

Effect of Acute Exercise on Postprandial Lipemia and Endothelial Function in Men with Peripheral Arterial Disease

Thesis submitted for the degree of Masters of Science

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of MSc is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

Introduction: Postprandial lipidemia (PPL), defined as an increase in plasma levels of triglyceride-rich lipoproteins following the consumption of a high fat meal (HFM) is associated with endothelial dysfunction. Acute exercise reduces PPL and maintains endothelial function (EF) in healthy adults. The effect of acute exercise on PPL and endothelial function has not been studied in patients with peripheral arterial disease (PAD).

Purpose: To examine the effect of an acute bout of exercise on PPL, vascular inflammation and endothelial function in men with PAD.

Methods: Men (n=8) with PAD underwent two oral fat tolerance tests (OFTT). On the evening prior to each OFTT, participants rested (control), or exercised until they expended 200 Kcal. Blood samples were obtained at baseline and 30 min, 1, 2, 3 and 4 h postprandial. Endothelial-dependent dilation (EDD) and endothelial-independent dilation (EID) were measured in the brachial artery using ultrasonography at baseline, 2 h and 4 h postprandial.

Results: Postprandial TG increased significantly and EDD decreased significantly following the OFTT. An acute bout of discontinuous exercise that resulted in a 200 Kcal expenditure did not significantly attenuate the post prandial TG response or significantly ameliorate the decrease in endothelial vasomotor function. Compared to baseline values, circulating leukocytes, and TNF- α increased ($p < 0.05$) in both conditions 4 h postprandial. There were no changes in C-Reactive Protein (CRP).

Conclusion: Prior exercise has no effect on PPL or EDD following an OFTT in men with PAD.

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LIST OF TERMS AND ABBREVIATIONS

Term	Description
PAD	Peripheral arterial disease
IC	Intermittent claudication
PPL	Postprandial lipemia
HFM	High fat meal
CVD	Cardiovascular disease
TG	Triglycerides
RLP	Remnant like parts
EDD	Endothelial dependant dilation
EID	Endothelial independent dilation
TEE	Total energy expenditure
CLI	Critical limb ischemia
ABI	Ankle brachial index
CRP	C-reactive protein
hsCRP	High sensitivity C-reactive protein
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol

Term	Description
OxLDL	Oxidised LDL
ACH	Acetylcholine
NO	Nitric oxide
TGRL	Triglyceride rich lipoprotein
AUC	Area under the curve
VLDL	Very low density lipoproteins
TNF- α	Tumour necrosis factor alpha
OFTT	Oral fat tolerance test
ECG	Electrocardiogram

Chapter 1

INTRODUCTION

Peripheral arterial disease (PAD) is a distinct atherothrombotic syndrome marked by stenosis of peripheral arteries, typically those in the lower extremities, causing inadequate blood flow to the limbs. Intermittent claudication (IC) is the most common symptom of PAD and is characterized by the onset of pain in the lower extremities during exercise that is relieved with rest (6).

Supervised aerobic exercise has been shown to increase maximum walking distance in patients with PAD [1]. However, recent studies indicate that acute bouts of exercise to the onset of intermittent claudication may cause a systemic thrombo-inflammatory response in this population [2,3]. This may be due to the fact that exercising to the onset of intermittent claudication, followed by reperfusion on rest, results in the formation of oxygen-derived free radicals (ODFR) and cytokines (3-5). Oxygen-derived free radicals cause lipid peroxidation, which results in structural damage to the vascular endothelium and increased vascular permeability.

The endothelium is a 0.2- to 4- μm -thick monolayer of squamous endothelial cells that line the lumen of the entire surface of the vascular tree and plays an important role in the regulation of vascular tone, haemostasis, immune and inflammatory responses (1). Damage to the endothelium from mechanical forces and processes related to cardiovascular disease risk factors and the resulting inflammatory response can generate a pro-thrombotic environment favourable for the initiation and progression of atherosclerosis

(1-4), the most frequent underlying cause of PAD. The risk factors for PAD include age, male gender, family history, diabetes, smoking, hypertension and hyperlipidaemia (5, 6).

Postprandial lipemia (PPL) describes the increase in plasma levels of triglyceride-rich lipoproteins for up to 8 h following the consumption of a high fat meal (HFM), and may represent an independent risk factor for atherosclerotic cardiovascular disease (ACVD)(7). In contemporary Western societies the vasculature is commonly exposed to prolonged postprandial hyperlipidemia. It has been estimated that individuals consuming a typical Western diet spend approximately 18 h per day in a postprandial state (8). The adverse effect of postprandial triglycerides (TG) is thought to be mediated by proatherogenic lipolysis products of nascent triglyceride-rich lipoproteins, such as remnant like particles (RLP) and fatty acids. Even a transient increase in these proatherogenic products may increase pro-coagulant and pro-inflammatory activity (7) and impair endothelial dependent vasodilatation (EDD) (9), a predictor of atherosclerosis and future cardiovascular events.

Physical activity and physical fitness are associated with a lower incidence of CVD. The relative risk of death from CVD in the most active individuals is half that of their sedentary counterparts (10). The cardioprotective effect of exercise may be mediated in part by an influence on TG metabolism. Acute exercise 1-16 h prior to feeding a standard HFM can significantly reduce the postprandial TG response in adults (11, 12).

The available evidence suggests that total energy expenditure (TEE) may be more critical than exercise intensity in influencing postprandial TG metabolism. In addition, the exercise benefits can be accumulated over the course of a day in two or three shorter bouts. The possibility of accumulating benefit with multiple short bouts is particularly important for

patients with PAD as in many instances the disease restricts their ability to exercise for prolonged periods. To date, no studies have evaluated the effect of exercise on postprandial lipemia in patients with PAD. The purpose of this study is to examine the effects of an acute bout of exercise on postprandial lipemia and endothelial function in men with PAD.

Specific Aims

1. To compare the effects of a standardised OFTT with and without a prior acute exercise bout on postprandial TG in men with PAD
2. To compare the effects of a standardised OFTT meal with and without a prior acute exercise bout on circulating markers of inflammation in men with PAD
3. To compare the effects of a standardised OFTT with and without a prior acute exercise bout on brachial artery flow mediated dilation in men with PAD

Study Hypothesis

1. In men with PAD, the postprandial TG response to a standardised OFTT will be significantly lower when the meal is preceded with an acute exercise bout compared to no exercise
2. In men with PAD, the circulating levels of inflammatory markers in response to a standardised OFTT will be significantly lower when the meal is preceded with an acute exercise bout compared to no exercise
3. In men with PAD, brachial artery flow mediated dilation following a standardised OFTT will be significantly greater when the meal is preceded with an acute exercise bout compared to no exercise

Chapter 2

LITERATURE REVIEW

Peripheral Arterial Disease

Peripheral arterial disease (PAD) has been defined as obstruction of blood flow into an arterial tree excluding the intracranial or coronary circulations (Figure 2.1) (13).

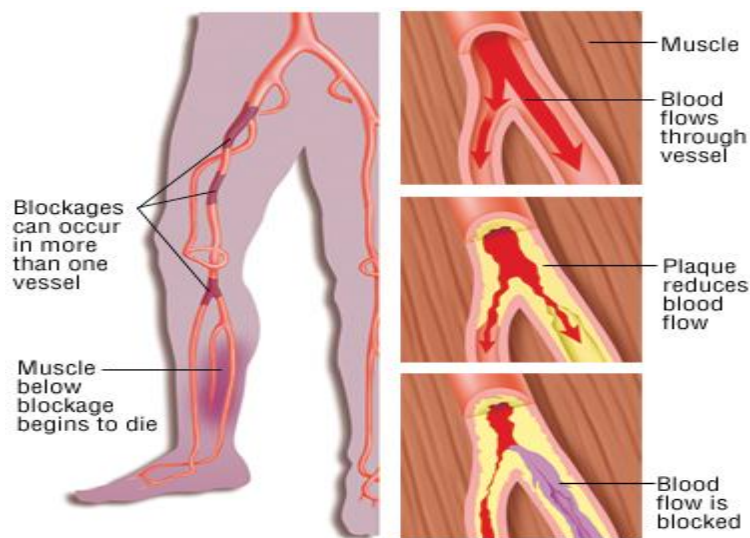


Figure 2.1: Development of atherosclerotic lower extremity PAD

It represents a continuum of disorders that range from asymptomatic PAD, symptomatic IC, and critical limb ischemia (CLI) (Figure 2.2). Patients with PAD develop atherosclerotic occlusive lesions in the arteries supplying the lower extremities, and limitation in blood flow to active muscles is the primary pathophysiologic event. A number of arterial segments can be affected, including the inflow vessels (aorta and iliac arteries), and also the femoral, popliteal, and tibial vessels in the leg (14).

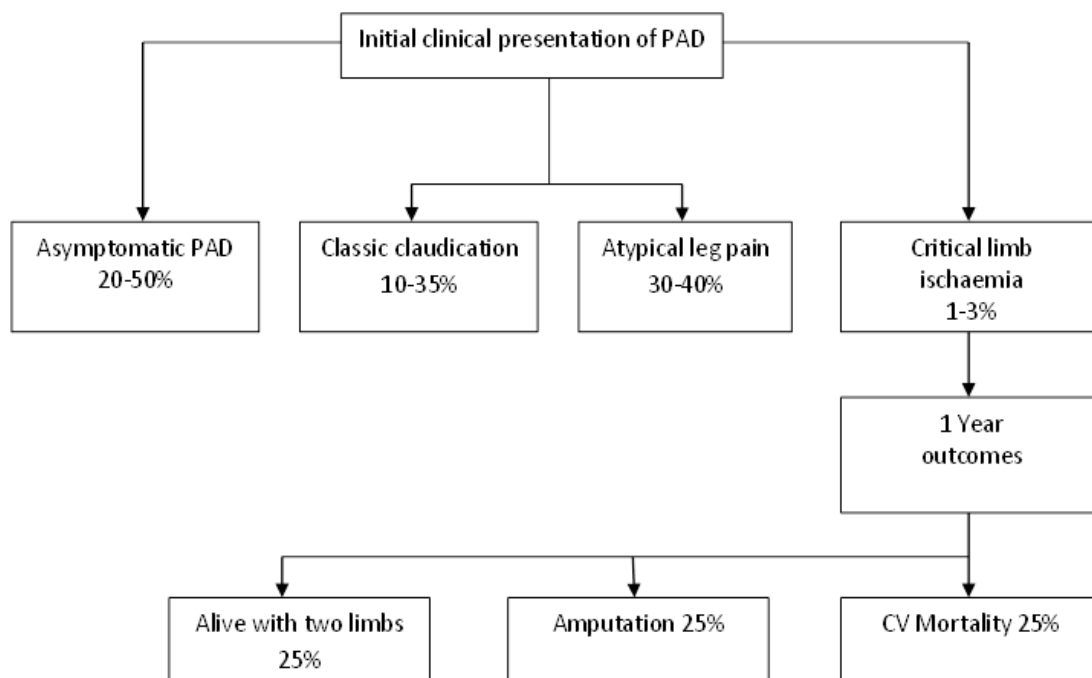


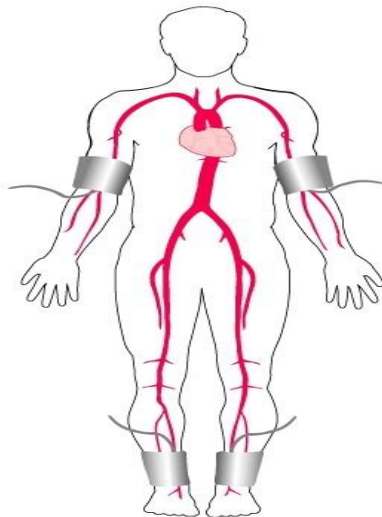
Figure 2.2: Natural history of atherosclerotic lower extremity PAD

The figure presents 1-year outcomes for patients who present with critical limb ischemia. As illustrated, all patients with lower extremity PAD are at risk of progressive limb ischemia and are at high risk of fatal and nonfatal atherothrombotic events, including myocardial infarction and stroke

PAD prevalence is 3-12% in the general population, with IC prevalence of 1-2% (15; 16). PAD prevalence increases significantly with age, affecting up to 20% of patients >75 years (17, 18). Coexistent coronary artery disease (CAD) and cerebrovascular disease (CBVD) are common in patients with PAD, particularly in the elderly population. The coexistence of CAD and stroke was found to be 68% and 42% respectively, among men > 50 years of age in an academic, hospital-based geriatric practice (19). The prevalence of PAD may be vastly underestimated due to the fact that the majority of individuals with lower extremity PAD do not experience recognisable ischemic symptoms in the limb, and are therefore classed as being 'asymptomatic' (5).

Diagnosis of PAD

The ankle brachial index (ABI) is a simple, reproducible, and cost-effective non-invasive assessment that is commonly used to detect lower-extremity arterial stenosis (5). It is determined by measuring systolic blood pressure in the posterior tibial and/or the dorsalis pedis arteries in both legs, with the lowest ankle pressure then divided by the brachial systolic blood pressure (Figure 2.3). When used by trained technicians, ABI is a reliable and valid assessment tool for detecting stenosis $\geq 50\%$ in leg arteries (20).



ABI is defined as:

Highest ankle systolic pressures (posterior tibial or dorsalis pedis) mmHg

Highest brachial artery systolic pressure mmHg

Figure x

Normal ABI ranges from 0.91 to 1.30. Decreases in ABI are consistent with PAD, and an ABI cut-off of ≤ 0.9 is used as the indicator of PAD (22) (Table 2 1). Mild to moderate PAD usually produces an ABI in the range of 0.41 to 0.90. A reading below 0.40 indicates the presence of severe PAD. Resnick et al (22) examined all-cause and CVD mortality in relation to low and high ABI in 4393 American Indians in the Strong Heart Study. They showed a U-

shaped association between ABI and mortality, with significantly increased risk in both the <0.90 and >1.40 groups.

ABI	Interpretation
>1.30	Normal, but considered incompressible (calcified) arteries
0.91–1.30	Normal range
0.41–0.90	Mild to moderate PAD
0.5–0.74	Consistent with moderate peripheral arterial disease (intermittent claudication or rest pain)
< 0.4	Severe PAD

Table 2.1: Interpretation of ankle-brachial index (ABI) values

Invasive methods such as duplex scanning, magnetic resonance imaging (MRI), and digital subtraction angiography are commonly used for anatomical localisation of arterial disease prior to intervention rather than for initial diagnosis.

Intermittent Claudication

Intermittent claudication (IC), defined as exercise-induced muscle pain that is relieved with rest, is one the most common symptoms in patients with lower extremity PAD (4). Symptoms may be described as pain, achiness, a sense of fatigue, or nonspecific discomfort that occurs with exercise. Symptoms normally dissipate following several minutes of rest (23).

The location of the pain is an indication of the site of arterial occlusion (Figure 2.4) (24). Claudication of the calf is usually the result of an occlusion in the superficial femoral artery. The most frequently affected artery in intermittent claudication is the popliteal artery; symptoms are most common in the calf muscles (25). This artery is an extension of the femoral artery and continues below the knee where it branches off and carries blood to

the muscles in the calf and foot. Hip, thigh, and buttock claudication are associated with occlusion of the aorta and iliac arteries (25).

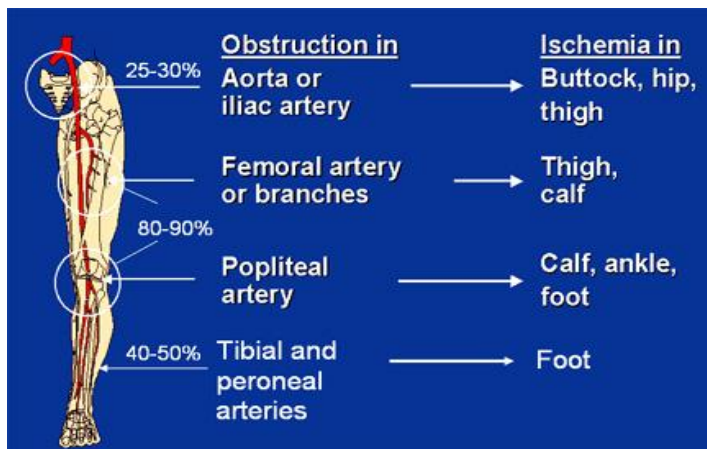


Figure 2.4: Location of the pain associated with PAD

Critical Limb Ischemia

CLI is defined as PAD causing lower-extremity pain at rest or imminent limb loss caused by severe impairment of blood flow to the affected limb, and is classified as Fontaine Class III (26). The Fontaine Classification is the method by which chronic peripheral ischaemia is classified. CLI is a manifestation of PAD that describes patients with chronic ischemic rest pain, foot and leg ulcers, or gangrene. Discomfort is often worse in a supine position and pain can be reduced when the limb is placed in a dependant position. CLI results from the presence of multilevel occluded vessels that impair blood flow and distal perfusion pressure to a level insufficient to satisfy the nutritive needs of the limb at rest (13). Approximately 500–1000 people per million of the population are diagnosed with CLI (27). PAD patients with diabetes are at a greater risk of CLI, and risk of amputation is increased 5 fold in this population (28).

The clinical diagnosis of CLI should be confirmed by haemodynamic parameters such as the ankle or toe systolic pressure. Ischemic rest pain most commonly occurs below an

ankle pressure of 50 mmHg or a toe pressure <30 mmHg. Up to 30% of patients with lower extremity PAD will progress from IC to CLI over the course of their disease (27, 29). Approximately 25% of patients die and a further 30% require a major amputation one year after CLI diagnosis (27). Owing to the high risk of limb loss and fatal and nonfatal vascular events, it is vitally important to diagnose CLI quickly. The severity of the symptoms of PAD can be classified according to either the Fontaine or Rutherford scales (Table 2.2).

Table 2.2: Classification of peripheral artery disease (Fontaine and Rutherford scale adapted from Norgren *et al*, 2007)

Fontaine		Rutherford		
Stage	Clinical	Grade	Category	Clinical
I	Asymptomatic	0	0	Asymptomatic
IIa	Mild claudication	I	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

Risk Factors in PAD

The primary etiology of PAD is atherosclerosis, and the risk factors are similar to those for coronary artery and cerebrovascular disease. Age, smoking and diabetes are the most powerful risk factors for PAD. Others include African American ethnicity, dyslipidemia, hypertension, hyperhomocysteinemia, elevated C-reactive protein (CRP) levels, and chronic renal insufficiency (30).

The prevalence of PAD increases with age. A strong association between advanced age (≥ 70 years) and PAD prevalence has been shown in the National Health and Nutrition Examination Survey (NHANES) 1999–2000. The prevalence of PAD was 4.3% in subjects aged 40 years or older compared with 14.5% in those aged 70 years or older (30). In 1985, Criqui *et al.*, reported the prevalence of PAD to be 2% to 3% in individuals aged 50 years or less compared with 20% in those aged greater than 75 years (33).

Diabetes and smoking are the two most common modifiable risk factors for PAD. Results from a survey conducted in Sweden found that 21% of diabetic patients had signs of PAD (34). In a cross-sectional analysis of a 4153 Greek adults (37), the odds ratio for vascular disease was 1.94 for patients with the metabolic syndrome, 3.04 for patients with the metabolic syndrome and diabetes, and 1.48 for patients with the metabolic syndrome but no diabetes. The association of smoking and PAD is dose-dependent; the risk of PAD increases with years smoked and packs smoked per year. (38). The incidence of PAD increases from 2.6% in never smokers to 4.5% in moderate smokers (0-25 pack/year) to 9.8% in heavy smokers (>25 pack/year) (39). More than 80% of individuals with PAD are current or former smokers (35, 40). Individuals of African American ethnicity are approximately 3-4 times more likely to develop PAD compared to their non-African white counterparts (31, 32).

The common dyslipidemia found in PAD is similar to insulin resistance, enforcing the strong association between diabetes with PAD (41). Patients with PAD have higher circulating levels of triglycerides (42), lower levels of high-density lipoprotein cholesterol (HDL-C) and higher levels of total cholesterol than healthy individual's subjects (42). The risk

of PAD increases by 5-10% for each 10mg/dL increase in total cholesterol (43). In a cross sectional study involving 708 men, aged 55-74., Planas *et al.*, (44) found that the waist-to-hip ratio was independently associated with PAD. CRP, an acute-phase reactant produced by the liver in response to inflammation, is one of many circulating inflammatory markers that is related to CAD and may play a role in its pathogenesis. There is evidence of a linear relation between CRP concentrations and the severity of PAD (34).

Treatment of PAD

The goals of treatment for individuals with PAD are to prevent progression of PAD, prevent coronary or cerebrovascular events, and for those with IC to relieve symptoms, improve functional capacity and improve quality of life (34). For all patients across the PAD spectrum risk factor modification is recommended, including weight loss, smoking cessation, lipid-lowering therapy, anti-platelet therapy and increased exercise (23). Recommendations for individuals with IC include supervised exercise interventions and pharmacological therapies (Cilostazol and pentoxifylline). Cilostazol reduces the pain of intermittent claudication by dilating the arteries, thereby improving the flow of blood and oxygen to the legs. Pentoxifylline improves blood flow by making it easier for red blood cells to pass through vessels. It also decreases the viscosity of blood. Surgical or endovascular intervention is usually reserved for patients with severe, lifestyle-limiting symptoms that do not adequately respond to conservative management, including pharmacologic treatment.

Treatment of PAD – Exercise

The physiological, metabolic, and mechanical alterations that occur during periods of exercise presumably stimulate an adaptive response that ultimately reduces claudication

symptoms and improves functional capacity. Exercise training improves maximal walking ability by an average of 150% (45-51). Improvements in walking ability are most often attained when exercise sessions >30 min are undertaken at least 3 times per week for 6 months and involve walking to near maximal pain (52, 53). The exact mechanisms by which exercise training yields improvements in walking ability remain unclear.

The increased blood flow in response to repeated periods of exercise-induced hyperemia may alter vascular structure and function (54). Angiogenesis in response to exercise training may increase blood flow to skeletal muscle, distal to the stenosis (55). However, studies examining the effect of exercise training on leg blood flow have been equivocal. Gardner *et al.*, 2001 found that 6 months of exercise training increased reactive hyperaemic blood flow by 27% and max calf blood flow by 30% in patients with PAD. The improvements in blood flow were associated with improved walking ability. However, resting blood flow and ABI were not altered in response to training (56). In contrast, a number of studies have reported no changes in leg blood flow in patients with improved walking ability after an exercise program (57, 58), suggesting that other mechanisms may be responsible for the large improvements observed following exercise training.

Impaired endothelial dependent vasodilation (EDD) has been demonstrated in patients with PAD (59) and in patients with risk factors for atherosclerosis, such as type 2 diabetes, (60) hypertension, and hyperlipidemia (61). Results from studies in patients with other chronic diseases, suggest that exercise training improves endothelial-dependent vasodilatation (62, 63). Brachial artery flow mediated dilation (FMD) was found to be

significantly decreased in men with PAD, 30 min and 2 h following 10 min of treadmill exercise to intolerable ischaemic pain in the affected lower extremity exercise (64).

Exercise training may improve abnormal hemorheology in patients with claudication, thereby facilitating oxygen delivery (65). Hemorheology refers to the properties of flowing blood and its elements specifically, plasma viscosity, hematocrit, red cell deformability, and red cell aggregation. Ernst & Matrai 1987 et al., found that treadmill walking at 3 km/hr to absolute claudication twice a day, five days per week for 2 months resulted in significant normalization of blood and plasma viscosity, blood cell filterability, and red cell aggregation. No changes occurred in a non-exercise control group (66).

Chronic ischemia of PAD results in metabolic abnormalities in the affected skeletal muscle. PAD is associated with increased plasma and skeletal muscle short-chain acylcarnitine content (23). Resting muscle short-chain acylcarnitine content is inversely correlated with claudication limited functional capacity (23). The degree of metabolic dysfunction is a better predictor of functional capacity than haemodynamic measurements. In patients with unilateral claudication, the increase in acylcarnitines only occurred in the affected skeletal muscle, indicating that this metabolic abnormality was specific to the limb with reduced blood flow (67). Patients with the greatest accumulation of acylcarnitines had the lowest treadmill exercise performance. Treadmill exercise training, but not resistance training, reduces plasma levels of acylcarnitine accumulation (68). Improvements in maximal walking ability may also be related to changes in walking efficiency or improvement in the tolerance of claudication pain (23).

Treatment of PAD - Pharmacotherapy

Effective drug therapies include aspirin, pentoxifylline and cilostazol (71). Pentoxifylline is a methylxanthine derivative with hemorrheologic and immunomodulating properties. It reduces blood viscosity, changes the morphology of red blood cells, and decreases the potential for platelet aggregation and thrombus formation. Cilostazol is a quinolinone derivative that inhibits cellular phosphodiesterase III. It suppresses platelet aggregation, activates lipoprotein lipase, and causes arterial dilation (69). A large randomly controlled prospective trial found that cilostazol was significantly better than pentoxifylline or placebo for increasing walking distances in patients with moderate to severe PAD (70). The improvement in treadmill walking performance with pentoxifylline was not significantly different from the placebo (70). Data supporting use of pentoxifylline for claudication is weak, and pentoxifylline is not generally accepted as efficacious.

Aspirin and clopidogrel are often used as antithrombotic therapies in PAD. They have not been shown to improve symptoms of intermittent claudication but are important in reducing cardiovascular complications associated with the presence of atherosclerosis and PAD. The Antithrombotic Trialist Collaboration (ATT) found that even a low dose of aspirin (75–150mg) reduced vascular events by 32% in patients with PAD (71). However, the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial found that clopidogrel treatment in patients with symptomatic PAD was more effective than aspirin in preventing ischemic events, 5.83% and 5.32% for clopidogrel and aspirin, respectively (72).

Treatment for CLI can be quite complex, but the primary aim should be to reduce the pain and improve blood flow in order to minimize the need for amputation. The majority of

patients with CLI will ultimately require a revascularization procedure. Endovascular therapy is now the primary strategy for management of CLI. Patients with CLI not eligible for arterial reconstruction, prostanoids are the only vasoactive drugs with proven efficacy. Creutzig et al., (73) concluded in a recent meta-analysis of randomized placebo-controlled trials, of patients with stage III or IV PAD that PGE1 therapy not only had significant beneficial effects over placebo on ulcer healing and pain relief, but also increased the number of patients surviving with both legs at 6-months follow-up.

Atherosclerosis and Endothelium

Atherosclerosis is the most frequent underlying cause of PAD (6). It is characterized by the accumulation of monocyte-derived macrophages within the vessel wall and the accompanying inflammatory response. This process is initiated by the transmigration and retention of low density lipoprotein cholesterol (LDL-C) in the sub-endothelial extracellular matrix, where it is subjected to chemical modification, and converted to oxidized LDL (OxLDL), a key pathogenic mediator of atherosclerosis (Figure 4.5) (107).

OxLDL stimulates inflammatory signaling by endothelial cells, resulting in the expression of membrane-bound adhesion molecules that facilitate the attachment of circulating leukocytes to the vessel wall, and induce smooth muscle cell proliferation. Attachment of monocytes facilitates their migration across the endothelium into the subendothelial space where they differentiate into macrophages. Macrophages engulf OxLDL, leading to the formation of large foam cells, a major component of early lesions. Systemic inflammatory markers of vascular inflammation include an elevated white blood cell count (WBC), and high circulating levels of CRP and TNF- α .

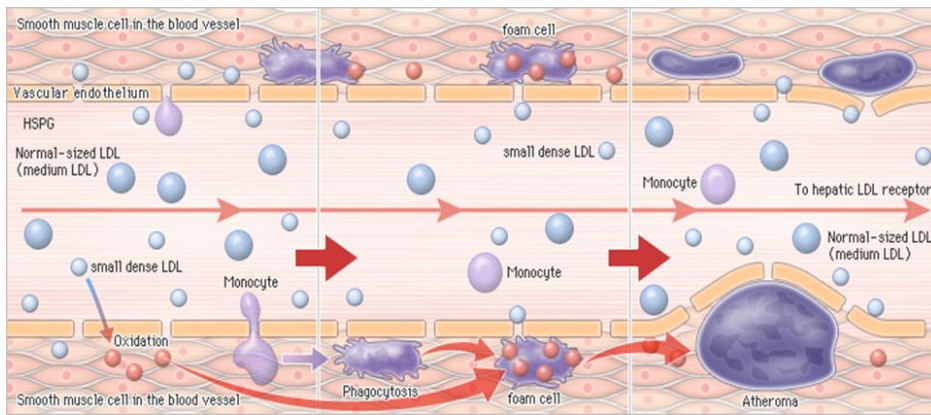


Figure 4.5: Summary of the atherosclerotic disease progression and development

Endothelium and Endothelial Function

Endothelial cells form a continuous monolayer that line blood vessels of the entire vascular tree, and represent a surface area of approximately 4000 to 7000 m². Malpighi's discovery in the 17th century of the endothelium as a physical separation between blood and tissue with no substantial functionality persisted throughout the nineteenth and twentieth centuries (74). Landmark studies in the 1980's demonstrated the obligatory role of endothelial cells in acetylcholine (ACh)-mediated vasodilation (75) and in the paradoxical ACh-mediated vasoconstriction of atherosclerotic vessels (76). The endothelium is now viewed as a complex dynamic barrier that plays a crucial role in maintaining vascular integrity.

The endothelium-dependent vasodilatory response to exogenously administered acetylcholine (ACh) is attributable to the production and diffusion of nitric oxide (NO), a hydrophobic diatomic gas produced in response to changes in shear forces, or via a variety of agonists acting on specific endothelial cell membrane receptors (Figure 4.6) (81). In addition to its vasodilatory effects, NO counteracts leukocyte adhesion to the endothelium, attenuates vascular smooth muscle proliferation and migration, influences the production

of superoxide anion, suppresses platelet aggregation and protects against vascular injury, inflammation, and thrombosis, key events in the development and progression of atherosclerosis (77).

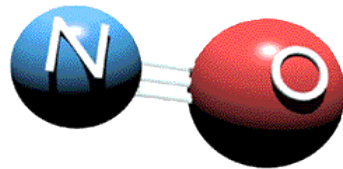


Figure 4.6: Molecular structure of nitric oxide

Cardiovascular disease risk factors such as aging and a family history of CVD, active and passive smoking, lipid disorders, hypertension, diabetes mellitus, obesity, and physical inactivity, among others, have been shown to promote the development of atherosclerosis through their deleterious effects on endothelial structure and function (78). Damage to the endothelium caused by cardiovascular disease risk factors results in the reduction of nitric oxide (NO) bioavailability. The progressive inability of endothelial cells, exposed to risk factors, to generate sufficient NO promotes a vascular phenotype prone to atherogenesis (79).

The functional and structural integrity of the endothelium is critical in maintaining vascular homeostasis, and the presence of atherosclerosis has been shown to affect the vasomotor responses of the diseased vessel to shear stress, and a number of vasoactive compounds (80). Flow-mediated dilation (FMD) is a commonly used non-invasive procedure to assess endothelial function. It is based on the assumption that healthy, intact endothelial cells can detect, and dilate, to changes in shear stress following a brief period of occlusion (62). The percentage change in arterial diameter in response to shear stress can be measured using high-resolution B mode ultrasonography, and is believed to be endothelial-

dependent and mediated by NO. Diameter changes are also measured after administration of glyceryl-trinitrate to assess the response of the vessel to endothelium-independent vasodilation (81).

The prognostic significance of endothelial function as a predictor of cardiovascular events in healthy individuals and in patients with CAD and those with normal coronary arteries and risk factors for atherosclerosis has been addressed in a number of studies (82, 83, 84,). Interventions proven to reduce cardiovascular risk, such as weight loss, smoking cessation, lipid-lowering therapy, and angiotensin-converting enzyme (ACE) inhibitors been shown to improve endothelial function and decrease cardiovascular risk (85).

Endothelial Function – Exercise

Evidence from both cross-sectional and longitudinal studies indicate that cardiorespiratory fitness delays the decrease in endothelial function associated with ageing (86) and reverses impaired endothelial function in individuals with atherosclerotic CVD (87). Exercise-induced improvements in vascular function appear to occur more readily and with remarkable consistency in vessels with antecedent functional impairment. Improvements in endothelial function induced by exercise training are attributable to a combination of enhanced vasodilatory capacity and arterial remodelling (62).

Observations that a single bout of exercise can transiently alter atherosclerotic CVD risk factors (89) have led to the notion that perhaps some of the effects of exercise on endothelial function may be attributable to the acute effect of exercise. A number of studies have investigated the effect of an acute bout of exercise on endothelial function in

healthy and disease populations. Table 2.4 provides a summary overview of studies that have examined the effect of acute exercise on endothelial function.

Cycling for 30 min at 50% VO_{2peak} increases brachial artery FMD in young female smokers (90), and treadmill exercise for 34 min at 60% VO_{2max} almost doubled brachial artery FMD in premenopausal women (91) but had no effect on brachial artery FMD in postmenopausal women. Both low volume high-intensity interval exercise and moderate-intensity endurance exercise significantly increased absolute FMD and normalized brachial artery FMD 1 h post exercise in men and women with CAD (92). Brachial artery FMD is enhanced 1 h following 45 min acute bouts of low-, moderate- and high-intensity treadmill exercise in active overweight men, but is attenuated in inactive overweight men (93).

In contrast a number of studies have found a transitory functional deterioration in FMD in healthy individuals 1 h after a single bout of high-intensity interval running (94), non-elite runners 1 h after completing a marathon, and healthy male smokers compared to non-smokers (7.7 v 4.1%) immediately following 40 min of submaximal steady-state exercise on a cycle ergometer (95).

Brachial artery FMD was found to be significantly decreased in men with PAD, 30 min and 2 h following 10 min of treadmill exercise to intolerable ischaemic pain in the affected lower extremity exercise (64). In contrast, Silvestro et al., found that an acute bout of treadmill exercise to the onset of claudication has no effect on brachial artery FMD measured 5 min post exercise in men and women (62 ± 2 year) with PAD (96). In contrast, FMD was significantly impaired following treadmill exercise to maximal claudication pain, demonstrating that exercise-induced ischemia further deteriorates FMD. Intravenous

administration of vitamin C ameliorates the impaired FMD response following treadmill exercise to maximal claudication pain only. Others have shown that antioxidant treatment can prevent acute impairment in endothelial function. The benefits of vitamin C on endothelial function are believed to be due to superoxide scavenging, and/or inhibition of LDL-C that takes place during high-intensity exercise. Vitamin C is also an important regulator of the intracellular redox (96).

Table 2.3: Studies that examined the effect of acute exercise on endothelial function

Author	Patient Cohort	Mode of Exercise	Duration	Intensity	FMD
Cosio-Lima (2006)	Renal Transplant	TM Walking	30 min	70 - 85% HRmax	—
Gaenger (2001)	Smoking	Cycling	40 min	50% VO ₂ peak	↓
Silvestro (2002)	PAD	TM Walking	Max claudication	3 km.h ⁻¹ @ 3%	↓
Harvey (2005)	Pre & post-menopausal	TM Walking			↑
Rooks (2011)	Smoking/non-smokers	Cycling	59% VO ₂ peak	30 min	↑
Jones (2008)	Diurnal Variation	Intermittent Cycling	3 x 10 min	70% VO ₂ peak	↑
Jones (2010)	Diurnal Variation	Intermittent Cycling	3 x 10 min	70% VO ₂ peak	↑
Rognmo (2005)	CV Fitness	HII Running	5 x 5 min	90% HRmax	↓ high fit — low fit
Harris (2008)	Overweight (8 active)	Intermittent Cycling	45 min	25% VO ₂ peak 50% VO ₂ peak 75% VO ₂ peak	↑
Llewellyn (2012)	Healthy	TM Running	30 min	60% VO ₂ max	↓
Currie (2012)	CAD	Intermittent vs. Continuous	10 x 1min vs 30 min	80% vs. 55% POpeak	↑
Dawson (2008)	Nonelite Runners	Marathon	42.2 km	n/a	—
Joras	PAD	TM Walking	Max claudication	3.2 km.h ⁻¹ @ 12%	↓

CAD, coronary artery disease; FMD, flow-mediated vasodilation; Tm, Treadmill; HII, High intensity interval; HRmax, Heart rate max; PAD, peripheral arterial disease; VO₂, oxygen consumption; PO, power output.

Supervised treadmill exercise has been shown to improve vascular function, in patients with PAD. McDermott *et al.*, randomly assigned 156 patients with PAD to 6 months of supervised treadmill exercise, lower extremity resistance training, or a sedentary control group. Improvements in brachial artery FMD were significantly greater at 6 months in the treadmill group than the resistance training, or the control group. Changes in brachial artery FMD in the resistance training group were not different from the control group. Improvements in the 6-min walk test and maximum treadmill walking time were significantly greater in the treadmill exercise group than the resistance training group (97). Intermittent treadmill walking at 2.0 mph to near-maximal claudication pain 3 days a week for 6 months has also been shown to significantly improve brachial artery FMD. Time to onset of IC pain increased by 94%, and the time to maximal claudication pain increased 43% with exercise (98).

Another acute study also showed reduced FMD response following acute exercise in patients with PAD (100). The exercise protocol involved walking at 3 km/hr and a 3% grade with a 3% grade increase every 2 minutes up to a maximum of 15%. Patients exercise was stopped when claudication pain became intolerable, and FMD was assessed 5 minutes later. In patients with peripheral arterial disease, calf pain during walking, followed by a period of rest to allow reperfusion of the ischemic limb, induces increased oxidative stress and a marked inflammatory response (42, 48, 100). By reducing nitric oxide availability both these events may be responsible for the acute systemic endothelial insult observed in the present acute studies.

Lipids

Lipids are a heterogeneous group of hydrophobic organic molecules that have a number of important physiological functions including the formation of membranes, energy storage, cellular signalling, protection, insulation and the production of steroid hormones and bile acids. They can be broadly classified as fatty acids, acylglycerols, phospholipids, eicosanoids, steroids and lipoproteins (128).

Acylglycerols are composed of a glycerol molecule with one (monoacylglycerol), two (diacylglycerol) or three (triacylglycerol) fatty acids attached at the hydroxyl group. Triacylglycerols, also termed triglycerides (TG), are the most abundant lipid in the body. Triglyceride and cholesterol are solubilised by their incorporation into small lipid-protein complexes termed lipoproteins. Lipoproteins are classified according to their density as chylomicrons, very low density lipoproteins (VLDLs), intermediate density lipoproteins (IDLs), low density lipoproteins (LDLs) or high density lipoproteins (HDLs). They contain different concentrations of protein, triglyceride, cholesterol, cholesterol esters and phospholipids (129).

Triglyceride is the principal form in which fat is present in the diet. Following their hydrolysis by pancreatic lipase, TGs combine with proteins, free cholesterol and phospholipids in the intestinal mucosal cells to form nascent chylomicrons. The TG rich nascent chylomicrons are secreted into the lymphatic system and enter the circulation at the thoracic duct (129).

Circulating nascent chylomicrons acquire additional apoproteins, Apo E and Apo C-II, from high density lipoprotein cholesterol (HDL-C) and become mature chylomicrons (128)

that are approximately 90% triglyceride, 5% cholesterol, 4% phospholipid and 1% protein. During their passage through the circulation, chylomicrons bind to lipoprotein lipase (LPL) on the surface of capillary endothelial cells resulting in the hydrolysis of TG to fatty acids and glycerol in a reaction that requires the presence of Apo C-II (128). Fatty acids can then be oxidised as fuel by muscle or other cells, or alternatively they can be re-esterified to triglycerides and stored in adipose tissue (128). Following removal of triglyceride the chylomicron is termed a chylomicron remnant. Remnants are removed from circulation by hepatic cell surface receptor proteins.

In addition to dietary TG, the liver also plays a key role in the synthesis and transport of lipids. Hepatic derived cholesterol and triglyceride along with Apo B-100, phospholipid and small amounts of Apo E and Apo C are packaged into nascent VLDL particles (128) that consists of approximately 50% triglyceride, 20% phospholipids, 20% cholesterol and 10% protein (130). Following their release into the circulation, nascent VLDL particles acquire Apo E and Apo C from circulating HDL. As with chylomicrons, TG is hydrolysed to free fatty acids and glycerol by lipoprotein lipase (130). Some of the VLDL remnants are cleared from circulation by binding of Apo B-100 and Apo E to hepatic LDL receptors (128). The remaining triglyceride-depleted VLDL remnants are termed intermediate density lipoprotein (IDL). Through the action of hepatic lipase (HL) IDL can be further metabolised to produce low density lipoprotein (LDL) (128).

LDL is made up of approximately 50% cholesterol, 25% protein, 20% phospholipids and 5% triglycerides (130) and functions to deliver free cholesterol to peripheral tissues (131). LDL particles are cleared from circulation primarily by hepatic LDL receptors.

Postprandial Lipemia

PPL is characterized by an increase in plasma levels of TGRL for up to 8 h following the consumption of a HFM. Studies in healthy populations and those with CVD and CVD risk factors and clinical populations have shown that the postprandial period has a significant deleterious effect on endothelial function, as evidenced in reduced brachial artery FMD, increases in circulating adhesion molecules, cytokines and endothelial microparticles (1, 102, 103, 9). The maximum impairment in EDD occurs 4 h postprandial, coinciding with the peak TG concentration, and continues for up to 8 h (9, 95). Consecutive HFM causes further dysfunction to the endothelium, and greater oxidative stress for each consecutive meal (11).

The concept of atherosclerosis as a postprandial phenomenon was first proposed by Zilversmit (1979) (104), and is related to the increased production of reactive oxygen species (ROS) including superoxide anion O_2^- , (105, 106, 103) which in turn reduces NO bioavailability. A reduction in NO bioavailability has been identified as a primary pathogenic factor responsible for endothelial dysfunction. Nitric Oxide (NO) is produced by nitric oxide synthase (NOS) which catalyzes the conversion of L-arginine to NO and L-citrulline. Shear stress produced by laminar blood flow stimulate eNOS phosphorylation and is responsible for basal endothelial NO tone. A decrease in eNOS activity or oxidative inactivation of NO have been shown to reduce NO bioavailability (38) Peluffo G, Radi R. Biochemistry of protein tyrosine nitration in cardiovascular pathology. *Cardiovasc Res* 75: 291–302, 2007.

The reaction of NO reacts with superoxide radicals (O_2^-) results in a decrease in its concentration and the formation of peroxynitrite ($-OONO$). Cardiomyocytes and endothelial and smooth muscle cells are major sites of O_2^- production in the cardiovascular system.

When O_2^- levels increase, NO is consumed and peroxynitrite is formed, hampering the diffusion of NO. The reaction of NO with O_2^- results in a loss of its protective action and the formation of peroxynitrite, a potent pro-oxidant. This may result in a shift in the anti-atherogenic actions of NO to a pro-atherogenic oxidative phenotype.

Prospective studies in healthy population groups indicate that the postprandial triglyceride (TG) level is a more reliable predictor of future CVD events than fasting TG levels. Specific TGRL remnants such as VLDL, LDL and lipoprotein B-containing particles are related to initiation and progression of atherosclerosis (107). Transmigration of LDL particles across the vascular endothelium and their subsequent oxidation is a primary event in the atherosclerotic process. Postprandial LDL particles are more easily oxidised than fasting LDL. Similarly, postprandial VLDL particles may also be more prone to oxidation than fasting VLDL (108).

The lipolysis products of oxidised chylomicrons have been identified as potential components of postprandial lipoproteins that increase endothelial permeability and stimulate adhesion molecule expression (132; 133). Chylomicrons obtained following meals rich in polyunsaturated fats were more easily oxidised in vitro, and consistently induced higher levels of adhesion molecule expression (132, 133).

Postprandial Lipemia and PAD

PAD is associated with several lipid abnormalities including increased levels of fasting TGs and low levels of HDL-C. In PAD patients with a normal lipid profile, administration of an oral fat load alters the lipid profile to one considered atherogenic, and characterized by

an increased magnitude of postprandial lipemia (increased AUC-TG) and a reduction of HDL-C (22).

Postprandial Lipemia and Exercise

Moderate-intensity exercise performed 18 to 20 h before a HFM meal attenuates the postprandial increase in plasma TG (23). The mechanisms responsible for the exercise related attenuation of postprandial TG are not fully understood. Exercise reduces the fasting TG pool size, but the incremental area (above baseline) under the TG-time curve is also reduced compared with control conditions. The exercise-induced plasma TG lowering effect may be due to increased activity of lipoprotein lipase, which could enhance the removal of TG-rich lipoproteins from the blood. LPL activity is up-regulated after exercise in a time-course consistent with the postprandial reduction of TG. Other putative mechanisms include hormonal changes (44), alterations in cellular homeostasis (45), and muscle contraction (43) may play a role in the exercise induced attenuation in TG following a HFM. The lower postprandial TG concentrations after exercise also reflect an enhanced rate of removal by peripheral tissues of TG-rich lipoproteins. A decreased rate of VLDL synthesis and secretion from the liver is also a contributing factor and accounts, and may also play a role in reducing circulating postprandial TG (24).

Postprandial lipemia and intermittent exercise

A number of studies have examined the effect of exercise intensity and durations on attenuating the postprandial increase in plasma TG (25, 26). Regardless of exercise duration and intensity, total energy expenditure (TEE) is the primary factor that influences PPL. Hardman(109) et al., compared the lipemic response to a standard OFTT consumed 16–18 h

following a moderate intensity (60% VO_2 max) and low intensity (30% VO_2 max) exercise bout. The moderate intensity exercise session resulted in a larger attenuation in postprandial TG-AUC when compared to the low intensity exercise. When the duration of low intensity exercise was increased so that the TEE was identical to the moderate intensity trial, each bout of exercise reduced lipemia to the same degree.

The energy expenditure can also be accumulated over a number of exercise sessions Gill et al., 1998 compared the accumulative effect of multiple short duration bouts of exercise to one continuous bout on PPL in healthy men. Participants exercised on a treadmill at 60% VO_2 max in either one 90-min session (continuous exercise trial), or three 30-min sessions at intervals of several hours (intermittent exercise trial). Total caloric expenditure was similar in both trials. The TG-AUC was similar in both trials, indicating that both intermittent and continuous exercise can similarly reduce PPL (109).

The possibility of an accumulating benefit with multiple short bouts is particularly important for patients with PAD, as in many instances the disease restricts their ability to exercise for extended periods. Individuals with PAD exhibit a postprandial lipid profile that is considered atherogenic, and also have an exaggerated postprandial lipemic response following a HFM. Based on findings from previous studies involving individuals with other forms of CVD, continuous and intermittent exercise may attenuate the postprandial response. To date, no studies have examined the acute effects of exercise on PPL and endothelial function in men with PAD.

Inflammation and Exercise

Inflammation plays an important role in the pathogenesis of cardiovascular disease. (Blum A, Miller HI. The role of inflammation in atherosclerosis. *Isr J Med Sci* 1996;32:1059–1065.) There is an inverse association between levels of physical activity and the blood concentration of inflammatory markers (Hammett et al 2006, Kohut et al 2006, Markovitch et al 2008, Plaisance et al 2007). The mechanisms responsible for the decrease in the circulating levels of inflammatory biomarkers in response to exercise are not completely understood (Kohut et al 2006). Inflammatory markers have been found to transiently increase after a single bout of high-intensity exercise (Markovitch et al 2008, Olson et al 2007, Plaisance et al 2007). This transient increase results in the subsequent up-regulation of anti-inflammatory pathways, such as CRP (Markovitch et al 2008). Plaisance et al., (2007) found no significant change in the circulating levels of selected serum inflammatory markers in response to an acute bout of exercise at 70% VO_2 (until they expended 500 kcal) in 21 moderately/highly fit men.

There is evidence that unhealthy individuals may express increased concentrations of inflammatory markers immediately post exercise (Plaisance et al 2007) and experience greater tissue damage. Obese individuals often exhibit elevated levels of inflammatory markers (Olson et al 2007). Waist circumference is positively associated ($p < 0.05$) with baseline levels of CRP (Plaisance et al 2007). Undertaking resistance training 2 times per week for 1 year had been shown to significantly increase lean body mass and significantly reduce the circulating levels of CRP in overweight women (Olson et al 2007). Relatively

small increased in lean body mass may decrease circulating inflammatory markers (Olson et al 2007).

Inflammatory biomarkers

Inflammation plays an important role in the onset and development of atherosclerotic lesions (110,111). C-reactive protein (CRP) is part of a group of substances known as “acute phase reactants” synthesised primarily by hepatic tissue, in response to tissue injury, including that caused by infection, malignant disease and chronic inflammatory conditions. It plays an active role in vascular inflammation and development of atherosclerosis (112). Elevated levels of CRP increase the expression of cell adhesion molecules and interleukin-6 (IL-6). CRP can also help facilitate LDL-C uptake by endothelial macrophages, (113-115) and decrease the expression of endothelial derived nitric oxide synthase (116). CRP also increases the expression and activity of the serine protease inhibitor plasminogen activator inhibitor (PAI-1).

Tumour necrosis factor alpha (TNF- α) is a proinflammatory cytokine produced by monocytes/macrophages (117), that is involved in the development of atherosclerosis. TNF- α stimulated the expression of adhesion molecules and IL-6, a major inducer of hepatic CRP synthesis (118). High circulating levels of TNF- α increases the risk of recurrent events in stable post-myocardial infarction patients (119).

Conclusion

PAD affects 3-12% and IC affects 1-3% of the general population, (15, 16) and its prevalence increases significantly with age, affecting up to 20% of patients over the age of 75 years. Patients with PAD have an exaggerated PPL response to a HFM. Exercise

attenuates the postprandial response to HFM in obese patients and those with CAD and diabetes. To date, no studies have examined the acute effects of exercise on PPL and endothelial function in men with PAD.

Chapter 3

METHODOLOGY

Participants

Eight men ≥ 50 year with diagnosed PAD who had been participating in a community-based phase IV cardiac rehabilitation programme, for a minimum of 6 months, were recruited. Participants were excluded if they had a ratio of arm blood pressure to ankle blood pressure ≥ 0.95 at rest or ≥ 0.85 after exercise, Fontaine Stage III/Fontaine Stage II PAD (intermittent claudication upon ambulation) for ≤ 3 months, unstable angina, uncontrolled hypertension (systolic blood pressure (BP) >180 mmHg, diastolic BP >100 mmHg), resting tachycardia, unstable/acute heart failure and in good health for a minimum of two weeks prior to beginning the study.

Research Design

The study used a randomised cross-over design. Participants visited the Cardiovascular Research Unit at Dublin City University on three occasions and involved one screening visit and two experimental trials. They subsequently underwent two oral fat tolerance tests (OFTT) with a 4 h observation period, separated by approximately 7 d. On the evening prior to the OFTT, the participants either rested at home (CON trial) or completed a 200 kcal treadmill walk (EX trial) at a self-regulated intensity. Participants did not participate in the community-based phase IV cardiac rehabilitation program for 2 d before each experimental trial. They recorded their normal diet the day prior to the first OFTT and repeated this diet on the day prior to the second OFTT.

Screening

The nature and risks of the study were explained. A plain language statement was read and informed consent was obtained in accordance with the Research Ethics Committee at Dublin City University. Participants then completed a general health questionnaire (appendix D), and had their blood pressure, height and weight measured.

Experimental Trials

Participants reported to the Cardiovascular Research Unit in DCU at 8 am following an overnight fast. An intravenous catheter (21G) was inserted into a prominent forearm vein. Following a 10 min rest period, a baseline blood sample was obtained. Participants underwent (under supervision) an OFTT. The test meal consisted of croissants, butter, chocolate and potato crisps with a macronutrient composition per 2m² body surface area of 38 g fat, 57 g carbohydrate, which amounted to a total meal count of 639 Kcal. Water was consumed ad libitum following the first OFTT. The volume of water consumed and the time(s) of consumption relative to the meal were recorded and replicated following the subsequent OFTT. Blood samples were obtained before the test meal was ingested and in the postprandial period at 30, 60, 120, 180, and 240 min. Participants rested quietly during this period, but were permitted to read or watch television.

Acute Exercise Bout

Participants exercised on a treadmill (Woodway ELG 55, Waukesha, WI) at a self-selected intensity until they expended 200 kcal. The velocity and gradient control arrows were visible and participants were allowed to alter the treadmill grade and velocity ad libitum. A discontinuous protocol was used due to nature of PAD. Energy expenditure and exercise intensity were recorded continuously throughout the exercise bout. Metabolic measurements were recorded throughout the exercise bout using open circuit spirometry (SensorMedics Vmax 229 metabolic system, SensorMedics Corp., Yorba Linda CA).

Blood Pressure

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

Height and Weight

Height and body mass were measured to the nearest 0.1 cm and 0.1 kg respectively using the SECA Stadiometer. Participants were barefoot for the measurement.

Overview of Endothelial Function Assessment

Flow mediated dilation (FMD) was assessed using high-resolution ultrasonography, by the same investigator, in a quiet, temperature-controlled room. Ultrasound measurements were performed on a SonoSite MicroMaxx® (SonoSite Inc., Bothell,

Washington, US) ultrasound system with a linear array transducer (Figure 3.1), operating at a frequency of 12.0 MHz.



Figure 3.1: SonoSite MicroMaxx[®] Ultrasound system and 12.0 MHz linear array transducer

Participants arrived to the Vascular Research Unit, DCU at approximately 8.00 am after 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.

All brachial artery images were acquired with the participants in a supine position. A baseline brachial artery image was acquired following a 10 min rest period and was immediately followed by assessment of FMD. The right arm of the participant rested on an examination table perpendicular to the bed, and was extended and externally rotated to permit imaging of the right brachial artery. An automated blood pressure cuff was placed on the right forearm, distal to the brachial artery (Figure 3.2) and electrodes for a 3-lead ECG were placed on their chest. The ECG tracing was activated and settings adjusted to ensure clear identification of the R wave which corresponds to the end of diastole in the cardiac cycle.



Figure 3.2: 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 maximize vessel diameter and provide optimal blood vessel wall definition. 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. The imaging plane should bisect the vessel in the longitudinal direction to ensure diameter measurements obtained from these images reflect the true diameter of the vessel (Corretti et al., 2002).

M mode Imaging

The brachial artery was imaged using M mode function to facilitate arterial diameter measurements at appropriate time points (figure 3.3). The baseline brachial artery image was named and saved for subsequent off-line analysis of arterial diameter

using a custom-designed, semi-automated ultrasound arterial measurement software. Each image acquired, incorporated a minimum of 2 ECG R waves. The diameter of the brachial artery was measured at a minimum of two and maximum of three consecutive R waves on the ECG, a process referred to as “gating”. The R wave represents the end of diastole in the cardiac cycle. Gating allows diameter estimates to be taken at specified vertical cross-sections of the artery. The mean of the 2-3 measurements was taken as the brachial artery diameter.

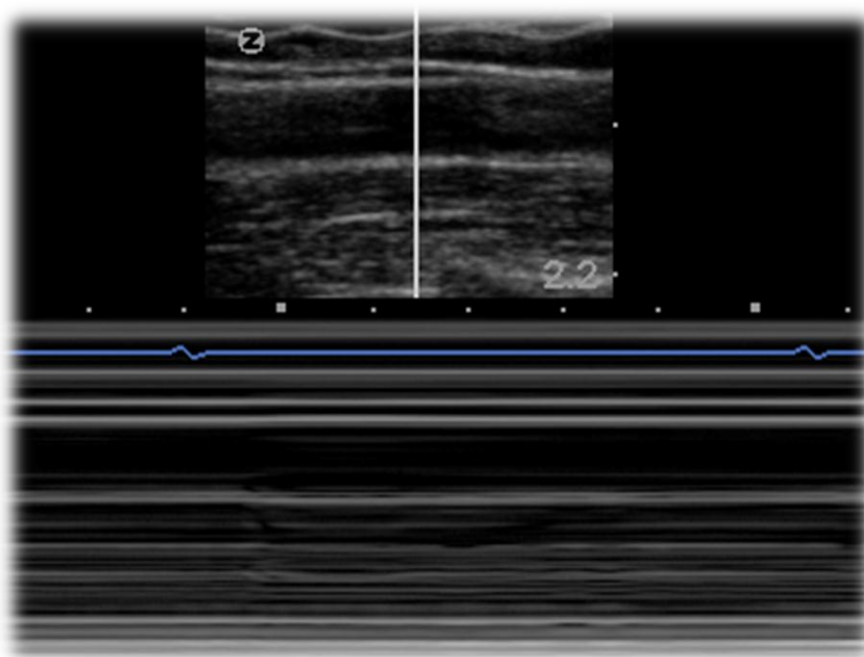


Figure 3.3: M mode ultrasound image of the brachial artery

Doppler Imaging

Doppler imaging was used to measure blood flow velocity (cm/s) 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 60°. 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°. The Doppler function traced the spectral wave form (Figure 3.4). The investigator froze the image and peak systolic velocity was manually measured using the in-built ultrasound calipers (SonoSite MicroMaxx®). The “Doppler” function traced the spectral wave form.

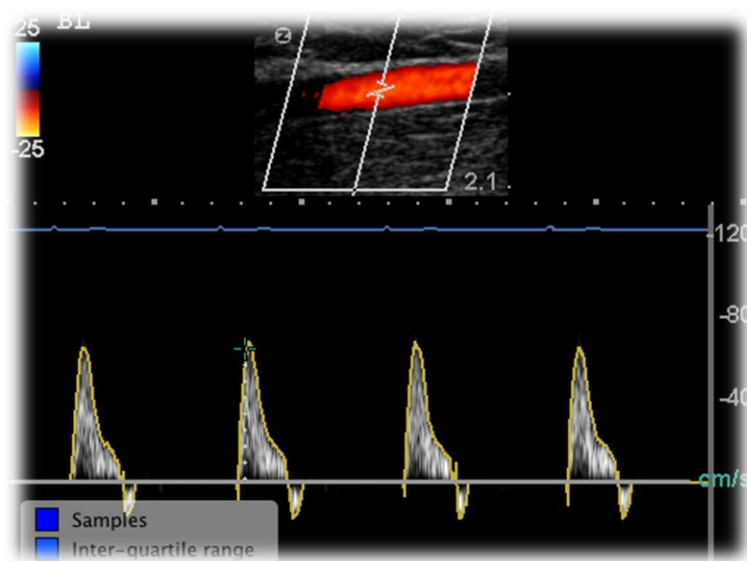


Figure 3.4: Frozen screen shot of a Doppler image

Endothelial-Dependent Dilation (EDD)

Figure 3.5 illustrates the endothelial-dependent dilation assessment protocol. After baseline measurements were recorded, the pneumatic cuff was inflated to 250 mmHg for 5.0 min. Following 5 min of occlusion, the cuff was rapidly released resulting in reactive

hyperemia of the hand and a subsequent increase in brachial artery blood flow. Post-deflation peak systolic velocity was measured within 15 seconds of cuff release and vessel diameter was recorded to allow calculation of hyperaemic flow. M-mode images of the brachial artery were named and recorded every 30 sec post-deflation for 5 min. These images were saved for post-analysis, using a custom-designed, semi-automated ultrasound arterial measurement software.

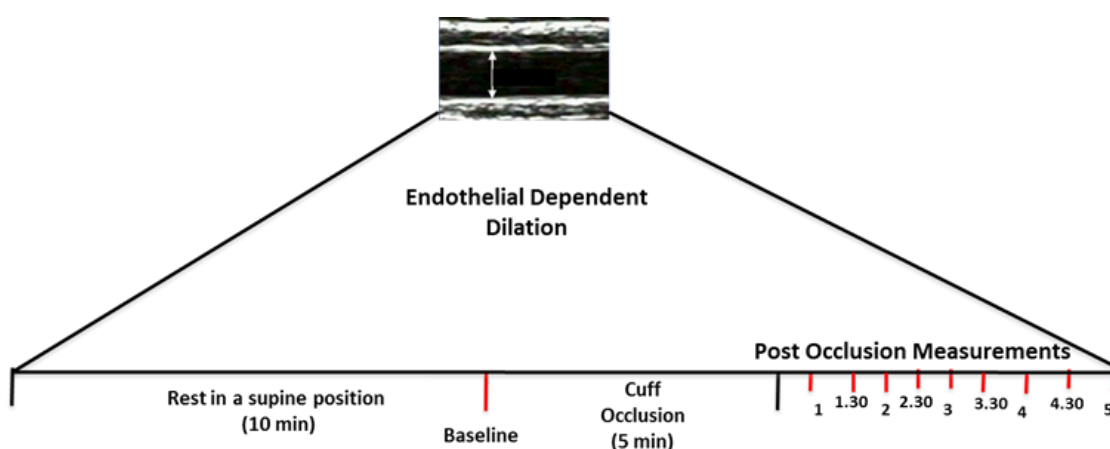


Figure 3.5: Overview of the protocol used to assess endothelial dependent dilation

Endothelial Independent Dilatation (EID)

Figure 3.6 illustrates the endothelial-independent dilatation assessment protocol after a resting period of 15 min post-deflation; the brachial artery was imaged using M-mode ultrasound. This image was named and recorded as a new baseline. Participants were administered with a single spray of sublingual glyceryl trinitrate (GTN) (400 μ g). M-mode images of the brachial artery were once again named and recorded every 30 sec post GTN for 5 min. Similarly, images were saved for post-analysis.

Effect of Acute Exercise on Postprandial Lipemia and Endothelial Dysfunction in Men with Peripheral Arterial Disease

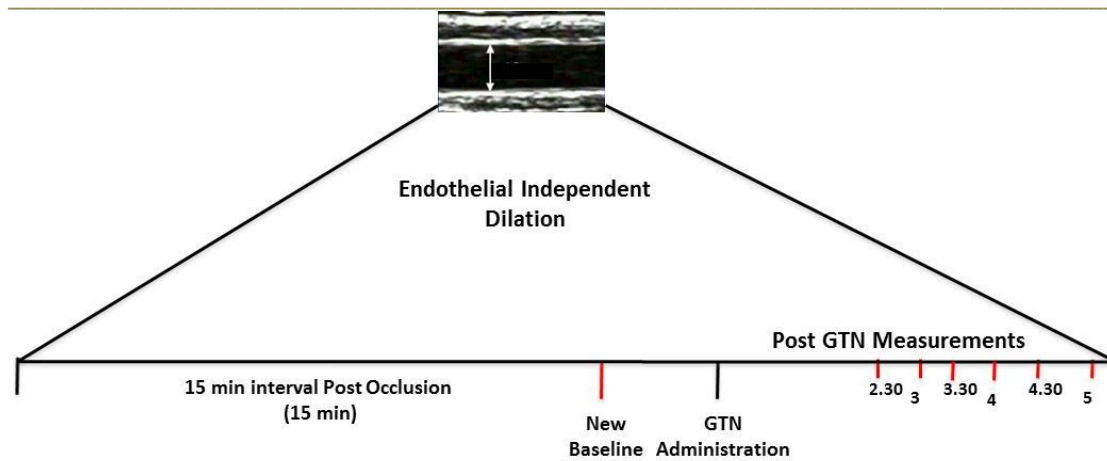


Figure 3.6: Overview of the protocol used to assess endothelial independent dilation

All images were selected for analysis using a standard dialog box (Figure 3.7). 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 using a slider to change the threshold intensity values of the segmentation.

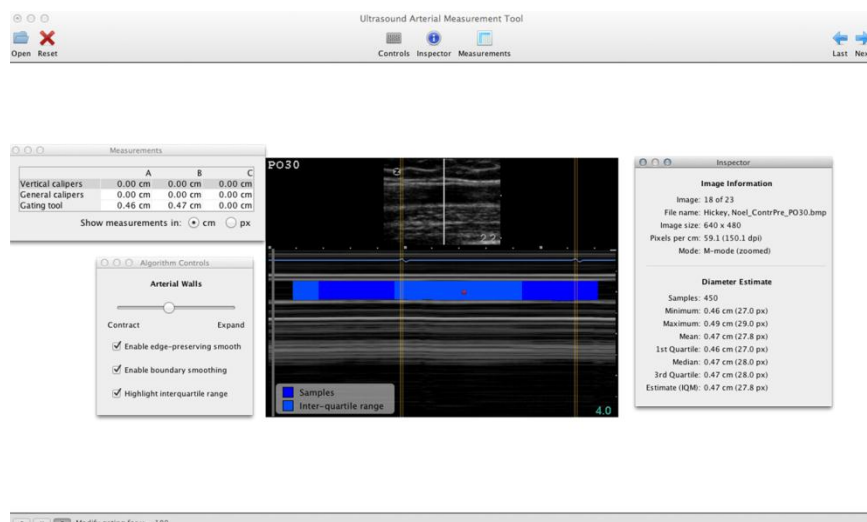


Figure 3.7: Standard dialog box

Gated measurements of the brachial artery diameter were recorded using a minimum of two and maximum of 3 consecutive R waves on an ECG. The mean of the 2-3 measurements was taken as the brachial artery diameter.

Cardiorespiratory and Metabolic Measures

Expired air was collected continuously during the exercise bout for determination of $\dot{V}O_2$ and $\dot{V}CO_2$ via a breath by breath metabolic system (Sensormedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analysers were calibrated with standard gases of known concentration. Energy expenditure and substrate oxidation was estimated using indirect calorimetry.

Energy expenditure during the acute bout of exercise was calculated using the equation of Weir:¹³⁴ $EE \text{ (kJ}\cdot\text{min}^{-1}) = 16.5 \dot{V}O_2 \text{ (L}\cdot\text{min}^{-1}) + 4.63 \dot{V}CO_2 \text{ (L}\cdot\text{min}^{-1})$. The rate of carbohydrate and fat oxidation was calculated from gas exchange measurements according to the table of non-protein respiratory quotient:¹³⁵ $\text{CHO oxidation} = 4.85 \times \dot{V}CO_2 - 3.226 \times \dot{V}O_2$, and $\text{lipid oxidation} = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2$, with mass expressed in $\text{g}\cdot\text{min}^{-1}$ and gas volume in $\text{L}\cdot\text{min}^{-1}$. The percentage of carbohydrate and lipid oxidation during exercise was calculated using the McGilvery & Goldstein equations:¹³⁶ $[\% \text{lipid oxidation} = ((1 - \text{RER})/0.29) \times 100$, and $[\% \text{CHO oxidation} = ((\text{RER} - 0.71)/0.29) \times 100$. Values for $\dot{V}O_2$, $\dot{V}CO_2$ and RER were averaged every minute throughout the exercise period.

Mass Flow Sensor Heated wire Anemometer - Mode of Operation

A mass flow sensor (Sensormedics, Loma Linda, CA, USA) was used to collect breath-by-breath measurement of ventilation. 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°C and -240°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 change, 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 the 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 artefact due to cooling of the gas as it passes through a breathing assembly. The mass flow meter responds to instantaneous flow rates between $0\text{-}16\text{ L}\cdot\text{sec}^{-1}$ and integrated flow between $0\text{-}350\text{ L}\cdot\text{min}^{-1}$ with flow resistance $<1.5\text{ cmH}_2\text{O}\cdot\text{L}^{-1}\cdot\text{sec}^{-1}$. The mass flow sensor was outputted to the analyser 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 used to calibrate the mass flow sensor prior to each test. The syringe 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 $< 0.5\text{L}\cdot\text{sec}^{-1}$, and at least one of the four strokes had to have an average flow $> 3.0\text{L}\cdot\text{sec}^{-1}$.

Gas Analysers

The Vmax 229 utilizes a rapid response infrared measurement technique. An O_2 and CO_2 analyzer 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 it 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 CO_2 in the sample cell. Based on measured levels of infrared light intensity, the analyzer computes the PCO_2 in the gas sample. The CO_2 analyzer is linearly scaled across the 0-100% range with a resolution of 0.01% CO_2 , and a response time of $< 130\text{ms}$ (10-90%) at $500\text{ml}\cdot\text{min}^{-1}$ flow. The O_2 analyzer is based on the high paramagnetic susceptibility of O_2 . A diamagnetic glass dumbbell suspended in a magnetic field rotates in proportion to the PO_2 . The analyzer is linearly scaled across the 0-100% range with a resolution of 0.01% O_2 and a response time of $< 130\text{ms}$ (10-90%) at $500\text{ml}\cdot\text{min}^{-1}$ flow.

Calibration of CO_2 and O_2 Analyzers

The gas analyzers were calibrated with standard gases of known concentration (BOC gases, Dublin, Ireland). The first calibration gas contained $26.00\pm 0.02\%$ oxygen (O_2) and the balance nitrogen (N_2). The second calibration gas contained $4.00\pm 0.02\%$ carbon

dioxide (CO₂), 16.00±0.02% O₂, and the balance N₂. A small bore drying tube connected to the CO₂ and O₂ 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 O₂ and CO₂ analyzers. The calibration gas was sampled at a rate of 125 Hz. The response time was similar between O₂ and CO₂ analyzer.

Blood Sampling and Processing

Blood samples were collected into vacutainers containing either potassium EDTA or without any additive (Becton Dickinson and Co., UK). Serum was separated by centrifugation (1620 g for 15 min at 4 °C) (PK121R centrifuge, ALC, VA) within 30 min of collection. Plasma and Serum samples were stored at -80°C. Repeated measures from all subjects were defrosted together and analysed in the same run.

Biochemical Assays

Immediately following collection, a complete blood count was performed on whole blood samples drawn at baseline, 30 min, 60 min, 120 min, 180 min, 240 min in an EDTA-coated tube using an automatic hematology analyser (Ac-T Diff 2, Beckman Coulter, CA). Serum total cholesterol, TG, HDL-C, LDL-C and high-sensitivity CRP were determined using enzymatic assays (Randox laboratories, Northern Ireland). Commercially available enzyme-linked immunoassays (R&D Systems, UK) were used to determine plasma TNF-α. Intra-assay coefficient of variation was 8.1%.

Statistical Analysis

Statistical analysis was performed using SPSS 19.0 computer software (SPSS, Chicago, IL). The dependent variables were EDD and EID, TG, total cholesterol, HDL-C, LDL-C, leukocytes, hsCRP, and TNF- α . A mixed model (time x experimental condition) repeated measures ANOVA was used to compare changes in endothelial function, blood lipids, leukocytes and inflammatory markers. Where indicated by a significant F value, post hoc tests, with a Bonferroni correction for multiple comparisons, was performed to compare specific group means. Data are presented as mean \pm standard deviation. A probability of $p \leq 0.05$ was accepted for statistical significance.

Summary postprandial responses to the test meal are reported as time-averaged postprandial values (Appendix B) using the approach of Gill et al (2006). Time-averaged postprandial values represent the total area under the concentration vs. time curve (AUC), calculated using the trapezium rule, and divided by the length of the postprandial period. The TG increment (Appendix B) represents the AUC above baseline, divided by the length of the postprandial period.

Chapter 4

RESULTS

Participant's physical characteristics are summarized in Table 1. Participants were classified as being overweight and borderline hypertensive. Fasting concentrations of total cholesterol, HDL-C, LDL-C and TG were within the normal range. The total caloric count of the meal was 639.5 ± 35.3 Kcal and consisted of 38.1 ± 2.2 g of fat and 57.0 ± 3.5 g of carbohydrate. Participants exercised for 38.0 ± 3.6 min and expended 195.2 ± 8.8 kcal·hr⁻¹ during the acute bout of intermittent exercise. The treadmill velocity and gradient was 4.7 ± 0.3 km·hr⁻¹ and $0.9 \pm 0.1\%$, respectively. Total oxidation of carbohydrate was 0.63 ± 0.10 g/min and fat was 0.25 ± 0.04 g/min.

Triglycerides

Circulating TG levels were significantly higher ($p < 0.05$) in both the exercise and control trials at 120 min, 180 min and 240 min compared to baseline (Figure 4.1). There was no significant difference in TG at any time points between the exercise and control trial. The TG-AUC was 12% lower in the exercise than the control trial (7.1 vs 8.1 mmol/L). This difference did not however reach statistical significance. There were no within or between trial differences in total cholesterol, LDL-C or HDL-C at any time point (Table 2).

Inflammatory Markers

Compared to baseline, circulating leukocytes were significantly higher ($p < 0.05$) in both the exercise and control trial at 120 min, 180 min and 240 min. There was no significant difference in leukocyte number between the exercise and control trial at any

time point during the study. Plasma levels of hsCRP did not change in response to the OFTT during the control or exercise condition. Plasma concentrations of TNF- α were significantly higher ($p < 0.05$) than baseline at 120 min, 180 min and 240 min following the OFTT in both the exercise and control trials. There was no significant difference in plasma concentrations of TNF- α between the exercise and control trial at any time point.

Endothelial Function

FMD results are presented in figure 4.4. A significant main effect for condition ($P < 0.05$) was observed but there was no significant condition-by-time interaction. There was a significant decrease ($p < 0.05$) in FMD 2 h postprandial in both conditions. There was no significant difference due to the exercise condition. There was no significant difference in endothelial-independent dilation in either condition. A significant percentage increase ($p < 0.05$) was observed in brachial artery Doppler flow between 2 h and 4 h postprandial in the control trial. Endothelial dependent dilation (EDD) and endothelial independent dilation (EID) were not significantly different between the groups.

Table 4.1: Subjects physical characteristics (n=8)

Age (yrs)	62.3 ± 2.4
Height (cm)	167.9 ± 8.9
Body mass (kg·m ⁻²)	28.8 ± 3.4
Ankle Brachial Index	0.6 ± 0.1
Body surface area (m ²)	1.9 ± 0.1
Systolic BP (mmHg)	138.0 ± 7.8
Diastolic BP (mmHg)	84.9 ± 3.6
Cholesterol (mmol/L ⁻¹)	5.0 ± 1.1
HDL-C (mmol/L ⁻¹)	3.0 ± 1.4
LD-C (mmol/L ⁻¹)	1.3 ± 0.2
Triglycerides (mmol/L ⁻¹)	1.1 ± 0.5

Values are mean ± SD

Table 4.2: Effect of acute exercise on lipids and inflammatory biomarkers

	Control (n=8)		Exercise (n=8)	
	0 h	4 h	0 h	4 h
Total cholesterol (mmol.L ⁻¹)	4.8 ± 1.2	4.9 ± 1.1	5.0 ± 1.2	4.8 ± 1.1
LDL-cholesterol (mmol.L ⁻¹)	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.3	1.1 ± 0.2
HDL-cholesterol (mmol.L ⁻¹)	3.0 ± 1.1	2.8 ± 1.1	3.0 ± 1.3	2.9 ± 1.1
TG (mmol.L ⁻¹)	1.2 ± 0.5	2.6 ± 0.8*	1.1 ± 0.4	2.1 ± 0.8*
CRP (mg.L ⁻¹)	3.5 ± 2.3	3.5 ± 2.4	3.6 ± 2.0	3.4 ± 2.1
TNF-α (pg.mL ⁻¹)	2.4 ± 1.2	2.6 ± 1.2*	2.4 ± 1.1	2.7 ± 1.2*
WBC (cells x 10e3.μL ⁻¹)	6.8 ± 1.3	8.3 ± 1.5*	6.9 ± 1.1	8.1 ± 0.8*

Values are mean ± SD; *p<0.05 vs. 0 h (within group)

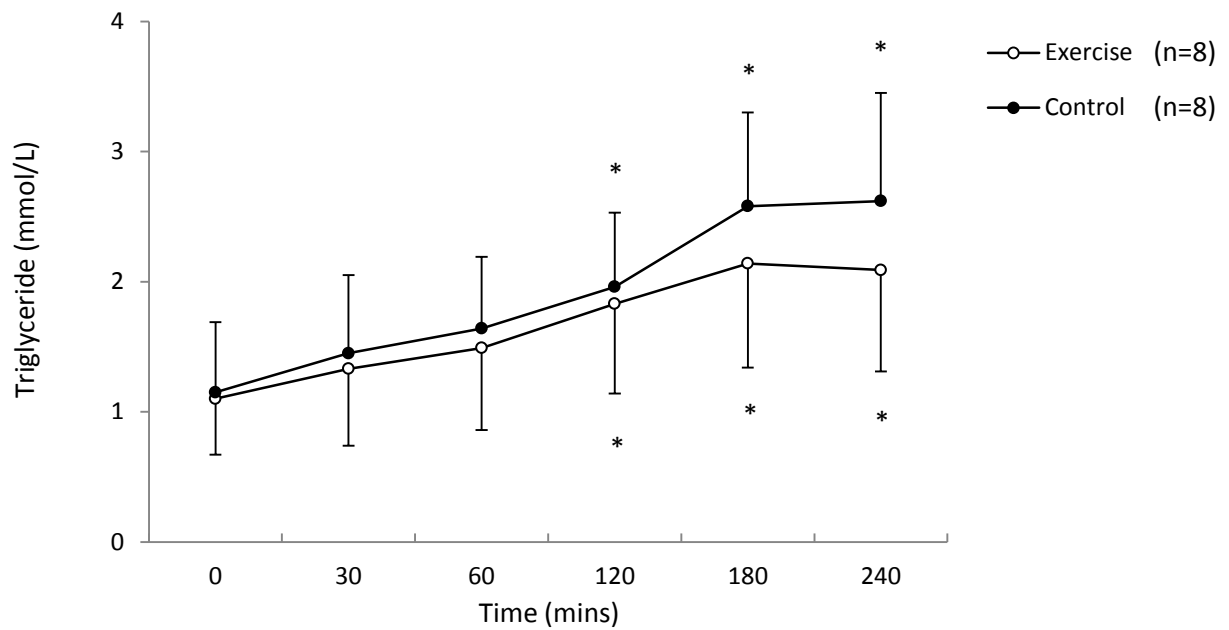


Figure 4.1 Circulating levels of TG in the postprandial period following the control and exercise trial. Values are mean \pm SD; *P<0.05 vs. baseline in exercise and control trial

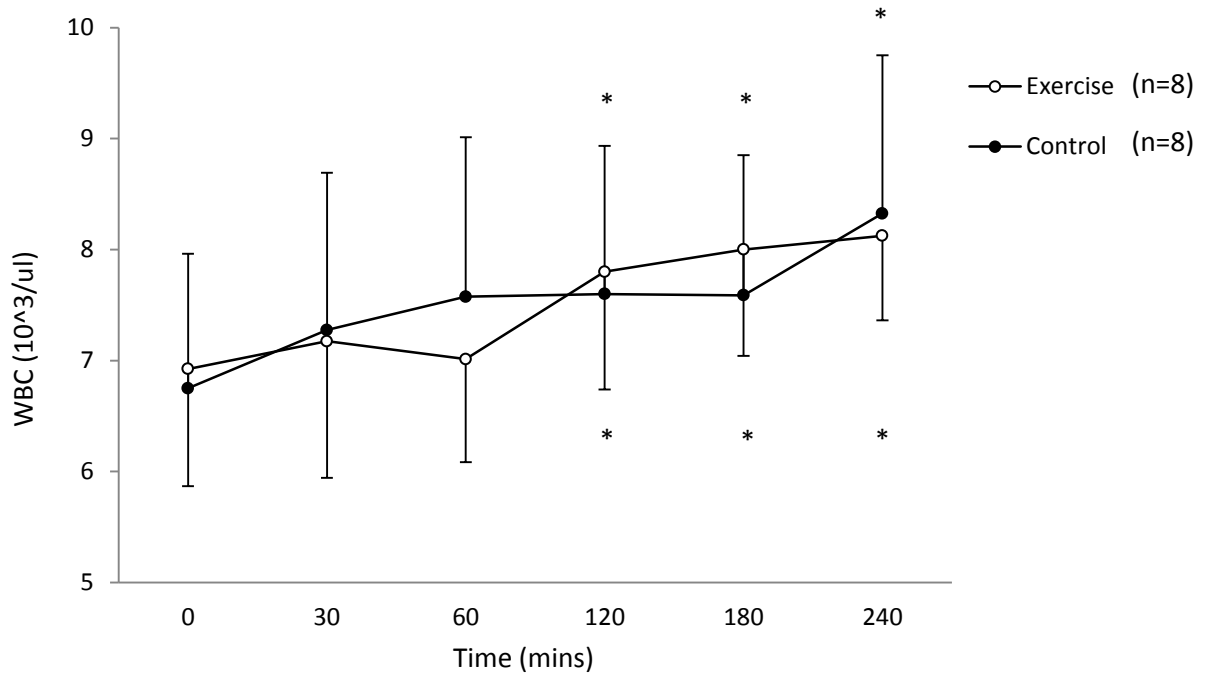


Figure 4.2 Circulating leukocytes in the postprandial period following the control and exercise trial. Values are mean \pm SD; * $P < 0.05$ vs. baseline in exercise and control trial

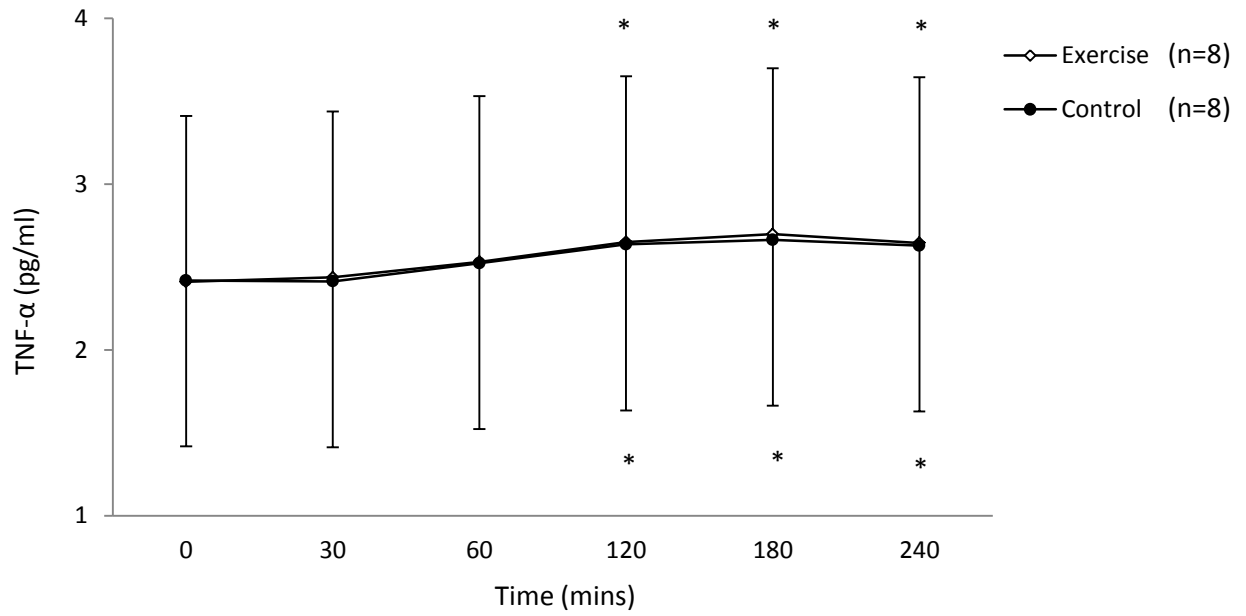


Figure 4.3. Circulating levels of TNF- α in the postprandial period following the control and exercise trial. Values are mean \pm SD; *P<0.05 vs. baseline in exercise and control trial

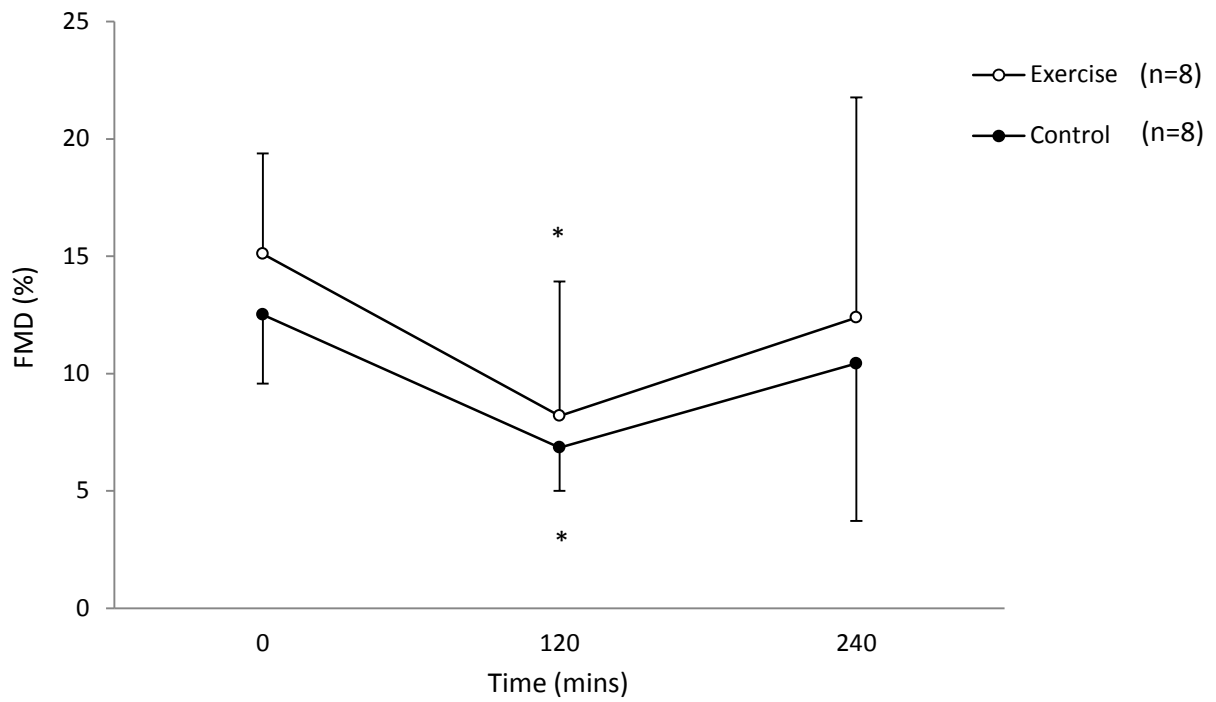


Figure 4.4 Brachial artery flow mediated dilation in control and exercise trial before the HFM, and 2 h and 4 h following the HFM. Values are mean \pm SD; * $P < 0.05$ vs. baseline in exercise and control trial

Chapter 5

DISCUSSION

Postprandial lipemia (PPL) is characterized by an increase in triglyceride-rich lipoproteins for up to 8 h following the consumption of a HFM. Regular exposure to high postprandial TG concentrations is positively associated with atherosclerosis through its adverse effect on vascular integrity and inflammation.¹ Epidemiological studies also indicate that elevated postprandial lipemia is an independent predictor of CVD².

Reducing the accumulation of TG rich lipoproteins during the postprandial period presents a viable target for lowering arteriosclerotic risk. The benefits of aerobic exercises in acute PPL have been documented in several studies (11, 100, and 120). Several exercise variations have been tested, such as light, moderate and intense continuous and intermittent exercises. To date, no studies have examined the effect of exercise on PPL in patients with PAD. It was hypothesised that in men with PAD, an acute exercise bout would reduce the postprandial TG response, decrease circulating levels of inflammatory markers and preserve brachial artery FMD in response to a standardised HFM.

The major findings of the present study are that acute exercise does not i) attenuate the PPL in response to a HFM in patients with PAD, and ii) ameliorate the reduction in brachial artery FMD in response to a HFM, or iii) alter the endothelial independent

¹ Patsch, J. R., Miesenböck, G., Hopferwieser, T., Mühlberger, V., Knapp, E., Dunn, J. K., Gotto, A. M. and Patsch, W. (1992) Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler. Thromb.* **12**, 1336-1345

² estgaard, B. G., Benn, M., Schnohr, P. and Tybjaerg-Hansen, A. (2007) Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA, J. Am. Med. Assoc.* **298**, 299-308

vasomotor responses. These findings may be partly explained by the fact that the total caloric expenditure (200 kcal) may have been insufficient to induce a significant decrease in PPL. Previous studies that have found an attenuated postprandial response to a HFM involved moderate to vigorous levels of exercise at 60% VO_2 max that resulted in a caloric expenditure between 600 – 900 kcal (1,109). The functional limitations associated with PAD precluded these individuals from undertaking a prolonged bout of exercise that would be sufficient to meet the caloric threshold required to attenuate the PPL response to a HFM. It was not possible to assess the relative metabolic demand during exercise due to the fact that participants were unable to undertake a maximal exercise test. However, the fact that the mean treadmill walking velocity was $4.7 \text{ km}\cdot\text{h}^{-1}$ would indicate that the exercise intensity was light to moderate. Pilot work, prior to the study indicated that on average, PAD patients were only able to sustain discontinuous exercise at light to moderate levels of activity for > 30 min.

Postprandial TG levels were similar in both the exercise and control trials up to 3 h. The fact that TG levels began to decrease towards baseline values at 4 h in the exercise group, but were still rising in the control group, suggests a delayed clearance of TG, in the control trial. The TG-AUC pattern was similar to that found in other studies that compared the effect of an acute bout of exercise on postprandial TG-AUC (120, 121).

Impaired EDD has been linked to the pathogenesis of atherosclerotic vascular diseases and acute cardiovascular events. A number of previous studies have found a transient impairment in endothelial vasomotor function in response to a high fat meal (HFM) (1). An acute bout of exercise performed prior to HFM can ameliorate this

impairment in endothelial vasomotor function (112). In the present study, brachial artery FMD was impaired for 4 h following consumption of a single HFM, and acute exercise had no effect on the FMD response. In fact, there is evidence that a single bout of exercise can transiently impair brachial artery FMD in patients with PAD. Elevated TG increases superoxide anion production and may reduce endothelial vasomotor function through direct inactivation of NO and increases in lipid oxidation (123).

In patients with PAD, calf-pain during walking, followed by a period of rest to allow reperfusion of the ischemic limb, induces an increase in oxidative stress and a marked inflammatory response (63,64). Oral administration of vitamins C and vitamin E ameliorates the decrease in endothelial function in response to a HFM in healthy individuals (96) indicating that an oxidative stress mechanism may be responsible for the impaired endothelial function after a HFM (21).

The increase in circulating levels of WBC and TNF- α at 4 h following a HFM are consistent with a number of previous studies (1,124), and have been shown to contribute to a pro-inflammatory environment. The inflammatory response in the postprandial state was not attenuated by acute exercise. Previous studies that have investigated the effects of a HFM on plasma levels of TNF- α have been equivocal. A study by Esposito *et al.*, (125) found that a single HFM further impaired endothelial function in individuals with the metabolic syndrome, and increased TNF- α levels were significantly related to this impairment. In contrast, others have found no change in TNF- α levels following a HFM. Gill *et al.*, (126) found that the decrease in endothelial function in the cutaneous microcirculation after a

HFM was not associated with increased circulating levels of TNF- α . However, this study involved endurance-trained athletes, and may not be representative of patients with CVD.

CRP is linked to endothelial cell activation (112). Studies examining CRP responses after an acute bout of exercise have used strenuous and prolonged activity bouts (112,114). A single session of exercise triggers an increase in circulating levels of CRP. There was no change in CRP at 4 h after the OFTT in either condition, in the present study. It is possible that the acute inflammatory response was much lower when compared to studies that used prolonged and/or high intensity exercise.

Study Limitations

- The most accurate method for normalizing FMD responses is believed to be the area under the curve shear response from post-occlusion to the time at which peak diameter occurs. Presently, the Vascular Research laboratory in DCU is not equipped to measure viscosity and the ultrasound system does not have the functionality for simultaneous acquisition of B-mode diameter and pulsed-wave Doppler velocity signals, or the continuous measurement of blood flow velocity. Duplex ultrasound for simultaneous capture is recommended where available. For these reasons shear rate was calculated as an accepted estimate of shear stress, using peak blood flow velocity (equation: shear rate = $4 \times \text{peak velocity} / \text{peak diameter}$)
- Due to dropouts only 8 participants completed the research study

Conclusion

In summary, the present study found that an OFTT significantly increases post prandial TG, leukocytes and TNF- α and significantly reduces brachial artery FMD in men with PAD. An acute bout of discontinuous exercise that resulted in a 200 Kcal expenditure does not attenuate the post prandial TG response or ameliorate the decrease in endothelial vasomotor function.

Chapter 6

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Appendices

Appendix A: Submission to Ethics Committee

Appendix B: Plain Language Statement

Appendix C: Informed Consent

Appendix D: Data Collection Sheets

Appendix E: Meal Composition

Appendix F: Endothelial dependant and independent dilation tables

PROJECT TITLE Effect of Acute Exercise on Postprandial Lipemia in 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.

	INCLUDED		NOT APPLICABLE
Bibliography	<input type="checkbox"/>		<input checked="" type="checkbox"/>
Recruitment advertisement	<input type="checkbox"/>		<input 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"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	draft	final	
Interview Schedule	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	draft	final	
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

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

(please give details)

- Research Masters Taught Masters
 PhD Undergraduate

Project Start Date: **01/02/11** **Project End date:** **01/07/12**

1.1 INVESTIGATOR CONTACT DETAILS *(see Guidelines)*

PRINCIPAL INVESTIGATOR(S):

TITLE	SURNAME	FIRST NAME	PHONE	FAX	EMAIL
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<i>TITLE</i>	<i>SURNAME</i>	<i>FIRST NAME</i>	<i>PHONE</i>	<i>FAX</i>	<i>EMAIL</i>
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Mr.	Kevin	O'Hara	085 1687637	01 7008888	kevin.ohara2@mail.dcu.ie

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

(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 investigator(s): *Niall Moyna*

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

Date: 01/12/10

2. PROJECT OUTLINE

2.1 LAY DESCRIPTION

High levels of fat in the blood following a meal can impair the ability of blood vessels to function properly. A single session of exercise that occurs 4-24 hours prior to a high fat meal can substantially reduce the subsequent increase in the amount of fat in the blood. Whether the impaired ability of blood vessels to function properly can be reduced with an acute bout of exercise has not been extensively investigated in patients with peripheral arterial disease (PAD). The purpose of this study is to investigate if a single bout of exercise prior to feeding can counteract the impairment in blood vessel function following a high fat meal in patients with PAD.

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH

The vascular endothelium is a primary target for injury from mechanical forces and processes related to cardiovascular risk factors including, aging, hypertension, dyslipidemia impaired fasting glucose, insulin resistance and smoking. Vascular injury provoked by pathophysiological stimuli reduces the bioavailability of endothelium-derived NO resulting in endothelial dysfunction. This in turn contributes to lesion development through inflammation, vasoconstriction, smooth muscle cell proliferation, platelet activation, leukocyte adhesion, mitogenesis, pro-oxidation impaired coagulation, and thrombogenesis. There is now accumulating evidence that endothelial dysfunction represents one of the earliest events in the pathogenesis of cardiovascular disease.

The available evidence suggests that high levels of circulating triglycerides in response to a fatty meal (postprandial triglycerides) impairs endothelial function for up to 6 h (Gaenger et al. 2001; Plotnick et al. 1997; Vogel et al. 1997). Since a significant part of the day is spent in the postprandial state (Sies et al. 2005), strategies that counteract this state may be effective in preventing cardiovascular disease. A single bout of prolonged exercise that occurs 4-24 hours prior to feeding can reduce postprandial triglycerides. This acute effect typically results in a 20-30% reduction in the area under the triglyceride-time curve. To our knowledge no studies have examined the efficacy of a single exercise bout in attenuating postprandial lipemia and endothelial dysfunction in patients with peripheral arterial disease. The purpose of this study is to investigate if a single bout of exercise prior to feeding can counteract the postprandial impairment on endothelial function 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 (VRU) in the School of Health and Human Performance on 4 separate occasions. During the first visit subjects will undergo a brief physical examination, have their body composition assessed and perform a treadmill exercise test. The second and third visit will take place within 12-14 h of each other. Subjects will visit the VRU between 6 and 7 pm to undertake a bout of exercise (visit 2). Subjects will return to the VRU the next morning to undergo a 4 h oral fat tolerance test (visit 3). On the evening prior the fourth visit the subjects will rest quietly at home and visit the VRU the next morning to undergo a 4 h oral fat tolerance test. Subjects will consume their normal diet for 2 d before and will not undertake strenuous physical activity for 24 h prior to each oral fat tolerance test (OFTT). They will fast for 12 h before the OFTT. Blood sample will be taken and endothelial function will be assessed at regular intervals for 4 hours.

Visit 1: The nature and risks of the study will be explained, and informed consent will be obtained. Subjects will undergo a brief physical examination. The treadmill test will involve an incremental walking to volitional fatigue (2mph: 0%grade, increasing 2% every 2min until absolute claudication distance). A physician will be present during the test. Subjects will wear a mouthpiece or facemask during the test to familiarize them with the equipment. A 12 lead ECG will be used to continuously monitor the electrical activity of the heart. Rating of perceived exertion will be measured every min.

Visit 2: During the second visit subjects will walk on a treadmill at a self selected intensity until they expend 300Kcal within 1hr. 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 300 kcal. Subjects will wear a mouthpiece or facemask during the test to measure caloric expenditure. Heart rate will be continuously measured and rating of perceived exertion will be recorded every 5 min.

Visit 3: Oral fat tolerance tests

Subjects will visit the VRU between 8-10 am the following morning in a fasted state. An intravenous catheter will be inserted into a forearm vein and a fasting blood sample obtained (0 h). This catheter will kept patent during the 4 h postprandial follow-up period by flushing regularly with a 0.9% saline solution. Endothelial dependent dilation (EDD) and endothelial independent dilation (EID) will then be measured. The test meal will consist of a macronutrient composition per 2m² body surface area of 97 g fat, 124 g CHO and 1450 kcal. Subjects will rest quietly in the laboratory during the observation period with blood sampled at 0.5, 1, 2, 4h postprandially. Endothelial function will be measured at 0.5, 2, and 4hr.

Visit 4: On the evening prior the fourth visit the subjects will rest quietly at home and visit the VRU the next morning to undergo a 4 h oral fat tolerance test. Subjects will undergo the same experimental protocol as described for visit 3.

Dietary Control

Subjects will be required to abstain from alcohol and not to engage in exercise or heavy physical work, for 3 d prior to each OFTT. On the day prior to each OFTT, diet will be strictly controlled with subjects consuming 3 meals provided by the laboratory. The meals will be individualized to provide an energy content equal to 1.4 times BMR, with 56% as CHO, 30% as fat and 30% as protein. BMR will be estimated from the Harris-Benedict equation (12). On the two days prior to this, subjects will consume their normal diet. Food items consumed along with portion size will be recorded on sheets provided, prior to the first OFTT. This diet was then replicated in advance of the second OFTT.

Brachial Artery Reactivity (BAR):

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.

2.4 PARTICIPANT PROFILE

Men and women aged ≥ 40 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 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

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. 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 masters 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

DCUREC/2005/004: Effect of acute exercise and muscle glycogen on postprandial lipemia

DCUREC/2006/? OPEEC Study - Obesity, Prandrandial Lipemia, Endothelial function and Exercise in Children Study

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	N
• 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 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. The amount of blood drawn is not harmful.

3. Assessment of BF, VR and PORH will require restriction of blood flow for up to 10 minutes. This may cause slight discomfort in the leg, which will go away after the blood pressure cuff is deflated.

Alternatives to the risks: Analysis of circulating levels of blood lipids cannot be undertaken without a sample of blood. The investigators are certified and experienced in phlebotomy and plethysmography.

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 cholesterol 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 (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

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

Mr. Kevin O'Hara is a graduate student in the School of Health and Human Performance, DCU. He has extensive experience in studies involving humans.

Ms. Sarah Hughes 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. Sarah is also a certified phlebotomist.

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.

Dr. Catherine Woods is Head of the School of Health and Human Performance, Catherine and Dr. Noel McCaffrey established the community based Smart Steps rehabilitation program in DCU. Dr Woods is actively involved in the co-ordination of the HeartSmart classes.

Brona Furlong has recently graduated first in her class with a 1st class honours degree in Sports Science and Health. She is currently enrolled as a PhD student in the School of Health and Human Performance. She has extensive experience in blood sampling and research involving human subjects. Brona will assist with data collection and supervising classes.

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 Cardiovascular 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.

7. FUNDING

7.1 HOW IS THIS WORK BEING FUNDED?

NA

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

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

NA

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.)*

APPENDIX: B

8. PLAIN LANGUAGE STATEMENT (Approx. 400 words – see Guidelines)

Plain Language Statement

Dublin City University

Project Title: Effect of Acute Exercise on Postprandial Lipemia in 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)

I. The way the body processes the fat we eat is one of the factors that influences our risk of developing heart disease. After a single fatty meal, the level of fat in the bloodstream increases at first and then decreases again over an 8 hour period. Individuals who are not capable of processing fatty meals efficiently have a high concentration of fat in their bloodstream over a prolonged period which may impair blood vessel function. This study will investigate if a single bout of exercise prior to feeding can counteract the impairment in blood vessel function following a high fat meal in patients with PAD. The study will require that you make 4 visits to the Vascular Research Unit in the School of Health and Human Performance in DCU.

II. Visit 1: During the first visit you will undergo a brief physical examination and have your weight, height and body fat measured using fat calipers. You will then undertake a treadmill test. This will involve you walking until the pain in your legs forces you to stop. 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're exercising. You will have electrodes placed on your chest to measure the electrical activity of my heart. For this, and all subsequent exercise tests, you will be fitted with a mouthpiece connected to a machine to measure the composition of gases in your breath.

Visit 2 and 3: The second and third visit will take place within 12-14 h of each other. You will visit the VRU between 6 and 7 pm and walk on a treadmill. You will walk until you get claudication pain and then *you will rest until it goes away*. You will repeat this cycle of walking and recovery until you burn *the 500 calories*.

You will return to the VRU the next morning to undergo a 6 h oral fat tolerance test (visit 3). Before the fat meal, you will have a small plastic tube, called a catheter, inserted into a vein in your arm to facilitate the taking of blood samples. You will eat a fatty meal and then have a blood sample taken and 30 min, 1 hour, 2 hours, 4 hours and 6 hours following the meal. The health of your arteries will be assessed at the same time that 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. During this time you will be free to read and work quietly. The total amount of blood taken during this visit will be approximately 7 tablespoons.

On the evening prior the fourth visit you will rest quietly at home and visit the VRU the next morning to undergo a 6 h oral fat tolerance test. The same measurements will be taken after the meal as in visit 3.

You will consume your normal diet for 3 d before and will not undertake strenuous physical activity for 24 hours prior to each fat test. You will fast for 12 hours before each fat test. For 2 days before the first fatty meal test, you will record the food you eat in a diary. You will follow the same diet for the 2 days before the second fatty meal test.

- III.
1. 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.
 2. 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.
 3. 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.

- VI.** Involvement in this study is completely voluntary. You may withdraw from the Research Study at any time. Withdrawal from the study will not affect your participation in the SmartSteps Programme or the medical management of your condition.

 - VIII.** 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
-

APPENDIX: C

9. INFORMED CONSENT FORM (Approx. 300 words – see Guidelines)

Informed Consent

Dublin City University

Project Title Effect of Acute Exercise on Postprandial Lipemia in Peripheral Arterial Disease

Principle Investigator Prof. Niall M. Moyna

Introduction to this study

The way the body processes the fat we eat is one of the factors that influences our risk of developing heart disease. After a single fatty meal, the level of fat in the bloodstream increases at first and then decreases again over an 8 hour period. Individuals who are not capable of processing fatty meals efficiently have a high concentration of fat in their bloodstream over a prolonged period which may impair blood vessel function. The purpose of this study is to investigate if a single bout of exercise prior to feeding can counteract the impairment in blood vessel function following a high fat meal in patients with PAD.

Participants Requirements

1. I will visit the Vascular Research Unit in the School of Health and Human Performance DCU on 4 separate days.
2. During the first visit I will undergo a brief physical examination and I will also have my weight, height and body fat measured using fat calipers. I will then walk on a treadmill. The treadmill speed will stay at 2.0 miles per hour and the incline will increase by 2.0% every 2 minutes. I will continue walking until the pain in my leg forces me to stop. 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. For this, and all subsequent exercise tests, I will be fitted with a mouthpiece connected to a machine to measure the composition of gases in my breath
3. The main part of the study will involve the collection of blood samples following consumption of two high fat meals. Before each fat meal, I will have a small plastic tube, called a catheter, inserted into a vein in my arm to facilitate the taking of blood samples. I will eat a fatty meal and then have a blood sample taken and 30 min, 1 hour, 2 hours, 4 hours and 6 hours following the meal. The health of my arteries will be assessed at the same time that the blood samples are taken. During

this time I will be free to read and work quietly. The total amount of blood taken during this visit will be approximately 100 ml or 7 tablespoons.

4. On the evening before one of the fatty meals, I will go to DCU to walk until I burn between 500 calories. I will walk until I get claudication pain and then I will rest until it goes away. I will repeat this cycle of walking and recovery until I burn the 500 calories. On the evening before the other fatty meal test, I will rest quietly at home.
5. For 2 days before each fatty meal test I will not be able to do any exercise or strenuous physical work (e.g. gardening). I will also not be able to drink alcohol.
6. For 2 days before the first fatty meal test, I will record the food I eat in a diary. I will follow the same diet for the 2 days before the second fatty meal test.
7. 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. If I am taking Viagra I will notify Kevin O'Hara. I will not take Viagra for at least 24 hours before the two visits involving the high fat meal.

Potential risks to participants from involvement in the Research Study

1. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Your heart will be continuously monitored using a 12 lead ECG and a physician will be present.
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 amount of blood drawn is not harmful; however, if I have a history of anaemia, I should inform the investigator.
4. To assess the resistance vessels in my calf it will require blocking the blood flow to my calf for 5 minutes. This may cause slight discomfort in the leg, which will go away after the blood pressure cuff is deflated

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 No

Do you understand the information provided? Yes No

Have you had an opportunity to ask questions and discuss this study? Yes No

Have you received satisfactory answers to all your questions? Yes No

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. Withdrawal from this study will not affect your participation in the HeartSmart programme or the medical treatment 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 Acute Exercise on Postprandial Lipemia in Peripheral Arterial Disease

Participants Signature: _____

Name in Block Capitals _____

Witness: _____

Date: _____

Appendix D

Data Collection sheets

Screening Visit

Last Name: _____

Date: _____

First Name: _____

Subject ID: _____

Date of Birth: ____/____/____

Age: _____ yrs

Contact Details

Contact number (home) _____

Contact number (mobile) _____

Email Address _____

Informed consent signed

Yes

No

Medications: _____

Anthropometrics

Height: _____ cm

BMI/BSA _____

Weight: _____ kg

BP: _____ mmHg

Any comments: _____

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

Have you ever been told you have high cholesterol or been on medication for cholesterol?

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? If yes, how many per day? Yes / No

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

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

Are you currently taking vitamin supplements? If so please state brand and dosage

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

.....

.....

.....

Date:

Signature:

Authorising Signature:

Oral Fat Tolerance Test

Date: _____ Trial (Control/Exercise): _____

Supervised by: _____ Canula Insertion: _____

Control of prior exercise, diet and alcohol

Have you consumed any alcohol during the last 48 hours? Yes / No

Apart from the treadmill walk in the laboratory, have you undertaken any exercise or prolonged strenuous physical work over the last 48 hours? Yes / No

Have you closely followed the diet you recorded prior to your fatty meal test? Yes / No

Test Meal

Body Surface Area (m^2) _____

Croissants ($100g/2m^2$) _____

Butter ($35g/2m^2$) _____

Chocolate ($45g/2m^2$) _____

Pringles ($24g/2m^2$) _____

Total fat content (g) _____

Total carbohydrate content (g) _____

Test meal started _____

Test meal finished _____

Water consumed with meal (ml) _____

Meal finished completely _____

Effect of Acute Exercise on Postprandial Lipemia and Endothelial Dysfunction in Men with Peripheral Arterial Disease

Blood Sample	Due time	Time taken if different	Vacutainers
0Hr	_____	_____	RR_P____
30min post meal	_____	_____	RR_P____
1Hr post meal	_____	_____	RR_P____
2Hr post meal	_____	_____	RR_P____
3Hr post meal	_____	_____	RR_P____
4Hr post meal	_____	_____	RR_P____

Self Regulated Exercise

Name: _____

Date: _____

Bout	Speed (km/hr)	Grade(%)	IC (mins)	AC (mins)	RPE	RQ	Rest	EE (Kcal)
1								
2								
3								
4								
5								
6								
7								

Total Energy expended (Kcal) _____

Total mins exercised _____

Total Fat Oxidation (g) _____

Total Carbohydrate oxidation (g) _____

Comments:

Appendix E

Meal composition and nutritional information

Name	Energy (KJ/100g)	Energy (Kcal/100g)	Protein (g/100g)	CHO (g/100g)	Fat (g/100g)
Cadburys Dairymilk Buttons	2205	525	7.5	56.8	30
Mayfair Croissants	1647	393	8.6	48.4	18.3
Kerrygold spreadable Butter	2982	725	0.7	0.6	80
Original Pringles	2192	526	3.9	52	34

APPENDIX F

Table 0.1: Exercise Brachial artery diameter (cm)

	Baseline (cm)	Peak Dilation (cm)	Absolute Δ (cm)	Percentage Δ (cm)
Baseline	0.43 \pm 0.06	0.50 \pm 0.07	0.07 \pm 0.02	15.1 \pm 4.3
2 h post	0.46 \pm 0.06	0.49 \pm 0.06	0.04 \pm 0.02	8.2 \pm 5.7*
4 h post	0.47 \pm 0.08	0.52 \pm 0.06	0.05 \pm 0.03	12.4 \pm 9.4

Values are mean \pm SD; *p<0.05 v Baseline

Table 0.2: Control Brachial artery diameter (cm)

	Baseline (cm)	Peak Dilation (cm)	Absolute Δ (cm)	Percentage Δ (cm)
Baseline	0.42 \pm 0.06	0.47 \pm 0.06	0.05 \pm 0.02	12.51 \pm 2.94
2 h post	0.45 \pm 0.06	0.48 \pm 0.07	0.03 \pm 0.01	6.85 \pm 1.85*
4 h post	0.46 \pm 0.08	0.50 \pm 0.06	0.04 \pm 0.02	10.43 \pm 6.7

Values are mean \pm SD; *p<0.05 v Baseline

Table 0.3: Exercise Brachial artery diameter (cm) GTN

	Baseline (cm)	Peak Dilation (cm)	Absolute Δ (cm)	Percentage Δ (cm)
Baseline	0.46 \pm 0.04	0.52 \pm 0.07	0.06 \pm 0.04	12.27 \pm 8.85
2 h post	0.46 \pm 0.07	0.54 \pm 0.05	0.08 \pm 0.02	17.36 \pm 6.92
4 h post	0.46 \pm 0.07	0.53 \pm 0.07	0.07 \pm 0.03	16.32 \pm 8.16

Values are mean \pm SD;

Table 0.4: Control Brachial artery diameter (cm) GTN

	Baseline (cm)	Peak Dilation (cm)	Absolute Δ (cm)	Percentage Δ (cm)
Baseline	0.46 \pm 0.05	0.53 \pm 0.06	0.07 \pm 0.03	14.25 \pm 6.10
2 h post	0.46 \pm 0.05	0.53 \pm 0.04	0.07 \pm 0.03	15.56 \pm 6.84
4 h post	0.47 \pm 0.07	0.53 \pm 0.07	0.07 \pm 0.05	14.58 \pm 11.36

Values are mean \pm SD;

Table 0.5: Percentage changes in brachial artery Doppler flow - Exercise

	Baseline (cm)	Peak Dilation (cm)	Absolute Δ (cm)	Percentage Δ (cm/s)
Baseline	60.79 \pm 10.62	99.68 \pm 27.02	38.89 \pm 20.50	62.94 \pm 16.63
2 h post	59.24 \pm 8.52	101.21 \pm 13.56	41.97 \pm 10.69	71.90 \pm 19.72
4 h post	54.10 \pm 8.50	89.63 \pm 19.94	35.54 \pm 19.80	67.85 \pm 36.43

Table 0.6: Percentage changes in brachial artery Doppler flow - Control

	Baseline (cm)	Peak Dilation (cm)	Absolute Δ (cm)	Percentage Δ (cm)
Baseline	56.53 \pm 13.73	104.96 \pm 18.83	48.43 \pm 19.69	92.98 \pm 52.60
2 h post	54.61 \pm 10.47	111.78 \pm 13.73	57.16 \pm 20.15	112.63 \pm 56.56*
4 h post	51.10 \pm 10.40	87.61 \pm 7.37	36.52 \pm 12.46	77.57 \pm 38.58

Values are mean \pm SD; *p<0.05 v Baseline

