with PAD, known as SmartSteps, was established in conjunction with the School of Health and Human Performance in DCU, DCU Sport, Beaumont Hospital and the Mater Misericordiae Hospital. Participants attended supervised exercise sessions 2 d·wk<sup>-1</sup> for 12 weeks. The exercise sessions involved 5 min of warm-up exercises, 30 min of intermittent treadmill walking, 15 min of resistance training and 5 min of cool down exercises. Participants were instructed to select a treadmill intensity that induced the onset of claudication after 3-5 min and walk to maximal claudication (Norgren, 2005). At maximal claudication participants rested in a seated position until the pain subsided before continuing. This pattern was continued until participants accumulated 30 min of exercise. Pain-free walking time and distance and maximal walking time and distance were recorded. When participants could successfully

complete 10 min of continuous walking, the treadmill intensity was progressed by increasing velocity by 0.5 km·h<sup>-1</sup> or grade by 1% as tolerated.

### **Statistical Analysis**

Prior to statistical analysis the data was checked for normality using the Shapiro-Wilks test. A dependent t-test (parametric) and the Wilcoxon signed-rank test (non-parametric) were used to compare mean differences in outcome variables at baseline and week 12. Statistical significance was accepted at the p<0.05 level of confidence. SPSS for Windows statistical software (V21.0, SPSS Inc, IL) was used to perform the statistical analysis.

## **Results**

### **Training Adherence**

Eleven participants completed the 12-week exercise programme. Four participants were lost to follow-up; 2 due to illness, 1 underwent revascularization, and 1 due to lack of interest. Training adherence for those who completed the programme was 83.9%.

### **Participant Characteristics**

Participant's physical and biological characteristics at week 1 and week 12 are summarized in table 5.1. Based on baseline BMI and blood pressure data the participants were on average overweight and stage I hypertensive <sup>375</sup>. Total cholesterol, LDL-C, HDL-C, and TGs were within the normal range <sup>376</sup>. Table 5.2 summarizes the medications taken by the participants. There was no change in body mass, BMI, BP, ABI, or blood lipids between week 1 and week 12.

Table 5.1: Physical and biological characteristics of the participants at week 1 and week 12 (n=11)

	<b>Week 1</b>	<b>Week 12</b>
Age (y)	67.55 ± 9.20	
Height (cm)	166.12 ± 6.40	
Body mass (kg)	77.96 ± 18.85	78.08 ± 18.79
BMI (kg·m <sup>-2</sup> )	28.13 ± 6.04	28.19 ± 6.11
Systolic blood pressure (mmHg)	157.60 ± 20.19	151.09 ± 11.95
Diastolic blood pressure (mmHg)	82.40 ± 14.66	79.00 ± 11.71
Ankle brachial index	0.70 ± 0.16	0.69 ± 0.18
Total cholesterol (mmol·L <sup>-1</sup> )	4.92 ± 1.23	4.91 ± 0.85
HDL-cholesterol (mmol·L <sup>-1</sup> )	1.27 ± 0.19	1.26 ± 0.23
LDL-cholesterol (mmol·L <sup>-1</sup> )	1.88 ± 0.59	1.92 ± 0.54
Triglycerides (mmol·L <sup>-1</sup> )	1.38 ± 0.55	1.99 ± 1.54

Values are means ± SD

Table 5.2: Medications taken by participants

<b>Drug class</b>	<b>N</b>	<b>%</b>
Statins	8	73
Anti-platelet	6	55
Beta blocker	4	36
Nonsteroidal anti-inflammatory drugs	4	36
Calcium channel blocker	4	36
Angiotensin-converting-enzyme inhibitor	3	27
Proton pump inhibitors	3	27
Diuretic	2	18
Angiotensin II receptor blocker	2	18
Hypoglycemic	2	18



## Endothelial Function

There was a significant increase in the percentage change in brachial artery diameter from  $2.71 \pm 5.56\%$  to  $7.35 \pm 4.76\%$  (figure 5.3) and absolute change in brachial artery diameter from  $0.01 \pm 0.02$  cm to  $0.03 \pm 0.02$  cm (figure 5.4) between week 1 and week 12. There was no significant change in percentage (figure 5.3) or absolute (figure 5.4) endothelial-independent dilation between week 1 and week 12. There was no significant change in baseline brachial artery diameter, or baseline or peak blood flow velocity (figure 5.5) between week 1 and week 12.

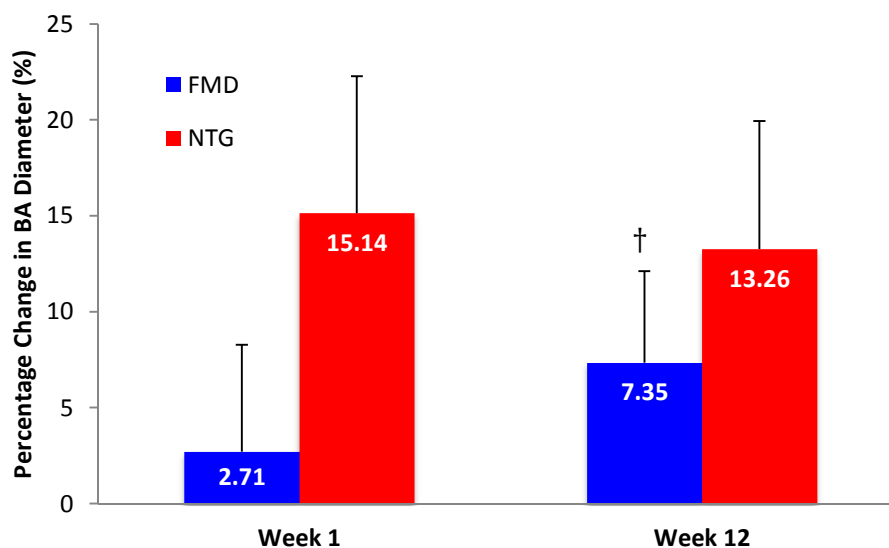


Figure 5.3: Percentage change in brachial artery diameter with flow-mediated dilation and following glyceryl-trinitrate administration at week 1 and week 12, †p<0.01 vs. week 1

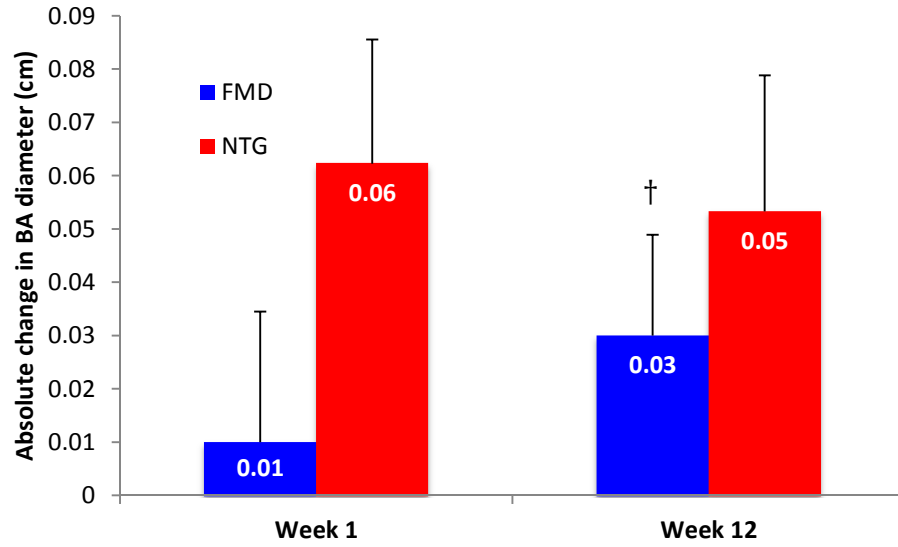


Figure 5.4: Absolute change in brachial artery diameter with flow-mediated dilation and following glyceryl trinitrate administration at week 1 and week 12, † p < 0.05 vs. week 1

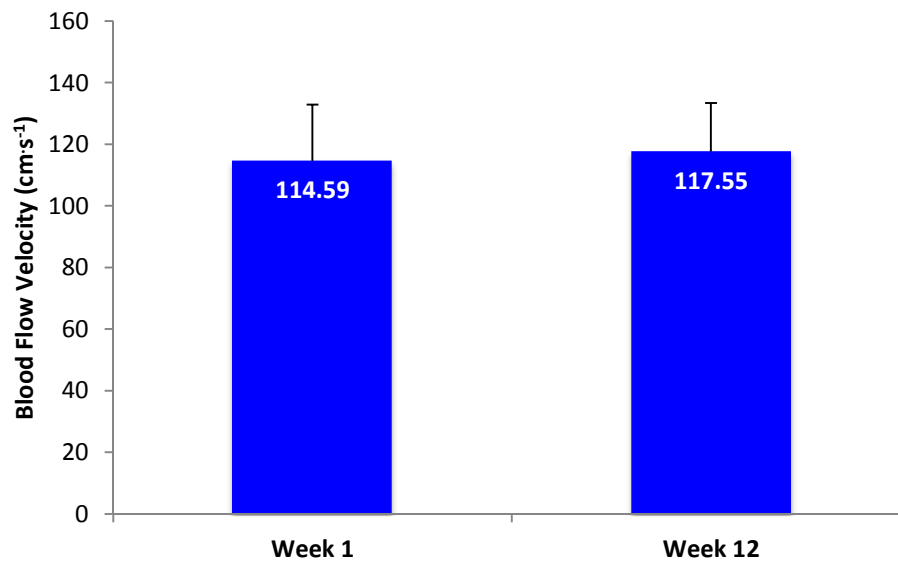


Figure 5.5: Peak blood flow velocity at week 1 and 12

## Walking Performance

Treadmill PFWT increased ( $p < 0.05$ ) from  $174.8 \pm 109.4$  s to  $247.8 \pm 115.6$  s and MWT increased ( $p < 0.01$ ) from  $259.5 \pm 155.7$  s to  $447.7 \pm 300.0$  s between week 1 and week 12 (figure 5.6). Compared with week 1, there was no significant change in peak  $\dot{V}O_2$ , ventilation, RER or HR during the treadmill test to maximal claudication at week 12 (table 5.3). Heart rate and the percentage of HRpeak at the onset of claudication were significantly lower at week 12 than week 1.

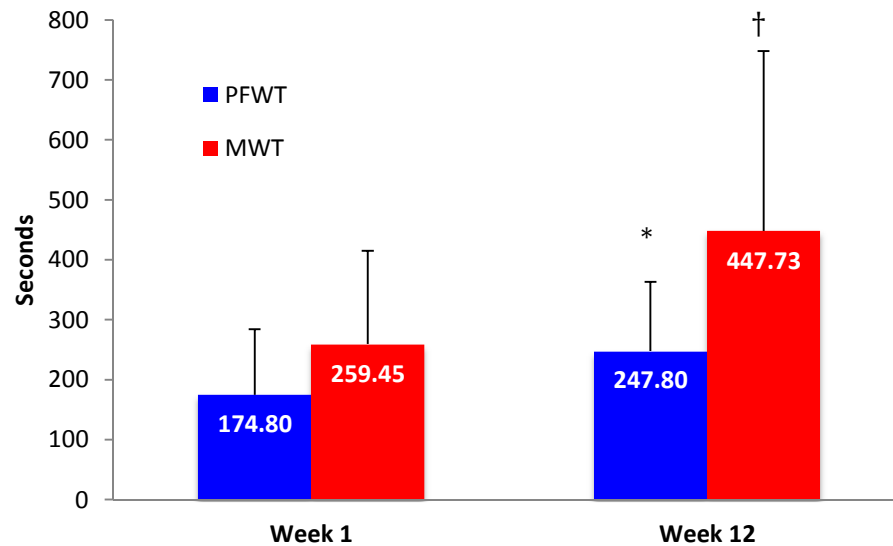


Figure 5.6: Pain free and maximal walking time at week 1 and week 12; \* $p < 0.05$  vs. week 1; † $p < 0.01$  vs. week 1

Table 5.3: Participant responses during the treadmill test to maximal claudication

	Week 1	Week 12
Treadmill grade (%)	2.91 ± 2.74	6.73 ± 5.16†
$\dot{V}O_2$ peak (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	15.84 ± 3.4	15.08 ± 2.76
$\dot{V}O_2$ peak (L·min <sup>-1</sup> )	1.22 ± 0.32	1.16 ± 0.24
Ventilation (L·min <sup>-1</sup> )	29.77 ± 7.63	30.56 ± 8.30
RER	0.85 ± 0.08	0.88 ± 0.10
HRpeak (beats·min <sup>-1</sup> )	106.30 ± 20.85	109.60 ± 19.54
HR at onset of claudication (beats·min <sup>-1</sup> )	102.38 ± 18.45	94.89 ± 15.80*
HR at onset of claudication (%HRpeak)	95.10 ± 3.61	89.14 ± 6.45*
HR at maximal claudication (beats·min <sup>-1</sup> )	105.10 ± 22.14	109.00 ± 20.02
HR at maximal claudication (%HRpeak)	98.65 ± 3.55	99.37 ± 1.34

Values are means ± SD; \*p<0.05 vs. week 1; †p<0.01 vs. week 1

The distance covered during the 6MWT increased significantly from 333.0 ± 52.7 m to 364.6 ± 52.4 m between week 1 and week 12 (figure 5.7). There was no significant change in HR response during the 6MWT between week 1 and week 12 (table 5.4).

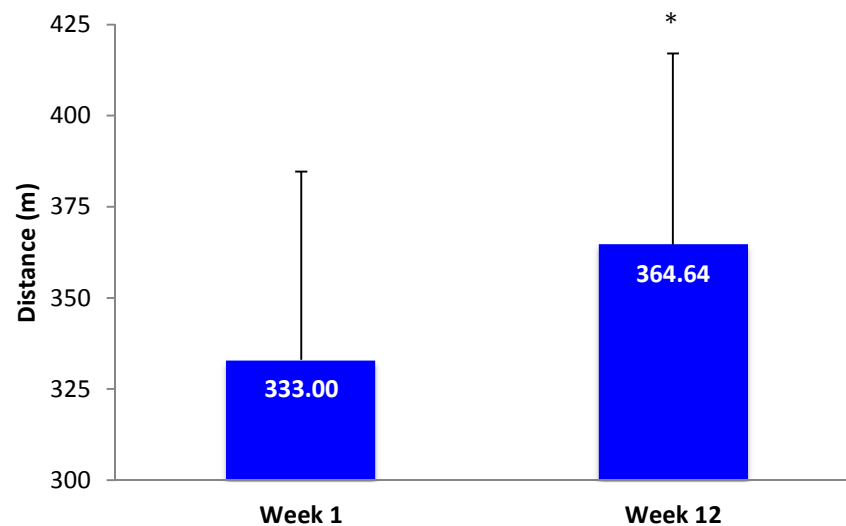


Figure 5.7: Six minute walk test distance at week 1 and week 12; \*p<0.05 vs. week 1

Table 5.4: Heart rate responses during 6MWT

	<b>Week 1</b>	<b>Week 12</b>
HR (beats·min <sup>-1</sup> )	89.37 ± 13.55	93.45 ± 15.64
HR (%HRpeak)	87.12 ± 6.35	87.10 ± 8.18
HRpeak (beats·min <sup>-1</sup> )	97.00 ± 13.31	100.44 ± 17.59
HR at onset of claudication (beats·min <sup>-1</sup> )	93.13 ± 12.33	93.63 ± 9.78
HR at onset of claudication (%HRpeak)	89.09 ± 6.03	86.67 ± 7.16
HR at onset of claudication (%6MWTpeak)	94.96 ± 4.14	96.40 ± 1.73

Values are means ± SD

There was no significant difference in the mean treadmill velocity and grade, PFWT, exercise bout time, number of exercise bouts, and total exercise time during training sessions at week 1 and week 12 (table 5.5).

Table 5.5: Average training session characteristics

	<b>Week 1</b>	<b>Week 12</b>
Treadmill velocity (km·h <sup>-1</sup> )	3.29 ± 0.60	3.61 ± 0.78
Treadmill grade (%)	0.10 ± 0.25	0.63 ± 0.95
Pain free walking time (s)	261.33 ± 136.48	251.11 ± 101.69
Exercise bout time (s)	474.82 ± 224.73	585.73 ± 217.67
Number of exercise bouts	2.82 ± 0.46	2.82 ± 0.41
Total exercise time (s)	1387.82 ± 485.11	1559.82 ± 278.77

Values are means ± SD

### Activity and Sedentary Behaviour

There was a significant decrease in total daily sedentary time, from 19.1 ± 2.6 h to 18.4 ± 2.9 h, and a significant increase in total daily standing time from 3.7 ± 2.0 h to 4.2 ± 2.2 h, and total daily ambulating time, from 1.2 ± 0.8 h to 1.4 ± 0.7 h, between week 1 and week 12 (figure 5.8). There was no significant change in step count or MVPA (table 5.4).

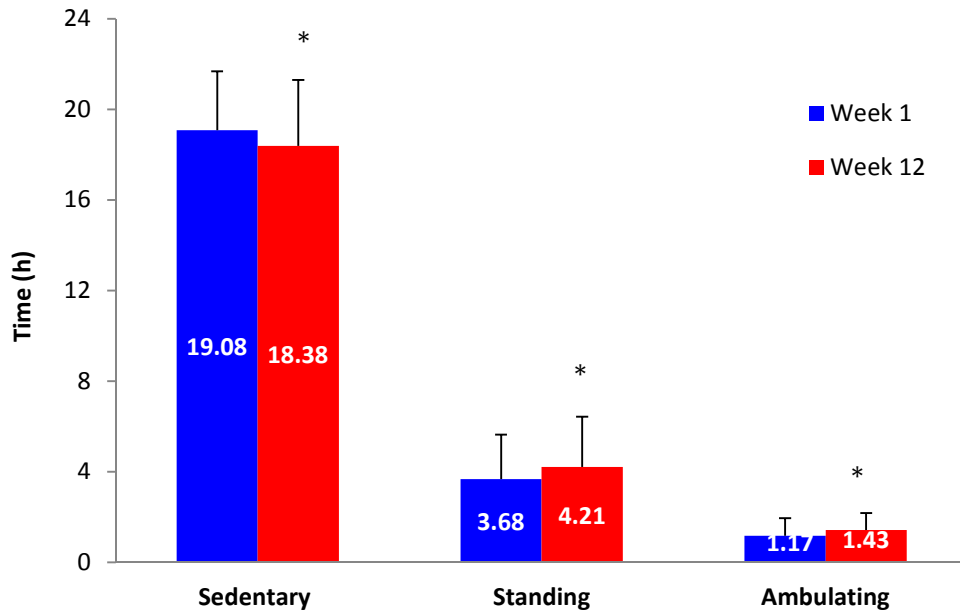


Figure 5.8: Total daily time (h) spent sedentary, standing, and ambulating at week 1 and week 12, \*p<0.05 vs. week 1

Table 5.6: Average daily step count and duration in moderate-to-vigorous physical activity

	Week 1	Week 12	Week 12 excluding training days
Step count	5657 ± 4303	6825 ± 4304	6103 ± 4835
MVPA (>25 steps·epoch <sup>-1</sup> ) (h)	0.25 ± 0.37	0.29 ± 0.39	0.23 ± 0.43
MVPA (>16 steps·epoch <sup>-1</sup> ) (h)	0.52 ± 0.51	0.66 ± 0.50	0.54 ± 0.58

Values are means ± SD

When the exercise training days were removed from the week 12 analysis, the significant decrease in total daily sedentary time and the significant increase in total daily standing time remained. However, there was no longer a significant change in total daily ambulating time (figure 5.9). There was no change in the number and duration of sedentary bouts.

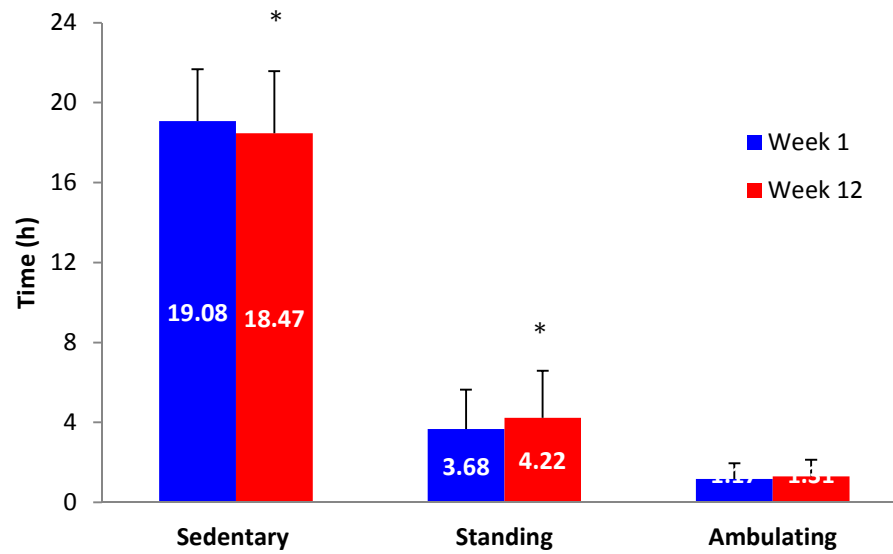


Figure 5.9: Total daily time (h) spent sedentary, standing, and ambulating at week 1 and week 12 excluding exercise training days, \*p<0.05 vs. week 1

### Quality of Life

There was a significant improvement in the PAQ summary score, due to a significant improvement in the symptom stability, symptom frequency/burden, QOL, and social function domains (figure 5.10). There was no significant change in the physical function or treatment satisfaction domains.

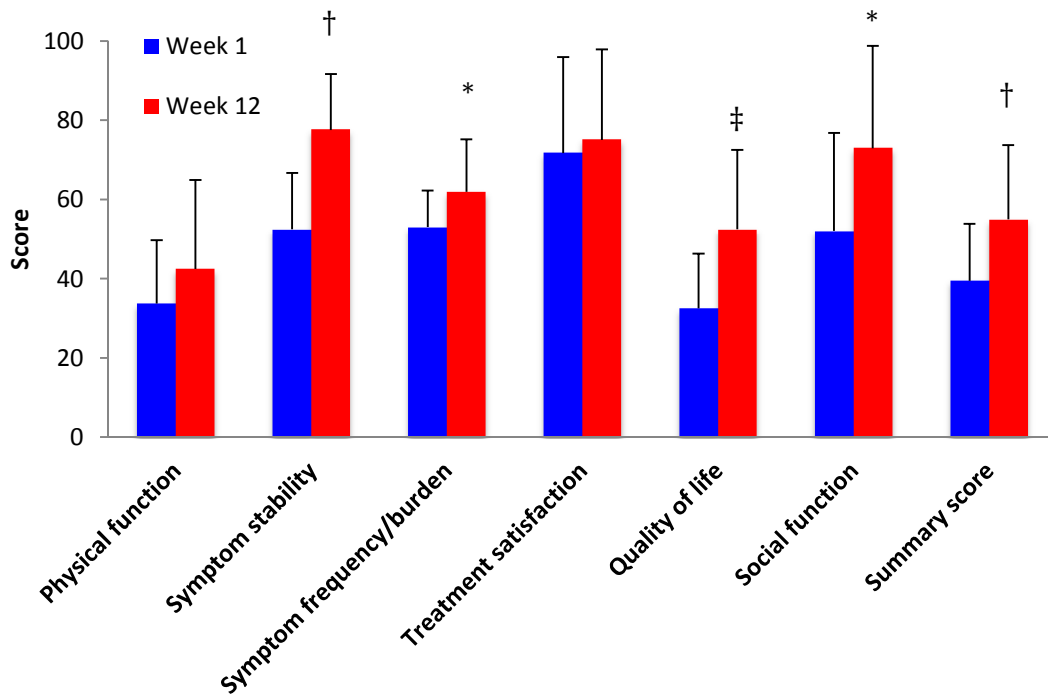


Figure 5.10: The Peripheral Artery Questionnaire domain scores at week 1 and week 12, \* $p < 0.05$  vs. week 1; † $p < 0.01$  vs. week 1; ‡ $p < 0.001$  vs. week 1

### Summary of Results

Twelve weeks of community-based exercise rehabilitation was associated with significant improvements in endothelial-dependent dilation, walking performance and QOL. There was no change in endothelial-independent dilation or ABI. There was a positive alteration in daily activity and sedentary behaviour patterns. Sedentary time decreased, standing time increased, and ambulating time increased on training days.



## Chapter VI

### DISCUSSION

#### Overview

Peripheral arterial disease and its symptom intermittent claudication can severely impair functional capacity and consequently diminish daily physical activity and quality of life <sup>140,236</sup>. Excessive sedentary behaviour may have detrimental effects on cardiometabolic health <sup>6,159</sup>, which may contribute to disease progression. Exercise can substantially improve symptom severity, functional capacity, daily physical activity levels, and quality of life in individuals with PAD <sup>8,9</sup>. However, the mechanisms through which exercise achieves these adaptations is not fully understood and further research is required to gain a greater understanding that can be used to optimize treatment strategies for PAD.

A series of studies were undertaken to determine sedentary behaviour and its effects, and the effects of acute and chronic exercise on endothelial function in men and women with PAD.

#### Study 1

Study 1 measured the daily sedentary, standing and ambulating time and the number and duration of sedentary bouts in men and women with PAD using an inclinometer-based motion sensor. Participants spent a mean of  $18.9 \pm 2.1$  h,  $3.9 \pm 1.8$  h, and  $1.2 \pm 0.6$  h of the total day sedentary, standing, and ambulating,

respectively. Gardner *et al.*, (2007) reported daily sedentary and ambulatory times of  $19.6 \pm 1.8$  h and  $4.4 \pm 1.8$  h, respectively in 98 men and women, mean age  $66 \pm 12$  yr, with PAD, measured using a step activity monitor<sup>144</sup>. The daily ambulatory time was significantly lower than 129 age- and gender-matched controls, whereas there was no difference in sedentary time between groups. The step activity monitor defined sedentary time as  $0 \text{ steps}\cdot\text{min}^{-1}$ , therefore sitting and standing were both classified as sedentary. The definition of sedentary behaviour is activities that do not increase energy expenditure substantially above the resting level<sup>380</sup>. Standing involves isometric contraction of the antigravity muscles and raises energy expenditure above resting levels, contributing to total daily energy expenditure<sup>188</sup>. Study 1 is the first study to directly and objectively measure sedentary time and differentiate between sitting and standing in individuals with PAD. Further research is required to compare “true” daily sedentary time between individuals with PAD and healthy controls.

The fact that the total daily ambulating time of individuals with PAD is lower in Study 1 than reported by Gardner *et al.* ( $1.2 \pm 0.6$  h vs.  $4.4 \pm 1.82$  h), but total step count is greater ( $5575 \pm 3197 \text{ steps}\cdot\text{d}^{-1}$  vs.  $3149 \pm 1557 \text{ steps}\cdot\text{d}^{-1}$ ), would indicate that participants in Study 1 ambulated at a higher cadence. Participants in Study 1 spent  $39 \pm 16\%$  of the total daily ambulating time at a cadence of  $>16 \text{ steps}\cdot\text{epoch}^{-1}$ . Gardner *et al.* defined high cadence as  $>15 \text{ steps}\cdot\text{epoch}^{-1}$  and only  $7 \pm 5\%$  of the total daily ambulating time was spent at this cadence. The difference in ambulating speed does not appear to be due to differences in ABI or age, but may result from

differences in functional capacity. Six minute walk distance was 11% longer in Study 1 compared with the study by Gardner *et al.* The step count in Study 1 was in line with findings by Sieminski and Gardner (1997) and Clarke *et al.*, (2013) of  $4737 \pm 2712$  steps·d<sup>-1</sup> and  $6524 \pm 2710$  steps·d<sup>-1</sup>, respectively<sup>140,145</sup>. Both of these studies found a significant difference in step count between individuals with PAD and healthy age-matched controls.

In Study 1, physical activity was associated with PAD severity. There was a significant relation between MVPA defined as >25 steps-epoch<sup>-1</sup> and resting ABI ( $r_s=0.44$ ,  $p<0.05$ ). This is in agreement with previous findings. Sieminski and Gardner (1997) reported a significant association between ABI and daily energy expenditure, measured by accelerometer ( $r=0.41$ ), and step count, measured by pedometer ( $r=0.41$ )<sup>140</sup>. For every 0.10 decrease in ABI, daily activity was 42 kcal·d<sup>-1</sup> or 612 steps·d<sup>-1</sup> lower. Gardner *et al.*, (1999) found no relation between EEPA, determined using both DLW or indirect calorimetry, and ABI or calf blood flow<sup>381</sup>. They did, however, find a significant relation between EEPA and markers of microcirculation, namely calf transcutaneous heating power at rest, with post-occlusion reactive hyperemia, and after maximal exercise.

Post-exercise change in ankle SBP is an indicator of the dynamic functional significance of an arterial stenosis. In Study 1, the post-exercise change in ankle SBP was inversely related with total daily sedentary time and positively related with total daily standing time. Excessive sedentary behaviour has been found to have detrimental effects on cardiometabolic health, which may contribute to disease

progression and, at least in part, account for the association between PAD severity and daily sedentary time. Epidemiological studies in the general population have reported significant associations between self-reported and accelerometer-derived sedentary time and cardiometabolic risk markers<sup>5,6,158–160,162,163,166,167</sup>. In Study 1 CV risk factors were not related to total daily sedentary time, but were related to the pattern of sedentary time accumulation. The pattern of sedentary time accumulation has been found to be of clinical relevance in addition to the detrimental effects of total daily sedentary time. Prolonged sedentary bouts appear particularly detrimental to health and the number of breaks in sedentary time has been found to be inversely related to cardiometabolic risk markers, independent of total sedentary time<sup>6,7,166</sup>. Study 1 was the first study to determine the pattern of sedentary behaviour in individuals with PAD. The greatest duration of daily sedentary time was spent in bouts >60 min. Prolonged sedentary bouts were associated with elevated DBP, total cholesterol, and CRP, and post-exercise change in ankle SBP.

The mechanisms responsible for the adverse cardiometabolic effects associated with prolonged sedentary behaviour are not fully understood. Potential mechanisms include the suppression of skeletal muscle LPL activity due to loss of local contractile activity leading to impairment in lipid metabolism<sup>181</sup>, a reduction in insulin action<sup>187</sup>, and the replacement of light-intensity activity<sup>157</sup>. Interrupting sedentary time with light or moderate-intensity exercise has been shown to lower glucose and insulin<sup>362</sup>. In Study 1, BMI, body mass, and post-exercise change in

ankle SBP were associated with the number or percentage of ambulatory breaks rather than total breaks, suggesting the role of light-intensity physical activity in the prevention of the adverse health effects associated with prolonged sedentary behaviour and potentially supporting the theory of the loss of low-intensity activity as the mechanism responsible for the negative molecular impact of inactivity.

In addition to an association with disease severity, previous studies have also found a relation between daily physical activity and functional capacity in individuals with PAD. Gardner *et al.*, (1998) found a significant association between EEPA, measured using both DLW and indirect calorimetry, and MWD and 6MWD. In Study 1, there was no significant relation between physical activity and PFWT, MWT, or 6MWT. This is an unexpected and difficult to explain finding. MVPA was derived from step cadence in Study 1 as accelerometer counts were unavailable and perhaps MVPA derived from accelerometer counts may have correlated with walking performance. However, Sieminski and Gardner (1997) found a significant relation between MWT and step count measured using a pedometer<sup>140</sup>. A significant relation has also been found between MWD and physical activity and step count, step cadence, and total activity time measured using a step activity monitor<sup>148</sup>.

In PAD the delivery of blood flow to the peripheral tissues depends not only on the severity of the stenotic occlusion but also on the regulatory mechanisms that control blood flow, specifically vascular tone<sup>292</sup>. Vascular reactivity, assessed by FMD, is impaired in individuals with PAD compared with healthy controls<sup>293,294</sup> and is associated with the severity of PAD, determined by ABI, in asymptomatic and

symptomatic individuals <sup>294</sup>. In addition, improvement in vascular reactivity is proposed as a mechanism through which exercise improves symptoms in individuals with PAD. In Study 1, FMD was significantly related with MVPA. Payvandi *et al.*, (2009) also found a significant relation between daily physical activity, measured by both accelerometer and pedometer, and FMD in individuals with PAD <sup>311</sup>. Endothelial dysfunction is a primary event in the pathogenesis of atherosclerosis. In PAD it can be exacerbated by chronic exposure to low-grade I-Rl during exercise. High levels of daily physical activity may have a protective effect against endothelial dysfunction.

Peripheral arterial disease and its associated symptoms can have a detrimental effect on personal, social, and occupational aspects of daily living, thus severely diminishing QOL <sup>234</sup>. Quality of life is related to ABI and leg symptom severity <sup>382</sup>. Study 1 is the first to examine the relation between QOL and both daily physical activity and sedentary behaviours. The PAQ scores were associated with overall activity and sedentary behaviours but not the pattern of accumulation of the sedentary behaviour. The overall PAQ summary score was significantly related with total daily sedentary, standing, and ambulating time, step count, robust MVPA, and PAD MVPA. The PAQ domain most affected by activity and sedentary behaviour was social function. It was inversely related with daily sedentary time and positively related with measures of activity. The physical function domain was related to measures of physical activity only and not sedentary behaviour.

Previous research has highlighted the relation between PAD severity and low levels of daily physical activity. Study 1 is the first to identify an association between

PAD severity and sedentary behaviour. The clinical implication of these findings is that in addition to targeting increases in physical activity, interventions in individuals with PAD should aim to decrease sedentary time. The association between PAD severity and sedentary time may be due to the adverse effects of prolonged sedentary bouts on CV risk factors and breaks in sedentary time may have a positive effect on these risk factors. Therefore, interventions should not only aim to reduce total sedentary time but also to break up sedentary time with frequent interruptions of light-intensity activity.

Further research is required to investigate the effect of reducing and breaking sedentary time on disease severity, CV risk factors, and QOL in individuals with PAD. There is a need to identify effective methods for altering sedentary behaviour. The development of a home-based monitoring system or stimulator device to inform patients of their sedentary behaviours or remind them to break sedentary behaviour may aid in altering behaviour patterns. The mechanisms underpinning the association between excessive and prolonged sedentary behaviour and adverse cardiometabolic effects are not fully understood and need to be further investigated. Research is needed to clarify if this association is due to the absence of NEAT or unique cellular and molecular processes.

## **Study 2**

Study 2 compared the effects of an acute bout of intermittent walking to OC and MC on endothelial-dependent and -independent dilation in men and women with PAD. There was no effect on endothelial-dependent or -independent dilation

following exercise under both conditions. Previous research found a transient impairment in FMD following acute exercise to maximal claudication in individuals with PAD<sup>12,14-16</sup>. This is in contrast to no change or significant increases in FMD following acute exercise in healthy individuals<sup>18</sup> and individuals with CAD<sup>19</sup>.

The impairment in FMD in individuals with PAD is dependent upon the degree of ischemia produced in the diseased limb during exercise and occurs following exercise to maximal claudication but not exercise to the onset of claudication<sup>14</sup>. The reduction in FMD with maximal exercise appears to be due to reduced NO bioavailability. Treatment with nitroaspirin, a NO-donating aspirin, but not aspirin, prevented the reduction in FMD following maximal exercise<sup>17</sup>. The ischemia induced by exercise to maximal claudication and the subsequent I-RI is likely responsible for the reduced NO bioavailability. The oxidative stress and inflammatory response associated with I-RI can cause both a reduction in NO synthesis and an increase in NO biodegradation. Plasma markers of oxidative stress<sup>14</sup>, oxidative damage<sup>95</sup>, and markers of inflammation<sup>17,254,255</sup> are significantly increased in PAD patients compared with healthy controls following acute exercise to maximal claudication. In a study by Silvestro *et al.*, (2002) exercise to maximal claudication was associated with a significant decrease in FMD and a significant increase in plasma levels of thiobarbituric acid-reactive substances (TBARS), an index of oxidative stress, and sICAM-1, compared with no change with exercise to the onset of claudication<sup>14</sup>. There was no significant relation between increases in TBARS and changes in FMD and sICAM-1. However, the impairment in FMD and



elevation of TBARs and sICAM-1 were eliminated by administration of an antioxidant, Vitamin C.

The absence of an impairment in FMD following acute intermittent exercise to either OC or MC in Study 2 is likely due to the absence of an acute increase in oxidative stress and inflammation. There was no change in plasma concentration of inflammatory markers INF- $\gamma$ , TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and WBCs with either exercise condition. In fact, both exercise conditions were associated with a significant decrease in INF- $\gamma$  1 h after exercise. Acute inflammation has been shown to impair FMD in humans <sup>383</sup>. These findings are in contrast to previous research that has shown significant increases in IL-6, IL-1 $\beta$ , TNF- $\alpha$ , leukocytes, sVCAM-1, and sICAM-1 in PAD <sup>17,254,255</sup>.

The cytokines INF- $\gamma$ , TNF- $\alpha$ , IL-6, IL-1 $\beta$  were selected as inflammatory markers because they are among the most commonly cited and researched inflammatory biomarkers in the study of both atherosclerosis <sup>91,384</sup> and exercise physiology <sup>385</sup>, which would allow for comparative analysis. There was no compelling reason to select other markers. The cytokines selected are expressed during the initial stages of the cytokine cascade of the fast innate inflammatory response and their production is upregulated rapidly in response to vigorous PA <sup>386</sup> and other forms of stress, including IR-I <sup>387</sup>. Previous research has shown significant elevations in circulating levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  following acute exercise in individuals with PAD <sup>254,388</sup>.

Additional inflammatory markers that could have been analyzed include IL-8, ICAM-1, VCAM-1, and P-, E-, and L-selectin, which have also been shown to increase following acute exercise in individuals with PAD<sup>17,388</sup>. CRP is a leading biomarker of inflammation but is not influenced by acute exercise in individuals with PAD<sup>389</sup>. Cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$  are released early in the cytokine cascade and are pleiotropic and have been implicated in the induction of an array of adhesion molecules, chemokines<sup>384</sup> and acute phase proteins<sup>390</sup>. Therefore they are upstream of these additional markers.

The absence of an inflammatory response is most likely due to the nature of the exercise protocol. Previous studies have employed acute exercise protocols consisting of a single short continuous bout of exercise, ranging in duration from approximately 2 min to 10 min. Current ACSM physical activity guidelines for adults, older adults, and adults with clinically significant chronic conditions or functional limitations recommend a minimum of 30 min moderate-intensity aerobic physical activity on 5 d·wk<sup>-1</sup> or a minimum of 20 min vigorous-intensity aerobic physical activity on 3 d·wk<sup>-1</sup><sup>378,379</sup>. Activity can be accumulated from bouts of  $\geq 10$  min. The functional limitation associated with intermittent claudication means that exercise rehabilitation programs designed for individuals with PAD often employ intermittent walking consisting of multiple exercise bouts to allow participants to accumulate minutes of exercise. In Study 2, the acute exercise protocol involved intermittent exercise to either OC or MC to accumulate 30 min of exercise.

The mechanisms through which multiple bouts of exercise may eliminate the detrimental effects of single bouts of exercise on inflammation and FMD are unclear. Putative mechanisms include the clearance of ROS and inflammatory mediators with repeated hyperemic episodes or the stimulation of antioxidant defenses. Laminar shear has been shown to upregulate superoxide dismutase and glutathione peroxidase expression in endothelial cells <sup>391,392</sup>. Repeated exposure to shear stress may also increase NO synthesis, which would increase vasodilation but may also inhibit ROS production through the direct inhibition of superoxide formation, possibly through the suppression of NADPH-oxidase upregulation <sup>393</sup> and/or inhibit leukocyte activation.

In Study 2, the mean exercise bout duration was significantly longer during the exercise to MC than the exercise to OC, and therefore fewer exercise bouts were required to accumulate 30 min of exercise and there were fewer recovery bouts during exercise. Interestingly, there was no difference in recovery duration during exercise bouts whether participants walked to OC or MC. Therefore, overall recovery time and hence, session time was significantly shorter in the MC condition than the onset of claudication condition. Given that a lack of time is a commonly cited barrier to cardiac rehabilitation exercise adherence <sup>394</sup>, the validation of effective, time-efficient exercise protocols provide advantageous treatment approaches.

Study 2 found no adverse effects on FMD or inflammation with acute intermittent exercise to maximal claudication. Previous exercise training studies

assessing the effect of exercise on endothelial function in PAD have used intermittent submaximal exercise protocols and have found significant improvements in FMD<sup>11–13,246,313</sup>. To date, no studies have determined the effect of exercise training involving intermittent exercise to maximal claudication on FMD in individuals with PAD. However, exercise training involving intermittent exercise to maximal claudication has been shown to have beneficial effects on pain-free and maximal walking performance in individuals with PAD<sup>207,346,395</sup>. Therefore, intermittent exercise to maximal claudication may represent an effective and more time efficient exercise prescription than exercise to the onset of claudication. However, according to the hedonic theory of motivation, people are likely to repeat an activity if they derive pleasure, a sense of energy, or enjoyment from their participation in the activity and are unlikely to repeat an activity if they derive displeasure, a sense of exhaustion, pain, or discomfort<sup>396</sup>. Exercise to maximal claudication pain may deter people from maintaining participation in an exercise programme and further research is required to determine the long-term adherence to such a protocol.

Further research is required to identify the reasons why, unlike acute short continuous exercise, acute intermittent exercise does not impair FMD in individuals with PAD. The effect of acute intermittent exercise on oxidative stress, antioxidant enzymes, and NO bioavailability needs to be further investigated.

### Study 3

Study 3 evaluated the effect of a 12-week community-based exercise rehabilitation programme on endothelial-dependent and –independent dilation in men and women with PAD. There was a significant increase in FMD between week 1 and week 12. The increase in FMD occurred in the absence of significant changes in endothelial-independent dilation, baseline brachial artery diameter, and baseline and peak blood flow velocity. Previous studies investigating the effect of exercise training on endothelial function in individuals with PAD have employed submaximal intermittent exercise protocols<sup>11-13,313</sup>. Study 3 is the first study to determine the effect of intermittent exercise to maximal claudication on FMD in individuals with PAD. In addition, all but one of the previous studies have failed to measure endothelial-independent dilation, which prohibits conclusions being drawn that the observed improvement in vasodilation is endothelial-dependent. In Study 3, there was no significant change in endothelial-independent dilation, indicating that the improvements in vasodilation were due to improvements in endothelial function.

The magnitude of improvement in FMD in Study 3 (158%) appears greater than previous studies. Andreozzi *et al.*, (2007) reported a significant improvement of 35% in FMD following 6 weeks of ITW at 60-70% of maximal walking ability 3 d·wk<sup>-1</sup><sup>12</sup>. The smaller improvement compared with Study 3 may be the result of a weaker exercise stimulus, a shorter exercise programme duration, or a better baseline FMD. Following a 12 week exercise programme involving ITW to moderately severe claudication in participants with similar baseline FMD to Study 3, Allen *et al.*, (2010)

reported a 79% increase in FMD from 2.4% to 4.3%<sup>11</sup>. Mika *et al.*, (2012) investigated the effects of differing exercise stimuli and compared the effects of pain-free and moderate pain intermittent walking training on FMD in individuals with PAD<sup>313</sup>. Flow-mediated dilation increased by 36% and 56% in the pain-free and moderate pain groups, respectively. However the difference between groups was not statistically significant. In Study 3, intermittent exercise to maximal claudication is likely to have induced a greater shear stress stimulus on the endothelium than previous studies involving submaximal exercise. Chronic exposure to this stress may profoundly upregulate mRNA expression of eNOS resulting in greater NO production and a greater improvement in FMD<sup>312</sup>.

Another potential mechanism that may explain the greater improvement in FMD with exercise to maximal claudication is an increased ischemic stimulus and subsequent ROS production. In low concentrations ROS are important redox signaling molecules involved in the regulation of cellular O<sub>2</sub> homeostasis. Some studies in animals have shown that the ROS hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) upregulates eNOS in response to exercise training<sup>397</sup>. In humans, H<sub>2</sub>O<sub>2</sub> has been shown to induce coronary arteriole vasodilation<sup>398</sup> and antioxidant administration has been shown to decreased exercise-induced brachial artery vasodilation<sup>399</sup>.

Previous research investigating the effect of exercise rehabilitation on FMD in individuals with PAD has involved primarily hospital- or laboratory-based exercise programmes. Study 3 is the first study to determine the effect of community-based exercise rehabilitation programme on FMD in individuals with PAD. The growing

epidemic of CVD and its escalating economic burden requires the employment of economically viable secondary prevention strategies. Home- and community-based exercise programmes provide an alternative to hospital-based programmes, but it is necessary to determine the efficacy of these alternatives if their use is to become more widespread in the treatment of PAD.

Study 3 also determined the effect of a 12-week community-based exercise rehabilitation programme on disease severity, walking performance, daily physical activity, and QOL in men and women with PAD. There was no significant change in ABI between week 1 and week 12. Previous research findings on the effect of exercise training on ABI have been equivocal, however the majority of studies have found no change in ABI and a Cochrane review of 22 RCTs investigating the effect of exercise rehabilitation in individuals with IC concluded that exercise did not significantly affect ABI <sup>9</sup>. The absence of a change in ABI in the presence of a significant improvement in FMD may appear conflicting. However, ABI is a measure of resting limb perfusion and FMD is measured in response to a hyperemic stimulus.

Pain-free and maximal walking time increased significantly by 60% and 84%, respectively in Study 3. A meta-analysis of 21 RCTs and non-randomized and uncontrolled trials reported a mean improvement of 179% and 122% in PFWD and MWD, respectively <sup>8</sup>. The apparently smaller improvements in Study 3 may be due to the exercise programme components. The meta-analysis determined the programme components that were most effective in improving walking ability and concluded improvements were significantly greater when participants exercised for

$\geq 3$  sessions·wk<sup>-1</sup> compared with  $< 3$  sessions·wk<sup>-1</sup> and for  $\geq 6$  months compared with  $< 6$  months. The exercise programme in Study 3 involved 2 sessions·wk<sup>-1</sup> for 12 weeks.

Other systematic reviews did not pool data because of differences in demographic data, baseline characteristics, exercise protocols, and testing protocols across studies. In a systematic review of 10 randomized controlled trials (RCTs) investigating the effect of exercise on walking performance in individuals with IC, Brandsma *et al.*, (1998) found improvements in MWT ranging from 28% to 210%<sup>192</sup>. A Cochrane review of 22 RCTs investigating the effect of exercise rehabilitation on relieving symptoms and improving walking ability in individuals with intermittent IC found the overall improvement in walking ability was 50% to 200%<sup>9</sup>.

Community-based exercise rehabilitation was associated with significant improvements in PFWD and MWD by 187% and 142%, respectively after 3 months and by 240% and 191% after 6 months in a study by Bendermacher *et al.*, (2007)<sup>265</sup>. However, adherence to the community-based programme was problematic, 40% of participants discontinued the exercise programme. Reasons for drop-out included satisfaction with the acquired improvements, unsatisfying results, lack of motivation, comorbidities, and other reasons. Kruidenier *et al.*, (2009) had similar difficulties during a year-long community-based exercise programme, with 53% of participants discontinuing the programme<sup>400</sup>. For the 129 men and women who did complete the programme significant improvements were found in PFWD and MWD. A recent study reported that only 66% of PAD patients accepted the invitation to attend a



community-based exercise programme <sup>401</sup>. Of these 35% failed to initiate attendance in the programme, 24% did not start the programme, 8% did not complete more than the initial 3 sessions, and over a 3 month period, regular attendance was registered for only 16%. In Study 3, 73% of participants completed the programme and training adherence was 83.91%. However, the sample size was small and the number of participants who were referred to the programme and did not attend is unknown.

Study 3 also determined the effect of exercise rehabilitation on 6MWD. The 6MWT may be a better measure of walking ability in a community setting than the treadmill test, given that participants select the walking speed and can alter their speed with the development of claudication pain. The 6MWT is also more strongly related to daily physical activity levels than treadmill test outcomes <sup>402</sup>. In Study 3, 6MWD increased significantly by 10% between week 1 and week 12. At baseline, 4 participants were unable to walk continuously and rested during the test and one participant stopped twice. At week 12, all participants completed the 6MWT without stopping.

Previous research in individuals with PAD has found similar results. Gardner *et al.*, (2001) and McDermott *et al.*, (2009) found a 12% and 11% improvement in 6MWD, respectively following 24 weeks of ITW 3 d·wk<sup>-1</sup> <sup>200,246</sup>. Tsai *et al.*, (2002) reported a larger improvement of 21% in 6MWD following 12 weeks of ITW 3 d·wk<sup>-1</sup> than controls <sup>209</sup>, which may be due to a lower baseline performance. An inverse

relation between baseline walking performance values and the change in walking performance has been previously shown in individuals with PAD<sup>13</sup>.

There was no change in  $\dot{V}O_{2\text{peak}}$  with exercise training in Study 3. The ambulatory limitation caused by intermittent claudication may restrict individuals with PAD from exercising at an intensity sufficient to induce central adaptations. Despite the majority, if not all, previous research reporting improvements in walking distances in individuals with PAD with chronic exercise, the findings on  $\dot{V}O_{2\text{peak}}$  have been controversial. Using the same training protocol, Hiatt *et al.*,<sup>101</sup> found a significant improvement in  $\dot{V}O_{2\text{peak}}$  in 19 men, mean age  $60 \pm 12.5$  yr, with PAD, whereas Gardner *et al.*,<sup>200</sup> reported no improvement in 61 men and women, mean age of  $70.5 \pm 1$  yr, with PAD. The lack of improvement in the study by Gardner *et al.*, may be due to the older age of the participants and a greater number of comorbidities, which may reduce the intensity at which they can exercise. In Study 3, there was a significant decrease in the HR at the onset of claudication pain with training, despite participants walking for longer and to a higher treadmill grade before the onset of claudication. This finding suggests the exercise training intensity was sufficient to induce some form of CV adaptation.

In Study 3, participation in the community-based exercise rehabilitation programme was associated with significant changes in daily activity and sedentary behaviours. The improvements in claudication symptoms and ambulatory capacity associated with exercise rehabilitation may remove the limitations preventing individuals with PAD from leading physically active lives. Total daily sedentary time

decreased by 3.7% or 42 min ( $p < 0.05$ ). Standing time increased by 13.5% or 30 min ( $p < 0.05$ ) and ambulating time increased by 16.7% or 12 min ( $p < 0.05$ ). The increase in ambulating time was attributed to attendance at the exercise programme sessions. There was no significant change in ambulating time on non-training days. In contrast, sedentary and standing times were significantly altered on non-training days. Study 3 is the first study to determine the effect of exercise rehabilitation on daily sedentary and standing time in individuals with PAD and demonstrates that community-based exercise rehabilitation is effective in decreasing total daily sedentary time and increasing standing time. However, there was no change in the number or duration of sedentary bouts, indicating the programme was not effective at breaking up sedentary time.

The effect of exercise rehabilitation on daily physical activity in individuals with PAD has been investigated by a small number of studies and findings are inconsistent. Following 6 months of ITW training to near-maximal claudication 3 d·wk<sup>-1</sup>, Gardner *et al.*, (2000) reported a significant improvement in daily physical activity, determined by both a Caltrac accelerometer worn over 2 d and the Minnesota LTPA questionnaire, in individuals with PAD<sup>245</sup>. Accelerometer-derived physical activity increased by 31% and the change was significantly related with a change in PFWD and MWD. Self-reported physical activity increased by 62% but the change was not associated with the improvements in walking performance. In a subsequent RCT using the same exercise protocol, Gardner *et al.*, (2001) found a significant increase in accelerometer-derived physical activity, but no change in self-

reported physical activity in individuals with PAD compared with controls<sup>200</sup>. These findings suggest the increase in physical activity was primarily unstructured activity, such as walking around the home.

In a study including both symptomatic and asymptomatic PAD patients, McDermott *et al.*, (2009) found no significant improvement in daily physical activity, determined using accelerometers worn over 7 d, following either ITW or lower extremity resistance training for 6 months<sup>246</sup>. It is possible that the lack of impairment in functional capacity experienced by asymptomatic participants at baseline may explain the findings. In Study 3, despite the significant increase in total daily ambulating time there was no significant change in step count. This may be attributable to the large inter-individual variation at baseline and week 12. Similarly, 2 studies using pedometers worn over 7 d found no change in step count following exercise rehabilitation<sup>198,218</sup>.

There was a significant improvement in the PAQ summary score between week 1 and week 12 in Study 3. This was accounted for by significant improvements in the symptom stability, symptom frequency/burden, QOL, and social function domains. There was no significant change in the physical function or treatment satisfaction domains. A recent systematic review of 23 exercise trials measuring the impact on QOL in patients with PAD found that all studies using the Medical Outcomes Study Short Form (SF-36) reported improvements in the physical functioning domain, with some also reporting improvements in the bodily pain, role functioning, vitality, general health and health transition domains<sup>241</sup>. In studies

using a disease-specific questionnaire, improvements have been found across a range of QOL domains. Using the PAQ, Murphy *et al.*, (2012) reported significant improvements across all domains except symptom stability and treatment satisfaction following 26 weeks of supervised exercise rehabilitation compared with optimal medical care <sup>218</sup>.

The reason for the lack of improvement in self-reported physical function in Study 3, despite significant improvement in objectively measured physical function, is unclear. Murphy *et al.*, (2012) found no relation between the PAQ physical function score and the change in MWT following exercise rehabilitation, although the score was strongly related with the change in PFWT <sup>218</sup>. Spertus *et al.*, (2004) found no relation between the PAQ physical limitation score and total exercise time at baseline but a significant relation at follow-up after revascularization, suggesting the PAQ domain is responsive to clinical change <sup>370</sup>. Using the Walking Impairment Questionnaire, Hiatt *et al.*, <sup>101</sup> found significant improvements, whereas Gardner *et al.*, <sup>200</sup> found no improvement following the same exercise protocol. Both studies reported improvements in treadmill walking performance. However  $\dot{V}O_{2peak}$  improved in the study by Hiatt *et al.*, only, which may suggest a link between improvements in aerobic fitness and QOL. Participants in the study by Gardner *et al.*, were also older and had a greater number of comorbidities, which may indicate the influence of additional factors on an individual's perception of physical function. Lastly, the improvements in walking performance in Study 3 were at the low end of

the range of improvement following exercise described by the Cochrane review <sup>9</sup>, which may call into question the clinical relevance of these improvements.

The findings of Study 3 give rise to a number of clinical implications and indications for further research. Firstly, intermittent exercise to maximal claudication may be a more effective training protocol to improve endothelial function in individuals with PAD. However, this needs to be investigated in a randomized controlled trial. If these findings are confirmed the mechanisms, responsible for the greater adaptation with maximal exercise will require further investigation. Future studies should measure NO bioavailability and markers of oxidative stress. The findings of Study 3 may also lend support to the theory that improvement in endothelial function is a mechanism through which exercise training improves symptoms in PAD. However, there was no relation between changes in endothelial function and changes in walking performance, indicating that other mechanisms are involved.

Study 3 adds to the very limited pool of research on the effect of community-based exercise rehabilitation in individuals with PAD. Community-based exercise may circumvent the limitations of hospital-based programmes such as treatment cost and transportation difficulty. It provides a patient and economically friendly alternative secondary prevention strategy and Study 3 provides evidence of its efficiency in improving functional capacity, daily physical activity and sedentary behaviours, and QOL in individuals with PAD. The limited research on community-based exercise programmes for PAD necessitates further study to determine the

optimal structure and protocol, participant adherence, and the duration of effects after such programs. Future research should also assess the effects of community-based exercise in direct comparison to hospital- or clinic-based exercise.

### **Future Research**

The findings of this series of studies provoke a number of interesting topics for future research. Firstly, the sedentary behaviour patterns of individuals with PAD need to be compared with that of non-PAD age-matched controls, to clarify if the observed behaviour is a consequence of PAD or typical of an elderly population. Research is required to identify effective methods for reducing sedentary behaviour and to investigate the effect of reducing sedentary behaviour. A greater understanding of the mechanisms underpinning the association between sedentary behaviour and cardiometabolic health not just in a PAD population but in the general population should be a major concern of future research. This would provide insight into whether and why altering sedentary behaviour can have beneficial effects on health and may highlight a promising treatment modality, as it is likely easier to motivate an individual to 'stop sitting rather than start walking'.

Study 2 found no adverse effects on FMD or inflammation with acute intermittent exercise to maximal claudication. Further research is required to identify the reasons why this form of exercise protocol had no adverse effects unlike previous protocols of short continuous maximal exercise in individuals with PAD. Intermittent exercise to maximal claudication represented a more time efficient exercise prescription compared with intermittent exercise to the onset of

claudication. A RCT should be performed to compare the training adaptations of these 2 intermittent protocols. Exercising to maximal claudication pain may cause displeasure and deter individuals with PAD from maintaining participation in an exercise programme. Further research is required to determine the long-term adherence to such a protocol.

Finally, Study 3 supported the effectiveness of community-based exercise rehabilitation in the treatment of PAD. Further study is required to determine the optimal structure and protocol, participant adherence, and the duration of effects after such programs. Future research should also assess the effects of community-based exercise in direct comparison to hospital- or clinic-based exercise.

### **Study Limitations**

The primary limitation in this series of studies is the absence of a control group in Study 1 and Study 3. In Study 1, it is not possible to determine whether the activity and sedentary behaviour patterns observed in individuals with PAD differ from the non-PAD population. In Study 3, it is possible, although unlikely, that the improvements in endothelial function, walking performance, daily physical activity and sedentary behaviours, and QOL were due to regression to the mean. Secondly, the sample size in each study was relatively small, which reflects the difficulty in recruiting a unique clinical population. Access to individuals with PAD was solely to those referred for exercise rehabilitation from local hospitals. We first had to establish the exercise rehabilitation programme and wait for hospital referrals. The initial referrals were recruited for pilot testing. The testing procedures for each



study were demanding and developing a rapport with the referrals was important for participation recruitment. Consequently, participant recruitment was slow and every effort was made to recruit the maximum number of participants within the given time-frame. Characterizing the behaviour patterns of a PAD population would benefit from a larger sample size. In addition, the number of participants falls short of previous studies investigating the effects of acute and chronic exercise on FMD.

Thirdly, a technical issue occurred during the analysis of the ActivPAL accelerometer counts and step cadence had to be used to identify MVPA. The use of step cadence, particularly at low speeds, may lead to reduced accuracy when assessing energy expenditure <sup>363</sup>. Finally, despite its widespread use and relation with invasive measures of endothelial function, there are a number of technical and interpretive limitations of the FMD technique <sup>366</sup>. These include the variability of FMD, the lack of a consensus on a normal FMD, and the lack of a standardized protocol with regard to cuff placement, occlusion time, timing post-occlusion, gating, and normalization for shear stress.

Medication use presents a challenge to the use and interpretation of FMD because many pharmacological stimuli may potentially influence vascular tone and endothelial function <sup>403</sup>. In line with current guidelines for FMD assessment <sup>366</sup>, all vasoactive medications were withheld for at least 4 half-lives, where possible. Vasoactive medications were defined as drugs that directly cause vasodilation or vasoconstriction, e.g. NTG, sildenafil citrate. Recent evidence suggests that the

continuation of non-nitrate vasoactive drugs has no significant effect on brachial reactivity<sup>404</sup>.

Conventional CV drugs have pleiotropic actions that may include improvement of endothelial function. The pleiotropic effects of drugs interfering with the renin–angiotensin–aldosterone system may prevent endothelial dysfunction and eNOS uncoupling. Both angiotensin-converting-enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) have been found to exert beneficial effects on endothelial function independent of their anti-hypertensive effects<sup>405</sup>. ACE inhibitors and ARBs have indirect antioxidant effects by preventing the activation of NADPH oxidase and increasing the activity of extracellular SOD<sup>406</sup>. ARBs may also increase BH4 levels<sup>407</sup> and reduce endothelin-1 activity<sup>408</sup>.

Statin therapy is associated with significant improvements in both peripheral endothelial function, assessed by FMD and venous occlusion plethysmography, and coronary endothelial function, assessed by angiography as the vasomotor response to intracoronary infusion of ACh<sup>409</sup>. In addition to their cholesterol lowering properties, statins can upregulate eNOS via several different mechanisms, including 1) increased eNOS mRNA stability through inhibition of Rho isoprenylation, 2) increased eNOS phosphorylation through PI3K-dependent signaling, 3) restoration of eNOS activity through reduction of caveolin-1 abundance<sup>410</sup>.

Beta-blockers are a heterogeneous group of antihypertensive agents with different selectivity for adrenergic receptors and/or additional effects in heart and peripheral circulation. Third generation  $\beta$ -blockers, including carvedilol and

nebivolol, display vasodilator properties. The non-selective agent carvedilol mediates vasodilation by blocking  $\alpha$ 1-adrenergic receptors located in SMC, which when stimulated trigger vasoconstriction<sup>411</sup>. Nebivolol is highly selective for  $\beta$ 1-adrenergic receptors and increases NO bioavailability by enhancing NOS activity and antioxidant effects via  $\beta$ 3-adrenergic receptor stimulation<sup>412</sup>.

Finally, calcium channel blockers (CCB) bind to voltage sensitive Ca channels and reversibly inhibit Ca entry into cardiac and vascular SMCs, thus decreasing intracellular Ca concentrations and causing SMC relaxation<sup>413</sup>. Additionally certain types of CCBs can enhance endothelial function by increasing eNOS activity and perhaps increasing antioxidant capacity.

## **Conclusion**

Peripheral arterial disease is a cause of increased risk of mortality and morbidity in the elderly population. With an ageing population and the growing epidemic of CVD, the need for an understanding of the factors that influence the progression and regression of PAD is ever more pertinent. This will allow the development of optimal primary and secondary prevention strategies. This series of studies is the first to identify the adverse effect of excessive daily sedentary time on PAD severity and QOL, whereas simply standing may have a protective effect. This adverse effect may at least in part be due to the detrimental association between prolonged sedentary bouts and CV risk factors, which may contribute to disease progression. Breaking up daily sedentary time may have positive implications on CV risk factors and PAD severity and interventions targeted at this population should

seek to alter their sedentary behaviour patterns in addition to increasing daily physical activity.

Exercise is a powerful treatment tool for PAD and is recommended as the first-line of therapy for the majority of PAD patients. In spite of this, the mechanisms that underpin the improvements incurred with exercise in individuals with PAD are not fully understood, which restricts the identification of the optimal exercise protocol. In this series of studies, intermittent exercise emerged as an ideal exercise prescription because in addition to allowing PAD patients accumulate minutes of exercise despite the limitations imposed by intermittent claudication, the multiple bouts may have a protective effect against the ischemia-reperfusion injury associated with exercise to maximal claudication. Adopting this intermittent strategy will allow individuals with PAD to exercise to maximal claudication and potentially stimulate greater improvements, without inducing an inflammatory response.

In this series of studies, community-based exercise rehabilitation also emerged as a potential effective treatment modality for PAD. Exercise rehabilitation in this format offers an economically viable alternative to hospital-based programmes. It may also remove barriers, e.g. transportation, and provide motivators, e.g. peer support, to exercise in older adults<sup>414</sup>, which in combination with its supervised and structured nature has the potential to lead to the long-term maintenance of exercise in individuals with PAD.

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## Appendices

## Appendix A

**Dublin City University**  
**RESEARCH ETHICS COMMITTEE**  
 APPLICATION FOR APPROVAL OF A PROJECT INVOLVING **HUMAN PARTICIPANTS**  
**Application No. (office use only)** DCUREC/2009/     
**Period of Approval (office use only)** ...../...../..... to...../...../.....

This application form is to be used by researchers seeking ethics approval for individual projects and studies. The **signed original and an electronic copy** of your completed application must be submitted to the DCU Research Ethics Committee.

**NB - The hard copy must be signed by the PI. The electronic copy should consist of one file only, which incorporates all supplementary documentation. The completed application must be proofread and spellchecked before submission to the REC. All sections of the application form should be completed. Applications which do not adhere to these requirements will not be accepted for review and will be returned directly to the applicant.**

Applications must be completed on the form; answers in the form of attachments will not be accepted, except where indicated. No handwritten applications will be accepted. **Research must not commence until written approval has been received from the Research Ethics Committee.**

**PROJECT TITLE**            Effect of a 12 Week Community-based Exercise Rehabilitation Programme on Vascular Health in Patients with Peripheral Arterial Disease

**PRINCIPAL INVESTIGATOR**            Prof. Niall M. Moyna

Please confirm that <u>all</u> supplementary information is included in your application (in both signed original and electronic copy). If questionnaire or interview questions are submitted in draft form, a copy of the final documentation must be submitted for final approval when available.	INCLUDED	NOT APPLICABLE
Bibliography	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Recruitment advertisement	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Plain language statement/Information Statement	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Informed Consent form	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Evidence of external approvals related to the research	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Questionnaire	<input type="checkbox"/> draft <input type="checkbox"/> final	<input checked="" type="checkbox"/>
Interview Schedule	<input type="checkbox"/> draft <input type="checkbox"/> final	<input checked="" type="checkbox"/>
Debriefing material	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Other	<input type="checkbox"/>	<input checked="" type="checkbox"/>

Please note:

1. Any amendments to the original approved proposal must receive prior REC approval.
2. As a condition of approval investigators are required to document and report immediately to the Secretary of the Research Ethics Committee any adverse events, any issues which might negatively impact on the conduct of the research and/or any complaint from a participant relating to their participation in the study

**1. ADMINISTRATIVE DETAILS**

**THIS PROJECT IS:**  Research Project  Funded Consultancy  
 (tick as many as apply)  Practical Class  Clinical Trial  
 Student Research Project  Other - Please Describe:

**Final Year Research Project**

Research Masters  Taught Masters  
 PhD  Undergraduate

**Project Start Date:** Start: 01/06/10 **Project End date:** End: 31/05/11

**1.1 INVESTIGATOR CONTACT DETAILS****PRINCIPAL INVESTIGATOR(S):**

TITLE	SURNAME	FIRST NAME	PHONE	FAX	EMAIL
Prof	Moyna	Niall	01 7008802	01 7008888	niall.moyna@dcu.ie

**OTHER INVESTIGATORS:**

TITLE	SURNAME	FIRST NAME	PHONE	FAX	EMAIL
Dr.	Mc Caffrey	Noel	087 2797597	01 7008888	<a href="mailto:noel.mccaffrey@dcu.ie">noel.mccaffrey@dcu.ie</a>
Dr.	Woods	Catherine	01 7008008	01 7008888	<a href="mailto:Catherine.woods@dcu.ie">Catherine.woods@dcu.ie</a>
Dr.	Murphy	Ronan	01 7008824	01 7008888	<a href="mailto:ronan.murphy@dcu.ie">ronan.murphy@dcu.ie</a>
Ms.	Furlong	Brona	086 3687961	01 7008888	<a href="mailto:brona.furlong2@mail.dcu.ie">brona.furlong2@mail.dcu.ie</a>
Ms.	Hughes	Sarah	086 8673608	01 7008888	<a href="mailto:sarah.hughes3@mail.dcu.ie">sarah.hughes3@mail.dcu.ie</a>
Ms.	Gray	Cleona	01 8034478	01 8034252	<a href="mailto:cgray@mater.ie">cgray@mater.ie</a>

**FACULTY/DEPARTMENT/SCHOOL/CENTRE:** School of Health and Human Performance

**1.2 WILL THE RESEARCH BE UNDERTAKEN ON-SITE AT DUBLIN CITY UNIVERSITY?**

YES  NO

**1.3 IS THIS PROTOCOL BEING SUBMITTED TO ANOTHER ETHICS COMMITTEE, OR HAS IT BEEN PREVIOUSLY SUBMITTED TO AN ETHICS COMMITTEE?**

YES  NO


**DECLARATION BY INVESTIGATORS**

*The information contained herein is, to the best of my knowledge and belief, accurate. I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures set out in the attached application in accordance with the guidelines, the University's policy on Conflict of Interest and any other condition laid down by the Dublin City University Research Ethics Committee or its Sub-Committees. I have attempted to identify all risks related to the research that may arise in conducting this research and acknowledge my obligations and the rights of the participants.*

*If there any affiliation or financial interest for researcher(s) in this research or its outcomes or any other circumstances which might represent a perceived, potential or actual conflict of interest this should be declared in accordance with Dublin City University policy on Conflicts of Interest.*

*I and my co-investigators or supporting staff have the appropriate qualifications, experience and facilities to conduct the research set out in the attached application and to deal with any emergencies and contingencies related to the research that may arise.*

**Signature(s):**

**Principal investigation:** 

**Print name(s) in block letters:** Niall M. Moyna

**Date:** 11 May 2010



## 2. PROJECT OUTLINE

### 2.1 LAY DESCRIPTION

Peripheral arterial disease is a form of cardiovascular disease that mainly affects the legs. One of the symptoms of PAD is muscle pain that develops in the legs during exercise. This is known as intermittent claudication. As a result, PAD patients tend to do relatively little exercise. It is possible that exercise training can improve walking ability and exercise ability in patients with PAD. The purpose of this study is to assess the effect of a 12 week community-based exercise rehabilitation programme (SmartSteps) on walking ability, aerobic fitness and the health of blood vessels in patients with peripheral arterial disease (PAD).

#### AIMS OF AND JUSTIFICATION FOR THE RESEARCH

PAD is a prevalent disease among people older than 55, and is a strong predictor of myocardial infarction, stroke and death from vascular causes. Patients with PAD are 6 times more likely to die from cardiovascular disease than individuals without PAD. PAD is a major cause of morbidity and mortality in older populations. People with PAD often experience a poor quality of life. One of the symptoms of PAD is muscle pain that develops in the lower extremities during exercise. This is known as intermittent claudication. Consequently, PAD patients tend to undertake relatively little exercise. Exercise may improve ambulatory function and exercise capacity and overall morbidity and mortality in patients with PAD. Damage to the endothelium layer of blood vessels is an early indicator of CVD. Endothelial dysfunction manifests as an inability of the blood vessel to dilate in response to a flow stimulus. Patients with PAD have impaired brachial artery reactivity and exercise training may help to improve endothelial function. The aim of this study is to assess the effect of a 12 week community-based exercise rehabilitation programme (SmartSteps) on ambulatory function, exercise capacity and vascular health in patients with PAD

### 2.3 PROPOSED METHOD

#### Overview

The study will take place in Dublin City University. Subjects will visit the Vascular Research Unit in the School of Health and Human Performance on 3 separate occasions.

**Visit 1:** Subjects will have the blood pressure in the arms and ankles measured at rest and immediately following a treadmill walking test.

**Visit 2:** Subjects will have a blood sample taken and endothelial dependent and independent dilation assessed. They will repeat the treadmill walking test.

**SmartSteps:** Subjects will then exercise 2d/wk for 12 week as part of a community based exercise rehabilitation programme called SmartSteps. The programme will take place in DCU.

**Visit 3:** After the 12 weeks subjects will return to the Vascular Research Unit. Subjects will have a blood sample taken, blood pressure in their arms and ankles measured and endothelial dependent and independent dilation assessed. They will repeat the treadmill walking test.

**Endothelial dependent and independent dilation:** Endothelial dependent dilation will be determined in response to reactive hyperemia following 5 min of lower arm occlusion. A blood pressure cuff will be placed on the left arm for blood pressure monitoring and another on the right lower arm for occlusion. ECG leads will be attached to monitor heart rate. Subjects will rest for 10 min in a supine position. Blood pressure will be determined during the final 2 minutes of the rest period. Baseline blood flow and brachial artery diameter (SonoSite, MicroMaxx) will be recorded. The right arm blood pressure cuff will then be inflated to approximately 220-230 mmHg and maintained at that pressure for 5 minutes. The cuff will then be rapidly deflated after 5 min of occlusion. Doppler blood flow measurement will be obtained during the first minute following cuff deflation. Brachial artery diameter will be assessed at one and three minutes post occlusion. Subjects will then rest for 15 minutes to eliminate endothelium dependent effects on brachial artery diameter. After this period, endothelial independent dilation will be assessed. Baseline blood flow and brachial artery diameter will be recorded and used as a baseline prior to sublingual nitroglycerine administration. Nitroglycerin (0.4mg) will be placed under the subjects tongue.

Doppler blood flow measurement will be obtained three minutes following the sublingual nitroglycerin administration and brachial artery diameter measurements will be assessed 3 and 5 minutes post nitroglycerin administration. If the subject is taking Viagra they will notify Brona Furlong. They will not be permitted to take Viagra for at least 24 hours before the administration of nitroglycerin.

**Treadmill test:** The treadmill test will involve an incremental walking protocol to volitional fatigue. Subjects will wear a mouthpiece or facemask during the test to measure oxygen uptake. A 12 lead ECG will be used to continuously monitor the electrical activity of the heart. Rating of perceived exertion will be measured every 5 min.

**SmartSteps:** SmartSteps is a community-based exercise rehabilitation programme aimed at individuals with PAD. DCU, Beaumont Hospital and the Mater Misericordiae Hospital are in the process of developing SmartSteps. The programme will be located at DCU Sports Complex. SmartSteps will be a sister programme of HeartSmart, the Phase IV community based cardiac rehabilitation programme in DCU. Phase IV cardiac rehabilitation aims to assist patients with the long-term maintenance of lifestyle changes. HeartSmart was established in 2006 by DCU, Beaumont Hospital, the Mater Misericordiae Hospital and Connolly Memorial Hospital. The programme is specifically designed for patients who have successfully completed hospital based phase III cardiac rehabilitation programme. Patients, who meet the inclusion criteria, are referred to the programme by the cardiac rehabilitation teams at the three partner hospitals. Participants undertaking the SmartSteps will be individuals with diagnosed PAD, who have been referred by vascular surgeons in the participating hospitals. Participants will attend supervised exercise sessions twice per week. The exercise sessions involve 5 minutes of warm-up exercises, 30 minutes of intermittent walking and 5 minutes of cool down exercises.

## 2.4 PARTICIPANT PROFILE

Men and women aged 40-65 yr with diagnosed PAD, who have been referred to the SmartSteps programme by the vascular surgeons in Beaumont Hospital and the Mater Misericordiae Hospital will be recruited.

### **Inclusion Criteria:**

- Referred by the vascular departments in Beaumont Hospital and The Mater Hospital
- Stable angina
- Ratio of arm blood pressure to ankle blood pressure  $<0.95$  at rest or  $<0.85$  after exercise
- Fontaine Stage II PAD (intermittent claudication upon ambulation) for  $> 3$  months
- Clinically stable and in good health for a minimum of two weeks prior to beginning the study

### **Exclusion Criteria:**

- Fontaine Stage I PAD (ambulation not limited by claudication)
- Fontaine Stage III PAD (pain at rest)
- Ulceration or gangrene
- Vascular surgery or angioplasty in past 6 months
- Co-morbidities contradictive to exercise
- Factors other than intermittent claudication limiting exercise tolerance
- Diabetes mellitus
- Unable to walk on a treadmill
- Current smoker
- Unstable angina
- Systolic blood pressure  $>180$  mmHg and/or diastolic blood pressure  $> 100$  mmHg
- Resting tachycardia
- Unstable or acute heart failure

**2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED**

Men and women referred to the SmartSteps programme by the vascular surgeons in Beaumont Hospital and the Mater Misericordiae Hospital will be informed of the research study. Participants must complete an induction day before commencing the SmartSteps programme. Participants will be informed of the research study at the induction day. A brief summary of the study will be provided to explain the study to the individuals and provide contact details. Following an expression of interest, potential subjects will be asked to visit the Vascular Research Unit in the School of Health and Human Performance. They will be told by agreeing to attend the first session they are not obligated to participate in the study. During the first visit blood pressure in the arms and ankles will be assessed in identify subjects with a ratio of arm to ankle pressure of <0.95 at rest and <0.85 after exercise. An explanation will be given to each potential subject to explain the nature, benefits, risks and discomforts of the study. They will be provided with a plain language statement, and the informed consent will be explained. They will be encouraged to ask questions, and any individual with doubts about participating in the study will have an opportunity to ask questions. Individuals who wish to participate in the study will have to provide written informed consent. Contact details will be provided to ensure all queries or concerns of the participant can be dealt with immediately.

**2.6 PLEASE EXPLAIN WHEN, HOW, WHERE, AND TO WHOM RESULTS WILL BE DISSEMINATED, INCLUDING WHETHER PARTICIPANTS WILL BE PROVIDED WITH ANY INFORMATION AS TO THE FINDINGS OR OUTCOMES OF THE PROJECT?**

The results will form the basis for a postgraduate thesis and will be presented at scientific meetings and published in scientific journals. The identity of individual participants will not be divulged. Group information will only be presented. Participants will be provided with a copy of their results, summarising information such as body mass index, blood pressure and cholesterol levels.

**2.7 OTHER APPROVALS REQUIRED** *Has permission to gain access to another location, organisation etc. been obtained? Copies of letters of approval to be provided when available.*

YES       NO       NOT APPLICABLE

*(If YES, please specify from whom and attach a copy. If NO, please explain when this will be obtained.)*

**2.8 HAS A SIMILAR PROPOSAL BEEN PREVIOUSLY APPROVED BY THE REC?**

<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
REC/2009/172 - Effect of Exercise Duration on Vascular Health in Patients with Cardiovascular Disease	

**3. RISK AND RISK MANAGEMENT**

**3.1 ARE THE RISKS TO SUBJECTS AND/OR RESEARCHERS ASSOCIATED WITH YOUR PROJECT GREATER THAN THOSE ENCOUNTERED IN EVERYDAY LIFE?**

YES       NO      *If YES, this proposal will be subject to full REC review*

*If NO, this proposal may be processed by expedited administrative review*

### 3.2 DOES THE RESEARCH INVOLVE?

	YES	NO
• use of a questionnaire? (attach copy)?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• interviews (attach interview questions)?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• observation of participants without their knowledge?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• participant observation (provide details in section 2)?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• audio- or video-taping interviewees or events?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• access to personal and/or confidential data (including student, patient or client data) without the participant's specific consent?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• administration of any stimuli, tasks, investigations or procedures which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• performance of any acts which might diminish the self-esteem of participants or cause them to experience embarrassment, regret or depression?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• investigation of participants involved in illegal activities?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• procedures that involve deception of participants?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• administration of any substance or agent?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
• use of non-treatment of placebo control conditions?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• collection of body tissues or fluid samples?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
• collection and/or testing of DNA samples?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• participation in a clinical trial?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• administration of ionising radiation to participants?	<input type="checkbox"/>	<input checked="" type="checkbox"/>

### 3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES

1. Exercise carries with it a very small risk of discomfort, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Subjects will continuously monitored using a 12 lead ECG.
2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks. The amount of blood drawn is not harmful
3. Assessment of endothelial dependent and independent dilation will require restriction of blood flow for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff is deflated. The nitroglycerin used may induce a headache that may last 5 - 10 minutes.

**Alternatives to the risks:** It is not possible to assess endothelial dependent and independent dilation without the use of brachial artery reactivity and the administration of nitroglycerin. Analysis of cardiovascular biomarkers cannot be undertaken without a sample of blood. The investigators are certified and experienced in phlebotomy and ultrasonography.

### 3.4 ARE THERE LIKELY TO BE ANY BENEFITS (DIRECT OR INDIRECT) TO PARTICIPANTS FROM THIS RESEARCH?

- YES    NO   Participants will be provided with a copy of their results, summarising information such as blood pressure and fitness levels

**3.5 ARE THERE ANY SPECIFIC RISKS TO RESEARCHERS? (e.g. risk of infection or where research is undertaken at an off-campus location)**

- YES    NO   Working with blood and needles carries risks, however the exposure to blood and needles is minimal and the School of Health and Human Performance has standard operating procedures for the handling of biological products.

**3.6 ADVERSE/UNEXPECTED OUTCOMES**

The School of Health and Human Performance has the facilities to implement all aspects of this study and has an emergency plan for adverse events. In the unlikely event of a major adverse outcome, an ambulance will be called and the participant will immediately be sent to Beaumont Hospital. In the unlikely event of a minor adverse outcome, the situation will be dealt with by the attending study physician with subsequent attention at the on-campus VHI SwiftCare clinic if required.

**3.7 MONITORING**

The principal investigator will be involved in all aspects of the research, including participant recruitment and data collection. The research team will have weekly meetings to update on all aspects of the study. The School of Health and Human Performance has a detailed list of Standard Operating Procedures for each of the protocols in this study. All researchers, including students, must be familiar with the procedures and the Safety Statement before beginning data collection.

**3.8 SUPPORT FOR PARTICIPANTS**

This project does not require additional support for participants

**3.9 DO YOU PROPOSE TO OFFER PAYMENTS OR INCENTIVES TO PARTICIPANTS?**

- YES    NO   (If YES, please provide further details.)

**4. INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS (Approx. 200 words – see Guidelines)**

Prof. Moyna is an exercise physiologist and has extensive experience in cardiovascular research.

Dr. Noel McCaffrey is a physician with extensive experience in exercise related research

Ms. Brona Furlong is a graduate student in the School of Health and Human Performance, DCU. She has extensive experience in studies involving human experimentation, and has undertaken extensive training in ultrasonography under the guidance of Cleona Gray, Chief Vascular Technologist in the Department of Vascular Surgery in the Mater Hospital, Dublin.

Dr Ronan Murphy has 12 years of post PhD experience and training in cell and molecular biology, vascular biology, and thrombosis & haemostasis. He received his undergraduate degree and Ph.D. with NUI Galway. Following this he worked for two years as a Clinical Research Scientist in the field of Pharmacogenomics. He was awarded a Fellowship from the HRB to work on bleeding disorders. Thereafter, he went to work for Prof. S.J. Shattil, at The Scripps Research Institute, San Diego (2000-2003). He has also been a visiting scientist to the Blood Research Institute, Milwaukee, USA.

**5. CONFIDENTIALITY/ANONYMITY**

**5.1 WILL THE IDENTITY OF THE PARTICIPANTS BE PROTECTED?**

- YES    NO

**IF YOU ANSWERED YES TO 5.1, PLEASE ANSWER THE FOLLOWING QUESTIONS:**

**5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED?**

Confidentiality is an important issue during data collection. Participant's identity and other personal information will not be revealed, published or used in further studies. Subjects will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password-protected file in a computer at DCU. The principal investigator, and collaborators listed on this ethics application will have access to the data.

**5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY:** *(Have you included appropriate information in the plain language statement and consent form? See Guidelines)*

YES       NO      *(If NO, please advise how participants will be advised)*

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**6 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL** *(see Guidelines)*

**6.1 HOW WILL THE DATA/SAMPLES BE STORED?** *(The REC recommends that all data be stored on campus)*

Stored at DCU   
Stored at another site  *(Please explain where and for what purpose)*

**6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?**

Access by named researchers only   
Access by people other than named researcher(s)  *(Please explain who and for what purpose)*  
Other :  *(Please explain)*

**6.3 IF DATA/SAMPLES ARE TO BE DISPOSED OF, PLEASE EXPLAIN HOW, WHEN AND BY WHOM THIS WILL BE DONE?**

The principal investigator will be responsible for security of the data. The data will be kept in locked cabinet in the Vascular Research Unit in the School of Health and Human Performance in DCU. Access to the data will only be attainable by the named researchers. Data will be kept for a minimum of five years from the date of publication of the research. Aside from the named researchers, no others will have access to the raw data. Data will be shredded by Prof. Moyna after 5 years.

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**7. FUNDING**

**7.1 HOW IS THIS WORK BEING FUNDED?**

Irish Research Council for Science, Engineering and Technology

**7.2 PROJECT GRANT NUMBER (If relevant and/or known)**

NA

**7.3 DOES THE PROJECT REQUIRE APPROVAL BEFORE CONSIDERATION FOR FUNDING BY A GRANTING BODY?**

YES       NO       Not Applicable

**7.4 HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING?**

YES       NO       Not Applicable

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Plain Language Statement

Dublin City University

Project Title: **Effect of a 12 Week Community-based Exercise Rehabilitation Programme on Vascular Health in Patients with Peripheral Arterial Disease**

The Research Study will take place in the School of Health and Human Performance, DCU.

The principle investigator is: Prof. Niall M. Moyna, (Tel: 7008802 Fax 7008888) EMAIL [niall.moyna@dcu.ie](mailto:niall.moyna@dcu.ie)

- I. Peripheral arterial disease (PAD) is a disease of blood vessels primarily in the legs. PAD is a common disease among people older than 55 years. It is a major cause of reduced quality of life and death in older populations. PAD increases the thickness of blood vessels, and also reduces the ability of blood vessel to dilate (get bigger). This can lead to blood flow restriction to the legs. We can use a simple ultrasound procedure to measure degree of blood flow restriction. Tiny pieces of the damaged blood vessel wall break off into the blood and these can be measured by taking a blood sample. Exercise can help to improve the walking ability of individuals with PAD. The purpose of this study is to evaluate the effect of a 12 week exercise programme on your walking ability, exercise capacity and the health of your blood vessels. You will be allowed to take part in the study if you meet the entry criteria and sign the informed consent.

If you agree to take part in the study you will be asked to make 3 visits to the Vascular Research Unit in the School of Health and Human Performance in DCU. You will fast for at least 12 hours and will not be allowed to exercise for at least 24 hours before these visits.

- II. **Visit 1:** You will walk on a treadmill. You will have electrodes placed on your chest to allow the researchers observe the electrical activity of your heart while you are walking. You will wear a mouthpiece to allow the researchers measure the amount of oxygen you use during the exercise. You will have the blood pressure in your arms and ankles measured before and after the exercise. This visit will last approximately 1 hour.
- Visit 2:** You will have a blood sample taken. About 2 tablespoons of blood will be taken. The health of a blood vessel in your arm will also be measured at the same times that blood samples are taken. This will be done by using an ultrasound to take an image of your blood vessel. This involves blocking the blood flow to your arm for 5 minutes using a blood pressure cuff and taking a nitroglycerin tablet under your tongue. You will repeat the treadmill walking test from the first visit. This visit will last approximately 2 hours.
- SmartSteps:** You will take part in SmartSteps exercise rehabilitation programme in the DCU Sports Complex. You will attend a SmartSteps exercise session twice per week for 12 weeks. After the 12 weeks of SmartSteps you will return to the Vascular Research Unit for Visit 3.
- Visit 3:** is the same Visit 2. You will have a blood sample taken, the blood pressure in your arms and ankles measured and the health of a blood vessel in your arm measured. You will repeat the treadmill walking test. This visit will last approximately 2 hours.
- III. Exercise carries with it a very small risk of discomfort, abnormal heart rhythms, heart attack, or death in less than 1 in 30,000 patients. Your heart rate will be continuously monitored using a 12 lead ECG.

Drawing blood may cause a slight pain where the needle is inserted and may leave a bruise. A person trained to take blood will be used to decrease these risks.

Taking an ultrasound image of your arm requires blocking the blood flow to your arm for 5 minutes using a blood pressure cuff. This may cause slight discomfort in your arm, which will go away after the blood pressure cuff is deflated. The nitroglycerin used in this study may cause a headache that could last 5 to 10 min.

- IV. Your confidentiality will be guarded. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate project and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. You will be assigned an ID number under which all personal information will be stored in the secure locked filing cabinet and saved in a password protected file in a computer at DCU. You need to be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.
- V. Involvement in this study is completely voluntary. You may withdraw from the Research Study at any point.
- VI. If you have concerns about this study and wish to contact an independent person, please contact: The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000



**Informed Consent**

**Dublin City University**

**Project Title**      **Effect of a 12 Week Community-based Exercise Rehabilitation Programme on Vascular Health in Patients with Peripheral Arterial Disease**

**Principle Investigator**      Prof. Niall M. Moyna

**Introduction to this study**

Peripheral arterial disease (PAD) is disease of blood vessels primarily in the legs. PAD is a prevalent disease among people older than 55 and is a major cause of reduced quality of life and death in older populations. PAD can be identified by measuring and comparing the blood pressure in your arms and ankles. PAD damages blood vessels and reduces the ability of blood vessel to dilate (get bigger). We can also use a simple ultrasound procedure to measure how much the blood vessels can dilate. Damaged blood vessels release cells into the blood which can be measured by taking a blood sample. Regular physical activity improves the health of blood vessels, and also has a beneficial effect on walking ability in individuals with PAD. This study will evaluate the effect of a 12 week exercise programme on your walking ability, exercise capacity and the health of your blood vessels.

**Participants Requirements**

1. I will visit the Vascular Research Unit in the School of Health and Human Performance DCU on 3 separate days. Each visit will last approximately 2 hours. During my first visit I will walk on a treadmill. I will have electrodes placed on my chest to allow the researchers observe the electrical activity of my heart during exercise. I will wear a mouthpiece to measure the amount of oxygen I use during the exercise. I will have the blood pressure in my arms and ankles measured before and after the treadmill exercise.
2. During my second visit I will have a blood sample taken. The total amount of blood drawn will be 2 tablespoons (30 cc). I will have the health of the arteries in my arm measured. I will repeat the treadmill walking test.
3. To test the health of the arteries in my arms I will lie on my back, and an ultrasound will be placed on my upper arm to create an image of my artery. After the first image is recorded, a blood pressure cuff will be inflated on my forearm to block blood flow for five minutes. This may be uncomfortable. The cuff will be released and the images of my arteries repeated. I will rest for 15 minutes and then have a nitroglycerin pill placed under my tongue. The nitroglycerin will cause my arm arteries to enlarge and how much they enlarge will again be documented by taking a third set of pictures.
4. I will take part in SmartSteps in DCU's Sports Complex. I will attend exercise sessions twice a week for 12 weeks. The sessions will involve walking until discomfort in my legs develops, resting until the discomfort disappears and then walking again. I will do this for approximately 30 minutes.
5. After the 12 week programme I will return to the Vascular Research Unit. A blood sample will be taken, the blood pressure in my arms and ankles will be measured and the health of the arteries in my arm will be measured. I will repeat the treadmill walking test.
6. I will fast for at least 12 hours and will not exercise for at least 24 hours before each visit. If I am taking Viagra I will notify Brona Furlong. I will not take Viagra for at least 24 hours before these two visits.

**Potential risks to participants from involvement in the Research Study**

1. Exercise carries with it a very small risk of discomfort, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. My heart rate will be continuously monitored using a 12 lead ECG.
2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks.
3. The pictures of my arm arteries require blocking the blood flow to my arm for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff is deflated. The nitroglycerin used in this study may induce a headache that could last 5 to 10 min.

**Benefits (direct or indirect) to participants from involvement in the Research Study**

After completing the study I will be provided with a copy of my results, summarising information such as my body mass index, blood pressure and cholesterol levels. There are no other direct benefits to me.

**Participant – please complete the following (circle Yes or No for each question)**

- |  |                              |                             |
|--|------------------------------|-----------------------------|
| Have you read or had read to you the Plain Language Statement?       | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Do you understand the information provided?                          | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Have you had an opportunity to ask questions and discuss this study? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Have you received satisfactory answers to all your questions?        | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

**Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations.**

Your identity and other personal information will not be revealed, published or used in further studies. You will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password protected file in a computer at DCU. The named investigators will have access to the data. Data will be shredded after 5 years by Prof. Moyna.

Confidentiality is insured, but you must be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

If you are in a dependent relationship with any of the researchers their involvement in the project will not affect ongoing assessment/grades/management or treatment of health at DCU.

**Signature:**

I have read and understood the information in this form. The researchers have answered my questions and concerns, and I have a copy of this consent form. Therefore, I (print name) \_\_\_\_\_ consent to take part in this research project entitled Effect of a 12 Week Community-based Exercise Rehabilitation Programme on Vascular Health in Patients with Peripheral Arterial Disease.

**Participants Signature:** \_\_\_\_\_

**Name in Block Capitals** \_\_\_\_\_

**Witness:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Appendix B**

**General Health Questionnaire**

Name: .....

Occupation: .....

Address: .....

Telephone: (Home) .....

(Work): .....

---

Do you have, or have you ever suffered from: Diabetes? Yes / No

Asthma? Yes / No

Epilepsy? Yes / No

Have you ever had pains in your chest or heart? Yes / No

Do you ever feel faint or have spells of dizziness? Yes / No

Do you have or have you ever had high blood pressure? Yes / No

Do you have a muscle, back or joint problem that could be aggravated by physical activity or made worse with exercise? Yes / No

Do you have any current injuries? Yes / No

In the past week, have you suffered from any illness which required you to be in bed or off work for one day or more? Yes / No

Do you smoke? Yes / No If yes, how many per day?

Do you drink? Yes / No If yes, how many units per week?

Is there a good physical reason not mentioned here why you should not carry out laboratory testing? Yes / No

Please provide any further information concerning any condition/complaints that you suffer from and any medication that you may be taking by prescription or otherwise:

.....

Date:

Signature:

Authorizing Signature:

## Appendix C

Physical Activity Readiness  
Questionnaire - PAR-Q  
(revised 2002)

# PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If  
you  
answered

### YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

### NO to all questions

- If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
  - take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

### DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

**Informed Use of the PAR-Q:** The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

**No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.**

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME \_\_\_\_\_

SIGNATURE \_\_\_\_\_

DATE \_\_\_\_\_

SIGNATURE OF PARENT  
or GUARDIAN (for participants under the age of majority) \_\_\_\_\_

WITNESS \_\_\_\_\_

**Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.**



© Canadian Society for Exercise Physiology www.csep.ca/forms

## Appendix D

### The Peripheral Artery Questionnaire

The following questions refer to blockages in the arteries of your body, particularly your legs, and how that might affect your life. Please read and complete the following questions. There are no right or wrong answers. Please mark the answer that best applies to you.

- Blockages in the arteries, often referred to as peripheral vascular disease, affect different people in different ways. Some feel cramping or aching while others feel fatigue. Which leg (or buttock) causes you the most severe discomfort, fatigue, pain, aching or cramps?

The **Right** leg (buttock)    The **Left** leg (buttock)    **Both** are the same    Neither

- Please review the list below and indicate how much limitation you have due to **your peripheral vascular disease** (discomfort, fatigue, pain, aching or cramps in your calves (or buttock)) over the past 4 weeks.

Place an **X** in one box on each line

Activity	Extremely Limited	Quite a bit Limited	Moderately Limited	Slightly Limited	Not at all Limited	Limited for other reasons or did not do the activity
Walking around your home	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking 100-200 yards on level ground	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking 100-200 yards up a hill	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking 300-400 yards on level ground	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hurrying or jogging (as if to catch a bus)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Vigorous work or exercise	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3. Compared with 4 weeks ago, have your symptoms of **peripheral vascular disease** (discomfort, fatigue, pain, aching, or cramps in your calves (or buttocks) changed?

My symptoms have become...

Much worse	Slightly worse	Not changed	Slightly better	Much better	I have had no symptoms over the past 4 weeks
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4. Over the past 4 weeks, how many times did you have **discomfort, fatigue, pain, aching, or cramps in your calves (or buttocks)**?

All of the time	Several times per day	At least once a day	3 or more times per week but not every day	1-2 times per week	Less than once a week	Never over the past 4 weeks
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

5. Over the past 4 weeks, how much has **discomfort, fatigue, pain, aching, or cramps in your calves (or buttocks)** bothered you?

It has been...

<b>Extremely bothersome</b>	<b>Moderately bothersome</b>	<b>Somewhat bothersome</b>	<b>Slightly bothersome</b>	<b>Not at all bothersome</b>	I've had <b>no leg discomfort</b>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

6. Over the past 4 weeks, how often have you been awakened with **pain, aching or cramps in your legs or feet**?

Every night	3 or more times per week but not every night	1-2 times per week	Less than once a week	Never over the past 4 weeks
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

7. How satisfied are you that everything possible is being done to treat your **peripheral vascular disease**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8. How satisfied are you with the explanations your doctor has given you about your **peripheral vascular disease**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

9. Overall, how satisfied are you with the current treatment of your **peripheral vascular disease**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

10. Over the past 4 weeks, how much has your **peripheral vascular disease** limited your enjoyment of life?

It has <b>extremely</b> limited my enjoyment of life	It has limited my enjoyment of life <b>quite a bit</b>	It has <b>moderately</b> limited my enjoyment of life	It has <b>slightly</b> limited my enjoyment of life	It has <b>not limited</b> my enjoyment of life at all
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

11. If you had to spend the rest of your life with your **peripheral vascular disease** the way it is right now, how would you feel about this?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

12. Over the past 4 weeks, how often have you felt discouraged or down in the dumps because of your **peripheral vascular disease**?

I felt that way <b>all of the time</b>	I felt that way <b>most of time</b>	I <b>occasionally</b> felt that way	I <b>rarely</b> felt that way	I <b>never</b> felt that way
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**13.** How much does your **peripheral vascular disease** affect your lifestyle? Please indicate how your **discomfort, fatigue, pain, aching or cramps in your calves (or buttocks)** may have limited your participation in the following activities over the past 4 weeks.

Please place an X in one box on each line

<b>Activity</b>	<b>Severely limited</b>	Limited <b>quite a bit</b>	<b>Moderately limited</b>	<b>Slightly limited</b>	<b>Did not limit at all</b>	Does not apply or did not do for other reasons
Hobbies, recreational activities	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Visiting family or friends out of your home	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Working or doing household chores	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



Appendix E



Dublin City University

RESEARCH ETHICS COMMITTEE

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING HUMAN PARTICIPANTS

Application No. (office use only) DCUREC/2010/ [ ][ ]

Period of Approval (office use only) .../.../... to .../.../...

This application form is to be used by researchers seeking ethics approval for individual projects and studies. The signed original and an electronic copy of your completed application must be submitted to the DCU Research Ethics Committee.

NB - The hard copy must be signed by the PI. The electronic copy should consist of one file only, which incorporates all supplementary documentation. The completed application must be proofread and spellchecked before submission to the REC. All sections of the application form should be completed. Applications which do not adhere to these requirements will not be accepted for review and will be returned directly to the applicant.

Applications must be completed on the form; answers in the form of attachments will not be accepted, except where indicated. No handwritten applications will be accepted. Research must not commence until written approval has been received from the Research Ethics Committee.

PROJECT TITLE Effect of Acute Exercise on Vascular Health in Patients with Peripheral Arterial Disease

PRINCIPAL INVESTIGATOR(S) Prof. Niall M. Moyna

Please confirm that all supplementary information is included in your application (in both signed original and electronic copy). If questionnaire or interview questions are submitted in draft form, a copy of the final documentation must be submitted for final approval when available.

Table with 3 columns: Item, INCLUDED, NOT APPLICABLE. Rows include Bibliography, Recruitment advertisement, Plain language statement, Informed Consent form, Evidence of external approvals, Questionnaire, Interview Schedule, Debriefing material, and Other.

Please note:

- 3. Any amendments to the original approved proposal must receive prior REC approval.
4. As a condition of approval investigators are required to document and report immediately to the Secretary of the Research Ethics Committee any adverse events, any issues which might negatively impact on the conduct of the research and/or any complaint from a participant relating to their participation in the study

Please submit the signed original, plus the electronic copy of your completed application to: Ms. Fiona Brennan, Research Officer, Office of the Vice-President for Research (fiona.brennan@dcu.ie, Ph. 01-7007816)

## 1. ADMINISTRATIVE DETAILS

**THIS PROJECT IS:**  
(tick as many as apply)

- |                                     |   |                          |                                 |
|-------------------------------------|---|--------------------------|---------------------------------|
| <input checked="" type="checkbox"/> | Research Project                                  | <input type="checkbox"/> | Funded Consultancy              |
| <input type="checkbox"/>            | Practical Class                                   | <input type="checkbox"/> | Clinical Trial                  |
| <input type="checkbox"/>            | Student Research Project<br>(please give details) | <input type="checkbox"/> | Other - Please Describe:        |
|                                     | Research Masters<br>PhD                           | <input type="checkbox"/> | Taught Masters<br>Undergraduate |

**Project Start Date:** 24/01/2011

**Project End date:** 31/07/2011

### 1.1 INVESTIGATOR CONTACT DETAILS (see Guidelines)

**PRINCIPAL INVESTIGATOR(S):**

TITLE	SURNAME	FIRST NAME	PHONE	FAX	EMAIL
Prof.	Moyna	Niall	01 7008802	01 7008888	<a href="mailto:niall.moyna@dcu.ie">niall.moyna@dcu.ie</a>

**OTHER INVESTIGATORS:**

TITLE	SURNAME	FIRST NAME	PHONE	FAX	EMAIL
Dr	McCaffrey	Noel	087 2797597	01 7008888	<a href="mailto:noel.mccaffrey@dcu.ie">noel.mccaffrey@dcu.ie</a>
Dr	Woods	Catherine	01 7008008	01 7008888	<a href="mailto:Catherine.woods@dcu.ie">Catherine.woods@dcu.ie</a>
Dr	Murphy	Ronan	01 7008824	01 7008888	<a href="mailto:ronan.murphy@dcu.ie">ronan.murphy@dcu.ie</a>
Ms	Furlong	Brona	01 7008472	01 7008888	<a href="mailto:brona.furlong2@mail.dcu.ie">brona.furlong2@mail.dcu.ie</a>
Ms	Hughes	Sarah	01 7008470	01 7008888	<a href="mailto:sarah.hughes3@mail.dcu.ie">sarah.hughes3@mail.dcu.ie</a>
Ms	Gray	Cleona	01 8034478	01 8034252	<a href="mailto:cgray@mater.ie">cgray@mater.ie</a>

**FACULTY/DEPARTMENT/SCHOOL/ CENTRE:**

School of Health and Human Performance

(NB – if Nursing, please note all students including PhD's must attach the letter from the Nursing Ethics Advisory Committee to this application)

### 1.2 WILL THE RESEARCH BE UNDERTAKEN ON-SITE AT DUBLIN CITY UNIVERSITY?

YES  NO (If NO, give details of off-campus location.)

### 1.3 IS THIS PROTOCOL BEING SUBMITTED TO ANOTHER ETHICS COMMITTEE, OR HAS IT BEEN PREVIOUSLY SUBMITTED TO AN ETHICS COMMITTEE?)

YES  NO (If YES, please provide details and copies of approval(s) received etc.)

**DECLARATION BY INVESTIGATORS**

*The information contained herein is, to the best of my knowledge and belief, accurate. I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures set out in the attached application in accordance with the guidelines, the University's policy on Conflict of Interest and any other condition laid down by the Dublin City University Research Ethics Committee or its Sub-Committees. I have attempted to identify all risks related to the research that may arise in conducting this research and acknowledge my obligations and the rights of the participants.*

*If there any affiliation or financial interest for researcher(s) in this research or its outcomes or any other circumstances which might represent a perceived, potential or actual conflict of interest this should be declared in accordance with Dublin City University policy on Conflicts of Interest.*

*I and my co-investigators or supporting staff have the appropriate qualifications, experience and facilities to conduct the research set out in the attached application and to deal with any emergencies and contingencies related to the research that may arise.*

**Signature(s):**

**Principal investigation:** 

**Print name(s) in block letters:** Niall M. Moyna

**Date:** 11 Dec 2010

## **2. PROJECT OUTLINE**

### **2.1 LAY DESCRIPTION** *(see Guidelines)*

Peripheral arterial disease (PAD) is a form of cardiovascular disease that manifests primarily in the lower limbs. PAD is prevalent in 3-12% of the general population  $\geq$  40 years of age and continues to increase with advancing age. Approximately 40-60% of PAD patients have concomitant coronary artery or cerebrovascular disease and are at a 2-3 fold increased risk of fatal or non-fatal heart attack, stroke and all-cause mortality. One of the primary symptoms of PAD is intermittent claudication, a form of muscle pain that develops in the lower limbs during exercise. Symptoms can be become so severe that they limit activities of daily living and thus diminish quality of life. Exercise improves symptoms and functional capacity in patients with PAD. DCU Sport has recently established SmartSteps, a community-based exercise rehabilitation programme aimed at individuals with PAD. Current ACSM physical activity recommendations advocate an accumulation of 200 kcal of energy expenditure through exercise five days a week in order to attain health benefits. The best possible way for patients with PAD to achieve this target has not been identified. High intensity exercise induces claudication symptoms more rapidly compared with low intensity exercise. This forces the patient to rest until the symptoms subside before resuming exercise. Low intensity exercise allows the patient to exercise for longer before the onset of claudication symptoms. This study will assess whether energy expenditure of 200 kcal is achieved more rapidly in patients with PAD by undertaking high intensity exercise with a greater number of rest periods or low intensity exercise with fewer rest periods.

The mechanisms through which exercise may improve claudication symptoms and functional capacity in patients with PAD are in part related to changes in vascular health and muscle metabolism. This study will compare the effects of exercise intensity on vascular health and muscle metabolism.

### **2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH** *(see Guidelines)*

High intensity exercise induces claudication symptoms more rapidly compared with low intensity exercise. This forces the patient to rest until the symptoms subside before resuming exercise. Low intensity exercise allows the patient to exercise for longer before the onset of claudication symptoms. Current ACSM physical activity recommendations advocate an accumulation of 200 kcal of energy expenditure through exercise five days a week in order to attain health benefits. The primary aim of this study is to determine whether energy expenditure of 200 kcal is achieved more rapidly by high intensity exercise with a greater number of rest periods or low intensity exercise with fewer rest periods.

The beneficial effects of exercise in PAD patients are related to improvements in vascular health and muscle metabolism. Endothelial function refers to the ability of the blood vessels to dilate and is an indicator of vascular health. Vascular health can also be assessed by measuring the number of microparticles circulating in the blood. These microparticles are released by the damaged endothelium, and currently little is known about the effect of exercise on their number and function in patients with PAD. PAD is associated with increased plasma acylcarnitine content. Exercise can reduce plasma acylcarnitine levels. This study will compare the effects of different exercise intensities on endothelial function and blood content of microparticles and acylcarnitine.

## 2.3 PROPOSED METHOD (see Guidelines)

### Overview

The study will take place in Dublin City University. Subjects will visit the Vascular Research Unit in the School of Health and Human Performance on 3 separate occasions.

**Visit 1:** Subjects will have their arm and ankle blood pressure measured at rest and immediately following a treadmill walking test.

**Visit 2:** Subjects will perform either low or high-intensity intermittent treadmill walking to expend 200 kcal. Before and after the exercise subjects will have a blood sample taken, endothelial dependent and independent dilation assessed and arm and ankle blood pressures measured

**Visit 3:** Same as Visit 2 except for the exercise intensity.

**Endothelial dependent and independent dilation:** Endothelial dependent dilation will be determined in response to reactive hyperemia following 5 min of lower arm occlusion. The increase in blood flow following 5 minutes of occlusion activates mechano-receptors on the surface of the vascular endothelium resulting in the conversion of L-arginine to nitric oxide. Nitric oxide is a potent vasodilator and its production is dependent on a healthy vascular endothelium. The degree of dilation in the brachial artery correlates with the degree of dilation in the coronary artery following infusion of acetylcholine allowing brachial artery reactivity to be used as a surrogate marker for coronary artery function. A blood pressure cuff will be placed on the left arm for blood pressure monitoring and another on the right lower arm for occlusion. ECG leads will be attached to monitor heart rate. Subjects will rest for 10 min in a supine position. Blood pressure will be determined during the final 2 minutes of the rest period. Baseline blood flow and brachial artery diameter (SonoSite, MicroMaxx) will be recorded. The right arm blood pressure cuff will then be inflated to approximately 220-230 mmHg and maintained at that pressure for 5 minutes. The cuff will then be rapidly deflated after 5 min of occlusion. Doppler blood flow measurement will be obtained during the first minute following cuff deflation. Brachial artery diameter will be assessed at one and three minutes post occlusion. Subjects will then rest for 15 minutes to eliminate endothelium dependent effects on brachial artery diameter. After this period, endothelial independent dilation will be assessed. Baseline blood flow and brachial artery diameter will be recorded and used as a baseline prior to sublingual glyceryl trinitrate administration. Glyceryl trinitrate (0.4mg) will be placed under the subjects tongue. Doppler blood flow measurement will be obtained three minutes following the sublingual glyceryl trinitrate administration and brachial artery diameter measurements will be assessed 3 and 5 minutes post glyceryl trinitrate administration. If the subject is taking Viagra they will notify Brona Furlong. They will not be permitted to take Viagra for at least 24 hours before the administration of glyceryl trinitrate.

**Treadmill test:** The treadmill test will involve an incremental walking protocol to volitional fatigue. The treadmill velocity will remain constant at 2.0 mph. The gradient will be 0%, and will be increased by 2% every 2 minutes. A physician will be present during the test. Subjects will wear a mouthpiece or facemask during the test to measure oxygen uptake. A 12 lead ECG will be used to continuously monitor the electrical activity of the heart. Rating of perceived exertion will be measured every 5 min.

**Intermittent treadmill walking:** Subjects will walk on a treadmill at 2 mph and at a grade equal to 40% or 80% of the maximum grade achieved during the treadmill test

in Visit 1. The treadmill exercise will be undertaken by having the subjects exercise until claudication symptoms develop, then rest until symptoms subside. The exercise-rest cycle will be repeated until the subjects expend 200 kcal. A physician will be present during the test. Subjects will wear a mouthpiece or facemask during the test to measure oxygen uptake. A 12 lead ECG will be used to continuously monitor the electrical activity of the heart. Rating of perceived exertion will be measured every 5 min.

#### **2.4 PARTICIPANT PROFILE** (see *Guidelines*)

Men and women  $\geq 40$  yrs of age with diagnosed PAD, who have been referred to the SmartSteps programme by the vascular surgeons in Beaumont Hospital and the Mater Misericordiae Hospital will be recruited.

##### **Inclusion Criteria:**

- Referred to the SmartSteps programme by the vascular surgeons in Beaumont Hospital and The Mater Hospital
- Ratio of arm blood pressure to ankle blood pressure  $<0.95$  at rest or  $<0.85$  after exercise
- Fontaine Stage II PAD (intermittent claudication upon ambulation) for  $> 3$  months
- Clinically stable and in good health for a minimum of two weeks prior to beginning the study

##### **Exclusion Criteria:**

- Fontaine Stage I PAD (ambulation not limited by claudication)
- Fontaine Stage III PAD (pain at rest)
- Ulceration or gangrene
- Vascular surgery or angioplasty in past 6 months
- Co-morbidities contradictory to exercise
- Factors other than intermittent claudication limiting exercise tolerance
- Diabetes mellitus
- Unable to walk on a treadmill
- Current smoker
- Unstable angina
- Systolic blood pressure  $>180$ mmHg and/or diastolic blood pressure  $>100$ mmHg
- Resting tachycardia
- Unstable or acute heart failure

#### **2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED** (see *Guidelines*)

20 men and women enrolled in the DCU SmartSteps programme will be recruited. Subjects referred to the SmartSteps programme by the vascular surgeons in Beaumont Hospital and the Mater Misericordiae Hospital will be informed of the research study. A brief summary of the study will be provided to explain the study to the individuals and provide contact details. Following an expression of interest, potential subjects will visit the Vascular Research Unit in the School of Health and Human Performance. They will be told that by agreeing to attend the first session they are not obligated to participate in the study. During the first visit blood pressure in the arms and ankles will be assessed to identify subjects with a ratio of arm to ankle pressure of  $<0.95$  at rest and  $<0.85$  after exercise. An explanation will be given to each potential subject to explain the nature, benefits, risks and discomforts of the study. They will be provided with a plain language statement, and the informed consent will be explained. They will be encouraged to ask questions, and any individual with doubts about participating in the study will have an opportunity to ask

questions. Individuals who wish to participate in the study will have to provide written informed consent. Contact details will be provided to ensure all queries or concerns of the participant can be dealt with immediately.

**2.6 PLEASE EXPLAIN WHEN, HOW, WHERE, AND TO WHOM RESULTS WILL BE DISSEMINATED, INCLUDING WHETHER PARTICIPANTS WILL BE PROVIDED WITH ANY INFORMATION AS TO THE FINDINGS OR OUTCOMES OF THE PROJECT?**

The results will form the basis for a postgraduate thesis and will be presented at scientific meetings and published in scientific journals. The identity of individual participants will not be divulged. Group information will only be presented. Participants will be provided with a copy of their results, summarising information such as body mass index, blood pressure and cholesterol levels.

**2.7 OTHER APPROVALS REQUIRED** *Has permission to gain access to another location, organisation etc. been obtained? Copies of letters of approval to be provided when available.*

YES       NO       NOT APPLICABLE

*(If YES, please specify from whom and attach a copy. If NO, please explain when this will be obtained.)*

**2.8 HAS A SIMILAR PROPOSAL BEEN PREVIOUSLY APPROVED BY THE REC?**

YES       NO

*(If YES, please state both the REC Application Number and Project Title)*

REC/2010/048 - Effect of a 12 Week Community-based Exercise Rehabilitation Programme on Vascular Health in Patients with Peripheral Arterial Disease

**3. RISK AND RISK MANAGEMENT**

**3.1 ARE THE RISKS TO SUBJECTS AND/OR RESEARCHERS ASSOCIATED WITH YOUR PROJECT GREATER THAN THOSE ENCOUNTERED IN EVERYDAY LIFE?**

YES       NO      *If YES, this proposal will be subject to full REC review*

*If NO, this proposal may be processed by expedited administrative review*

**3.2 DOES THE RESEARCH INVOLVE:**

	<b>YES</b>	<b>NO</b>
• use of a questionnaire? (attach copy)?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• interviews (attach interview questions)?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• observation of participants without their knowledge?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• participant observation (provide details in section 2)?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• audio- or video-taping interviewees or events?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• access to personal and/or confidential data (including student, patient or client data) without the participant's specific consent?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• administration of any stimuli, tasks, investigations or procedures which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
• performance of any acts which might diminish the self-esteem of participants or cause them to experience embarrassment, regret or depression?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• investigation of participants involved in illegal activities?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• procedures that involve deception of participants?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• administration of any substance or agent?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
• use of non-treatment of placebo control conditions?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• collection of body tissues or fluid samples?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
• collection and/or testing of DNA samples?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• participation in a clinical trial?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
• administration of ionising radiation to participants?	<input type="checkbox"/>	<input checked="" type="checkbox"/>

**3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES** (see *Guidelines*)

1. Exercise carries with it a very small risk of discomfort, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Subjects will continuously monitored using a 12 lead ECG.
2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks. The amount of blood drawn is not harmful.
3. Assessment of endothelial dependent and independent dilation will require restriction of blood flow for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff is deflated. The glyceryl trinitrate used may induce a headache that may last 5 - 10 minutes.

**Alternatives to the risks:** It is not possible to assess endothelial dependent and independent dilation without the use of brachial artery reactivity and the administration of glyceryl trinitrate. Analysis of cardiovascular biomarkers cannot be undertaken without a sample of blood. The investigators are certified and experienced in phlebotomy and ultrasonography.

**3.4 ARE THERE LIKELY TO BE ANY BENEFITS (DIRECT OR INDIRECT) TO PARTICIPANTS FROM THIS RESEARCH?**

- YES     NO    (If YES, provide details.) Participants will be provided with a copy of their results, summarising information such as blood pressure and fitness levels

**3.5 ARE THERE ANY SPECIFIC RISKS TO RESEARCHERS?** (e.g. risk of infection or where research is undertaken at an off-campus location)

- YES     NO    (If YES, please describe.) Working with blood and needles carries risks, however the exposure to blood and needles is minimal and the School of Health and Human Performance has standard operating procedures for the handling of biological products.

**3.6 ADVERSE/UNEXPECTED OUTCOMES** (see *Guidelines*)

The School of Health and Human Performance has the facilities to implement all aspects of this study and has an emergency plan for adverse events. In the unlikely event of a major adverse outcome, an ambulance will be called and the participant will immediately be sent to Beaumont Hospital. In the unlikely event of a minor adverse outcome, the situation will be dealt with by the attending study physician with subsequent attention at the on-campus VHI SwiftCare clinic if required.

**3.7 MONITORING** (see *Guidelines*)

The principal investigator will be involved in all aspects of the research, including participant recruitment and data collection. The research team will have weekly meetings to update on all aspects of the study. The School of Health and Human Performance has a detailed list of Standard Operating Procedures for each of the protocols in this study. All researchers, including students, must be familiar with the procedures and the Safety Statement before beginning data collection.

**3.8 SUPPORT FOR PARTICIPANTS** (see *Guidelines*)

This project does not require additional support for participants.



**3.9 DO YOU PROPOSE TO OFFER PAYMENTS OR INCENTIVES TO PARTICIPANTS?**

YES  NO (If YES, please provide further details.)

**4. INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS** (Approx. 200 words – see Guidelines)

Prof. Moyna is an exercise physiologist and has extensive experience in cardiovascular research.

Dr. Noel McCaffrey is a physician with extensive experience in exercise related research

Ms. Brona Furlong is a graduate student in the School of Health and Human Performance, DCU. She has extensive experience in studies involving human experimentation, and has undertaken extensive training in ultrasonography under the guidance of Cleona Gray, Chief Vascular Technologist in the Department of Vascular Surgery in the Mater Hospital, Dublin.

Dr Ronan Murphy has 12 years of post PhD experience and training in cell and molecular biology, vascular biology, and thrombosis & haemostasis. He received his undergraduate degree and Ph.D. with NUI Galway. Following this he worked for two years as a Clinical Research Scientist in the field of Pharmacogenomics. He was awarded a Fellowship from the HRB to work on bleeding disorders. Thereafter, he went to work for Prof. S.J. Shattil, at The Scripps Research Institute, San Diego (2000-2003). He has also been a visiting scientist to the Blood Research Institute, Milwaukee, USA.

**Ms. Cleona Gray is the chief vascular sonographer in the Mater Hospital. Her role in the study is to train Brona Furlong in ultrasonography techniques.**

**5. CONFIDENTIALITY/ANONYMITY**

**5.1 WILL THE IDENTITY OF THE PARTICIPANTS BE PROTECTED?**

YES  NO (If NO, please explain)

**IF YOU ANSWERED YES TO 5.1, PLEASE ANSWER THE FOLLOWING QUESTIONS:**

**5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED?** (see Guidelines)

Confidentiality is an important issue during data collection. Participant's identity and other personal information will not be revealed, published or used in further studies. Subjects will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password-protected file in a computer at DCU. The principal investigator, and collaborators listed on this ethics application will have access to the data.

**5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY:** (Have you included appropriate information in the plain language statement and consent form? See Guidelines)

YES  NO (If NO, please advise how participants will be advised.)

**6 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL** (see Guidelines)

**6.1 HOW WILL THE DATA/SAMPLES BE STORED?** (The REC recommends that all data be stored on campus)

Stored at DCU   
Stored at another site  (Please explain where and for what purpose)

**6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?**

Access by named researchers only

Access by people other than named researcher(s)  (Please explain who and for what purpose)

Other :  (Please explain)

**6.3 IF DATA/SAMPLES ARE TO BE DISPOSED OF, PLEASE EXPLAIN HOW, WHEN AND BY WHOM THIS WILL BE DONE?**

The principal investigator will be responsible for security of the data. The data will be kept in locked cabinet in the Vascular Research Unit in the School of Health and Human Performance in DCU. Access to the data will only be attainable by the named researchers. Data will be kept for a minimum of five years from the date of publication of the research. Aside from the named researchers, no others will have access to the raw data. Data will be shredded by Prof. Moyna after 5 years.

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**7. FUNDING**

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**7.1 HOW IS THIS WORK BEING FUNDED?**

Irish Research Council for Science, Engineering and Technology

**7.2 PROJECT GRANT NUMBER (If relevant and/or known)**

**7.3 DOES THE PROJECT REQUIRE APPROVAL BEFORE CONSIDERATION FOR FUNDING BY A GRANTING BODY?**

YES  NO

**I.4 HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING?**

Plain Language Statement

**7.5 DO ANY OF THE RESEARCHERS, SUPERVISORS OR FUNDERS OF THIS PROJECT HAVE A PERSONAL, FINANCIAL OR COMMERCIAL INTEREST IN ITS OUTCOME THAT MIGHT COMPROMISE THE INDEPENDENCE AND INTEGRITY OF THE RESEARCH, OR BIAS THE CONDUCT OR RESULTS OF THE RESEARCH, OR UNDULY DELAY OR OTHERWISE AFFECT THEIR PUBLICATION?**

YES  NO (If Yes, please specify how this conflict of interest will be addressed.)

**8. PLAIN LANGUAGE STATEMENT** (Approx. 400 words – see Guidelines)

Project Title: Effect of Acute Exercise on Vascular Health in Patients with Peripheral Arterial Disease

**The Research Study will take place in the School of Health and Human Performance, DCU.**

**The principal investigator is Prof. Niall M. Moyna (Tel: 7008802 Fax: 7008888)  
Email: [niall.moyna@dcu.ie](mailto:niall.moyna@dcu.ie)**

- I. Peripheral arterial disease (PAD) is a disease of the blood vessels that primarily affects the legs. PAD is a common disease among people older than 55 years. A common symptom of PAD is leg pain during exercise that disappears with rest. This is known as intermittent claudication and it can severely diminish quality of life. Exercise can help to improve symptoms and walking ability in individuals with PAD. Current guidelines recommend you expend 200 kcal through exercise five days a week in order to achieve health benefits. On account of intermittent claudication, the best way for individuals with PAD to achieve the 200 kcal target is unknown. High intensity exercise brings on claudication pain more rapidly compared with low intensity exercise. This forces the individual to rest until the pain disappears before starting to exercise again. Low intensity exercise allows the individual to exercise for longer before the onset of claudication. This study will assess whether 200 kcal is expended more rapidly in patients with PAD by undertaking high intensity exercise with a greater number of rest periods or low intensity exercise with fewer rest periods.

PAD reduces the ability of blood vessels to dilate (get bigger). This can lead to blood flow restriction to the legs. We can use a simple ultrasound procedure to measure the degree to which the blood vessels can dilate. Tiny pieces of the damaged blood vessel wall break off into the blood and these can be measured by taking a blood sample. From the blood sample we can also measure the extent to which your muscles use the oxygen that the blood supplies to the legs.

You will be allowed to take part in the study if you meet the entry criteria and sign the informed consent. If you agree to take part in the study you will be asked to make 3 visits to the Vascular Research Unit in the School of Health and Human Performance in DCU. You will fast for at least 12 hours and will not be allowed to exercise for at least 24 hours before these visits.

- II. **Visit 1:** You will walk on a treadmill. The electrical activity of your heart will be assessed by a 12 lead electrocardiogram. This is a special machine that takes 12 different views of your heart (like photographs) while you are exercising. You will have electrodes placed on your chest to measure the electrical activity of your heart. You will wear a mouthpiece to allow the researchers measure the amount of oxygen you use during the exercise. You will have the blood pressure in your arms and ankles measured before and after the exercise. This visit will last approximately 1 hour.

**Visit 2:** You will walk on a treadmill at either a low or high-exercise intensity. Before and after the exercise, you will have a blood sample taken. About 2 tablespoons of blood will be taken. The health of a blood vessel in your arm will also be measured after the blood samples are taken. This will be done by using an ultrasound to take an image of your blood vessel. This involves blocking the

blood flow to your arm for 5 minutes using a blood pressure cuff and taking one spray of glyceryl trinitrate under your tongue. You will have the blood pressure in your arms and ankles measured before and after exercise. This visit will last approximately 2 hours.

**Visit 3:** is the same as Visit 2 except you will exercise at the other intensity. You will have a blood sample taken, the blood pressure in your arms and ankles measured and the health of a blood vessel in your arm measured. This visit will last approximately 2 hours.

- III. Exercise carries with it a very small risk of discomfort, abnormal heart rhythms, heart attack, or death in less than 1 in 30,000 patients. Your heart rate will be continuously monitored using a 12 lead ECG.

Drawing blood may cause a slight pain where the needle is inserted and may leave a bruise. A person trained to take blood will be used to decrease these risks.

Taking an ultrasound image of your arm requires blocking the blood flow to your arm for 5 minutes using a blood pressure cuff. This may cause slight discomfort in your arm, which will go away after the blood pressure cuff is deflated. The glyceryl trinitrate used in this study may cause a headache that could last 5 to 10 min.

- IV. Your confidentiality will be guarded. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate project and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. You will be assigned an ID number under which all personal information will be stored in the secure locked filing cabinet and saved in a password protected file in a computer at DCU. You need to be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.
- V. Involvement in this study is completely voluntary. You may withdraw from the Research Study at any point. Withdrawal from the study will not affect your participation in the SmartSteps Programme or the medical management of your condition.
- VI. This study is funded by the Irish Research Council for Science, Engineering and Technology (IRCSET).
- VII. **If you have concerns about this study and wish to contact an independent person, please contact: The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000**

## **Informed Consent**

### **Dublin City University**

**Project Title:** Effect of Acute Exercise on Vascular Health in Patients with Peripheral Arterial Disease

**Principal Investigator:** Prof. Niall M. Moyna

#### **Introduction to this study**

Peripheral arterial disease (PAD) is a disease of the blood vessels that primarily affects the legs. PAD is a common disease among people older than 55 years. A common symptom of PAD is leg pain during exercise that disappears with rest. This is known as intermittent claudication and it can severely diminish quality of life. Exercise can help to improve symptoms and walking ability in individuals with PAD. Current guidelines recommend that adults burn 200 kcal through exercise five days a week in order to achieve health benefits. On account of intermittent claudication, the best way for individuals with PAD to achieve the 200 kcal target is unknown. High intensity exercise brings on claudication pain more rapidly compared with low intensity exercise. This forces the individual to rest until the pain disappears before starting to exercise again. Low intensity exercise allows the individual to exercise for longer before the onset of claudication. This study will assess whether 200 kcal is burned more rapidly in patients with PAD by undertaking high intensity exercise with a greater number of rest periods or low intensity exercise with fewer rest periods.

PAD reduces the ability of blood vessels to dilate (get bigger). This can lead to blood flow restriction to the legs. We can use a simple ultrasound procedure to measure the degree to which the blood vessels can dilate. Tiny pieces of the damaged blood vessel wall break off into the blood and these can be measured by taking a blood sample. From the blood sample we can also measure the extent to which your muscles use the oxygen that the blood supplies to the legs.

#### **Participants Requirements**

1. I will visit the Vascular Research Unit in the School of Health and Human Performance DCU on 3 separate days. Each visit will last approximately 2 hours. During my first visit I will walk on a treadmill. The electrical activity of my heart will be assessed by a 12 lead electrocardiogram. This is a special machine that takes 12 different views of my heart (like photographs) while I am exercising. I will have electrodes placed on my chest to measure the electrical activity of my heart. I will wear a mouthpiece to measure the amount of oxygen I use during the exercise. I will have the blood pressure in my arms and ankles measured before and after the treadmill exercise.
2. During my second visit I will walk on a treadmill at low or high intensity. The electrical activity of my heart will be assessed by a 12 lead electrocardiogram. I will wear a mouthpiece to measure the amount of oxygen I use during the exercise. Before and after the exercise I will have a blood sample taken. The total amount of blood drawn will be 2 tablespoons (30 cc). I will have the health of the arteries in my arm assessed and the blood pressure in my arms and ankles measured.

3. To test the health of the arteries in my arms I will lie on my back, and an ultrasound will be placed on my upper arm to create an image of my artery. After the first image is recorded, a blood pressure cuff will be inflated on my forearm to block blood flow for five minutes. This may be uncomfortable. The cuff will be released and the images of my arteries repeated. I will rest for 15 minutes and then have one spray of glyceryl trinitrate sprayed under my tongue. The glyceryl trinitrate will cause my arm arteries to enlarge and how much they enlarge will again be documented by taking a third set of pictures.
4. My third visit will be same as the second visit with the exception of the intensity at which I walk.
5. I will fast for at least 12 hours and will not exercise for at least 24 hours before each visit. If I am taking Viagra I will notify Brona Furlong. I will not take Viagra for at least 24 hours before these two visits.

**Potential risks to participants from involvement in the Research Study**

1. Exercise carries with it a very small risk of discomfort, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. My heart rate will be continuously monitored using a 12 lead ECG.
2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks
3. The pictures of my arm arteries require blocking the blood flow to my arm for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff is deflated. The glyceryl trinitrate used in this study may induce a headache that could last 5 to 10 min.

**Benefits (direct or indirect) to participants from involvement in the Research Study**

After completing the study I will be provided with a copy of my results, summarising information such as my body mass index, blood pressure and cholesterol levels. There are no other direct benefits to me.

**Participant - please complete the following (Circle Yes or No for each question)**

<i>Have you read or had read to you the Plain Language Statement</i>	<i>Yes/No</i>
<i>Do you understand the information provided?</i>	<i>Yes/No</i>
<i>Have you had an opportunity to ask questions and discuss this study?</i>	<i>Yes/No</i>
<i>Have you received satisfactory answers to all your questions?</i>	<i>Yes/No</i>
<i>Are you aware that your interview will be audiotaped?</i>	<i>Yes/No</i>

**Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations.**

Your identity and other personal information will not be revealed, published or used in further studies. You will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password protected file in a computer at DCU. The named investigators will have access to the data. Data will be shredded after 5 years by Prof. Moyna.

Confidentiality is insured, but you must be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

If you are in a dependent relationship with any of the researchers their involvement in the project will not affect ongoing assessment/grades/management or treatment of health at DCU. Involvement in this study is completely voluntary. You may withdraw from the Research Study at any point. Withdrawal from the study will not affect your participation in the SmartSteps Programme or the medical management of your condition.

**Signature:**

I have read and understood the information in this form. The researchers have answered my questions and concerns, and I have a copy of this consent form. Therefore, I (print name) \_\_\_\_\_ consent to take part in this research project entitled Effect of a 12 Week Community-based Exercise Rehabilitation Programme on Vascular Health in Patients with Peripheral Arterial Disease.

**Participants Signature:**

\_\_\_\_\_

**Name in Block Capitals**

\_\_\_\_\_

**Witness:**

\_\_\_\_\_

**Date:**

\_\_\_\_\_

Appendix F

**Perceived Claudication Pain Scale**

- |            |  |
|------------|--|
| <b>0</b>   | <b>No Symptoms</b>                           |
| <b>0.5</b> | <b>Tiredness, Heaviness or<br/>Tightness</b> |
| <b>1</b>   | <b>Onset of Pain</b>                         |
| <b>2</b>   | <b>Mild Pain</b>                             |
| <b>3</b>   | <b>Moderate Pain</b>                         |
| <b>4</b>   | <b>Maximal Pain</b>                          |



## Appendix G

Table: Daily activity and sedentary behaviour

Total sedentary time (h)	18.92 ± 2.11
Total standing time (h)	3.87 ± 1.75
Total ambulating time (h)	1.17 ± 0.57
Total non-sedentary time (h)	4.98 ± 2.13
Total no. of steps	5575.02 ± 3197.28
MVPA defined as >25 steps/epoch (h)	0.24 ± 0.30
MVPA defined as >16 steps/epoch (h)	0.50 ± 0.38
Percentage of total day spent sedentary (%)	78.99 ± 8.98
Percentage of total day spent standing (%)	16.13 ± 7.29
Percentage of total day spent ambulating (%)	4.89 ± 2.37
Percentage of total day spent non-sedentary (%)	21.02 ± 8.98
Percentage of waking day spent sedentary (%)	67.56 ± 13.34
Percentage of waking day spent standing (%)	24.89 ± 10.99
Percentage of waking day spent ambulating (%)	7.57 ± 3.45
Percentage of waking day spent non-sedentary (%)	32.46 ± 13.35

Values are means ± SD

Table: Number and duration of sedentary bouts

Total no. of sedentary bouts	39.61 ± 11.99
Total no. of sedentary bouts of <5 min	18.88 ± 9.24
Total duration spent in sedentary bouts of <5 min (h)	0.55 ± 0.29
Total no. of sedentary bouts of 5-10 min	6.08 ± 2.96
Total duration spent in sedentary bouts of 5-10 min (h)	0.71 ± 0.33
Total no. of sedentary bouts of 11-20 min	5.98 ± 1.92
Total duration spent in sedentary bouts of 11-20 min (h)	1.42 ± 0.48
Total no. of sedentary bouts of 21-30 min	3.17 ± 1.04
Total duration spent in sedentary bouts of 21-30 min (h)	1.30 ± 0.44
Total no. of sedentary bouts of 31-40 min	1.66 ± 0.68
Total duration spent in sedentary bouts of 31-40 min (h)	0.94 ± 0.40
Total no. of sedentary bouts of 41-60 min	1.60 ± 0.74
Total duration spent in sedentary bouts of 41-60 min (h)	1.30 ± 0.61
Total no. of sedentary bouts of >60 min	2.24 ± 1.14
Total duration spent in sedentary bouts of >60 min (h)	3.98 ± 2.33
Total no. of sedentary bouts of >90 min	1.15 ± 0.79
Total duration spent in sedentary bouts of >90 min (h)	2.63 ± 1.95
Total no. of sedentary bouts of >2 h	0.63 ± 0.62
Total duration spent in sedentary bouts of >2 h (h)	1.70 ± 1.73
Total no. of sedentary bouts of >3 h	0.16 ± 0.23
Total duration spent in sedentary bouts of >3 h (h)	0.60 ± 0.88
Total no. of sedentary bouts of >4 h	0.04 ± 0.09
Total duration spent in sedentary bouts of >4 h (h)	0.19 ± 0.41

Values are means ± SD

Table 3.4: Patterns of sedentary behaviour

Total day spent in sedentary bouts of <5 min (%)	2.30 ± 1.2
Waking day spent in sedentary bouts of <5 min (%)	3.54 ± 1.74
Total sedentary time spent in sedentary bouts of <5 min (%)	3.10 ± 2.95
Waking sedentary time spent in sedentary bouts of <5 min (%)	6.05 ± 4.60
Total day spent in sedentary bouts of 5-10 min (%)	2.94 ± 1.40
Waking day spent in sedentary bouts of 5-10 min (%)	4.55 ± 2.07
Total sedentary time spent in sedentary bouts of 5-10 min (%)	3.95 ± 2.32
Waking sedentary time spent in sedentary bouts of 5-10 min (%)	7.73 ± 5.34
Total day spent in sedentary bouts of 11-20 min (%)	5.93 ± 2.01
Waking day spent in sedentary bouts of 11-20 min (%)	9.19 ± 3.03
Total sedentary time spent in sedentary bouts of 11-20 min (%)	7.70 ± 2.83
Waking sedentary time spent in sedentary bouts of 11-20 min (%)	14.55 ± 5.69
Total day spent in sedentary bouts of 21-30 min (%)	5.43 ± 1.84
Waking day spent in sedentary bouts of 21-30 min (%)	8.58 ± 3.08
Total sedentary time spent in sedentary bouts of 21-30 min (%)	6.89 ± 2.22
Waking sedentary time spent in sedentary bouts of 21-30 min (%)	13.03 ± 4.35
Total day spent in sedentary bouts of 31-40 min (%)	3.92 ± 1.65
Waking day spent in sedentary bouts of 31-40 min (%)	6.09 ± 2.50
Total sedentary time spent in sedentary bouts of 31-40 min (%)	5.03 ± 2.14
Waking sedentary time spent in sedentary bouts of 31-40 min (%)	9.35 ± 3.86
Total day spent in sedentary bouts of 41-60 min (%)	5.43 ± 2.55
Waking day spent in sedentary bouts of 41-60 min (%)	8.56 ± 4.17
Total sedentary time spent in sedentary bouts of 41-60 min (%)	6.79 ± 2.99
Waking sedentary time spent in sedentary bouts of 41-60 min (%)	12.63 ± 5.44
Total day spent in sedentary bouts of >60 min (%)	16.59 ± 9.71
Waking day spent in sedentary bouts of >60 min (%)	25.87 ± 14.64
Total sedentary time spent in sedentary bouts of >60 min (%)	20.02 ± 10.93
Waking sedentary time spent in sedentary bouts of >60 min (%)	35.59 ± 17.82
Total day spent in sedentary bouts of >90 min (%)	10.96 ± 8.14
Waking day spent in sedentary bouts of >90 min (%)	17.12 ± 12.28
Total sedentary time spent in sedentary bouts of >90 min (%)	13.13 ± 9.30
Waking sedentary time spent in sedentary bouts of >90 min (%)	23.31 ± 15.29
Total day spent in sedentary bouts of >2 h (%)	7.08 ± 7.19
Waking day spent in sedentary bouts of >2 h (%)	11.19 ± 11.30
Total sedentary time spent in sedentary bouts of >2 h (%)	8.34 ± 8.18
Waking sedentary time spent in sedentary bouts of >2 h (%)	14.83 ± 14.35
Total day spent in sedentary bouts of >3 h (%)	2.50 ± 3.65
Waking day spent in sedentary bouts of >3 h (%)	4.06 ± 5.93
Total sedentary time spent in sedentary bouts of >3 h (%)	2.91 ± 4.21
Waking sedentary time spent in sedentary bouts of >3 h (%)	5.40 ± 7.92
Total day spent in sedentary bouts of >4 h (%)	0.79 ± 1.71
Waking day spent in sedentary bouts of >4 h (%)	1.35 ± 2.94
Total sedentary time spent in sedentary bouts of >4 h (%)	0.95 ± 2.07
Waking sedentary time spent in sedentary bouts of >4 h (%)	1.95 ± 4.30

Values are means ± S

## Appendix H

Table: The Peripheral Arterial Questionnaire scores at week 1 and following 12 weeks of community-based exercise rehabilitation

Domain	Week 1	Week 12
Physical function	33.71 ± 15.97	42.42 ± 22.5
Symptom stability	52.5 ± 14.19	77.5 ± 14.19 <sup>†</sup>
Symptom frequency/burden	53.05 ± 9.21	61.94 ± 13.23 <sup>*</sup>
Treatment satisfaction	71.67 ± 24.28	75.0 ± 22.91
Quality of life	32.5 ± 13.86	52.50 ± 20.05 <sup>†</sup>
Social function	52.08 ± 24.70	72.92 ± 25.88 <sup>*</sup>
Summary Score	39.47 ± 14.35	55.01 ± 18.69 <sup>†</sup>

Values are means ± SD; <sup>\*</sup>p < 0.05 vs. week 1; <sup>†</sup>p < 0.01 vs. week 1