CARDIFF UNIVERSITY

Clinical Effectiveness of the Angiotensin Converting Enzyme Inhibitor, Ramipril, in Patients with Intermittent Claudication: Randomised, Double-blind, Placebo-controlled Trial

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- **Shahin Y**, Barakat H, Barnes R, Chetter I. The Vicorder device compared to SphygmoCor in the assessment of carotid-femoral pulse wave velocity in patients with peripheral arterial disease. *Hypertension Research* 2013; 36(3):208-12.
- **Shahin Y**, Chetter I. Aortic augmentation index is independently associated with N-terminal pro B-type natriuretic peptide in patients

with peripheral arterial disease. *Vascular Endovascular Surgery* 2012; 46(8):648-53.

- **Shahin Y**, Hatfield J, Chetter I. C-reactive protein and the Framingham coronary risk score in patients newly diagnosed with intermittent claudication: A prospective study. *Vascular and Endovascular Surgery* 2012; 46(3): 242-5.

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- **Shahin Y**, Cockcroft JR, Chetter I. (Abstract) Improvement of walking distance in patients with intermittent claudication is partly due to its effect on arterial stiffness: Results form a randomized controlled trial. *Journal of Vascular Surgery* 2013; 57(5):97S.

- **Shahin Y**, Chetter I. (Abstract) Effect of the angiotensin converting enzyme inhibitor, ramipril, on walking distance, arterial stiffness and quality of life in patients with peripheral arterial disease: Randomized, double-blind, placebo-controlled trial. *British Journal of Surgery* 2013; 100(S2):1–8

- **Shahin Y**, Barakat H, Chetter I. (Abstract) Arterial stiffness is associated with intermittent claudication walking distance and estimated glomerular filtration rate in patients with symptomatic peripheral arterial disease. *European Surgical Research* 2013; 50:81.

- **Shahin Y**, Barakat H, Barnes R, Chetter I. (Abstract) The Vicorder device compared to SphygmoCor in the assessment of carotid-femoral pulse

wave velocity in patients with peripheral arterial disease'. *European Surgical Research* 2013; 50:89.

- **Shahin Y**, Chetter I. (Abstract) A comparison between aortic pulse wave analysis indices obtained by the Vicorder device and the SphygmoCor in patients with peripheral arterial disease. *European Surgical Research* 2013; 50:89.

- **Shahin Y**, Chetter I. (Abstract) Aortic augmentation index is associated with N-terminal pro B-type natriuretic peptide in patients with symptomatic peripheral arterial disease. *European Surgical Research* 2013; 50:151.

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- **Shahin Y**, Khan JA, Samuel N, Chetter I. (Abstract) Angiotensin converting enzyme inhibitors effect on endothelial dysfunction: A meta-analysis of randomized controlled trials. *Atherosclerosis supplement* 2011; 12(1):13-184.

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Abstract

Background

The HOPE trial showed that ramipril reduced cardiovascular morbidity and mortality in patients with peripheral arterial disease (PAD). However, evidence regarding the effect of angiotensin converting enzyme (ACE) inhibitors on walking distance, ankle brachial pressure index (ABPI), arterial stiffness and quality of life (QoL) in this group of patients is limited.

Objective

The aim of this study is to investigate ACE inhibitors effect on clinical parameters of PAD, arterial stiffness and QoL in patients with intermittent claudication (IC).

Methods

33 patients (25 males, mean age: 65+/-7.8) with IC (Fontaine stage II or higher) were randomised to receive ramipril (5 mg once daily for 2 weeks increased to 10 mg once daily for 22 weeks, n=14) or placebo (n=19) for 24 weeks in a double-blind study. Walking distance was assessed using a standard laboratory treadmill test (2.5 km/hr at 10% incline). ABPI was assessed pre (r-ABPI) and post-exercise (t-ABPI). Arterial stiffness indices were measured using the SphygmoCor device.

Results

After 24 weeks, ramipril improved maximum treadmill walking distance; adjusted mean change difference (95% confidence interval); by 130.5 (61.8 to 199.2) m longer than placebo (P=0.001), improved treadmill intermittent claudication distance by 121.9 (55.9 to 187.8) m longer than placebo (P=0.001) and improved patient reported walking distance by 159 (5.5 to 313) m compared to placebo (P=0.040). Ramipril reduced carotid femoral pulse wave velocity (a measure of arterial stiffness) by -1.47 (-2.4 to -0.57) m/s compared to placebo (P=0.002). However, r-ABPI and t-ABPI minimally changed in both groups (Ramipril 0.02 (-0.08 to 0.11) vs. placebo 0.03 (-0.05 to 0.10, P=0.830) and (Ramipril 0.04 (-0.04 to 0.12) vs. placebo 0.02 (-0.04 to 0.09), P=0.720), respectively. Ramipril had a slight insignificant effect on QoL physical domains compared to placebo.

Conclusion

Ramipril improves walking distance in patients with IC; however, this improvement is not related to improvement in ABPI but might be due to ramipril ability to reduce arterial stiffness. ACE inhibitors effect on QoL needs to be validated in a larger randomised controlled trial.

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Glossary of Abbreviations

95% CI	95% confidence interval
ABPI	Ankle brachial pressure index (See p21)
ACE	Angiotensin converting enzyme
AIx	Augmentation index (See p76)
AIx@75	Augmentation index adjusted to 75 beats/min (See p74)
ANCOVA	Analysis of covariance – statistical test (See p138)
ANOVA	Analysis of variance – statistical test (See p138)
AoBP	Aortic (Central) blood pressure
AP	Augmentation pressure (See p76)
ARB	Angiotensin receptor blocker (See p88)
AUC	Area under the curve (See p75)
BP	Blood pressure
bBP	Brachial blood pressure
CAD	Coronary artery disease (See p33)
CCBs	Calcium channel blockers (See p45)

CENTRAL	Cochrane Central Register of Controlled Trials (See p56)
CRP	C-reactive protein (See p33)
DM	Diabetes mellitus
ECM	Extracellular matrix (See p25)
ED	Ejection duration (See p73)
ED %	Ejection duration index (See p74)
eGFR	Estimated glomerular filtration rate (See p122)
EQ5D	EuroQol utility index- generic quality of life instrument (See p124)
FRS	Framingham risk score (See p38)
GENOA	Genetic Epidemiology Network of Arteriopathy study (See p31)
HDL-C	High density lipoprotein cholesterol (See p34)
HR	Heart rate
IC	Intermittent claudication
IQR	Interquartile range (See p136)
LDL-C	Low density lipoprotein cholesterol (See p53)
MAP	Mean arterial pressure (See p76)
MHRA	Medicinal Health Regulatory Agency (See p133)

MI	Myocardial infarction
MMPs	Matrix metalloproteinases (See p27)
MRA	Magnetic resonance angiography (See p40)
MTMMPs	Membrane-type metalloproteinases (See p27)
MWD	Maximum treadmill walking distance (See p54)
MWT	Maximum treadmill walking time (See p54)
MWU	Mann Whitney U test – statistical test (See p138)
NHANES	National Health and Nutrition Examination Survey (See p31)
NTproBNP	N-terminal pro B-type natriuretic peptide (See p106)
NO	Nitric oxide (See p79)
OR	Odds ratio
PAD	Peripheral arterial disease (See p21)
PFWD	Pain-free treadmill walking distance (See p54)
PFWT	Pain-free treadmill walking time (See p54)
PP	Pulse pressure (See p73)
PTA	Percutaneous transluminal angioplasty (See p42)
PWA	Pulse wave analysis (See p65)

PWVcf	Carotid-femoral pulse wave velocity (See p70)
QoL	Quality of life (See p41)
r	Pearson's correlation coefficient (See p139)
RAS	Renin angiotensin system (See p46)
RCT	Randomised controlled trial
RR	Relative risk
SEM	Standard error of mean (See p 136)
SEP	Supervised exercise programme (See p41)
SEVR	Subendocardial viability ratio (See p73)
SF-36	Short form-36 questionnaire- generic quality of life instrument (See p122)
TIMPs	Tissue inhibitor metalloproteinases (See p27)
tt	Transit time (See p70)
VasculQol	King's College vascular quality of life questionnaire- disease specific quality of life instrument (See p127)
$\chi 2$	Pearson's Chi-Square test – statistical test (See p139)

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…"Show me a thoroughly satisfied man and I will show you failure. I believe that restlessness is discontent and discontent is merely the first necessity of progress"

Thomas Alva Addison

1.1. Lower Limb Peripheral Arterial Disease and Intermittent Claudication

Opening Statement

Peripheral arterial disease (PAD) of the lower limbs is a common disorder and an important marker of cardiovascular risk. It is associated with mortality 3-5 times that of an age-matched population mainly due to cardiovascular and cerebrovascular events such as myocardial infarction (MI) and stroke. PAD can be classified into: asymptomatic and symptomatic or intermittent claudication (IC). Asymptomatic PAD is usually suspected during clinical examination of the lower limbs pedal pulses. It can be diagnosed by measuring the Ankle Brachial Pressure Index (APBI). APBI measurement is a simple and usually confirms the presence of the disease. It is calculated by dividing the ankle pressure by the highest measured systolic brachial blood pressure (bBP). An ABPI of ≤ 0.9 is usually diagnostic of PAD of the lower limbs. IC, which is the focus of this thesis, is a clinical diagnosis given to muscle ache or pain classically in the calf during a period of exercise and is relieved after a short period of rest.

Epidemiology

The annual incidence of IC is difficult to measure; however, the prevalence of the disease is more important and ranges from 3% in patients aged 40 to 6%

in patients aged 60 [1]. Based on several large population studies which looked at prevalence of IC, the disease is more prevalent in men in the younger age groups but occurs equally in men and women at older ages.

Aetiology

IC can be defined as exercise-induced pain or fatigue that affects a group of muscles in the lower limb which is relieved after a short period of rest. Other features of IC include: Progression over time, the pain increases and becomes severer while doing the same usual functions due to disease progression, the anatomical correlations of some risk factors for PAD with some segments of the arterial tree, for instance, diabetes mellitus (DM) with infrapopliteal arteries and smoking and hypertension with aortoiliac arteries and the level of stenosis or arterial involvement is usually above the area of pain. However, classical symptoms of claudication are not always featured leading to a 90% of cases with PAD of the lower limbs being missed [1-3].

Risk Factors

Several risk factors have been associated with an increased risk of developing PAD leading to IC. These risk factors include non-modifiable risk factors such as race, gender and age and modifiable risk factors such as DM, hypertension, smoking and hypercholesterolaemia.

Non-modifiable Risk Factors

I. Race: The GENOA (Genetic Epidemiology Network of Arteriopathy) study [4] showed differences in the prevalence of PAD among different ethnic groups and that this difference is independent of the difference in classical risk factors for atherosclerosis among these groups. Furthermore, the National Health and Nutrition Examination Survey (NHANES) in the United States [5] showed that the percentage of non-Hispanics Blacks who had an ABPI ≤ 0.9 was more than Whites (7.8% versus 4.4%, respectively).

II. Gender: The prevalence of PAD of the lower limbs is more common in men especially in the younger age groups; however, the prevalence decreases and becomes similar in both men and women in the older age groups. In patients with IC, the ratio of men to women is between 1:1 and 2:1 and sometimes reaches 3:1 in more advanced stages of the disease.

III. Age: Newman [6] found that an ABPI which is diagnostic of PAD is evident in 12% of adults above the age of 50. Of those about a third suffers from IC.

Modifiable Risk Factors

i. DM: Diabetes is one of the strongest risk factors associated with PAD. IC is twice as common in patients with DM as in non-diabetic patients. There is an increased risk of PAD of about 26% with every 1% increase in HbA1c in patients with DM [7]. PAD in patients with DM is more aggressive than in non-diabetic patients with early involvement of large arteries and distal symmetrical neuropathy. In a cross-sectional study of 4153 Greek adults [8], the odds ratio (OR) for vascular disease was 1.94 (95 per cent confidence interval (95% CI) 1.35 to 2.47) in patients with metabolic syndrome and 3.04 (1.98 to 4.11) in patients with metabolic syndrome and DM. Thus, the risk of PAD increased with a combination of metabolic syndrome and DM. Moreover, the need for amputation is five to ten times higher in patients with DM than in non-diabetics. This could be attributed to neuropathy and increased infection rate in diabetic patients.

ii. Hypertension: The National Health and Nutrition Examination Survey [5] showed an association between hypertension and PAD. In brief, some 2174 patients more than 40 years old were included in the survey. The prevalence of PAD was 4.3%. In patients above the age of 70 years the prevalence was 14%. There was a strong association between hypertension and PAD among other risk factors assessed in this survey (OR 1.75, 95% CI 0.97 to 3.13).

iii. Smoking: Smoking is another important risk factor for PAD. It promotes endothelial dysfunction and affects lipid metabolism and coagulation [9]. The Edinburgh artery study [10] involved 1592 men and women aged 57-74 years old randomly selected from several general practices across Scotland showed that smoking is strongly associated with PAD and that the association was stronger than with coronary artery disease (CAD). Cigarette smoking also increased biomarkers of coagulation and endothelial function. The OR of PAD increased 7.3 times (95% CI 4.2 to 12.8) by smoking 25 cigarettes of more.

iv. Hypercholesterolaemia: High cholesterol concentration and familial hyperlipidaemia are significant risk factors for atherosclerosis and PAD. The prevalence of PAD increases by 5 folds to 10 folds in patients with familial hypercholesterolaemia. Treatment with statins, therefore, has the potential to decrease the risk of PAD and increasing the walking distance in patients with IC. Ridker et al.[11] compared the predictive value of 11 lipid and non-lipid biomarkers as risk factors for PAD in 14916 healthy U.S male physicians aged 40 to 84 years. Multivariable analysis showed that the total cholesterol-high density lipoprotein cholesterol (HDL-C) ratio (Relative risk (RR) for those in the highest versus lowest quartile, 3.9; 95% CI 1.7 to 8.6) and C - reactive protein (CRP) (RR for the highest versus lowest quartile, 2.8; 95% CI 1.3 to 5.9) were the strongest independent predictors for development of PAD.

Arterial Wall Structure and the Extracellular Matrix

The structure of arteries and veins is generally similar with the walls comprising three concentric layers. The tunica adventitia is the strong outermost layer of both veins and arteries. It comprises connective tissue, collagen and elastin as well as nerves which supply the vessel and its nutrient vessels.

The tunica media is the middle, thickest layer and contains a circular arrangement of elastin, connective tissue and particularly in arteries vascular smooth muscle, which is responsible for the calibre of the vessel.

The tunica intima is the thinnest layer and in arteries this layer is composed of an internal elastic membrane lining and a single layer of smooth endothelial cells. The difference between veins and arteries is that veins do not contain the elastic membrane lining and in some veins the tunica intima is folded back on itself to form valves (Figure 1).

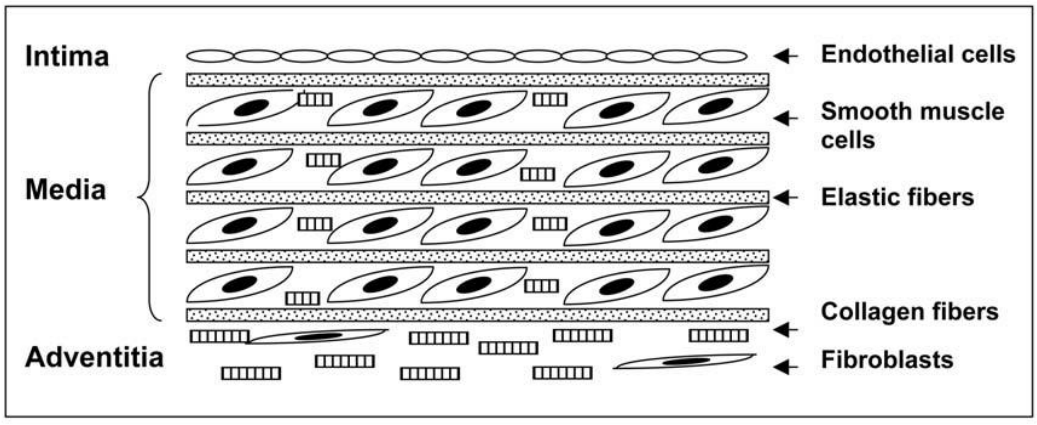

Figure 1: ***Arterial wall structure. Adapted from Jacob 2003***[12]

The extracellular matrix (ECM) plays a vital role in the structure of the arterial wall. The cells of the three layers of the arterial wall are embedded within their ECM. Endothelial cells lie on the basement membrane of the intima. This basement membrane consists of collagen type IV. Collagen and Elastin are the most important components of the arterial wall and they regulate the arterial wall function and the biomechanical properties of the arterial wall are influenced by the quantities of these two constituents.

The arterial wall is a dynamic organ composed of endothelium, medial smooth muscle and fibroblast cells invested in ECM (Figure 1). Vascular smooth muscle cells not only control wall tension but also synthesize the major structural components of the vessel wall [13].

The ECM consists of the scaffolding elements of collagen and elastin embedded in glycoproteins and proteoglycans (Table 1). Therefore, the stability, resilience and compliance of the vascular wall are dependent on the contribution of these imminent components. The relative content of these molecules is normally held stable by a slow but dynamic process of degradation and production. Dysregulation of this balance, mainly by stimulation of an inflammatory milieu, leads to overproduction of abnormal collagen and diminished quantities of normal elastin, resulting in an increase in arterial stiffness.

Structural Component	Molecules	Main Properties
Collagen fibres	Type I and III collagens (90%) Types V, VI, VIII, XII, XIV, XV and XVIII collagens	Tensile strength
Elastic fibres	Elastin (90%) Fibrillin-1 and -2, MAGP-1 et -2, Emilin-1 et -2, Fibulin-5	Elasticity
Basement membrane of endothelial cells and smooth muscle cells	Type IV collagen Laminin, Entacten Perlecan (HS-PG)	Filtration and resistance
Other components	Proteoglycans: Versican (CS-PG), hyaluronan Biglycan (DS-PG), decorin (DS-PG), lumican (KS-PG) (Small PG) Syndecans (HS-PG), glypican (HS-PG) (Cell-associated PG)	Hydration, filtration, interaction with other ECM components, reservoir of growth factors
	Structural glycoproteins: Fibronectins, Vitronectin	Interactions between ECM molecules via integrins
	Matricellular proteins: Thrombospondins, Tenascins, Osteopontin	Structure, bioreactivity

Table 1: ***Structural components and main properties of the extracellular matrix. ECM: Extracellular matrix, MAGP: Microfibrillar – associated glycoproteins, HS-PG: Heparan sulphate proteoglycan, CS-PG: Chondroitin sulphate proteoglycan, DS-PG: Dermatan sulphate proteoglycan, KS-PG: Keratan sulphate proteoglycan. Adapted from Jacob 2003***[12]

Angiotensin II stimulates both myocardial and vascular collagen expression [14] and inhibition of the renin-angiotensin system (RAS) will reduce the collagen deposition [15,16] and increase the elastin/collagen ratio leading to a reduction in arterial stiffness [16-21].

The regulation of the changes in ECM structure is partly mediated by matrix metalloproteinases (MMPs) which not only play a role as extracellular degrading enzymes but also exert effects on cellular growth and proliferation[11]. MMPs include collagenases (MMP-1, expressed by endothelial cells and MMP-8), gelatinases (MMP-2, expressed by vascular cells, MMP-9, expressed in macrophages and inducible in vascular cells), elastases (MMP-7, expressed by the vascular wall, MMP-12, synthesized during the differentiation of monocytes into macrophages), stromelysins and membrane-type matrix metalloproteinases (MT-MMPs). The proteolytic activity of each MMP is regulated at three levels. Firstly, gene expression and protein secretion levels, secondly, activation of the inactive pro-enzyme and thirdly, inhibition of tissue inhibitors of MMPs (TIMPs) or other inhibitors such as α2- macroglobulin.

Collagen and elastin are potently regulated by the catabolic MMPs which degrade the ECM by creating uncoiled less effective collagen and broken and frayed elastin molecules [22-24] increasing the stiffening of the arterial wall. (Arterial stiffness will be discussed in more detail in c chapter 3).

Clinical Features of IC

Symptoms and signs

The majority of patients with PAD have limited exercise performance and walking ability which can have a huge effect on their quality of life. The classical symptom in patients with PAD of the lower limbs is IC. This means that these patients limb due to the pain they experience while walking. This exercise-induced pain is caused by ischemia and is relieved after a short period of rest. The pain is most commonly localised to the calf but can affect the thigh and buttocks depending on the artery affected by atherosclerosis. It is worth mentioning that patients without classical symptoms of IC can also have walking limitations and experience atypical or no limb symptoms [25]. Patients also might not experience typical claudication symptoms due to them having conditions such as congestive heart failure, pulmonary disease or musculoskeletal disease which will prevent them from exercise and producing the symptoms of claudication. PAD is caused by atherosclerosis which causes stenosis and/or occlusion of the major arteries that supply the lower limbs. Patients with IC have no limbs symptoms at rest because they will have a normal and sufficient blood flow to the lower limbs. However, during exercise, the increased blood flow to the lower limbs is limited by the occlusive lesions in the arteries causing a mismatch between oxygen supply and the muscles metabolic demand which will produce the symptoms of claudication.

Clinical signs that are found in a patient with IC include, but are not limited to, changes in skin colour and temperature of the lower limbs, muscle atrophy due to lack of exercise, decreased hair growth and slow-growing and hypertrophied nails. Bruits could be auscultated over the major arteries supplying the lower extremities and indicate the presence of a turbulent blood flow due to stenosis and/or occlusion. The absence of bruits, however, does not exclude arterial disease. Examination of the pulses of the lower limbs is of paramount importance in the assessment of a patient with IC. A diminished or absent femoral pulse would suggest aortoiliac disease which suggests diminished blood inflow and a palpable femoral pulse and an absent pedal pulse would suggest arterial disease in the lower leg with preserved inflow. Moreover, isolated lesions in the internal iliac arteries could be associated with normal femoral and pedal pulses at rest but associated with buttock claudication and (impotence in males). Similarly, isolated lesions in the external iliac arteries produce similar signs of normal femoral and pedal pulses in the lower limbs at rest. Nevertheless, these pulses will not be maintained during exercise due to peripheral occlusive lesions limiting blood flow to the lower limbs.

Diagnosis

Figure 3 illustrates an algorithm for the diagnosis of PAD.

Ankle Brachial Pressure Index

Measurement of ABPI has become a standard part of the initial evaluation of a patient with PAD of the lower limbs. The method of measurement is simple and involves using a 10–12 cm sphygmomanometer cuff placed just above the ankle and a Doppler instrument used to measure the systolic pressure of the posterior tibial and dorsalis pedis arteries of each leg. The resulting ankle pressure values from each leg are then divided by the highest systolic bBP of either arm. The index leg is the leg with the lower ABPI. The ABPI provides important information in the assessment of a patient with symptomatic PAD (IC). A reduced ABPI confirms the presence of an occlusive disease between the heart and the ankle of the affected leg. An ABPI ≤0.9 is usually diagnostic of PAD and the lower the ABPI, the more severe the occlusion would be. ABPI also aids in the differential diagnosis of patients presenting with pain of the lower limbs induced by exercise; a normal ABPI will rule out the presence of arterial occlusive disease and other causes of exercise-induced pain in the lower limbs should be sought. Additionally, ABPI provides valuable prognostic information about the risk of developing cardiovascular events which is independent of other standard risk factors. For instance, an ABPI ≤ 0.9 is associated with a three to six fold increased risk of cardiovascular mortality. Therefore, ABPI has the potential to provide additional prognostic information when combined with other cardiovascular risk predictors such as the Framingham risk score (FRS).

Treadmill exercise test

Treadmill exercise testing is used mainly in patients with normal ABPI at rest such as patients with iliac artery disease where exercise can induce a decrease in ABPI which is measured immediately after walking on a treadmill and once detected confirms the diagnosis of PAD. These patients will walk on a treadmill at a typical speed of 3.2 km/h at an incline of 10%-12% until claudication pain occurs or for a maximum of 5 minutes[1]. A decrease in ABPI of 10%-15% is normally diagnostic of PAD.

Colour-assisted Duplex Ultrasonography

Duplex ultrasonography provides an attractive alternative to angiography. It is non-invasive, less expensive and in the correct hands can provide most of the anatomical information needed. Moreover, it can provide additional information such as velocity gradients across stenosis. The whole arterial tree can be visualized and assessed for the presence of occlusive lesions. However, crural arteries are challenging and cannot be visualised easily. Other limitations include the long time the test needs and the need for experience and training.

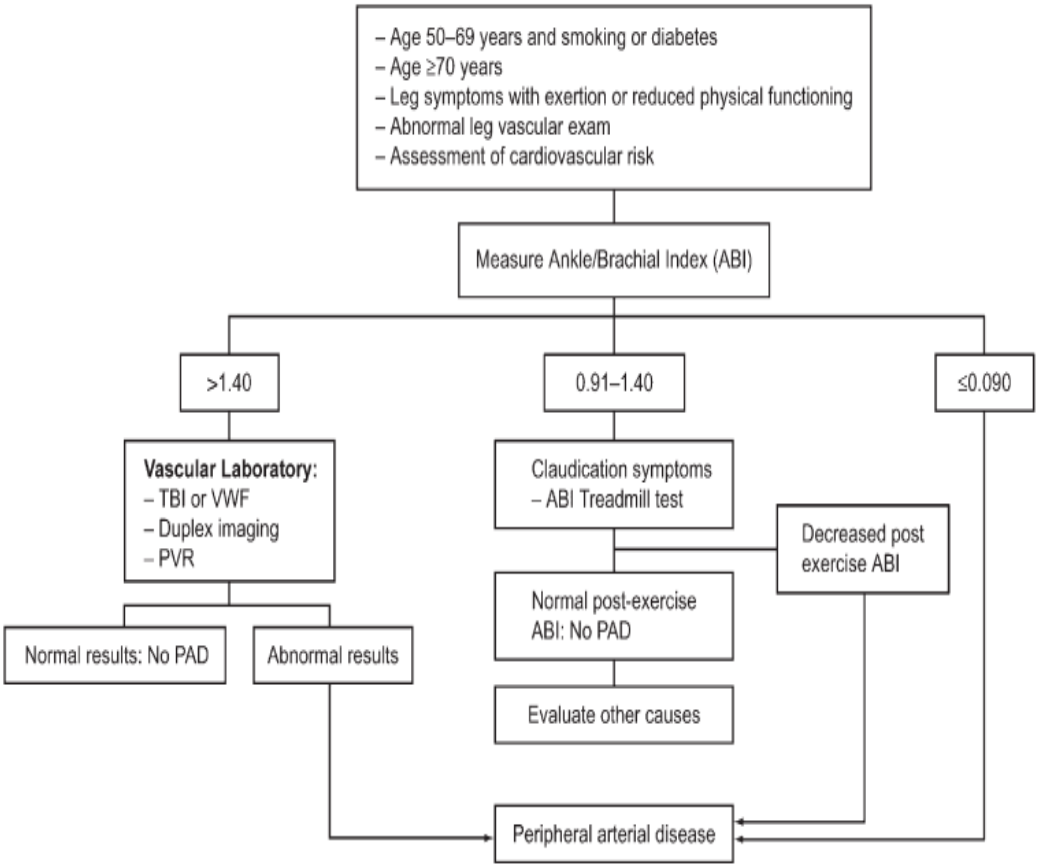

Figure 2: ***Algorithm for the diagnosis of PAD. TBI: toe brachial index, VWF: velocity wave form, PVR: pulse volume recording. This algorithm has been adapted from Hiatt et al.***[26]

Angiography

Angiography is considered the gold standard test but has some risks. These include severe reaction to the contrast material which can cause renal failure in patients with deranged renal function, bleeding, infection, pseudoaneurysm, haematoma, atherothrombotic events and carries a risk of mortality of about 0.17% [1]. Nevertheless, despite all the previously mentioned complications, digital subtraction angiography of the arterial tree from the renal to the pedal arteries remains the investigation of choice in patients with PAD.

Magnetic Resonance Angiography

Magnetic resonance angiography (MRA) is rapidly becoming the preferred diagnostic and treatment planning technique in patients with PAD. It produces a 3D image of the arterial tree which can be rotated and studied in detail enabling pre-procedural treatment planning. To this end, it has been widely used in the planning for endovascular procedures.

Disadvantages of MRA include the exclusion of some patients with metal implants due to the high magnetic field, claustrophobia and the artifacts produced by some stents in the peripheral arteries which makes assessment of these segments difficult. However, calcified arteries such as in patients with DM or renal failure can be visualised on MRA in contrast to other imaging techniques.

Biomarkers Associated with PAD

PAD is associated with increased cardiovascular morbidity and mortality. There have been several biomarkers associated with PAD, however, a few are used in routine clinical practice. The most important biomarkers associated with PAD are C-reactive protein (CRP), N-terminal pro B natriuretic peptide (NTproBNP), Fibrinogen and urine albumin creatinie ratio which is an indicator of urine albumin excretion rate.

Patients with PAD have several vascular beds affected by the disease such as; coronary, cerebral and renal ones. This may account for the increased mortality and morbidity associated with PAD due to cardiovascular and cerebrovascular events. It is essential to stratify and treat cardiovascular risk factors early to prolong survival in this patients' cohort. Biomarkers, therefore, can serve as indicators of the disease helping clinicians to identify patients with PAD at increased risk of cardiovascular, cerebrovascular or renovascular events.

C - reactive protein

CRP which is a marker of low- grade inflammation is mainly produced in the liver in response to pro-inflammatory cytokines such as IL-6[27] and is widely used in evaluating patients suffering from various inflammatory disorders. There is a link between the severity of PAD and the inflammation caused by

atherosclerosis where a number of pro-inflammatory cytokines such as: IL-6 and tumor necrosis factor-α are produced [28] causing an increase in serum CRP levels. There also seems to be an inverse relationship between ABPI and CRP levels[29]. Burke et al.[30] have also shown that CRP is a marker of vascular inflammation and that the higher the CRP levels the higher the number of vascular beds affected by atherosclerosis. Moreover, high serum CRP levels have shown to be associated with increased risk of cardiovascular events in patients with PAD[31,32]. Beckman et al[31] demonstrated that CRP and ABPI independently or in combination predicted acute coronary syndrome, stroke or death in patients with PAD. Furthermore, The Pravastatin or Atorvastatin Evaluation and Infection Therapy—Thrombolysis in Myocardial Infarction 22 study[33] showed that lowering CRP with statin therapy improves cardiovascular outcomes independent of the reduction in serum cholesterol levels. Serum CRP levels adds prognostic information to clinicians that might guide clinical work-up and therapy intensity. In our trial we hypothesized ,as reported in several previous observational studies[34-37] that Ramipril reduces CRP serum levels in patients with PAD, therefore, reducing inflammation associated with PAD which leads to a reduction in cardiovascular morbidity and mortality.

Fibrinogen

The components of the clotting cascade can be hypothesized to be useful predictors of atherosclerosis and subsequent complications. Ridker et al. [38] have shown that elevated fibrinogen levels are a consistent risk factor for atherothrombosis. Smoking, DM, hypertension, hypercholesterolaemia and inflammation are all causes for elevated fibrinogen levels. In patients with PAD, elevated fibrinogen levels are associated with increased cardiovascular events[39]. Measurement of fibrinogen in patients with PAD allows for identifying patients with increased risk of cardiovascular events who could be targeted with fibrate lowering medications which has a lipid and fibrate lowering effect[40]. In this trial, we hypothesized that ramipril reduces fibrinogen levels resulting in a reduction in cardiovascular events in patients with PAD.

Urine Albumin Creatinine Ratio

Shearman et al.[41] and Hickey et al.[42] have reported that urinary microalbumin concentrations increase following exercise in patients with intermittent claudication. The reason behind this is that severe ischaemia produced by exercise can initiate an acute inflammatory response which causes endothelial cell damage and a systemic increase in vascular permeability. Microalbuminuria results from an increase in the glomerular filtration of albumin and probably reflects a generalised increase in vascular permeability

to macromolecules. Additionally, Matsushita et al.[43] showed in a cohort study of 15 patients with IC that the post-exercise urinary microalbumin elevation was related to the MWD. For patients with an elevated urinary microalbumin concentration, microalbuminuria can be used as a marker for IC and help to evaluate the effects of treatments. It can also be a predictor of cardiovascular prognosis and a manifestation of widespread atherosclerosis. In this trial we hypothesized that ramipril reduces microalbuminuria in patients with PAD resulting in decreased cardiovascular events and a reduction in atherosclerosis.

N-terminal pro B-type natriuretic peptide

The amino-terminal fragment of B-type natriuretic peptide prohormone (NTproBNP) is a marker of cardiac function and is increased in symptomatic and asymptomatic heart disease[44,45]. NTproBNP has been shown to be a robust and independent predictor of all-cause mortality in patients with PAD and is considered a valuable tool for risk stratification in this patients' cohort[46]. Although NTproBNP is a surrogate marker of left ventricular systolic dysfunction and is increased in patients with heart failure, some studies have shown that NTproBNP plasma levels are increased in patients with atherosclerosis irrespective of left ventricular function[45,47]. Therefore, finding potential causes that may contribute to increased NTproBNP levels in patients with atherosclerosis and understanding the mechanism behind this

increase could be essential to decrease the associated high cardiovascular morbidity and mortality.

NTproBNP serum levels were measured in this trial to assess the impact of ramipril on cardiac function. The rationale behind this is that ramipril by reducing serum levels of NTproBNP would result in improved cardiac function and prognosis.

The Fate of the Leg

The overall prognosis for a patient with PAD is poor with a cumulative annual mortality of about 5%. In patients with IC, however, the prognosis for the limb is more benign with 75% of patients' symptoms remaining stable/ improving with only a small minority progressing to critical limb ischaemia. This stabilization of symptoms may be due to the development of collaterals, metabolic adaptation of ischaemic muscle or the alteration of gate in favour of the non-ischaemic muscle groups.

In addition, major amputations are a rare outcome of IC. A large scale study[48] found that the rate of amputation in unselected patients with PAD was less than 2% (Figure 4) [49].

Treatment

The treatment options for IC can be classified into non-invasive (Conservative) and invasive.

Non-invasive (Conservative) Management

Supervised Exercise Programme

Supervised exercise programmes (SEP) have a well-established role in the treatment of patients with IC with significant clinical and quality of life (QoL) improvements [50,51].

Two Cochrane reviews have shown that SEP is as effective as percutaneous transluminal angioplasty (PTA) in the treatment of stable claudicants [52,53]. SEP works by a combination of factors including optimised structural and metabolic performance of skeletal muscle, improvement in rheological parameters, development of collaterals, improved walking technique and marked psychological benefit [54]. In prospective studies, involving SEP, which have been conducted for 3 months or longer, there have been significant increases in treadmill walking distances and a reduction in claudication pain during exercise [55].

Pharmacotherapy

The focus of the pharmacotherapy is to reduce the symptoms of claudication and decrease the risk of cardiovascular morbidity and mortality associated with PAD.

Cilostazol

Cilostazol is a vasodilator drug with antiplatelet properties, and was approved for treatment of intermittent claudication in 1999 [26]. A meta-analysis of eight randomised control trials [56] (RCTs) and a more recent Cochrane review [57] concluded that cilostazol demonstrated significant improvement in walking distances and QoL for these patients as compared to placebo. Dawson et al.[58] investigated the effect cilostazol and pentoxifylline in 698 patients. Treatment with pentoxifylline improved walking distance to a similar extent as placebo. However, cilostazol improved walking distance by 54% from baseline. Cilostazol is a selective type III phosphodiesterase inhibitor, thereby increasing the intracellular concentration of cyclic adenosine monophosphate. This results in vasodilatation improving blood flow through the peripheral arteries. Additionally, it inhibits platelet aggregation, thrombus formation and vascular smooth muscle proliferation [26,56].

Lipid Lowering Drugs

Statins have shown to improve walking distance and decrease cardiovascular morbidity and mortality in PAD patients. Aronow et al.[59] found that simvastatin significantly increased treadmill claudication time by 24% at 6 months and 42% at 1 year compared to placebo. Mondillo et al.[60] reported similar improvement after short term therapy with simvastatin in 43 patients with IC and elevated cholesterol. Treadmill walking distance significantly

improved after simvastatin therapy by 126 metres and this was the case for ABPI which improved by 0.09.

Antithrombotic Agents (Aspirin or ADP-receptor Antagonists Clopidogrel or Ticlopidine)

Antithrombotic agents play an essential role in reducing the increased risk of cardiovascular events associated with PAD[61]. However, they have shown no benefit in improving the symptoms associated with PAD[62].

Vasodilators

This group of therapeutic agents were the first to be used for treatment of IC. Examples of these drugs include: α-blockers, direct vasodilators, β2 agonists, calcium channel blockers (CCBs) and ACE inhibitors. This group of drugs, apart from ACE inhibitors showed no benefit in improving claudication symptoms. The clinical effectiveness of ACE inhibitors in patients with IC is the main focus of this thesis. Summary of studies with their conclusions which assessed the effect of ACE inhibitors in IC is provided in chapter 2.

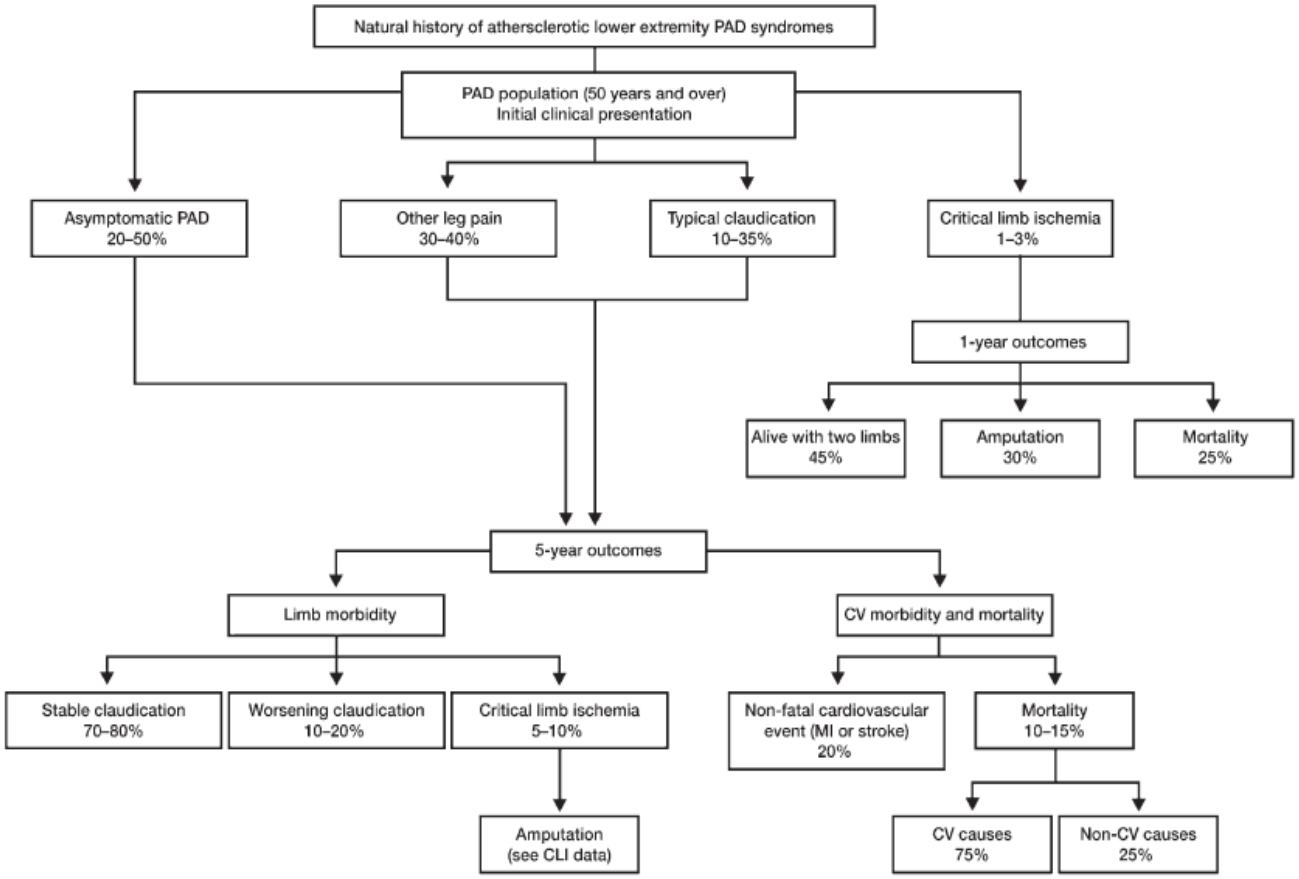

Figure 3: ***The fate of the claudicant over 5 years. CLI: critical limb ischaemia; CV: cardiovascular; MI: myocardial infarction. Adapted from Hirsch et al.***[49]

Invasive Management

Percutaneous Transluminal Angioplasty

PTA is the first line invasive treatment for patients with IC with significant clinical and QOL improvements[63,64]. However, long term results for PTA are highly variable depending upon the level of lesion; with better outcomes for aortoiliac disease as compared to infrainguinal disease[65]. More recent trials have demonstrated that the beneficial effects of PTA can be increased by combining it with other treatment modalities for sustained improvement in clinical and QoL parameters with reduction in need for re-intervention[66,67]. PTA works by increasing the blood flow to the limb by physically opening the narrowing in blood vessel.

1.2 Angiotensin Converting Enzyme Inhibitors

The rennin angiotensin system (RAS) plays a major role in cardiovascular disease, and over the last decade intensive research has investigated the possible clinical benefit of ACE inhibitors. They competitively inhibit ACE, a non-specific enzyme involved in the metabolism of many small peptides, including the conversion of angiotensin I, an inactive octapeptide, into angiotensin II, the actions of which are summarised in table 1.

Pharmacokinetics

ACE inhibitors are classified in 3 categories according to the group which binds the zinc atom of the ACE molecule into those containing a sulfhydril, a carboxyl or a phosphoryl group as the zinc ligand. Absorption is variable amongst ACE inhibitors, but peak plasma concentrations are generally reached 1-4 hours after ingestion [68]. Some ACE inhibitors are pro drugs and remain inactive until they are converted into active metabolites by hydrolysis in the liver or gastrointestinal system. Most ACE inhibitors & their metabolites are excreted via the renal route but some have a balanced elimination through the renal and hepatic routes. Captopril has a very short elimination half-life, accounting for its short duration of action whereas ramiprilat (the active metabolite of ramipril) is eliminated more slowly than the other ACE inhibitors. In patients with renal impairment (Creatinine clearance <30ml / min) renal excretion is reduced thus dose reductions are usually necessary to prevent elevated maximum plasma drug levels and toxicity [69-71].

Mechanism of Action

RAS has traditionally been regarded as a circulating endocrine system, however 90% of ACE is tissue bound. There are important differences in the binding affinity to tissue ACE between ACE inhibitors, with some demonstrating high affinity (tissue ACE inhibitors e.g. – perindopril, ramipril) and others low affinity (plasma ACE inhibitors e.g. captopril, enalapril,

lisinopril). It may be hypothesised, with some supporting evidence that the clinical effects in excess of that which may have been anticipated by BP lowering effects frequently observed in ACE inhibitor trials, are more common in trials of tissue than plasma ACE inhibitors[72-75].

ACE inhibitors also inhibit kininase, and increase bradykinin levels which in turn stimulates the B2 receptors, leading to the release of nitric oxide and vasoactive prostaglandins (prostacyclin & prostaglandin E2)[76,77].

ACE Inhibitors Effects

Haemodynamic Effects

ACE inhibitors decrease total peripheral vascular resistance and promote natriuresis but cause little change in heart rate (HR) – no reflex tachycardia or impaired HR during exercise / postural changes are observed. ACE inhibitors reduce endothelial dysfunction in patients with coronary artery disease, hypertension, non-insulin dependent DM and heart failure via the kininase inhibition pathway.

Vessels	**Vasoconstriction** **Stimulates noradrenaline, aldosterone, vasopressin & endothelin I release**
Heart	**Inotropic and chronotropic effects** **Coronary vasoconstriction**
Adrenal gland	**Aldosterone & adrenaline release**
Brain	**Vasopressin, Substance P, LHRH & ACTH release** **Thirst centre stimulation** **Increased sympathetic activation**
Kidney	**Vasoconstriction** **Contraction of mesangial cells** **Increased Na reabsorption in proximal tubule** **Increased K excretion in distal nephron** **Decreased rennin release**
Platelets	**Stimulates adhesion & aggregation**
Endothelial Cells	**Inactivation of NO (inhibits endothelial nitric oxide synthase)** **Expression of endothelial oxLDL receptor (LOX-1)**
Sympathetic outflow	**Enhancement of peripheral noradrenergic neurotransmission** **Catecholamine release from adrenal medulla**
Fibrinolysis	**Increased expression of PAI 1 & 2**
Inflammation	**Activation & migration of macrophages** **Increased expression of adhesion molecules (VCAM 1, ICAM 1 & P selectin)** **Increased expression of chemotactic proteins (MCP 1) & cytokines (IL6)**
Trophic effects	**Hypertrophy of cardiac myocytes** **Stimulation of vascular smooth muscle migration, proliferation & hypertrophy** **Stimulates proto oncogenes and MAPKs** **Increased production of growth factors (PDGF, bFGF, IGF-1)** **Increased synthesis of ECMPs (fibronectin, collagen type I & III) and MMPs** **Stimulation of NADH / NADPH oxidase activity, superoxide anion production and lipid peroxidation**
Atherosclerosis	**Stimulation of NADH / NADPH oxidase activity, superoxide anion production and lipid peroxidation**

Table 2: ***Effects of Angiotensin II. LHRH: Luteinizing hormone releasing hormone, ACTH: Adrenocorticotropin hormone, NO: Nitric oxide, PA: Plasminogen activator inhibitor, VCAM: Vascular cell adhesion molecule, ICAM: Intracellular adhesion molecule, MCP: Monocyte chemo-attractant protein, IL6: Interleukin 6, MAPK: Mitogen activated protein kinase, PDGF: Platelet derived growth factor, IGF: Insulin like growth factor, ECMP: Extracellular matrix proteins, MMP: Matrix metalloproteinases, NADH / NADPH: Nicotinamide adenine dinucleotide / nicotinamide adenine dinucleotide phosphate***

Renal Effects

ACE inhibitors decrease renal vascular resistance, increase renal blood flow and promote excretion of sodium & water, whilst maintaining glomerular filtration rate.

Neurohormonal Effects

Short-term ACE inhibitor treatment decreases plasma levels of angiotensin II, aldosterone, noradrenaline, adrenaline & vasopressin. During chronic ACE inhibitor therapy angiotensin and aldosterone levels return to pre-treatment values via aldosterone "escape" pathway.

Side Effects of ACE Inhibitors

a. Hypotension: symptomatic hypotension due to the blockade of angiotensin II mediated vasoconstrictor tone is relatively common especially following first dose and is dose dependant.
b. Dry Cough: Appears in 5-10% of patients, is not dose dependant and is more common in women and Asian populations. It usually develops between 1 week and 2 months of treatment initiation and may necessitate treatment withdrawal, following which the cough disappears within 3-5 days. It is due to the increase in bradykinin levels and

demonstrates no difference in propensity amongst the different ACE inhibitors.

c. Hyperkalaemia: Rare among patients with normal renal function, but more frequent in the elderly, patients with heart failure, impaired renal function, receiving potassium or potassium sparing diuretics, heparin or NSAIDs.

d. Acute renal failure: Occurs more frequently in patients with volume depletion (high diuretic doses), bilateral renal artery stenosis, stenosis of the dominant renal artery or a single kidney and renal transplant recipients.

e. Angioedema: A rare but life threatening side effect. Generally occurs within the first month of treatment and disappears within hours of ACE inhibitor withdrawal.

f. Teratogenic effect: When ACE inhibitors are administered during the 2nd or 3rd trimester of pregnancy.

g. Other side effects: Ageusia and other taste disturbances, neutropenia (rare) and maculopapular rash.

Clinical Indications

I. **Heart Failure:** There is clear level 1 evidence (based on data from multiple RCTs & meta-analyses) that ACE inhibitors are first line therapy in patients with chronic heart failure of all degrees of severity[78-81].

II. **Acute MI – Early Intervention:** In high risk patients (Clinical signs of heart failure [82] or left ventricular dysfunction [80,81]) following acute MI, there is clear level 1 evidence that ACE inhibitors reduce mortality. In low risk unselected patients following acute MI, clinical trials have demonstrated benefit in terms of mortality to be marginal [83-85] or negligible[86,87]. Indeed in the meta-analysis of the ACE Inhibitor in Myocardial Infarction Collaborative Group[88], including over 100 000 patients, 30 day mortality was reduced from 7.6% in the placebo group to 7.1% in the ACE inhibitor group (Adjusted RR (ARR) 0.5%, number needed to treat (NNT) to prevent 1 death = 200). No benefit was observed in low risk groups (inferior MI, no heart failure) or diabetics and ACE inhibitors were associated with an excess of persistent hypotension (17.6% versus 9.3%) and renal dysfunction (1.3% versus 0.6%). A consensus document clearly states that this small benefit may not be sufficient enough to recommend the use of ACE inhibitors in large groups of low risk, unselected patients[89].

III. **Hypertension**: A number of large, long term follow up trials compared different therapeutic strategies and could not demonstrate an unequivocal difference in favour of a particular antihypertensive agent[90-92]. A meta-analysis, including 9 RCTs, comparing old drugs (diuretics and β-blockers) against CCBs and ACE inhibitors in 62, 605 hypertensive patients, found no difference in outcome between the different antihypertensives[93]. It seems that the level of blood pressure reduction is more important than the antihypertensive used, therefore the selection of a specific antihypertensive must be based on individual patient profiles.

IV. **Stroke:** ACE inhibitors are superior to placebo in reducing recurrent stroke (ARR 4%) but not all cause mortality in patients with a history of stroke or transient ischaemic attack over a 4 year follow up period[94]. This may be attributed to the significant fall in blood pressure in the ACE inhibitor group as a reduction in stroke as an endpoint has not been observed in other RCTs in high risk patients comparing different antihypertensive agents[91,95].

V. **Chronic Kidney Disease:** Interim analysis in a RCT comparing ACE inhibitors to CCBs in 1094 African Americans with hypertensive nephrosclerosis suggested retarded renal disease progression, but evidence for long term benefit was lacking[96].

VI. **DM:** In a study (ADVANCE) of 11, 140 type 2 diabetic patients, with a history of major cardiovascular disease or at least one other risk factor for cardiovascular disease, randomised to perindopril and indapamide demonstrated a reduction in major macro or micro vascular events after a mean of 4.3 years follow up (ARR 1.3%, P = 0.040, NNT over 5 years to prevent 1 death = 79)[97]. Moreover in 13 101 adults with hypertension and type 2 diabetes enrolled in the antihypertensive and lipid lowering treatment to prevent heart attack (ALLHAT) trial, diuretics were equivalent to ACE inhibitors in reducing cardiovascular complications[98]. However evidence suggests that in type 2 diabetics with hypertension and microalbuminuria, ACE inhibitors provide combined cardiovascular and renal protection and are therefore recommended[99,100]. In the diabetic population of the HOPE study significant differences in the primary outcome only became evident 2 years after treatment initiation[101].

VII. **High Risk Cardiovascular Patients with no Left Ventricular Dysfunction or Heart Failure:** The HOPE study randomised 9297 patients with high risk of cardiac events (History of CAD, stroke, PAD, or DM plus at least one other cardiovascular risk factor – hypertension, elevated total cholesterol / Low density lipoprotein cholesterol (LDL-C), smoker, microalbuminuria) without evidence of heart failure or left ventricular dysfunction to an ACE inhibitor

(ramipril) or placebo. Ramipril was associated with a significant reduction in the composite primary outcome of MI, stroke or cardiovascular death (ARR 3.8% - P < 0.001)[102].

VIII. **Low Risk Cardiovascular Patients**: The Prevention of Events with Angiotensin Converting Enzyme inhibition (PEACE) trial in 8290 patients with stable coronary disease randomised to ACE inhibitor or placebo demonstrated no significant difference in primary outcome (cardiovascular death, MI and coronary revascularisation) after a median follow up of 4.8 years (ARR 0.6%, P = 0.430)[103]. The EUROPA trial in 13 655 patients with stable coronary disease without evidence of heart failure or substantial hypertension found a small but significant benefit in primary end point (cardiovascular death myocardial infarction or cardiac arrest) in patients randomised to ACE inhibitor (ARR 2%, P = 0.003) over a mean follow up period of 4.2 years[104].

Introduction

Several studies (RCTs) investigated the effect of ACE inhibitors on walking ability in patients with PAD of the lower limbs. These studies examined the effect of a variety of ACE inhibitors on walking distance and ABPI. However, they lacked power and the duration of treatment with ACE inhibitors was short. In order to increase the power of these studies we performed a meta-analysis of published data to investigate the effect of ACE inhibitors on maximum walking distance (MWD) or time (MWT) as a primary outcome measure and pain-free walking distance (PFWD) or time (PFWT) and ABPI as secondary outcome measures in patients with IC[105].

Systematic Review and Meta-analysis

The literature was searched using MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) databases on RCTs written in English from 1966 to 2010. Studies were eligible to be included in our review if they were: 1. RCTs which compared any kind of ACE inhibitors with placebo or no treatment 2. Included patients with symptomatic PAD of the lower limbs (IC) as either the study population or a subgroup 3. Used MWD and/or PFWD and/or ABPI as outcome measures. Minimum period of treatment with ACE inhibitors was one month.

Overall, studies included a total of 576 patients. There were 334 (58%) males and 242 (42%) females, with a mean age of 60.7 years, age range (58-66). Of

which, 137 (24%) patients had symptomatic PAD of the lower limbs. Patients' characteristics are summarised in table 3 and RCTs which were included in the systematic review are summarised in table 4.

Description of Included Studies

A double blind, placebo- controlled, crossover trial of antihypertensive treatment in 23 patients with hypertension and PAD, failed to demonstrate any statistically significant benefit of captopril 25 mg twice daily over placebo in terms of PFWD and MWD (149 +/- 71.5 metres vs. 145 +/- 89.4 metres and 228 +/- 143 metres vs. 226 +/- 151.9 metres, respectively)[106] . Captopril preserved lower limb arterial circulation possibly by maintaining the collateral blood supply which could be attributed to the lack of angiotensin II vasoconstriction effect caused by ACE inhibition and reduced breakdown of bradykinin.

Indeed, a placebo controlled, crossover, RCT of ACE inhibition with cilazapril (2.5mg/day) for 8 weeks in 23 claudicants demonstrated a deleterious effect on treadmill walking time[107]. Mean MWT was longer in the placebo group 8.04 +/- 6.39 min (431 +/- 343 meters) than the cilazapril group 6.05 +/- 5.01 min (325 +/- 269 meters), $P < 0.009$. ABPI was higher, but statistically insignificant, after treatment in the placebo group than the cilazapril group (0.69 +/- 0.12 vs. 0.66 +/- 0.15), $P > 0.050$.

A multicentre, double blind, RCT of 54 patients with essential hypertension and claudication (Fontaine IIb) randomised to perindopril 4mg once daily (n= 26) or matching placebo (n=28) for 6 weeks found that there was a slight but not a statistically significant increase in PFWD in favour of the perindopril group (173 +/- 17 meters vs. 165 +/- 10 meters)[108]. In terms of MWD, patients in the placebo group walked a significantly longer distance than the perindopril group (369 +/- 46 meters vs. 323 +/- 43 meters, respectively). This difference was found to be statistically insignificant according to the authors. There was no difference in the ABPI in both groups after treatment (0.75 +/- 0.05).

Finally, a further double blind trial[109] randomised 40 intermittent claudicants with superficial femoral artery disease to either ramipril 10 mg once daily or matched placebo for 24 weeks (20 patients per group) and found ramipril in comparison to placebo to be associated with statistically significant improvements in PFWT, 381 +/- 124 sec (339 +/- 110 meters) in the ramipril group vs. 161 +/- 29 sec (143 +/- 26 meters) in the placebo group, $P < 0.001$. This was also the case for MWT, 687 +/- 181 sec (611 +/- 161 meters) in the ramipril group vs. 234 +/- 31.4 sec (208 +/- 28 meters) in the placebo group, $P < 0.001$, ankle brachial pressure indices at rest and post exercise and Walking Impairment Questionnaire scores. The changes in ABPI with ramipril (0.73 +/- 0.09 in the ramipril group vs. 0.50 +/- 0.10 in the placebo group, $P < 0.001$) were found to be due to a reduction in brachial systolic blood pressure at rest

and both a reduction in brachial systolic blood pressure and an increase in ankle pressure post exercise. Ahimastos et al.[109] used a strict inclusion criteria which limited the applicability of the results to non-diabetic patients with infrainguinal arterial disease.

Results of Systematic Review

ACE Inhibitors Effect on MWD

Maximum walking distances/times were pooled successfully from all four studies[106-109]. After analysing these data, we found significant heterogeneity among the groups and no significant difference in the pooled treatment effect (standard mean difference = 0.46, 95 per cent CI (-0.99 to 1.92), P = 0.530, I^2=95%) (Figure 5).

ACE Inhibitors Effect on PFWD

Pain free walking distance/time could be pooled from three studies[106,108,109]. Analysis of these data showed significant heterogeneity and no significant differences in the pooled treatment effect (standard mean difference 0.97, 95 per cent C.I. (-0.24 to 2.18), P=0.120, I^2= 90%) (Figure 6).

ACE inhibitors effect on ABPI

Ankle Brachial Pressure Indices were also pooled from three studies[107-109] and analysis of data showed significant heterogeneity and no significant differences in the pooled treatment effect (Standard mean difference 0.68, 95 per cent C.I. (-0.70 to 2.06), P=0.330, I^2=93%) (Figure 7).

Discussion of Systematic Review

This systematic review and meta-analysis has highlighted the contradicting evidence regarding the impact of ACE inhibition on treadmill walking distances in patients with IC; as 3 out of 4 studies reported negative results; however, these studies were not properly powered and two of them were crossover trials[106,107]. Crossover trials are not likely to be suitable for evaluating disease progression and severity because treatment effects are not fully reversible after each treatment. Moreover, the variation in findings may reflect variability in patient subgroups, disease distribution, and dose/duration of treatment. Long term (>24 weeks) treatment of non-diabetic patients with isolated infrainguinal disease would seem to infer maximal benefit.

Further data from RCTs is required to analyse the effectiveness of ACE inhibitors for symptom relief, generic and disease specific QoL and perhaps the vascular endothelium in patients with IC.

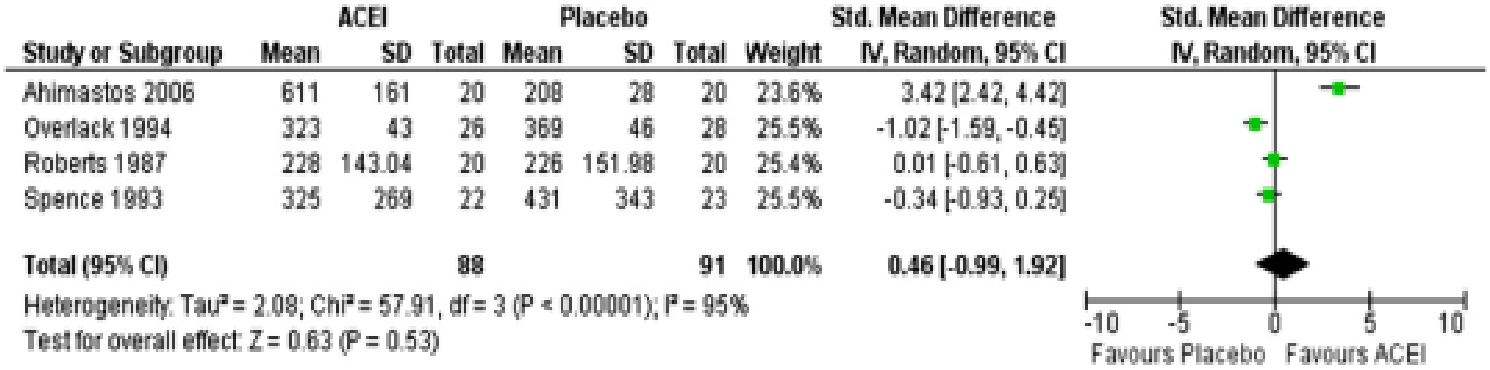

Figure 4: ***Forest plot illustrating ACE inhibitors effect on MWD. CI: confidence interval, SD: standard deviation, IV: inverse variance***

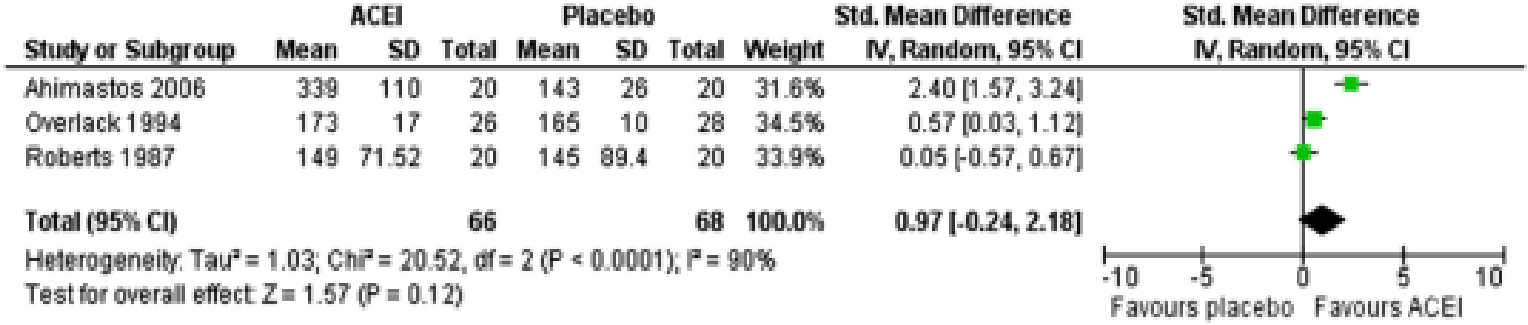

Figure 5: ***Forest plot illustrating ACE inhibitors effect on PFWD. For further explanation see legend for figure 5***

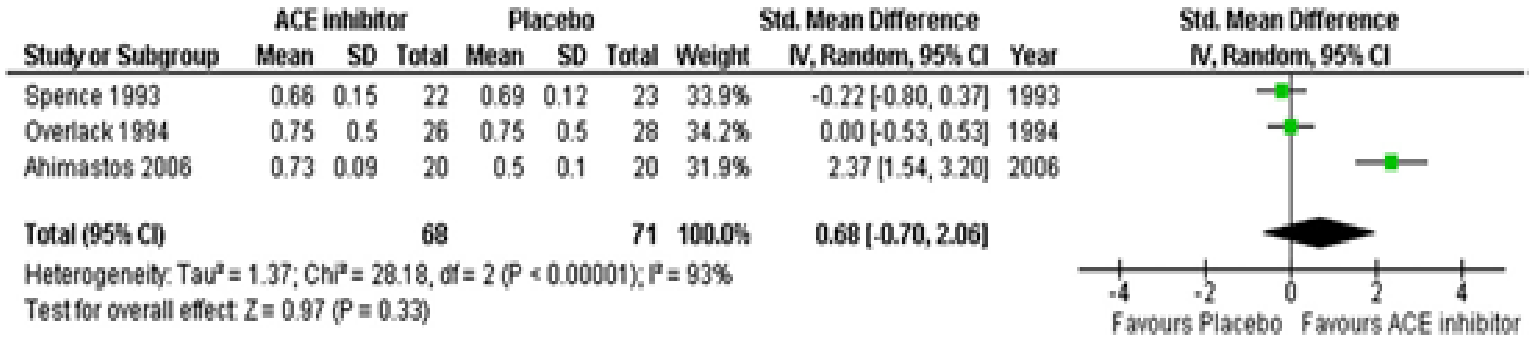

Figure 6: ***Forest plot illustrating ACE inhibitors effect on ABPI. For further explanation see legend for figure 5***

First Author	*Year*	*Age, y*	*Male n (%)*	*Baseline ABPI*	*Diabetes Mellitus n(%)*	*BMI Kg/m²*	*Current Smokers n(%)*	*SBP mmHg*	*DBP mmHg*
Roberts[106]	1987	58	17(74)	<0.9	0(0)	NR	9(39)	NR	115
Spence[107]	1993	60	16(70)	0.69	NR	NR	NR	138	83
Overlack[108]	1994	59	263(54)	0.68	106(22)	27	NR	160	100
Ahimastos[109]	2006	66	38(95)	0.56	0(0)	24	17(43)	139	85

Table 3: ***Characteristics of patients included in the systematic review. ABPI: Ankle brachial pressure index, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, NR: Not reported***

First Author	*Year*	*Type*	*Jaddad Score*	*Total No. of patients*	*Type of ACEI*	*Dose*	*Follow up*	*Outcome*
Roberts[106]	1987	RCT	4/5	23	Captopril	50mg/day	6 months	No effect on pain free and maximum walking distances
Spence[107]	1993	RCT	4/5	23	Cilazapril	2.5mg/day	8 weeks	No effect on leg blood flow & adverse effect on walking time on a treadmill
Overlack[108]	1994	RCT	4/5	490 (54 with clinical PAD)	Perindopril	4mg/day	6 weeks	No effect on pain free and maximum walking distances
Ahimastos[109]	2006	RCT	5/5	40	Ramipril	10mg/day	24 weeks	Improved pain free and maximum walking distances

Table 4: ***Summary of RCTs which assessed ACE inhibitors effect on walking distance and/or ABPI. RCT: Randomised controlled trial, PAD: Peripheral arterial disease***

History

The history of the clinical importance of arterial stiffness goes back to as early as the 200 BC in the 'Yellow Emperor's Classic of Internal Medicine'. Chinese physicians identified the association of pulse change with disease; however, their method of assessment of the pulse did not go beyond manual palpation[110].

In 1880, the English psychologist C.S.Roy described that elastic vessels which are found mainly in children are more desirable than stiff ones[111] which was a remarkable finding given the era. In 1863, more significant advances in the area of arterial stiffness were made by Etienne Marey, a French chronophotographer, by developing the Sphygmograph[112] (Figure 7). This device was made of brass, ivory, steel and wool. The arterial pulse waveform was recorded using a steel spine tipped with ivory which was applied over the radial artery. He then studied the correlation between pulse wave progression, cardiac cycle and heart sounds [113].

Fredrick Akbar Mahomed established the foundation of pulse wave analysis (PWA) in 1872. He studied the radial artery waveform and illustrated a difference between it and the carotid waveform. He also studied the effect of hypertension on the radial artery waveform [114]. Sir William Broadbent (1890) and Mackenzie (1902) went on to develop the device further and thus began the era of sphygmocardiography.

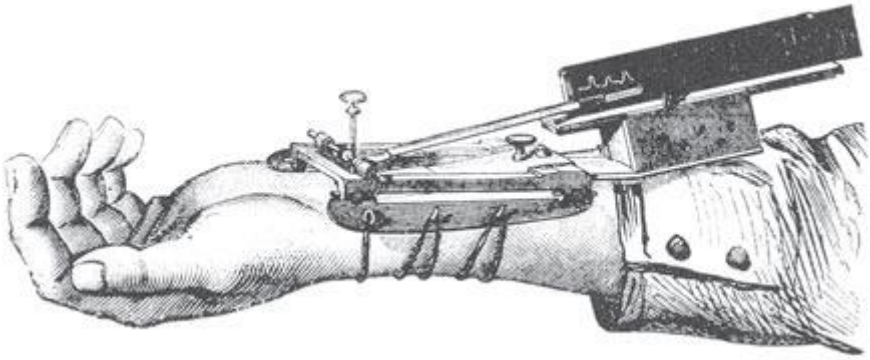

Figure 7: ***Etienne Marey Sphygmograph (Taken from Nelson et al[112])***

Definition

Arterial stiffness describes the rigidity of the artery. It can be defined as the resistance of an elastic body to deflection by an applied force [115]. It is one of the earliest detectable manifestations of structural and functional changes within the vessel wall. Arterial stiffness is associated with several pathological conditions[116-119] and has been identified as an independent risk factor for cardiovascular disease[120].

Understanding Arterial Stiffness

To better understand arterial stiffness, there are some key concepts that need to be highlighted:

1. **The Windkessel principle:** Large arteries have the function of being a conduit for blood to reach peripheral tissues; however, another function is to provide adequate buffering for each ventricular systole through arterial-ventricular coupling. Therefore, the aorta varies in its histology dependent on the function of its sections. For instance, proximal aorta is more elastic which allows it to act as a reservoir for blood produced after each stroke volume and the distal aorta is stiffer as it is more collagenous allowing it to mainly act as a conductive system to peripheral tissues[121].

2. **Stress, Laplace's law and strain:** Stress (σ) can be defined as the force applied to an area of an object (F/A). Laplace's law determines the circumferential stress in a blood vessel which is directly proportional to the vessel pressure and its radius and inversely proportional to the vessel thickness. On the other hand, strain (ϵ) can be defined as the percentage change in the length of an object which has been subjected to stress. It can be calculated as:

   $$\epsilon = L - L0 / L0$$

   where L0 is the initial length and L is the final length.

3. **Young's elastic modulus (E):** This is defined as the ratio between stress and strain. The relationship between stress and strain is nonlinear in most studies where the slope determines the intrinsic elastic properties of the vessel wall. Young's elastic modulus can be calculated as:

   $$E = \sigma / C$$

   where C is the vessel compliance.

4. **Arterial compliance (C):** Compliance is defined as the change in a vessel diameter (ΔD) at a given pressure (ΔP) at a fixed vessel length. It is the reciprocal of stiffness and can be calculated as:

   $$C = \Delta D / \Delta P$$

5. **Distensibility:** Defined as relative change in compliance and it is the inverse of the elastic modulus.

6. **Stiffness:** Defined as the resistance to deformation. Stiffness measurement depends on three variables of any studied vessel (E, h and r) according to the Moens-Korteweg equation which calculates pulse wave velocity (PWV) considering there are no significant changes in vessel wall thickness as:

$$PWV=\sqrt{(E_{inc}.h)/(2rp)}$$

where E_{inc} is the incremental elastic modulus, h is the vessel wall thickness, r is the vessel radius and p is the density of the blood. From the equation we note that PWV is proportional to the square root of the stiffness of the vessel and that it is not sensitive to changes in the vessel wall thickness or dimensions. This formula illustrates that the most important determinant of stiffness in a blood vessel is the percentage of elastin to collagen within its wall, hence the more elastic the blood vessel is the lesser the stiffness.

How to Measure Arterial Stiffness?

There are several methods to assess arterial stiffness in clinical and research settings. These include measurement of arterial compliance and distensibility

by ultrasound, cardiac MRI and the oscillometric or applanation tonometry method. Arterial stiffness can be quantified using PWV and PWA. The applantation tonometry technique will be explained in further detail in the methods section (Chapter 5).

Pulse Wave Velocity

The gold standard method of quantifying arterial stiffness is PWV and more specifically the carotid-femoral PWV (PWVcf) or otherwise called aortic PWV. This is due to its simplicity, accuracy and reproducibility [122-124]. Other suggested methods of measurement include the brachial-ankle PWV or brachial-radial PWV, which to date, have shown no predictive value in terms of cardiovascular risk.

PWV is an indirect measure of arterial stiffness which is based on estimating the velocity of the pressure wave travelling between two arterial sites. The velocity is related to the stiffness of the arterial segment between the two measurement sites such that increased arterial stiffness is associated with increased PWV. It is calculated as the distance travelled between the two arterial sites divided by the time required for the pulse waveform to travel between these two sites:

PWV (metres/second) =Distance (metres)/tt (second)

where tt is transit time.

PWV is a regional functional assessment of stiffness over a certain arterial length, whereas, strain, compliance and distensibility are local measures of elasticity. It is best described by the Moens-Korteweg equation (Mentioned above). A more useful equation is the Bramwell-Hill equation[125] which describes the relationship between distensibility and PWV and expressed in meters/second:

$$PWV=\sqrt{1/Dp}$$

where D is distensibility and p is blood density.

Pulse Wave Analysis

Pulse can be described as a series of harmonics travelling the arterial tree which can be described in terms of dimension, flow and pressure. These pulse waveforms transmit energy rather than matter along the arterial tree. Therefore, and as the arterial tree is a closed circuit, when the pulse waveform reaches a point of resistance such as a bifurcation in the arterial tree or an atheroma, this will generate a waveform which is reflected back to the origin (the heart) during diastole. Thus, the pulse waveform is formed from two components: the incident wave which is generated by the left

ventricle and the reflected or returned wave. The summation of both waves will form the arterial pulse waveform (Figure 9)[126].

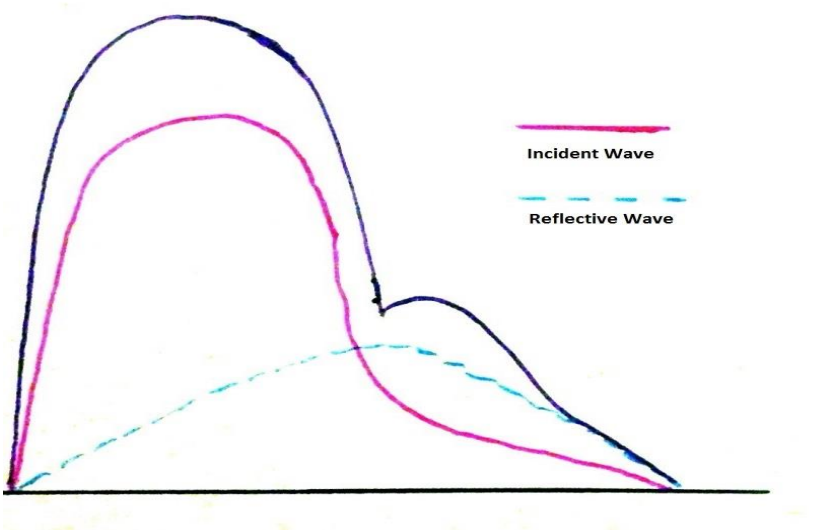

Figure 8: ***Arterial waveform divided into forward travelling incident wave and a reflected wave***

Wave reflection is an integral part of the arterial pulse waveform. This is important in relation to the timing of return of the waveform in relevance to the cardiac cycle. For instance, a wave reflected during the cardiac ejection systolic period due to increased PWV or reflection from a more proximal arterial site or prolonged ejection time will augment systole. Systolic augmentation will lead to increased cardiac loading which potentially can cause left ventricular hypertrophy, systolic or diastolic cardiac failure or left atrial strain and enlargement which will lead to atrial fibrillation with

thromboembolic events[112]. Conversely, a decreased PWV, a more distal wave reflection arterial site and a shorter ejection time will result in the reflected wave arriving during diastole. Augmentation of diastole will result is increased coronary perfusion and decreases left ventricular systolic workload.

Multiple indices can be derived from the pulse waveform (Figure 10) including: Augmentation pressure (AP), augmentation index (AIx), aortic (central) systolic and diastolic blood pressures, mean arterial pressure (MAP), ejection duration (ED) and subendocardial viability ratio (SEVR).

Augmentation Pressure and Augmentation Index

Wave reflection generates an amount of pressure which augments the aortic or central pressure; this is called AP. AIx is the ratio between AP and central pulse pressure (PP). AIx is primarily a measure of wave reflections; however, due to its correlation with PWV[127], it has been considered a measure of arterial stiffness too. Therefore, AIx is a composite measure of wave reflection and arterial stiffness. Nevertheless, Kelly et al. [128] found that AIx correlated with PWV, BP and age in a univariate analysis but only with age in multivariate analysis. This suggests that the correlation becomes stronger as the aorta ages and becomes stiffer. Therefore, in an elastic aorta, the AIx is more likely to be representative of the wave reflection rather than its

velocity[126]. As AIx is influenced by variations in HR[129], it has been adjusted to 75 beats/min which can be calculated by some devices that use the applanation method in AIx measurement such as the SphygmoCor device. This measurement is called AIx@75.

AIx is the most important derivative of the arterial pulse waveform with several studies indicating its cardiovascular prognostic value[130,131].

Subendocardial Viability Ratio or Buckberg Ratio

The area under the curve (AUC) of the systolic and diastolic portions of the central aortic pulse waveform can be measured using PWA[132]. During diastole blood flow occurs in the coronary artery. The diastolic-AUC represents myocardial perfusion (Oxygen supply) while the systolic-AUC represents myocardial contraction (Oxygen-demand). SEVR is the ratio between supply and demand or diastolic-AUC divided by systolic-AUC. In healthy coronary arteries, subendocardial ischaemia occurs at levels below 50%[133].

Ejection Duration

PWA can also be used to calculate the duration of the left ventricular systolic ejection duration or the systolic time interval in milliseconds[132]. The ratio of the systolic ejection duration to the total duration of the cardiac cycle is

called ED index (ED %). It has been shown that patients with systolic dysfunction have higher ED% than patients with diastolic dysfunction[132].

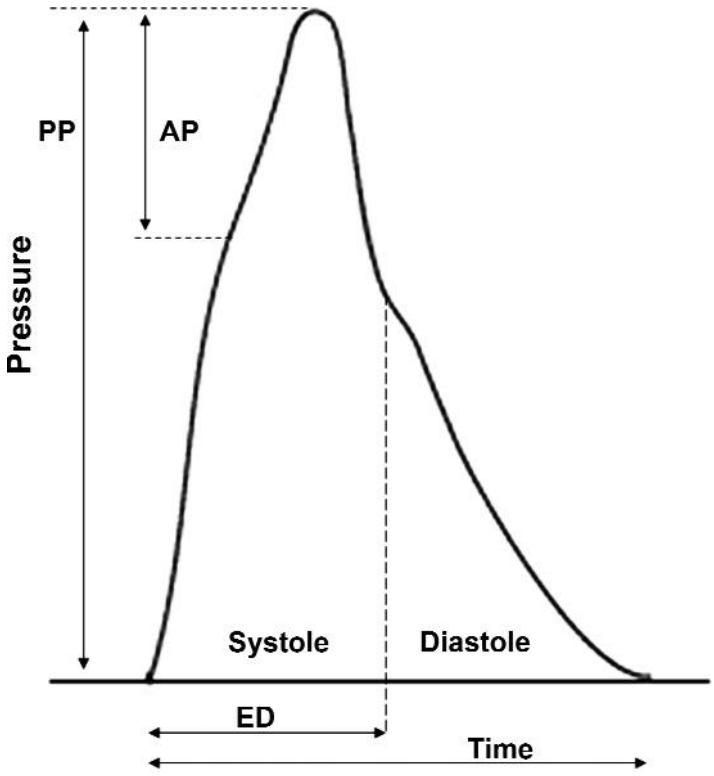

Figure 9: ***Arterial pulse wave with indices derived from PWA. PP: Pulse pressure, AP: Augmentation pressure, ED: Ejection duration or time to reflected wave. Adapted from Crilly et al[134]***

Factors that Affect Pulse Wave Analysis and Velocity

Several factors can affect PWA and PWV including:

I. **Age:** Arteries become stiffer as they age. This is due to hyperplasia of the intima and loss of elastin in the media layer and its replacement with collagen. These changes in the arterial wall structure that occur with ageing will result in an increase in PWV and AIx[135,136]. In healthy middle

aged adults, the normal values of PWVcf vary according to the arterial site. These are 4m/s in the ascending aorta, 5m/s in the carotids and abdominal aorta, 7m/s in the brachial artery and 8m/s in the iliac arteries[137]. On the other hand, AIx normal values range from 5% in healthy females less than 20 years old to 37% in females aged from 80 to 90 years[135]. In healthy males, however, AIx values range from -2% in the less than 20 years old to 30% in males aged between 80 and 90 years old[135].

II. **Height:** An inverse relationship exists between body height and AIx[127,128]. Yasmin and Brown[127] explained this by the shorter distance that the reflected wave has to travel back to its origin at a given PWV. This can explain the increase of AIx in women compared to men. Nonetheless, this inverse relationship between AIx and short stature could be due to the increase of cardiovascular risk in patients with decreased height[138,139].

III. **HR:** It is well known that AIx is influenced by changes in HR. This was demonstrated in patients with normal left ventricular function who underwent diagnostic cardiac catheterisation[140]. The right atrium was paced to increase the heart rate and this produced a decrease in the AIx by 5.6% for each 10 beats/min increase in the heart rate. The relationship between PWV and HR, however, is not as clear. While some studies did not show a relationship[127,141], other studies did[142-144].

IV. **Atherosclerosis and DM:** Several studies have shown that PWV correlates with treated and non-treated cardiovascular risk factors of atherosclerosis included in the FRS[145,146]. PWV also correlated with insulin, cholesterol and triglycerides in other studies[147,148]. Carotid intima thickness is a recognised marker of atherosclerosis, not only in the carotid artery, but also a reflection of atherosclerosis in the coronary arteries[149], have also shown a positive correlation with PWV[150]. Therefore, increased atherosclerosis risk will increase PWV and arterial stiffness.

PWV is also increased in patients with DM type 2. McVeigh et al have shown that arterial stiffness, assessed invasively in the brachial artery, was increased in a population of patients with DM type 2 before cardiovascular manifestations of DM were apparent[151]. Furthermore, arterial stiffness was increased in healthy offspring of patients with DM type 2 in other studies[152,153]. Certainly, the evidence suggests that increased arterial stiffness (PWV) and endothelial dysfunction precedes the development of DM[154].

While the evidence is clear about the association of DM types 1 and 2 with increased arterial stiffness, it is uncertain about the relationship with AIx. O'Brien and colleagues showed no increase of AIx after controlling for HR in a large cohort of patients with DM[155]. Other studies showed heterogeneous results mainly due to the different methods or devices used to measure AIx[151,156,157].

V. **PAD:** Arterial stiffness prognostic value is poorly studied in patients with PAD. Although some studies have shown that patients with PAD have stiffer carotid and femoral[158] arteries as well as increased aortic stiffness[159], there are no studies in the literature which evaluate whether increased arterial stiffness in this patients' cohort has any prognostic value. In 4159 randomly selected patients in Denmark, AIx had the same predictive power as ABPI and CRP[160]. AIx also predicted patients with abnormal ABPI in a study of 475 adults without cardiovascular disease[161]. Additionally, AIx, not PWV, was associated with aortic atherosclerosis in a cohort of African American patients who underwent transesophageal echocardiography[162].

VI. Drugs:

i. **β-Blockers:** Different β-Blockers have different effects on arterial stiffness dependent on their receptor affinities. For instance, bisoprolol has shown to decrease PWV in the short term[163] but not in the long term[164]. Some β-Blockers also increase AIx by decreasing HR and others decrease AIx by decreasing vascular tone[165]. B-Blockers prolong the cardiac diastolic duration which leads to increased coronary perfusion and a decrease in AIx.

ii. **Antioxidants:** Vitamin C, E and xanthines such as allopurinol have potential positive effects on the cardiovascular system. This is

believed to be due to their role in decreasing endothelial dysfunction by their action upon nitric oxide (NO) by decreasing its degredation by free radicals[166-170]. Regarding arterial stiffness, vitamin E improved arterial compliance in middle aged men and women[171] and vitamin C has been associated with a decreased arterial stiffness[172].

iii. **ACE inhibitors:** The effect of ACE inhibitors will be discussed in detail in the following section.

iv. **Other antihypertensives:** Different antihypertensive agents have different effects on arterial stiffness[163] which is independent of their effect on BP reduction. For example, two antihypertensive agents (Fosinopril and atenolol) which reduce BP to a similar degree were compared in terms of their effect on AIx. Fosinopril reduced AIx more than atenolol[173]. This is however expected due to the effect of atenolol on HR which is considered a disadvantage as decreasing HR will result in an increase in AIx. In the CAFE trial, AIx and AP decreased to more extent in the amlodipine with or without perindopril treatment arm than the atenolol with or without thiazide treatment arm[174]. Klingbeil et al[175] have also shown that treatment with valsartan reduced AIx more than hydrochlorothiazide in a double-blind randomised trial.

Introduction

Several recent trials tested the effect of ACE inhibitors on arterial stiffness as measured by PWV and wave reflections as measured by AIx in patients with several pathological conditions.

In this meta-analysis we investigated the effect of ACE inhibitors compared with placebo or no treatment on arterial stiffness as measured by PWV and on arterial wave reflections as measured by AIx in patients with several pathological conditions as a primary outcome measure. We also studied the effect of ACE inhibitors compared with other antihypertensive agents: Angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs), β-blockers and diuretics on arterial stiffness and wave reflections as a secondary outcome measure. As a tertiary outcome measure, we investigated this effect when ACE inhibitors are combined with other antihypertensive agents and when they are compared to a combination of antihypertensive agents.

Systematic Review and Meta-analysis

A systematic review was conducted to investigate the effect of ACE inhibitors on arterial stiffness assessed by PWV and/or AIx. The literature was searched on RCTs that assessed this effect from inception until July 2011 using the medical databases: MEDLINE, EMBASE and CENTRAL. In addition, we

searched the reference lists of relevant articles to identify articles missed by the electronic searches.

We used the MeSH and free keywords 'angiotensin converting enzyme inhibitors', 'angiotensin converting-enzyme inhibitors', 'ACE inhibitors', 'arterial stiffness', 'pulse wave velocity', 'PWV', 'augmentation index', and 'AIx'. An expanded search was used using Boolean operators.

The search was limited to RCTs which were published in English and involved humans. It is worth mentioning that we only included studies where the minimum treatment period with ACE inhibitor was one month.

Results of Systematic Review and Meta-analysis

Our analysis included 23 RCTs[173,176-197] and 1645 patients. Characteristics of trials have been summarized in tables 5 and 6.

In all trials, major characteristics of patients at baseline were similar between study groups. The mean age of patients ranged from 33[177] to 69[192] years. The mean systolic BP ranged from 120[177] to 176[197] mmHg and the mean diastolic BP ranged from 69[176] to 105[173] mmHg at baseline. Mean follow-up duration ranged from 1 month [181,187] to 52 months[179]. Patients' characteristics have been summarized in table 7.

ACE Inhibitors Effect on Carotid-Femoral Pulse Wave Velocity

ACE Inhibitors vs. Placebo

Overall, 5 trials[176-180] assessed the effect of ACE inhibitors on PWVcf compared to placebo. These studies included a total of 469 patients. 227 patients received ACE inhibitor and 216 patients received placebo. Across the 5 trials we found insignificant heterogeneity (I^2=52%, P=0.060). A fixed effect model showed that treatment with ACE inhibitor significantly improved PWVcf (Pooled mean change difference=-1.69 m/s, 95% CI -2.05 to -1.33, P<0.00001) (Figure 10).

Author/Year	Country	PWV	Design	Masking	Participants (n)	Assessment method	ACEI (n)	Control (n)	ACEI	ACEI d/d	Control	Control d/d	ΔSBP ACEI	ΔSBP Control	ΔDBP ACEI	ΔDBP Control	outcome
ACEI vs. placebo																	
Yu/2006 [176]	Taiwan	CF	Parallel	Double	46	Applanation tonometry*	24	22	Ramipril	7.5 mg	Placebo	----------	-10	-5	-9	-3	No significant effect on PWV
Ahimastos/ 2007 [177]	Australia	CF, FA	Parallel	Double	17	Applanation tonometry*	10	7	Perindopril	8 mg	Placebo	----------	-4	1	-4	1	Reduced arterial stiffness
Rahman A/2007[178]	Malaysia	CF	Parallel	Double	33	SphygmoCor	11	10	Ramipril	5 mg	Placebo	----------	-7	-9.9	-6	-5.4	Reduced arterial stiffness
Rahman B/2007[178]	Malaysia	CF	Parallel	Double	33	SphygmoCor	10	9	Ramipril	5 mg	Placebo	----------	-13	-3	-9.6	-2.7	Reduced arterial stiffness
Mitchell/ 2007 [179]	Canada	CF	Parallel	Open	300	Applanation tonometry*	152	148	Trandolapril	4 mg	Placebo	----------	NR	NR	NR	NR	Modest reduction in arterial stiffness
Ahimastos/ 2008[180]	Australia	CF	Parallel	Double	40	Applantion tonometry*	20	20	Ramipril	10 mg	Placebo	----------	-5.4	-0.5	-6.3	-0.7	Reduced arterial stiffness
ACEI vs. other antihypertensives																	
1.ACEI vs. ARB																	
Mahmud/ 2002[181]	Ireland	CF	Cross-over	Single	12	Complior	12	12	Captopril	100 mg	Valsartan	160 mg	-19	-23	-13	-13	Both treatments reduced arterial stiffness
Rajzer /2003[182]	Poland	CF	Parallel	Open	118	Complior	38	24	Quinapril	20 mg	Losartan	100 mg	-41	-30	-11	-7	PWV decreased only under Quinapril
Rehman/ 2007[183]	Malaysia	CF	Parallel	Double	39	Complior	20	19	Perindopril	4 mg	Losartan	50 mg	-18	-14	-9	-8	PWV decreased under both
Ali/2009[184]	UK	CF, FD	CR, Cross-over	Double	15	Complior	15	15	Lisinopril	20 mg	Irbesartan	300 mg	-17.2	-27.9	-14.6	-20.1	PWV decreased under both
2. ACEI vs. CCB																	
London/ 1994[185]	France	CF	Parallel	Single	32	Applanation tonometry*	14	10	Perindopril	4 mg	Nitrendipine	40 mg	-26	-19.6	-14.9	-9.8	PWV decreased under both
Rajzer /2003[182]	Poland	CF	Parallel	Open	118	N/A	38	37	Quinapril	20 mg	Amlodipine	10 mg	-41	-27	-11	-9	PWV decreased only under Quinapril
Mackenzie/ 2009[186]	UK	CF	Parallel	Double	59	SphygmoCor	15	14	Perindopril	4 mg	Lercanidipine	10 mg	-17	-13	-5	-1	PWV did not change by any
3.ACEI vs. β-blocker																	
Pannier/ 2001[187]	France	CF	Cross-over	Double	20	Complior	20	20	Perindopril	8 mg	Atenolol	100 mg	-13.8	-16.5	-9.6	-10.3	PWV decreased under both
Mackenzie/ 2009[186]	UK	CF	Parallel	Double	59	N/A	15	17	Perindopril	4 mg	Atenolol	50 mg	-17	-18	-5	-8	PWV did not change under both

Table 5: ***Studies investigating ACE inhibitors effect on PWV***

Author/Year	Country	PWV	Design	Masking	Participants (n)	Assessment method	ACEI (n)	Control (n)	ACEI	ACEI d/d	Control	Control d/d	ΔSBP ACEI	ΔSBP Control	ΔDBP ACEI	ΔDBP Control	outcome
4. ACEI vs. Diuretic																	
Breithaupt-Grogler/ 1996[188]	Germany	CF	Parallel	Double	17	Applanation tonometry*	9	8	Cilazapril	5 mg	Hydrochlo ro-thiazide	25 mg	-11	-25	-11	-17	PWV decreased under both
Mackenzie /2009[186]	UK	CF	Parallel	Double	59	N/A	15	13	Perindopril	4mg	Bendroflu- azide	2.5 mg	-17	-14	-5	-3	No change in PWV under both
Kostka-Jeziorny/ 2011[189]	Poland	CF	Parallel	Open	66	Complior	35	31	Perindopril	10 mg	Hydrochlo ro-thiazide	25 mg	-23	-24	-14	-13	PWV decreased only under Perindopril
5. ACEI vs. ACEI and ARB																	
Mahmud/ 2002[181]	Ireland	CF	Cross-over	Single	12	N/A	12	12	Captopril	100 mg	Captopril Valsartan	100mg 160mg	-19	-28	-13	-18	PWV decreased more under combined therapy
ACEI and other antihypertensives vs. other antihypertensives																	
Asmar /2001[196]	France	CF	Parallel	Double	406	Complior	204	202	Perindopril Indapamide	2mg 0.625mg	Atenolol	50mg	-23.1	-16.2	-13.3	-12.9	PWV decreased under both to a similar degree

Table 5 (Continued): ***CF: Carotid-femoral, CR: Carotid-radial, FA: Femoral-ankle, FD: Femoral-dorsalis pedis, ARB: Angiotensin receptor blocker, CCB: Calcium channel blocker, N/A: Not applicable as reported elsewhere in the table, d/d: Dose per day, *Studies which measured PWV using sequential applanation tonometry without mentioning the name of the device used***

Author/ Year	Country	Design	Masking	Participants (n)	Assessment method	ACEI (n)	Control (n)	ACEI	ACEI d/d	Control	Control d/d	ΔSBP ACEI	ΔSBP Control	ΔDBP ACEI	ΔDBP Control	ΔHR ACEI	ΔHR Control	outcome
ACEI vs. placebo																		
Dart/2001[190]	Australia	Parallel	Open	111	PWV Medical BP Analysis System	50	61	Perindopril	8 mg	Usual treatment	----------	-13	-7	-10	-4	3	-1	Perindopril reduced AIx
Deary/2002[191]	UK	Cross-over	Double	30	SphygmoCor	30	30	Lisinopril	10 mg	Placebo	----------	-15♂ -21♀	0 0	-11 -9	0 0	1 1	0 0	Lisinopril reduced AIx
Tsang/2006[192]	USA	Parallel	Double	21	SphygmaCor	9	12	Quinapril	60 mg	Placebo	----------	-12	-9	NR	NR	NR	NR	Quinapril reduced AIx
Yu/2006 [176]	Taiwan	Parallel	Double	46	Applanation tonometry*	24	22	Ramipril	7.5 mg	Placebo	----------	-10	-5	-9	-3	3	0	No significant effect on AIx
Rahman A/2007[178]	Malaysia	Parallel	Double	33	SphygmoCor	11	10	Ramipril	5 mg	Placebo	----------	-7	-9.9	-6	-5.4	1.5	1.4	Ramipril reduced AIx
Rahman B/2007[178]	Malaysia	Parallel	Double	33	SphygmoCor	10	9	Ramipril	5 mg	Placebo	----------	-13	-3	-9.6	-2.7	9.8	2.7	Ramipril reduced AIx
Mitchell/2007[179]	Canada	Parallel	Open	300	Applanation tonometry*	152	148	Trando-lapril	4 mg	Placebo	----------	NR	NR	NR	NR	NR	NR	Trandolapril reduced AIx
Ahimastos/2008[180]	Australia	Parallel	Double	40	Applanation tonometry*	20	20	Ramipril	10 mg	Placebo	----------	-5.4	-0.5	-6.3	-0.7	NR	NR	Ramirpil reduced AIx
ACEI vs. other antihypertensives																		
1.ACEI vs. ARB																		
Mahmud/2002[181]	Ireland	Cross-over	Single	12	SphygmoCor	12	12	Captopril	100 mg	Valsartan	160 mg	-19	-23	-13	-13	-1	0	AIx decreaed under both
2. ACEI vs. CCB																		
Deary/2002[191]	UK	Cross-over	Double	30	N/A	30	30	Lisinopril	10 mg	Amlodipine	5 mg	-15♂ 21♀	-9 -26	-11 -9	-8 -5	1 1	3 -1	AIx decreased under both
Mackenzie/2009[186]	UK	Parallel	Double	59	SphygmaCor	15	14	Perindopril	4 mg	Lercani-dipine	10 mg	-17	-13	-5	-1	2	2	Lercanidipine reduced AIx more than Perindopril
3.ACEI vs. β-blocker																		
Chen/1995[173]	China	Parallel	Double	79	Applanation tonometry*	41	38	Fosinopril	20 mg	Atenolol	100 mg	-23	-28	-14	-16	1	-15	Fosinopril reduced AIx more than Atenolol

Author/ Year	Country	Design	Masking	Participants (n)	Assessment method	ACEI (n)	Control (n)	ACEI	ACEI d/d	Control	Control d/d	ΔSBP ACEI	ΔSBP Control	ΔDBP ACEI	ΔDBP Control	ΔHR ACEI	ΔHR Control	outcome
Pannier/2001[187]	France	Cross-over	Double	20	SphygmoCor	20	20	Perindopril	8 mg	Atenolol	100 mg	-13.8	-16.5	-9.6	-10.3	-0.5	-10.2	AIx decreased significantly under Perindopril
Deary/2002[191]	UK	Cross-over	Double	30	N/A	30	30	Lisinopril	10 mg	Bisoprolol	5 mg	-15♂ -21♀	-19 -21	-11 -9	-11 -12	1 1	-8 -14	Only Lisinopril reduced AIx
Neal/2004[193]	UK	Cross-over	Double	12	SphygmoCor	12	12	Lisinopril	20 mg	Bisoprolol	20 mg	-24	-12	-11	-7	-1	-19	Lisinopril only reduced AIx
Kaiser/2006[194]	Germany	Cross-over	Double	10	SphygmoCor	10	10	Enalapril	10 mg	Nebivolol	5 mg	-15	-17	-7	-6	NR	NR	No significant difference between both
Mackenzie/2009[186]	UK	Parallel	Double	59	N/A	15	17	Perindopril	4 mg	Atenolol	50 mg	-17	-18	-5	-8	2	-10	AIx decreased only under Perindopril
4.ACEI vs. diuretic																		
Deary/ 2002[191]	UK	Cross-over	Double	30	N/A	30	30	Lisinopril	10 mg	Bendroflu-azide	2.5 mg	-15♂ -21♀	-6 -19	-11 -9	-3 -4	1 1	1 0	AIx decreased only under Lisinopril
Jiang/2007[195]	China	Parallel	Double	101	SphygmoCor	51	50	Enalapril	10 mg	Indapa-mide	2.5 mg	-16.9	-14	-9.9	-7.8	-0.5	0.6	Only Enalapril decreased AIx
Mackenzie/2009[186]	UK	Parallel	Double	59	N/A	15	13	Perindopril	4mg	Bendroflu-azide	2.5 mg	-17	-14	-5	-3	2	2	Perindopril reduced AIx more than Bendrofluazide
5. ACEI vs. ACEI and ARB																		
Mahmud/ 2002[181]	Ireland	Cross-over	Single	12	N/A	12	12	Captopril	100mg	Captopril Valsartan	100mg 160mg	-19	-28	-13	-18	-1	-1	AIx decreased more under combination therapy
ACEI and other antihypertensives vs. other antihypertensives																		
1. ACEI & diuretic vs. β-blocker																		
Asmar/ 2001[196]	France	Parallel	Double	406	Applanation tonometry*	65	64	Perindopril Indapa-mide	2mg 0.625mg	Atenolol	50mg	-23.1	-16.2	-13.3	-12.9	-1.4	-7.8	AIx decreased more under ACEI & diuretic

Author/ Year	Country	Design	Masking	Participants (n)	Assessment method	ACEI (n)	Control (n)	ACEI	ACEI d/d	Control	Control d/d	ΔSBP ACEI	ΔSBP Control	ΔDBP ACEI	ΔDBP Control	ΔHR ACEI	ΔHR Control	outcome
2. ACEI & diuretic vs. CCB																		
Ferguson/ 2008[197]	Australia	Cross-over	Double	28	SphygmoCor	22	22	Fosinopril HCT	20mg 12.5 mg	Amlodipine	10mg	-22	-16	-9.2	-5	NR	NR	AIx decreased more with combination therapy
3. ACEI & diuretic vs. diuretic																		
Ferguson/ 2008[197]	Australia	Cross-over	Double	28	SphygmoCor	22	22	Fosinopril HCT	20mg 12.5 mg	Indapa-mide	2.5mg	-22	-23	-9.2	-8	NR	NR	AIx decreased more with combination therapy

Table 6: ***Studies investigating ACE inhibitors effect on AIx. CCB: Calcium channel blocker, ARB: Angiotensin receptor blocker, ♂: Male, ♀: Female, HCT: Hydrochlorothiazide, N/A: Not applicable, NR: Not reported, d/d: Dose per day, * Studies which measured AIx using sequential applanation tonometry without mentioning the name of the device used***

Author/Year	Patients	Mean Age,y	Male n (%)	Mean SBP	Mean DBP	MAP	Diabetes Mellitus n(%)	Follow-up Months
Yu/2006[176]	ESRD	47	30(65)	123	69	NR	3(7)	12
Ahimastos/2007[177]	Marfan's	33	14(82)	120	72	82	0(0)	6
Rahman/2007[178]	DM type 2, IGT	47	NR	126	80	NR	33(50)	12
Mitchell/2007[179]	CAD	63	271(90)	133	79	97	26(9)	52
Ahimastos/2008[180]	PAD	66	38(95)	140	87	92	0(0)	6
Mahmud/2002[181]	Hypertension	49	NR	157	96	NR	NR	1
Rajzer/2003[182]	Hypertension	54	54(46)	154	97	116	NR	6
Rehman/2007[183]	Hypertension	52	NR	151	93	NR	NR	4
Ali/2009[184]	Hypertension	66	NR	162	99	NR	NR	3
London/1994[185]	ESRD	53	14(44)	176	98	124	NR	12
Mackenzie/2009[186]	Hypertension	68	31(53)	159	84	107	NR	2.5
Pannier/2001[187]	Hypertension	49	13(65)	164	99	NR	NR	1
Breithaupt-Grogler/1996[188]	Hypertension	45-67	NR	150	99	121	NR	3
Kostka-Jeziorny/2011[189]	Hypertension	46	40(61)	157	97	NR	0(0)	2
Dart/2001[190]	Hypertension	60	53(48)	147	88	NR	NR	3
Deary/2002[191]	Hypertension	47	22(73)	157	99	NR	NR	1.5
Tsang/2006[192]	DD	69	10(48)	127	71	NR	0(0)	12
Chen/1995[173]	Hypertension	45.8	52(66)	151	105	NR	NR	2
Neal/2004[193]	Transplant	60	6(46)	154	92	114	0(0)	3
Kaiser/2006[194]	DM type 2	62	7(70)	155	90	NR	10(100)	3
Jiang/2007[195]	Hypertension	54	55(54)	155	95	NR	NR	2
Asmar/2001[196]	Hypertension	NR	NR	162	99	119	NR	12
Ferguson/2008[197]	Hypertension	62	14(50)	164	86	NR	0(0)	2

Table 7: ***Patients' characteristics. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, ESRD: End-stage renal disease, DM: Diabetes mellitus, CAD: Coronary artery disease, PAD: Peripheral arterial disease, DD: Diastolic dysfunction, NR: Not reported***

ACE Inhibitors vs. Other Antihypertensives

9 trials [181-189] which included 378 patients assessed the effect of ACE inhibitors (n=178) versus other antihypertensives (n=220) including: ARBs, CCBs, β-blockers, diuretics and a combination of ACE inhibitor and ARBs. Compared with other antihypertensive agents, ACE inhibitors insignificantly reduced PWVcf (Pooled mean change difference= -0.19 m/s, 95% CI -0.59 to 0.21, P=0.360, I^2= 0%) (Figure 11).

ACE Inhibitors vs. ARBs

4 trials [181-184] which included 184 patients assessed the effect of ACE inhibitors (n=85) versus ARBs (n=70) on PWVcf. ACE inhibitors showed no significant effect on PWVcf when compared with ARBs (Pooled mean change difference = -0.36 m/s, 95% CI -0.92 to 0.20, P=0.210, I^2=0%) (Figure 11).

ACE Inhibitors vs. CCBs

ACE inhibitors effect compared with CCBs effect on PWVcf was evaluated in 3 trials[182,185,186]. Studies included a total of 209 patients. ACE inhibitors (n=67) showed insignificant effect on PWVcf when compared with CCBs (n=61) (Pooled mean change difference = -0.62 m/s, 95% CI -2.37 to 1.13, P=0.490, I^2=0%) (Figure 11).

ACE Inhibitors vs. β-Blockers

2 trials [186,187] which included 79 patients evaluated the effect of ACE inhibitors (n=35) versus β-blockers (n=37) on PWVcf. Treatment with ACE inhibitors showed no significant effect on PWVcf when compared with β-blockers (Pooled mean change difference= 0.44 m/s, 95% CI -0.69 to 1.57, P=0.440, I^2=0%) (Figure 11).

ACE Inhibitors vs. Diuretics

3 trials[186,188,189] which included 142 patients evaluated the effect of ACE inhibitors versus diuretics on PWVcf. Treatment with ACE inhibitors insignificantly reduced PWVcf compared to diuretics (Pooled mean change difference= -0.69 m/s, 95% CI -1.6 to 0.21, P=0.130, I^2=0%) (Figure 11).

ACE Inhibitors vs. ACE Inhibitors and ARBs

One RCT [181] which included 12 patients assessed the effect of ACE inhibitor versus a combination of ACE inhibitor and ARB on PWVcf. Treatment with ACE inhibitor and ARB insignificantly reduced PWVcf when compared to ACE inhibitor alone (Mean change difference= -1 m/s, 95% CI -2.2 to 0.2, P=0.100) (Figure 11).

ACE Inhibitors Combined with Other Antihypertensive Agents vs. Other Antihypertensive Agents

ACE Inhibitor and Diuretic vs. β-Blocker

Asmar et al[196] evaluated the effect of ACE inhibitor combined with a diuretic versus β-blocker. Treatment with β-blocker insignificantly reduced PWVcf more than a combination of ACE inhibitor and diuretic (Mean change difference=-0.19 m/s, 95% CI -0.14 to 0.53, P=0.260).

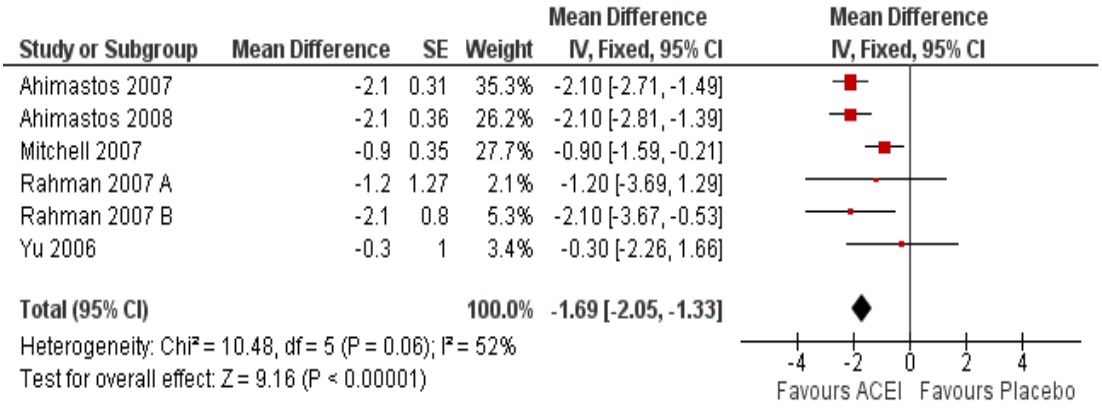

Figure 10: ***Forest plot illustrating ACE Inhibitors effect on changes in PWVcf compared with placebo. The x-axis is in m/sec. Small squares represent the differences in mean changes in PWVcf across individual studies between study groups (ACE Inhibitor minus placebo or no treatment). The 95 per cent confidence intervals for individual studies are represented by a horizontal line and by a diamond for pooled effect. CI: Confidence interval, SE: Standard error, IV: Inverse variance***

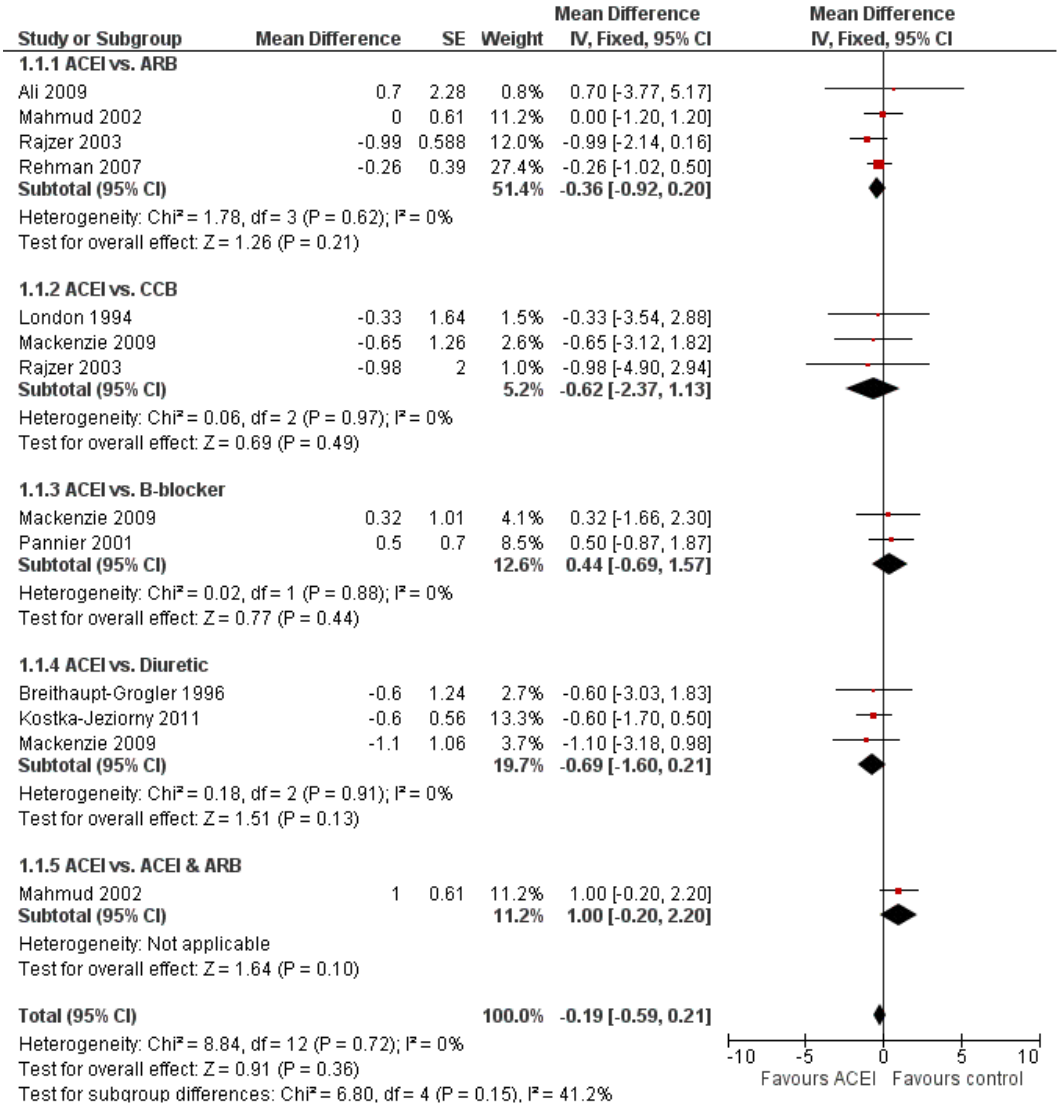

Figure 11: ***Forest plot illustrating ACE inhibitor effect on changes in PWVcf compared with other antihypertensive agents. For further detail see legend for figure 11. ARB: Angiotensin receptor blocker, CCB: Calcium channel blocker, CI: Confidence interval, SE: Standard error, IV: Inverse variance***

ACE Inhibitors Effect on Augmentation Index

ACE Inhibitors vs. Placebo

7 trials [176,178-180,190-192] assessed the effect of ACE Inhibitors on AIx versus placebo. These studies included 614 patients. 306 patients received ACE inhibitor and 312 patients received placebo or their usual treatment. Treatment with ACE inhibitor significantly reduced AIx when compared to placebo or usual treatment (Pooled mean change difference=-3.79%, 95% CI -5.96 to -1.63, P=0.0006, I^2=87%, P for heterogeneity < 0.00001) (Figure 12).

ACE Inhibitors vs. Other Antihypertensives

8 trials[173,181,186,187,191,193-195] which included 323 patients evaluated the effect of ACE inhibitors versus other antihypertensives. Treatment with ACE inhibitor significantly reduced AIx compared to other antihypertensives as a whole (Pooled mean change difference= -1.84%, 95% CI -3 to-0.68, P=0.002) with insignificant heterogeneity (I^2=32%, P=0.110) (Figure 13).

ACE Inhibitors vs. ARBs

ACE inhibitors effect on AIx compared with ARBs was only assessed in one trial [181] which included 12 patients. Treatment with ACE inhibitor showed no significant effect on AIx when compared with ARB (Pooled mean change difference=-3%, 95% CI -25.54 to 19.54, P=0.790) (Figure 13).

ACE Inhibitors vs. CCBs

Two trials [186,191] which included 89 patients evaluated the effect of ACE inhibitors compared to CCBs on AIx. Treatment with ACE inhibitors showed no significant effect on AIx when compared to CCBs (Pooled mean change difference= -2.96%, 95% CI -8.54 to 2.63, P=0.300) with no heterogeneity (Figure 13).

ACE Inhibitors vs. β-Blockers

6 trials[173,186,187,191,193,194] assessed the effect of ACE inhibitors on AIx compared to β-blockers. Treatment with ACE inhibitors significantly reduced AIx when compared with β-blockers (Pooled mean change difference= -1.6%, 95% CI -2.84 to -0.36, P=0.010) with significant heterogeneity (I^2=67%, P=0.006) (Figure 13).

ACE Inhibitors vs. Diuretics

ACE inhibitors effect on AIx compared to diuretics was assessed in 3 trials[186,191,194]. Treatment with ACE inhibitors showed no significant effect on AIx when compared to diuretics (Pooled mean change difference= -4.1%, 95% CI -8.3 to 0.1, P=0.060) with no heterogeneity (Figure 13).

ACE Inhibitors vs. ACE Inhibitors and ARBs

One RCT[181] which included 12 patients assessed the effect of ACE inhibitor versus a combination of ACE inhibitor and ARB on AIx. Treatment with ACE inhibitor and ARB significantly reduced AIx when compared to ACE inhibitor (Pooled mean change difference= -3%, 95% CI -22.4 to 16.4, P= 0.760) (Figure 13).

ACE Inhibitors Combined with Other Antihypertensive Agents vs. Other Antihypertensive Agents

ACE Inhibitors and Diuretics vs. Other Antihypertensive Agents

Overall, 2 trials [196,197] evaluated the effect of ACE inhibitors combined with diuretics versus other antihypertensive agents on AIx. The combination therapy significantly reduced AIx when compared with other antihypertensives as a whole (Pooled mean change difference= -4.68%, 95% CI -7.94 to -1.78, P=0.002) with no heterogeneity (Figure 14).

ACE Inhibitor and Diuretic vs. β-Blocker

ACE inhibitor combined with a diuretic versus β-blocker was assessed in one RCT[196]. Treatment with a combination of ACE Inhibitor and diuretic significantly reduced AIx when compared to β-blocker (Mean change difference=-5.57%, 95% CI -10.77 to -0.36, P=0.040) (Figure 14).

ACE Inhibitor and Diuretic vs. CCB

Ferguson et al[197] assessed the effect of ACE inhibitor combined with diuretic versus CCB on AIx. The combined treatment significantly reduced AIx compared to CCB (Mean change difference=-6.5%, 95% CI -12.7 to -0.22, P=0.040) (Figure 14).

ACE Inhibitor and Diuretic vs. Diuretic

Ferguson et al[197] also assessed the effect of a combination therapy of ACE inhibitor and diuretic versus diuretic on AIx. The combination therapy insignificantly reduced AIx when compared to diuretic alone (Mean change difference=-3.2%, 95% CI -8.1 to 1.7, P=0.200) (Figure 14).

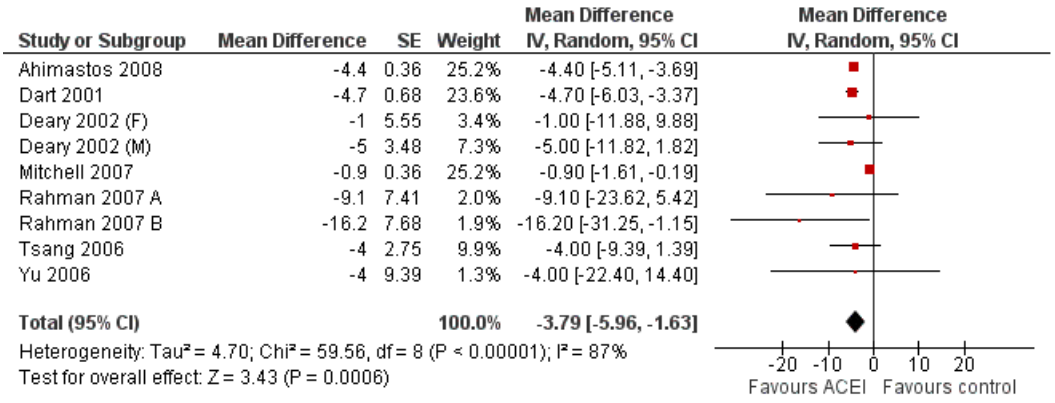

Figure 12: ***Forest plot illustrating ACE inhibitors effect on changes in AIx compared with placebo or usual treatment. The x-axis is in percentage (%). For further detail see legend for figure 11. CI: Confidence interval, SE: Standard error, IV: Inverse variance***

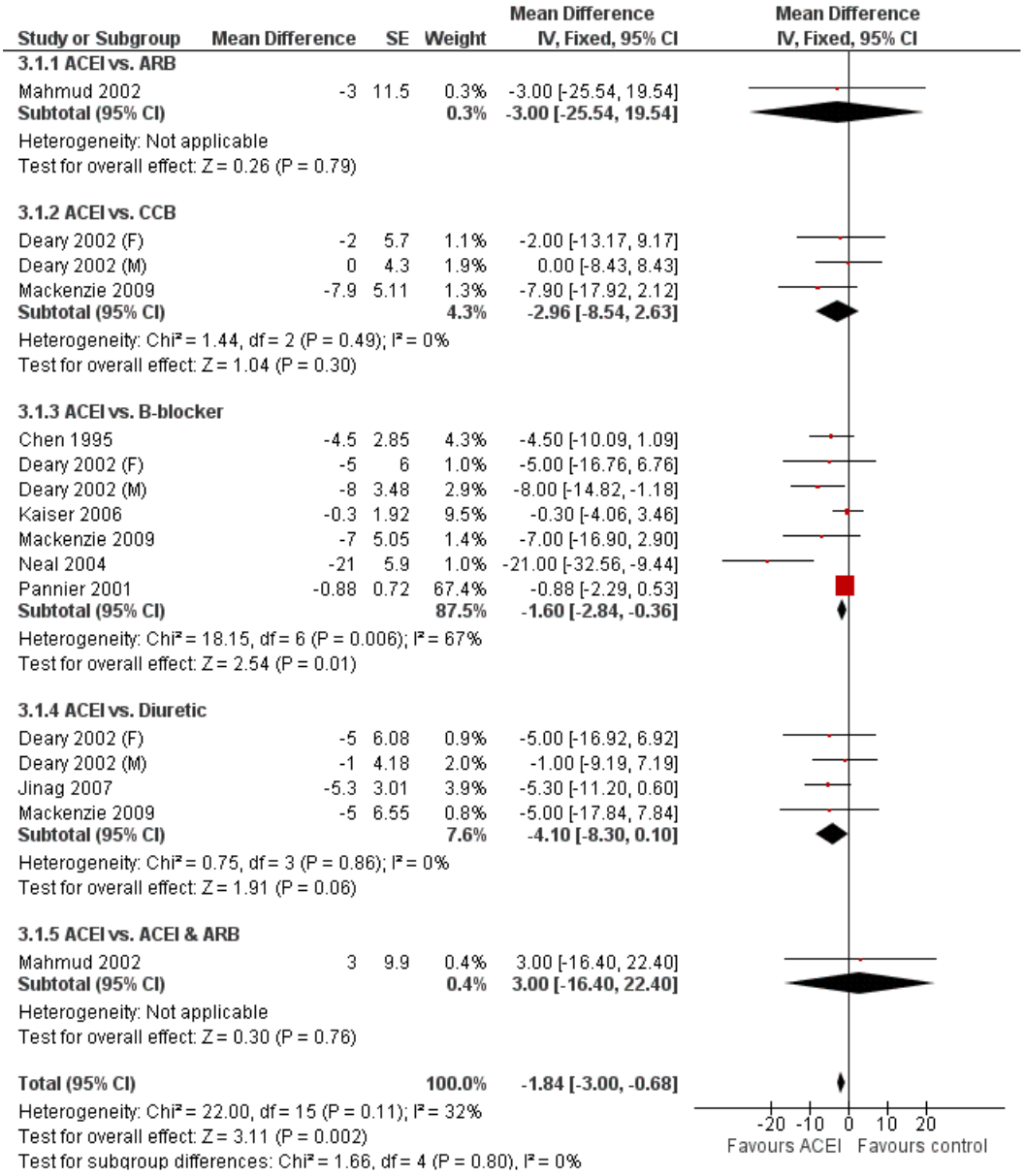

Figure 13: ***Forest plot illustrating ACE inhibitors effect on changes in AIx compared with other antihypertensive agents. For further detail see legend for figure 11. ARB: Angiotensin receptor blocker, CCB: Calcium channel blocker, CI: Confidence interval, SE: Standard error, IV: Inverse variance***

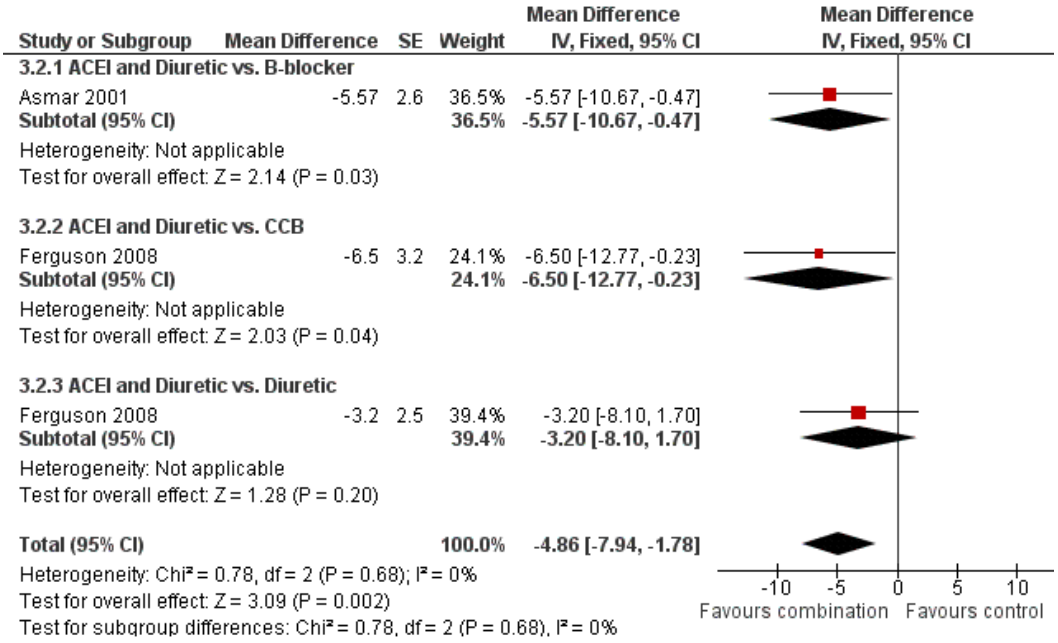

Figure 14: ***Forest plot illustrating the effect of a combination therapy of ACE inhibitor and diuretic compared with other antihypertensive agents on AIx. For further detail see legend for figure 11. CCB: Calcium channel blocker, CI: Confidence interval, SE: Standard error, IV: Inverse variance***

Discussion of Systematic Review

The main finding of this extensive systematic review and meta-analysis on the effect of ACE inhibitors on arterial stiffness is that treatment with ACE inhibitors in patients with arterial stiffness caused by different pathological conditions improved the stiffening of the arteries as assessed by PWVcf and reduced arterial wave reflections as assessed by AIx when compared with placebo. Furthermore, ACE inhibitors significantly reduced AIx and insignificantly PWVcf when compared with other antihypertensive agents as a whole. ACE inhibitors were not superior to ARBs, CCBs, β-blockers and diuretics in their effect on PWVcf when compared separately and as a whole. However, their effect on AIx was superior to the combined effect of other antihypertensives but only superior to β-blockers when compared separately. Furthermore, there is lack of evidence regarding trials assessing the effect of ACE inhibitors on arterial stiffness in patients with PAD with only one RCT assessing this effect[180]. Ahimastos and colleagues[180] assessed arterial stiffness using the ultrasound method which is not the gold standard. Their study also was limited to non-diabetic patients with infrainguinal disease which limits their findings. Hence, the need for further trials to confirm the effect of ACE inhibitors on arterial stiffness in patients with PAD.

5.1 Hypotheses Testing

The role of the ACE inhibitor, ramipril, in reducing cardiovascular morbidity and mortality and health economic analysis of cost effectiveness in patients with PAD with no evidence of left ventricular dysfunction or heart failure is supported by level-1 evidence. However, the evidence regarding the effect of ramipril on walking distance in patients with IC is limited with only one published randomised controlled trial which was limited to non-diabetic patients with infrainguinal disease. Additionally, there are no published randomised controlled trials evaluating this effect on quality of life (QoL) in patients with IC.

Therefore, through a double blind, randomised, placebo controlled clinical trial; we aim to test the following hypotheses:

Hypothesis (1): *ACE inhibitor improves clinical indicators of lower limb ischaemia in patients with IC.*

Hypothesis (2): *ACE inhibitor improves arterial stiffness and wave reflections in patients with IC.*

Hypothesis (3): *ACE inhibitor improves cardiovascular prognosis in patients with IC.*

Hypothesis (4): *ACE inhibitor improves biochemical markers of inflammation and ischemia reperfusion in patients with IC.*

Hypothesis (5): *ACE inhibitor improves generic and disease specific QoL in patients with IC.*

5.2 Study Design

A double blind, randomised, placebo controlled clinical trial of two way design with participants randomised to placebo group or ramipril group.

5.3 Sample Size

Based on a previous RCT[109] which was done to examine the effects of ACE inhibitor on walking in patients with PAD, 40 patients were randomised to achieve a power of 86%. Therefore, to detect a change of 150 meters MWD with ramipril, with a 5% two tailed significance level assuming a pooled standard deviation (SD) of 125 metres, 12 patients in each group (a total of 24 patients) will achieve a power of 96%. However, with an estimate of 30% loss to follow up/withdrawals, we aimed to recruit a total of 32 patients.

5.4 Assessment of Eligibility

Inclusion Criteria

- Patients with unilateral or bilateral intermittent leg claudication which was stable for the last 6 months.
- Patients with ABPI < 0.9 at rest at least in one leg.
- BP ≤ 160/90 and a stable medication regimen for the last 6 months.
- Able to give informed consent

- Able to comply with study protocol

Exclusion Criteria

- Documented bilateral renal artery stenosis
- Unlikely to be compliant with medication or follow up as determined by the recruiting institution
- Pregnancy and breast feeding
- Women of child bearing age not having a method of contraception
- Patients with critical limb ischemia (This includes patients with ischaemic rest pain and ulceration > 2 weeks and/or a resting ankle pressure < 50mmHg- Grades II and III according to Rutherford et al 1997[198]
- Patients who had a recent (less than 3 months) angioplasty or bypass surgery
- Patients who are unable to perform a treadmill test due to a limiting heart, respiratory or arthritic disease
- History of angioneurotic oedema
- Currently taking ACE inhibitor or Angiotensin receptor blocker
- Contraindication to ACE inhibitor

- History of ACE inhibitor intolerance
- A creatinine rise of > 30% from baseline and/or Potassium > 5.9 mmol/l
- Unwillingness to participate
- Level 1 evidence for ACE inhibitor treatment, including:

a. Documented heart failure, left ventricular dysfunction or ejection fraction <35% on previous echocardiography

b. Uncontrolled hypertension, BP > 160mmHg systolic or 100mmHg diastolic on 3 separate readings measured after 10 minutes rest on 2 separate occasions

c. Recent (< 3months) myocardial infarction or stroke

d. Chronic renal impairment (serum creatinine > 250 mmol/l).

5.5 Randomisation

Patients were randomised to receive either placebo or ramipril. Randomisation was undertaken by pharmacy department at Hull Royal Infirmary and was done through a computer generated blocks of 10s. At randomisation participants were given a unique identification number to be used for identification purposes through-out the trial.

5.6 Trial Phases

'Run in' Phase

All consenting patients undertook a 'run in' phase prior to randomisation, in which they received ramipril 2.5mg orally o.d. for 1 week, increased to 5mg orally o.d. for a further week. At the end of each week clinical status, adverse events (dizziness, symptomatic hypotension, cough), drug compliance, BP, and renal function were assessed. At this stage participants were excluded if they were non-compliant (< 60% of medication taken), suffered disabling side effects, demonstrated significant deterioration in renal function (A creatinine rise of >30% from baseline, potassium > 5.9mmol/l) or withdrew consent. Patients then had a 2-week wash out period.

Randomisation Phase

After the washout period, patients willing to continue the trial were randomised on a 1:1 ratio into one of the following groups:

- Placebo group
- Ramipril group: Patients will be given Ramipril 5 mg once daily for 2 weeks increased to 10 mg once daily for 22 weeks.

5.7 Trial Treatments

Ramipril and placebo were encapsulated and identical in shape. Study medication was stored and dispensed by the trial site's pharmacy department in accordance with Good Clinical Practice and Good Manufacturing Practice. At the 'run in' phase, each patient was dispensed one container of 7 capsules of ramipril 2.5 mg for a week and one container of 7 capsules of ramipril 5 mg for another week at another visit.

At the randomisation phase each patient was dispensed one container which had 14 capsules of either ramipril 5 mg or placebo for 14 days and another container which had 154 capsules of either Ramipril 10 mg or placebo for 154 days at another visit.

Each container was tamper evident and child resistant. All containers were labelled with a double blind label printed in black ink. The label included the protocol number, drug name written as 'ramipril/placebo', subject number, container quantity, storage conditions, expiration date, randomisation or run in phase and investigator's name. Code break envelopes were also provided and were kept in pharmacy.

5.8 Double Blinding

Both patients and the principal investigator were blinded to study treatments. The dispensing pharmacists were also blinded to study treatments.

5.9 Trial Follow-up

Patients were followed up at 2 weeks, 6 weeks and 24 weeks using the trial specific proforma.

5.10 Trial Outcome Measures

Primary Outcome Measure

The primary outcome measure for this trial is the treadmill MWD.

Secondary Outcome Measures

Other Clinical Indicators of Lower Limb Ischaemia

a) Patient Reported Walking Distance (PRWD)

b) Treadmill ICD or the distance associated with the onset of claudication pain measured on the same standardised treadmill test

c) ABPI at rest (ABPI- r) and following treadmill testing (ABPI – t)

QoL

a) Generic – measured using the SF36 and EuroQol (EQ5D) instruments

b) Disease specific – measured using the King's college VascuQol

Biomarkers of Inflammation, Ischaemia Reperfusion and Cardiovascular Prognosis

a) CRP

b) Fibrinogen

c) Urine Albumin Creatinine Ratio (UACR)

d) N-terminal pro B-type natriuretic peptide (NTproBNP)

Arterial Effects

a) Arterial stiffness by measuring PWV

b) Wave reflections by measuring AIx

5.11 Trial tests

Blood Pressure Measurement

BP was measured from each arm at both seated and supine positions at every visit using an automated oscillometric device (Welch Allyn, Arden, NC, USA). This device has been validated in terms of BP measurement in several previous studies[199-201].

Ankle Brachial Pressure Index Measurement

ABPI measurement is a simple method of quantifying the severity of arterial occlusion in the leg and was first described in 1968 [202]. The index is measured by measuring the maximum systolic BP at the ankle using a hand-held Doppler (Recording the highest of either dorsalis pedis (Figure 16) or posterior tibial artery (Figure 17)) and dividing this by the highest value of systolic pressure recorded from the right and the left brachial arteries. In a healthy person, ABPI is > 1.0. ABPI between 0.5 and 0.95 is indicative of PAD or IC. ABPI <0.5 is indicative of critical limb ischaemia. ABPI is considered an independent predictor of cardiovascular events[203] and a low ABPI is associated with increased all-cause and cardiovascular mortality, MI and stroke [204-212].

ABPI Measurement Technique Using Doppler

Patients were asked to rest in a supine position for 5 minutes to allow BP to settle. A cuff was placed around the upper arm and the brachial pulse was located. Ultrasound gel was then applied and a highly sensitive 8 MHz Doppler probe (Parks Medical Electronics, Inc. Aloha, Oregon, USA) was placed over the brachial pulse. The cuff was then inflated until the signal disappeared and deflated until the systolic signal was regained. The same procedure was performed on the opposite arm and the higher of the two values was recorded as the systolic bBP. The same procedure was performed

to obtain ankle systolic pressure with the cuff placed above the malleoli after locating the dorsalis pedis pulse and then repeated for the posterior tibial pulse with the higher of the two values recorded. In order to obtain ABPI, this value was then divided by the brachial systolic pressure. ABPI of the most symptomatic leg was entered into analysis. ABPI was measured at rest and after undertaking a treadmill exercise test. Patients underwent ABPI measurements at baseline, 2 weeks, 6 weeks and 24 weeks.

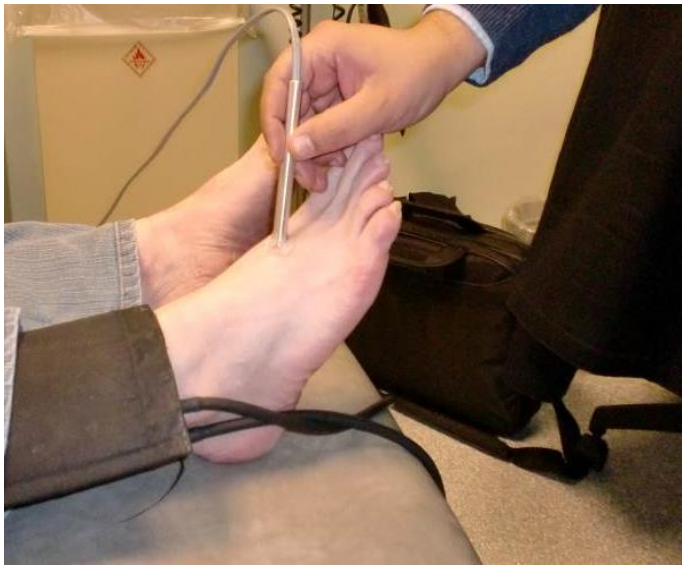

Figure 15: ***Detecting dorsalis pedis artery pulse using hand-held Doppler***

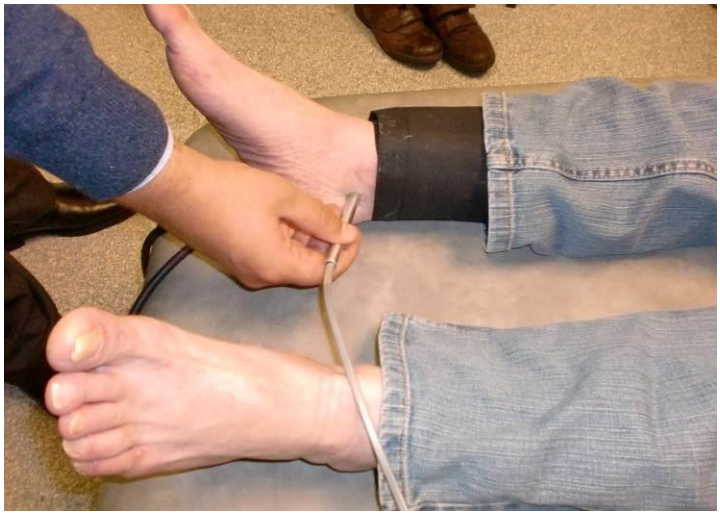

Figure 16: ***Detecting posterior tibial artery pulse using hand-held Doppler***

Treadmill Exercise Test

Treadmill exercise testing is mainly used for patients with PAD with a normal resting ABPI such as patients with iliac artery disease where a significant drop in ABPI post exercise is diagnostic of PAD. However, it has been shown that walking impairment is a strong predictor of long term outcome in patients with impaired ABPI and normal ABPI[213].

Treadmill Exercise Test Protocol

Patients underwent treadmill exercise testing at a speed of 1.6 mph with a 10 degree incline and for a maximum of 10 minutes according to a standard constant-loading protocol in our laboratory. The limitations of this protocol are discussed in the limitation of the study section of this thesis. Patients were asked to walk on the treadmill to familiarize them with the test during the 'run in' phase of the trial before conducting a formal test in the

'randomization phase'. Furthermore, patients were given clear verbal instructions to what exactly expected of them during conducting a treadmill exercise test. Patients' familiarization with the treadmill exercise test is important to reduce interventional bias in the trial. Patients were also instructed to avoid walking long distances before their lab visit to avoid claudication, avoid smoking or drinking alcohol and wear comfortable clothes. During the test, patients were asked not to hold onto the treadmill bars for support or off-loading as this will alter patients' performance and introduces variability in the test. The timing device used to record walking time was hidden from patients to avoid introducing bias in their performance. ICD was defined as the distance patients with IC can walk until they start experiencing pain in the affected leg. MWD was defined as the maximum treadmill walking distance that patients with IC can walk until they are unable to walk anymore.

Pule Wave Velocity and Augmentation Index Measurement

PWV was calculated between the carotid and femoral arteries (PWVcf) as this measurement technique is considered by many to be the gold standard of PWV measurement[214]. Several devices are available in the market for PWV measurement including: The SphygmoCor (AtCor Medical Pvt. Ltd, Sydney, Australia), the Complior (Artech Medical, Pantin, France), PulsePen (DiaTence, Milan, Italy) and most recently the Vicorder (Skidmore Medical, Bristol, UK). The SphygmoCor system, which is considered the gold standard,

uses sequential applanation tonometry of the arterial waveform with electrocardiogram (ECG) gating. It is easily applied and has been widely used in adults with excellent reproducibility[215,216]. The Complior system, on the other hand, uses mechanotransducers to detect the pressure waveforms and allows simultaneous recordings. The latter two devices have shown differences in PWV values when compared with each other mainly due to differences in the methods used to measure transit time rather than in the methods used to acquire the waveforms[217,218]. Nevertheless, to date, there is no agreement to which device is more accurate in terms of PWV measurement[219].

SphygmoCor Device

The SphygmoCor (Model SCOR-Pvx, software version 8; AtCor Medical Pvt. Ltd, Sydney, Australia) was used for PWVcf measurements (Figure 18). It uses the applanation tonometry technique to acquire the pulse waveform using one tonometric Miller transducer. In order to obtain the PWVcf, the carotid and femoral pulse waveforms are recorded sequentially using the transducer (Figures 19 and 20) and at the same time an ECG is recorded (Figure 21) as a reference to calculate tt using the foot-to-foot method. The distance the pulse waveform travels between the two recording sites (Carotid and femoral) is measured using a tape measure over the body area. The distance between the suprasternal notch and the carotid recording site (Proximal) is measured (Figure 22) as well as the distance between the suprasternal notch

and the femoral recording site (Distal) (Figure 23). The difference between the proximal and distal distances is then calculated automatically by the device upon entering the former distances. The PWVcf is then calculated as PWVcf= Distance (m)/ tt (s). All PWVcf measurements acquired in our trial had an operator index range of 85-100%.

Figure 17: ***The SphygmoCor device***

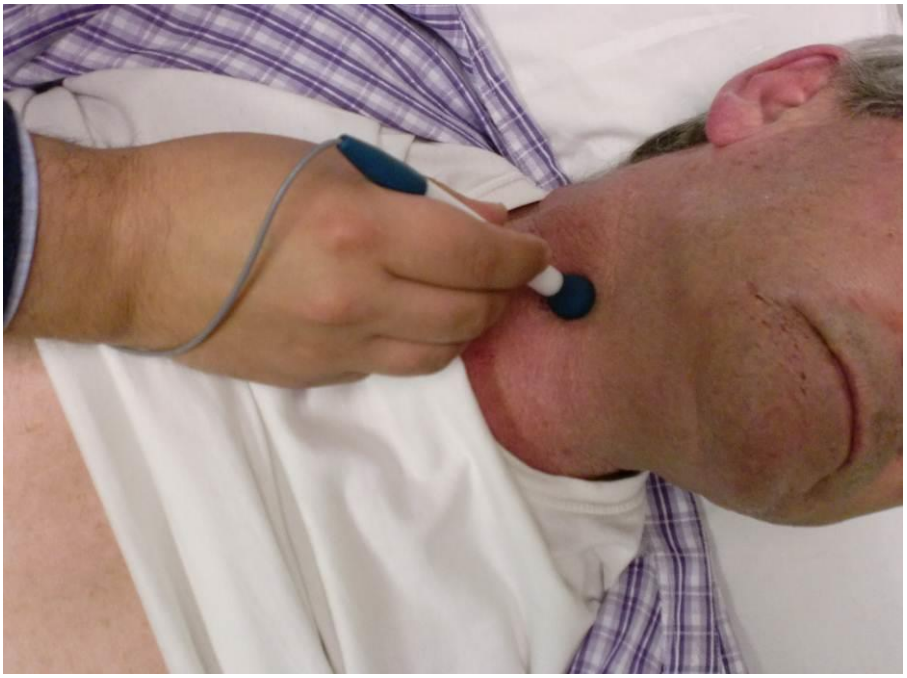

Figure 18: ***Recording the carotid pulse using Miller transducer***

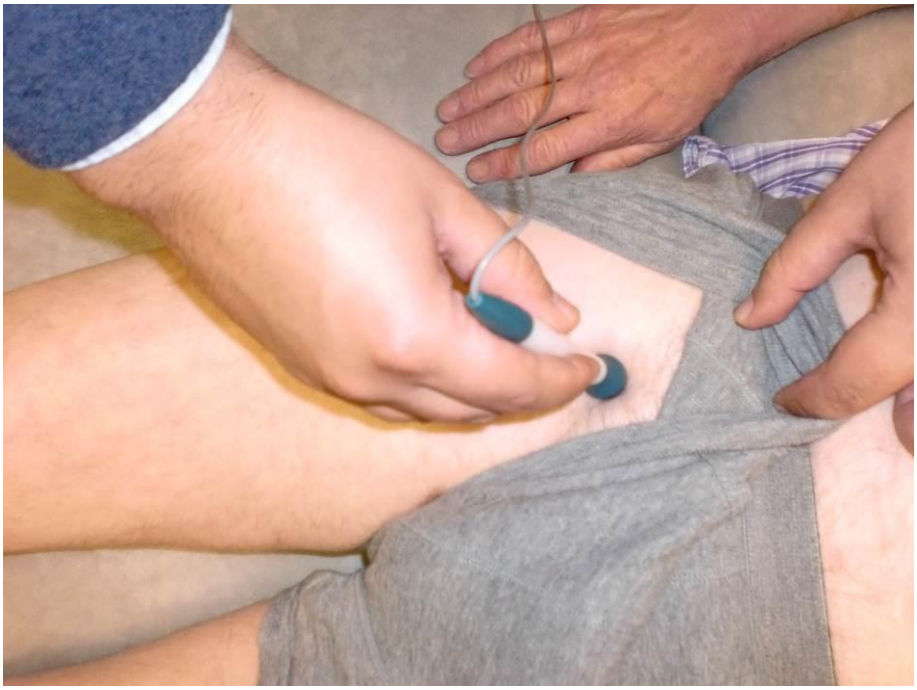

Figure 19: ***Recording the femoral pulse using Miller transducer***

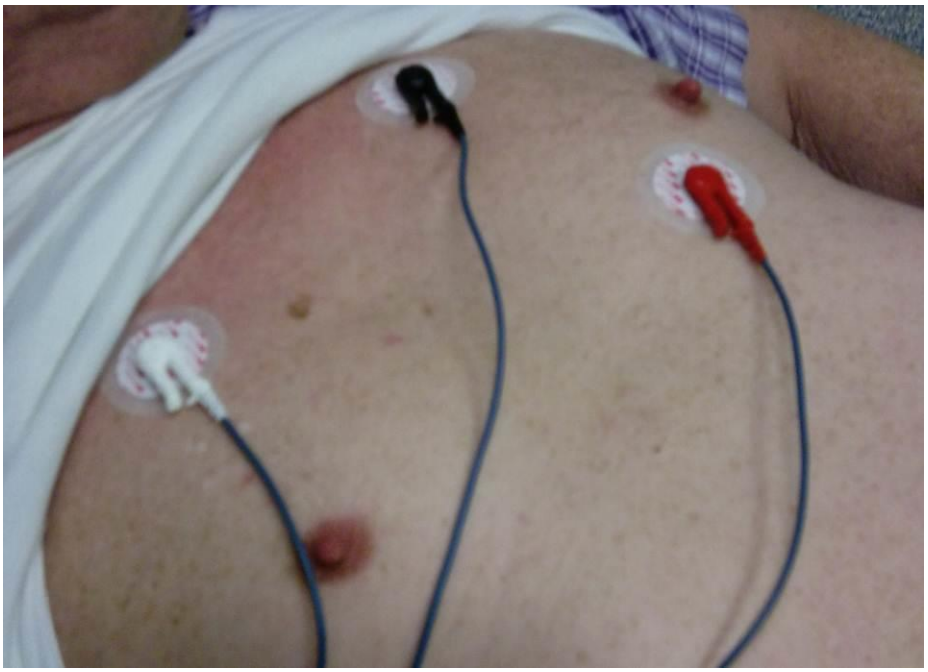

Figure 20: ***Recording of the ECG***

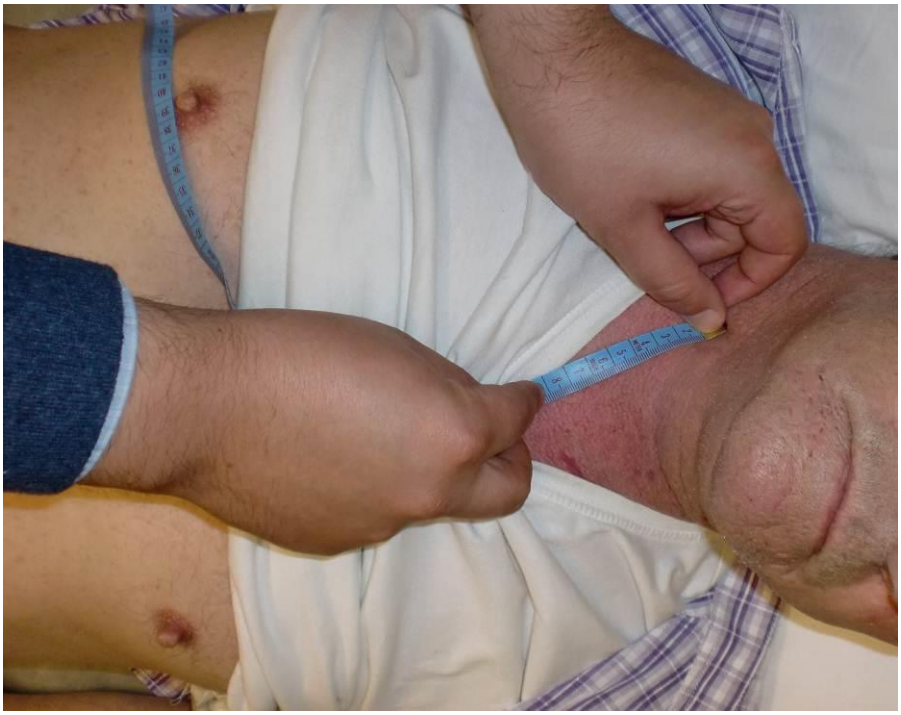

Figure 21: ***Proximal distance between suprasternal notch and carotid pulse detection point***

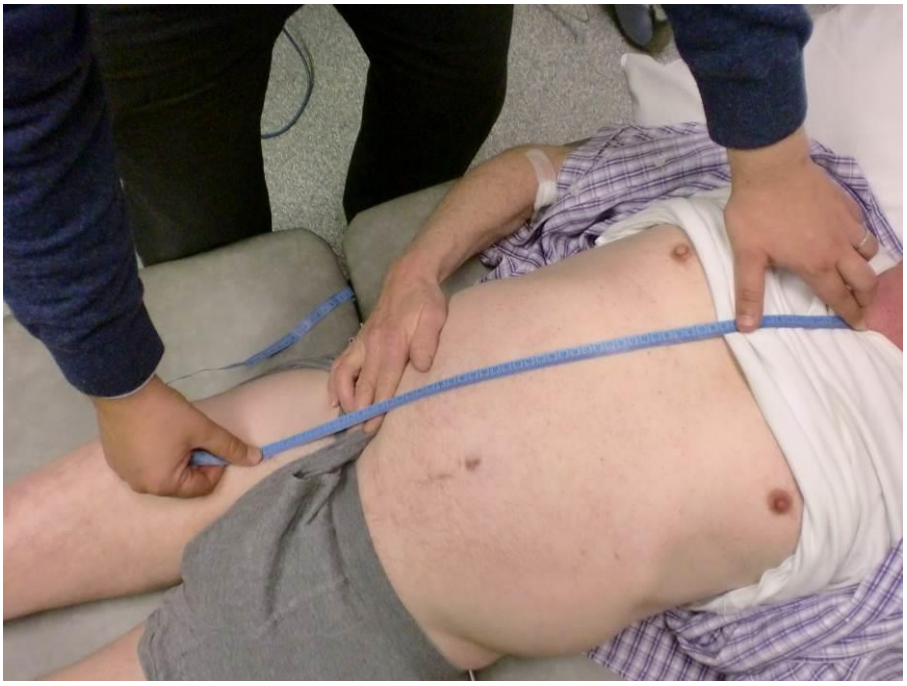

Figure 22: ***Distal distance between suprasternal notch and femoral pulse detection point***

Furthermore, the coefficient of variation (CoV) for the SphygmoCor repeated measurements was 5%. In addition, limits of agreement (LoA) for the SphygmoCor repeated measurements ranged between -1.79 to 1.85 m/s with 93% of the measurements falling within 2 SDs of the mean difference using Bland-Altman plot[220] (Figure 24).

The same device was used for PWA. A hand-held high-fidelity tonometer (Miller tonometer, Houston, Texas, USA) was used for applanation tonometry of the right radial artery (Figure 25). The SphygmoCor device generated an average radial pulse wave contour after a 10 second recording period. This was then converted to a central pulse wave using a general transfer function

available within the SphygmoCor device[221,222]. AIx and ED % were then derived from PWA.

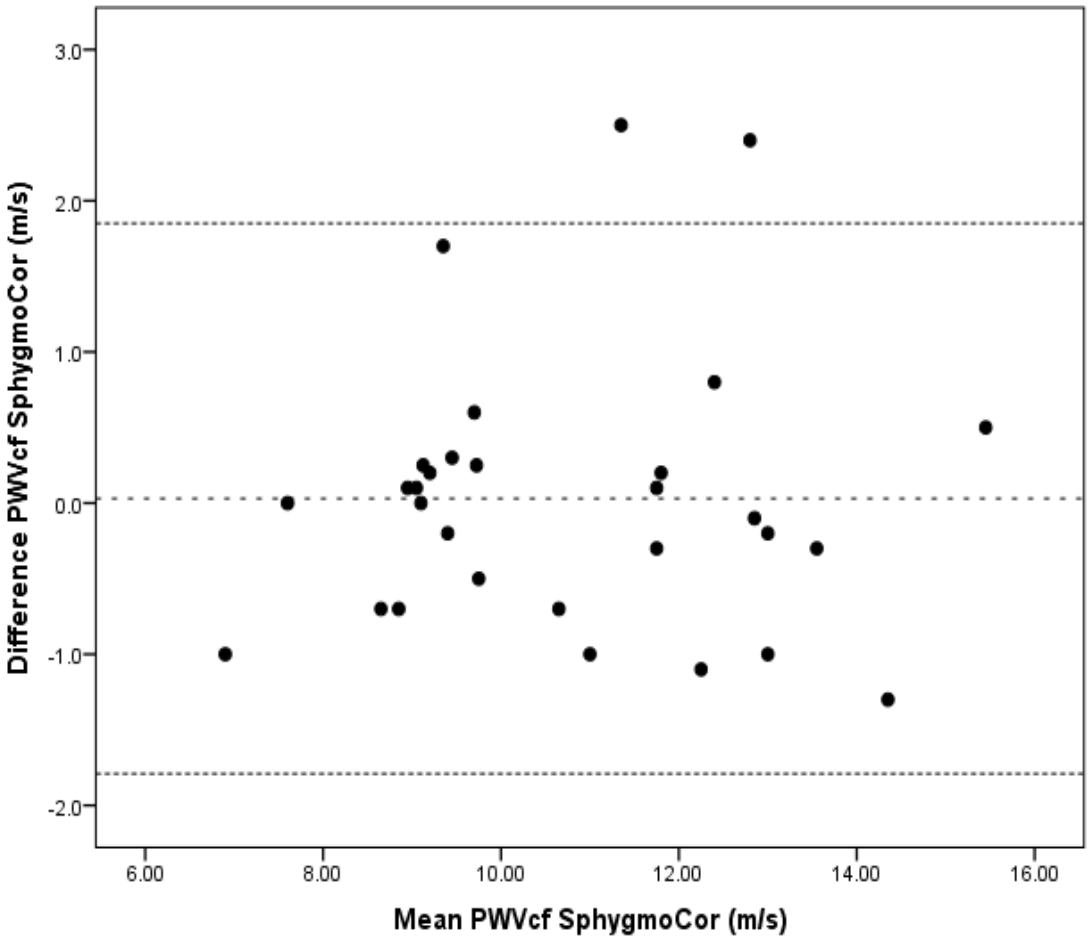

Figure 23: ***Bland-Altman plot illustrating intra-rater reproducibility of the PWVcf measurements by the SphygmoCor device. The upper and lower dotted lines represent the level of agreement between repeated PWVcf measurements (mean difference +/- 2 SDs). The middle dotted line represents the mean of the difference between all repeated PWVcf measurements generated by the device***

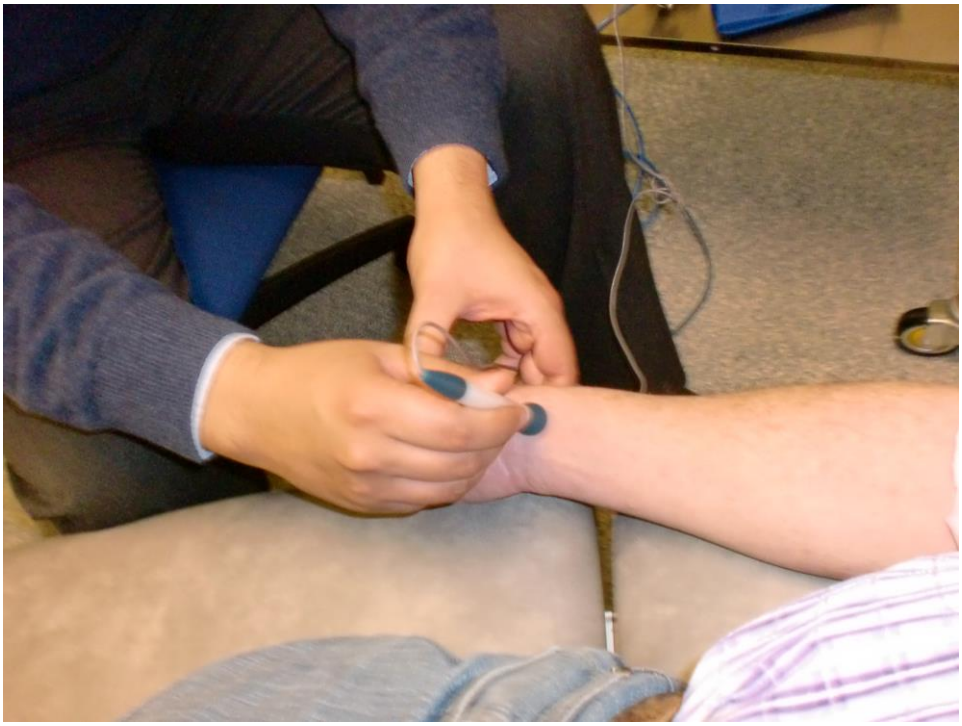

Figure 24: ***Applanation tonometry of the right radial artery using a high fidelity monometer***

On the other hand, for the AIx measurements the LoA for the SphygmoCor repeated measurements ranged between -3 and 2% with 96% of the measurements falling within 2 SDs of the mean difference (Figure 26).

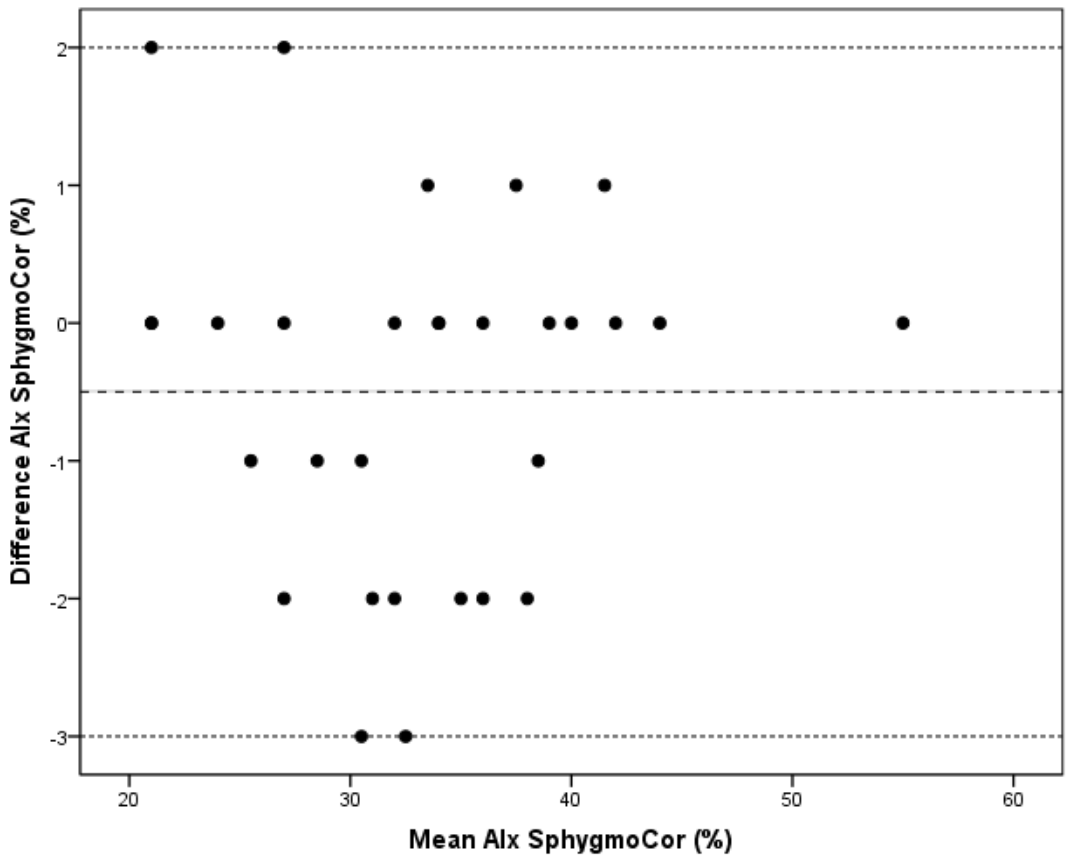

Figure 25: ***Bland-Altman plot illustrating intra-rater reproducibility of the AIx measurements by the SphygmoCor device. For further detail, please see legend for figure 24***

The Vicorder Device Compared to SphygmoCor in the Assessment of Carotid Femoral Pulse Wave Velocity in Patients with PAD

In order to establish which device has more reliability and validity in estimating PWVcf in patients with IC, an observational study was conducted[220] comparing the Vicorder (Bristol, UK) which is a new device in the market for measuring PWVcf with the SphygmoCor which is considered by many as the gold standard for PWVcf measurement.

To this end, 30 patients with PAD (23 men, mean age 64.9 +/-7.5), who are some of the patients who took part in the original trial, underwent PWVcf measurement twice by a single investigator during one visit (Baseline) using the Vicorder and SphygmoCor according to manufacturers' instructions. Intra-rater reproducibility for each device was assessed using intraclass correlation coefficients (ICC) and Bland-Altman method. The latter was also used to compare between the two devices.

Measurements of PWVcf were highly reproducible using both devices (ICC=0.94 and 0.92, for the Vicorder and SphygmoCor, respectively). LoA using the Bland-Altman method were-1.07 to 1.09 m/s and -1.79 to 1.85 m/s for the Vicorder and SphygmoCor, respectively. Bland-Altman plots indicated that 90% of PWVcf measurements using the Vicorder and 93% of the measurements using the SphygmoCor fell within two standard deviations of the mean difference. Transit time differed significantly between the two devices (mean difference 30+/-9.2 ms, P<0.001) with the Vicorder recording

higher values. Nevertheless, the two devices recorded nearly similar PWVcf measurements (mean difference -0.69+/-1.6 m/s, P= 0.020) with 97% of PWVcf values falling within two standard deviations of the mean difference on Bland-Altman plot.

To conclude, both devices generated highly reproducible PWVcf measurements in patients with PAD and were in good agreement when compared with each other. However, the discrepancy in transit time between the two devices lead to the Vicorder producing lower values of PWVcf at high values produced by SphygmoCor. This discrepancy in transit time generated by the Vicorder lead us to use the SphygmoCor for measuring PWVcf in the trial.

Biomarkers of Inflammation, Ischaemia Reperfusion and Cardiovascular Prognosis

The rationale behind measuring the following biomarkers has been discussed extensively in the introduction. Biomarkers of inflammation (CRP and Fibrinogen), biomarkers of ischaemia reperfusion (UACR at rest (r-UACR) and after exercise (t-UACR) and biomarker of cardiovascular prognosis (NTproBNP) were measured in the trial at baseline, 2,6 and 24 weeks follow-up. The CRP assay had a sensitivity of 0.2 mg/l with a standard reference

range of 0.5-8mg/l and a CoV of <6%. To measure NTproBNP, blood samples were taken in EDTA Vacutainers and centrifuged at 4°C. The resultant plasma was analysed using an Elecsys 2010 analyser (Roche diagnostics). For the purpose of diagnosing heart failure, patients with NTproBNP plasma levels < 300 pg/ml were unlikely to have heart failure and patients with levels > 500 pg/ml were very likely to have heart failure[223].

Other Biomarkers

Blood samples were obtained for a serum lipid profile including: Total cholesterol, triglyceride, LDL-C and HDL-C which was analysed using an enzymatic method. Furthermore, renal function was monitored closely throughout the trial by measuring urea, creatinine and estimated glomerular filtration rate (eGFR). All these measurements were obtained at baseline, 2,6 and 24 weeks.

Generic Quality of Life Assessment

Short Form-36

The generic SF-36 instrument (Figure 26-Appendix A) resulted from the Medical Outcomes Survey and the RAND health insurance experiment[224-227]. The aim was to produce a comprehensive, standardised, psychometrically sound practical instrument. This was to reduce the burden of prolonged

health surveys on both researchers and trial participants. The SF-36 has now become the most used health questionnaire in the world[228]. It produces a comprehensive profile of eight domains covering the range of physical and psychological well-being: physical function, role limitation due to physical disability (Role – physical), bodily pain, general health perception, vitality, social function, role limitation due to emotional problems (Role – emotional) and mental health. Item scores for 36 questions are coded, summed and transformed on to a scale from 0 (Worst health) to 100 (Best health) in each domain.

SF-36 has been extensively shown to be both valid and reliable in several health groups including patients with PAD[229-236]. Its global popularity has resulted in it being translated into 130 languages and norm based scores produced for a range of populations based upon population responses, making it a patient centred questionnaire.

EuroQoL

The EuroQol (EQ5D™; EuroQol Group, Rotterdam, The Netherlands) was developed by a multidisciplinary group of researchers from five European centres[237] (Figure 27-Appendix A). It is an index scale, mapping three available responses to five questions onto a utility scale with 245 possible states. The UK weights were derived from TTO responses from 3000 adults from the general population[238] with statistical modelling. The EQ5D has been

shown to be valid[237,239,240] and is the recommended utility measure for cost utility analysis in the UK[241]. It is also a popular system world-wide with 150 languages available. The EQ5D also includes a 100 point visual analogue scale, from "Worst imaginable health state" to the "Best imaginable health state".

Disease Specific Quality of Life Assessment

King's College VasculQoL

The vascular QoL (VasculQol) questionnaire (Figure 28-Appendix A) was developed by a group of clinicians in the academic vascular department at King's College Hospital[242]. The aim was to produce a disease specific QoL questionnaire for patients with chronic lower limb ischaemia and to design an evaluative instrument which is responsive to within subject variability that adds to clinical measures of outcome when comparing different treatment options for patients with lower limb ischaemia.

The VascuQol questionnaire consists of 25 questions which cover five domains (Activities, symptoms, pain and emotional and social items) with each domain scored on a scale of 0 (Worst score) to 7 (Best score).

5.12 Quality Control and Quality Assurance

Monitoring

The study was monitored in accordance with the Hull and East Yorkshire Trust Research and Development (R&D) department's standard operating procedures to ensure compliance with the UK clinical trials regulations. All trial related documents were made available upon request for monitoring by R&D monitors and for inspection by the Medicinal Health Regulatory Agency (MHRA). Monthly monitoring reports were completed and sent to R&D for regular trial up-dates.

Ethical Review

The final study protocol, including the final patient information and consent documents were given a favourable opinion by Ethics Committee prior to the study commencing. In addition Hull and East Yorkshire Trust R&D approval, Site Specific Assessment (SSA) approval and MHRA clinical trial authorisation was also acquired prior to commencing the study.

Progress reports and notification of serious adverse events will be provided to the Ethics Committee according to local regulations and guidelines.

Ethical Conduct of the Study

The study was conducted in accordance with The Medicines for Humans Use (Clinical trials) Regulations 2004 and Amendment Regulations 2006 and subsequent amendments; the International Conference for Harmonisation and Good Clinical Practice (ICH/GCP) guidelines; and the Research Governance Framework for Health and Social Care.

All patients with IC were invited to enter the study if they fitted the inclusion/exclusion criteria. They were given a patient information sheet to read and a verbal discussion about the study by one of the study doctors. Patients who decided to enter the study were asked to sign a consent form.

Patients were informed that they can leave the study at any time and that their future medical care will not be affected.

All information collected about participants was collated using unique research numbers to ensure patients confidentiality. No patients were named in any report or were identifiable.

Anonymised data was stored on an NHS computer compliant with regulatory requirements in respect of patients' confidentiality. Data will be kept for a maximum of 5 years.

Data Handling and Record Keeping

The chief investigator was responsible for data collection, recording and quality. Data was collected and retained in accordance with the Data Protection Act 1998. Study documents (paper and electronic) were retained

in a secure (kept locked when not in use) location during and after the trial has finished. All essential documents including source documents will be retained for a minimum period of 5 years after study completion (last patient, last visit). A label stating the date after which the documents can be destroyed was placed on the inside front cover of the case notes of trial participants.

Indemnity

This was an NHS –sponsored research study. If there was negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS indemnity covers NHS staff and medical academic staff with honorary contracts only when the trial has been approved by the R&D department. NHS indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm.

5.13 Statistical Analysis

All data was transcribed and recorded in a secured and dedicated database (Microsoft Excel®, Redmond, WA, USA). A Statistical Package for the Social Sciences Program (SPSS) version 18 for Windows (SPSS Inc. Chicago, IL) was used for statistical analysis.

The statistical analysis was performed according to a standardised prospectively determined protocol. No assumptions were made at any time as to the direction of any relationships and no imputation of missing data was

attempted. Any key assumptions of the statistical techniques used were tested as appropriately.

Continuous Data

Continuous variables were expressed as mean +/- SD or adjusted mean changes (95% CI) or adjusted mean changes +/- standard error (SEM) for normally distributed data. If data was not normally distributed it was expressed as median (interquartile range (IQR)).

Adjusted mean changes (95% CI) for outcome measures are presented using box graphs and tables.

Analysis

The distribution of continuous data was explored using histogram analysis prior to performing any statistical analysis. Any data which appeared to be normally distributed underwent further testing using the Kolmogorov-Smirnov statistic. This test assesses the normality of the distribution of values. For the purposes of this test, a non-significant result (P value > 0.050) indicates normality.

Intragroup comparisons, featured the analysis of paired data (i.e. before and after in the same patient) and intergroup comparisons used unpaired data (i.e. from different patients). Hypothesis testing was performed comparing groups according to the data distribution and whether it was paired or

unpaired. The quoted "P-value" represents the probability of having observed the data if the null hypothesis were true[243] (i.e. there is no actual differences between the data). "P-values" are quoted to three decimal places and a value of less than 0.050 was regarded as "significant" and led to rejection of the null hypothesis.

Comparison between the groups at baseline was performed using the unpaired Student t-test (t test)[244] for normally distributed data and Mann-Whitney U (MWU)[245] test for non-normally distributed data.

Intragroup analysis of mean changes from baseline was tested using one-way repeated measures analysis of variance (ANOVA)[246,247]. Intergroup analysis was tested by means of one-way analysis of covariance (ANCOVA) model using baseline variables as the covariates in the model[247]. Therefore, adjusted mean changes +/- SEM or (95% CI) are displayed.

Non-parametric baseline variables were log-transformed before being entered as covariates in the ANCOVA model[247]. Post-hoc comparisons were analysed using the Bonferroni correction.

To examine the relationship between changes in walking distance from baseline at 24 weeks with corresponding changes in arterial stiffness indices, Pearson's correlation coefficient (r) was used for univariate analysis.

Categorical Data

Simple categorical data is presented as percentages (x/y) where x represents the number of cases in a category and y represents the total number of cases under consideration.

Analysis

The primary hypothesis test used in categorical analysis is Pearson's Chi-square test ($\chi 2$ test)[248]. If greater than 20% of expected frequencies are less than 5 or any are below 1, then Fisher's exact test (FET)[249] was used.

Linear Regression Analysis

Linear regression analysis was used to further examine covariates which were significant in the univariate analysis using Pearson's correlation. Covariates which were statistically significant on univariate analysis were entered into the regression model with adjustment for HR and MAP.

Linear regression was also performed using the enter method to determine whether ramipril effect on walking distance is related to its effect on systolic bBP and diastolic bBP.

6.1 Study Population:

Flow of patients through the trial is presented in Figure 30. Overall, 166 patients were assessed for eligibility over a period of 10 months from January 2011; 38 patients were recruited and started on the run-in phase following which 5 patients withdrew and 33 patients were double-blind randomised to receive ramipril (n=14) or placebo (n=19), of whom, 29 patients (ramipril, n=12; placebo, n=17) completed all trial follow-ups.

The most frequent reason for failing inclusion into the trial was being treated with ACE inhibitor or ARB (n=59) followed by declining to participate (n=27) in the trial and having an ABPI >0.9 (n=25).

Baseline patients' characteristics are summarised in table 8. There was no significant difference between the two groups in terms of demographics, cardiovascular risk factors, site of arterial disease or concomitant medications.

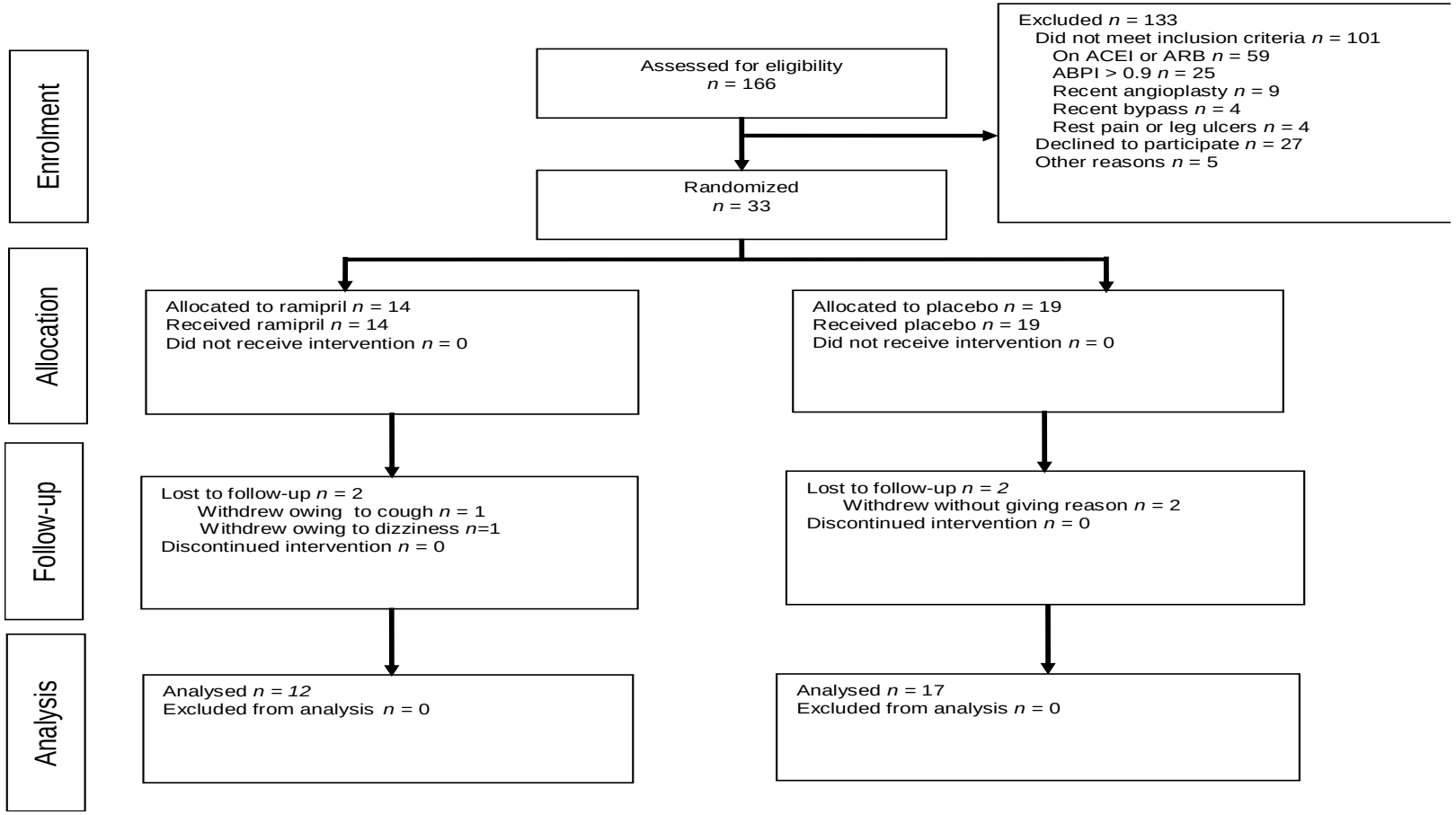

Figure 29: ***Consort diagram for the trial. ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blocker, ABPI: Ankle brachial pressure index***

	Ramipril (*n* = 14)	Placebo (*n* = 19)	*P*†
Age (years)*	64.4(8.2)	64.7(7.7)	0.892‡
Sex ratio (M : F)	11 : 3	14 : 5	1.000
Body mass index (kg/m^2)	28.1(3.8)	28.3(4.2)	0.885‡
Cardiovascular risk factors			
Smoking			
Current smoker	7	9	1.000
Ex-smoker	7	6	0.472
Never smoker	0	4	0.119
Diabetes mellitus	4	7	0.738
Dyslipidaemia	12	18	0.561
Hypertension	7	13	0.472
Coronary artery disease	2	2	1.000
Previous PAD treatment			
Angioplasty	4	7	0.719
Peripheral bypass surgery	1	0	0.424
Concomitant medications			
Antiplatelet agents	14	17	0.496
Lipid-modifying agents	12	16	1.000
Beta-blockers	2	1	0.561
Calcium channel blockers	6	9	1.000
Diuretics	2	3	1.000
Arterial stenosis site			
Infrainguinal	12	15	1.000
Suprainguinal	0	2	0.496
Mixed	2	2	1.000

Table 8: ***Patients' baseline characteristics. *Values are mean (SD).PAD: Peripheral arterial disease, †Fisher's exact test except ‡unpaired t test***

6.2 Primary Outcome Measure: Maximum Walking Distance

Ramipril versus Placebo Effect on Maximum Walking Distance

There was no significant difference between the ramipril and placebo groups at baseline in terms of MWD (median (IQR), 137 (110-213) *versus* 143 (72-213) metres; P=0.760).

Intragroup analysis showed that the mean change in MWD in the ramipril group increased significantly at 24 weeks compared to 2 weeks (P=0.006) and 6 weeks (P=0.010 (P=0.226 for comparison between mean changes at 2 and 6 weeks).

In the placebo group, however, there was no significant increase in mean change in MWD at 24 weeks compared to 2 weeks (P=1.000) and 6 weeks (P=0.937) (P=0.134 for comparison at 2 and 6 weeks).

Intergroup analysis showed that the adjusted mean change from baseline in MWD after 24 weeks of treatment with ramipril was 130.5 (95% CI 61.8 to 199.2) metres longer than with placebo (P = 0.001) (Figure 31a).

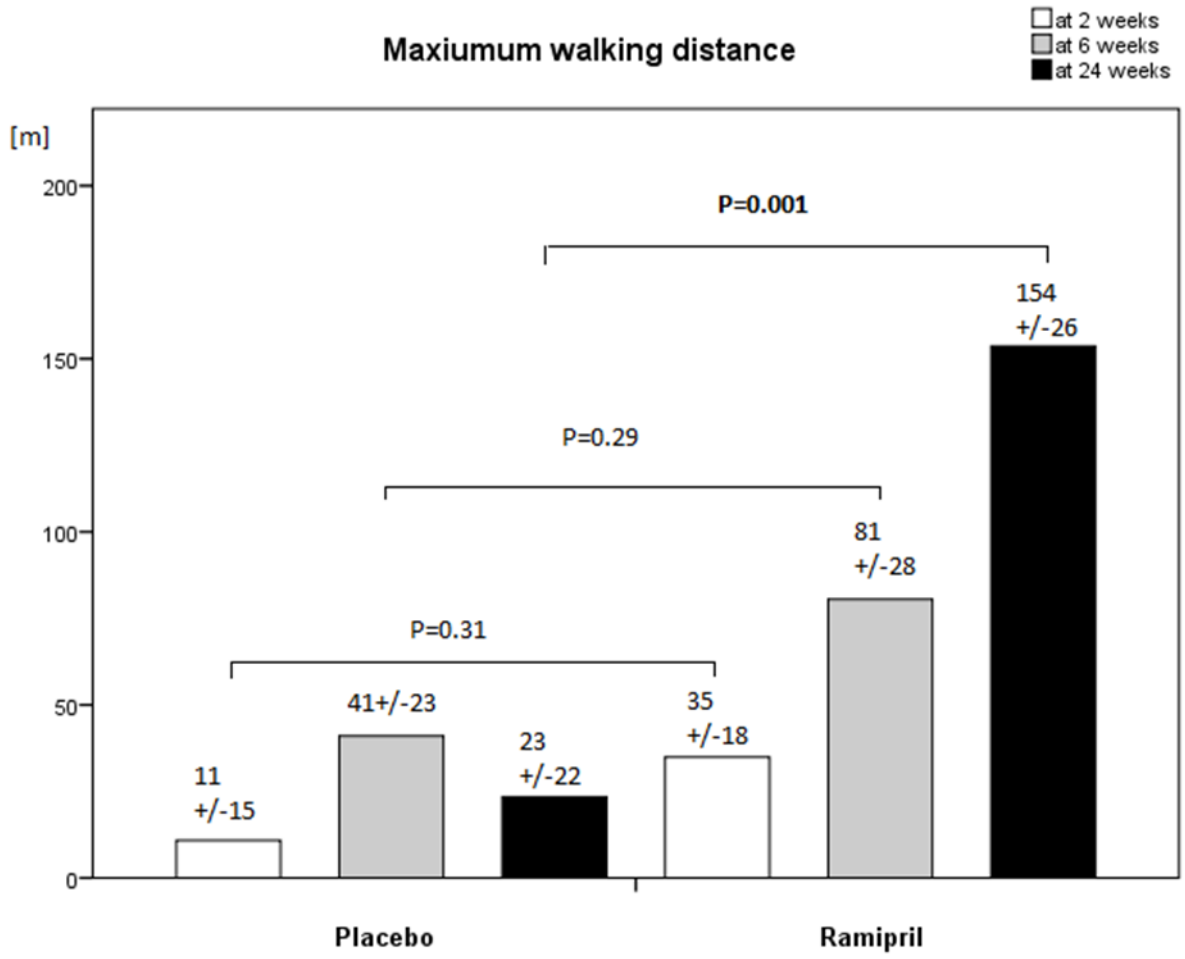

Figure 30a: ***Adjusted mean (SEM) changes in maximum walking distance in the ramipril and placebo groups at 2, 6 and 24 weeks. *P = 0.001 (1-way ANCOVA)***

6.3 Secondary Outcome Measures:

Ramipril versus Placebo Effect on Intermittent Claudication Distance

There was no significant difference between the ramipril and placebo groups at baseline in terms of ICD (median (IQR) 81 (48–114) versus 94 (32–163) metres; P = 0.986).

In the ramipril group, ICD increased significantly at 24 weeks compared with 2 weeks (P = 0.020) and 6 weeks (P = 0.042) (P = 0.076 for comparison between mean changes at 2 and 6 weeks).

In the placebo group, there was no significant change at 24 weeks compared with 2 weeks (P = 0.693) and 6 weeks (P = 1.000) (P = 0.377 for comparison between changes at 2 and 6 weeks).

The adjusted mean change in ICD with ramipril was 122 metres (95% CI 56 to 188) metres longer than with placebo after 24 weeks (P = 0.001) (Figure 31b).

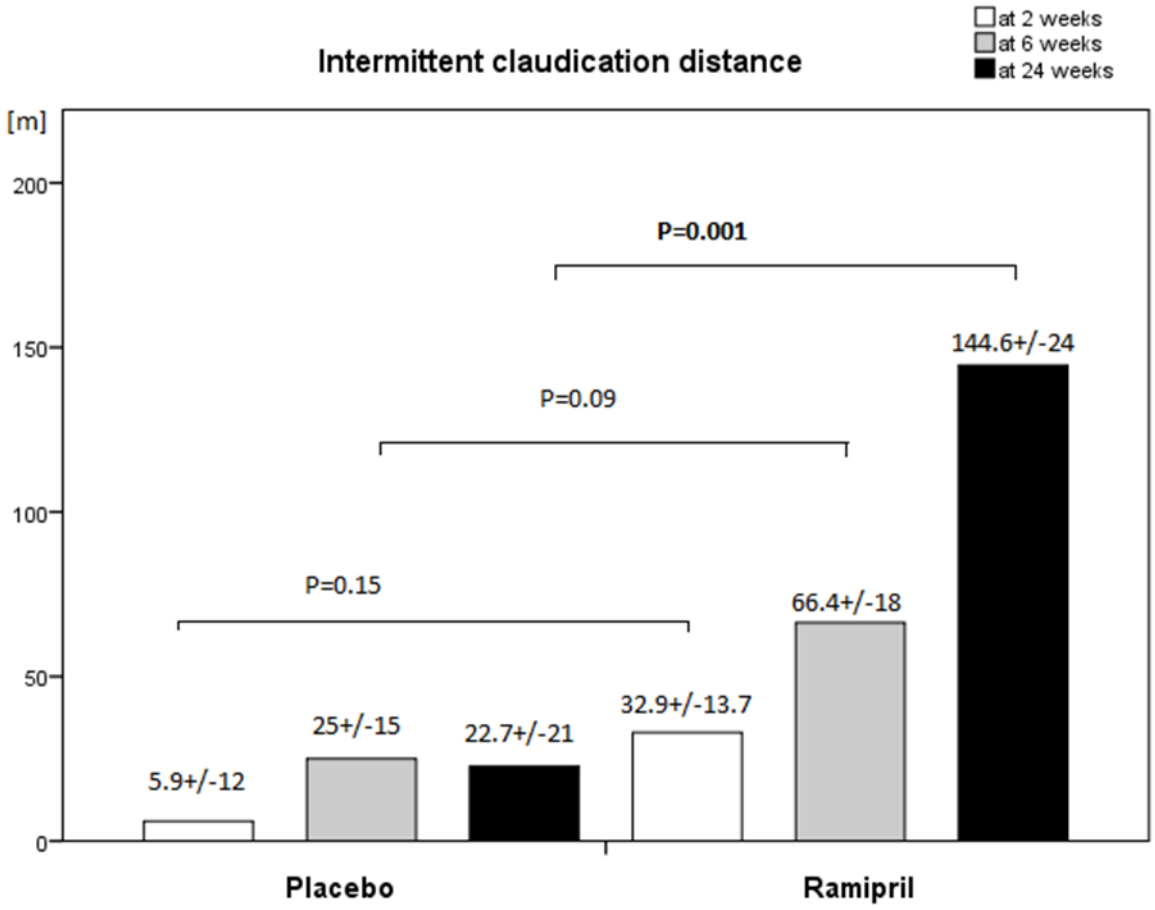

Figure 30b: ***Adjusted mean (SEM) changes in intermittent claudication distance in the ramipril and placebo groups at 2, 6 and 24 weeks. *P = 0.001 (1-way ANCOVA)***

Ramipril versus Placebo Effect on Patient Reported Walking Distance

The ramipril and placebo groups had significantly different baseline PRWD (median (IQR) 100 (87–263) and 229 (200–457) metres respectively; P = 0.011).

In the ramipril group, PRWD was non-significantly longer at 24 weeks compared with 2 weeks (P = 0.312) and 6 weeks (P = 1.000) (P = 0.585 for comparison between changes at 2 and 6 weeks).

In the placebo group, there was no significant change in PRWD at 24 weeks compared with 2 weeks (P = 1.000) and 6 weeks (P = 0.816) (P = 1.000 for comparisons between mean changes at 2 and 6 weeks). Nonetheless, PRWD significantly improved after 24 weeks of treatment with ramipril, by 159 metres (95% CI 66 to 313) metres compared with placebo (P = 0.043) (Figure 31c).

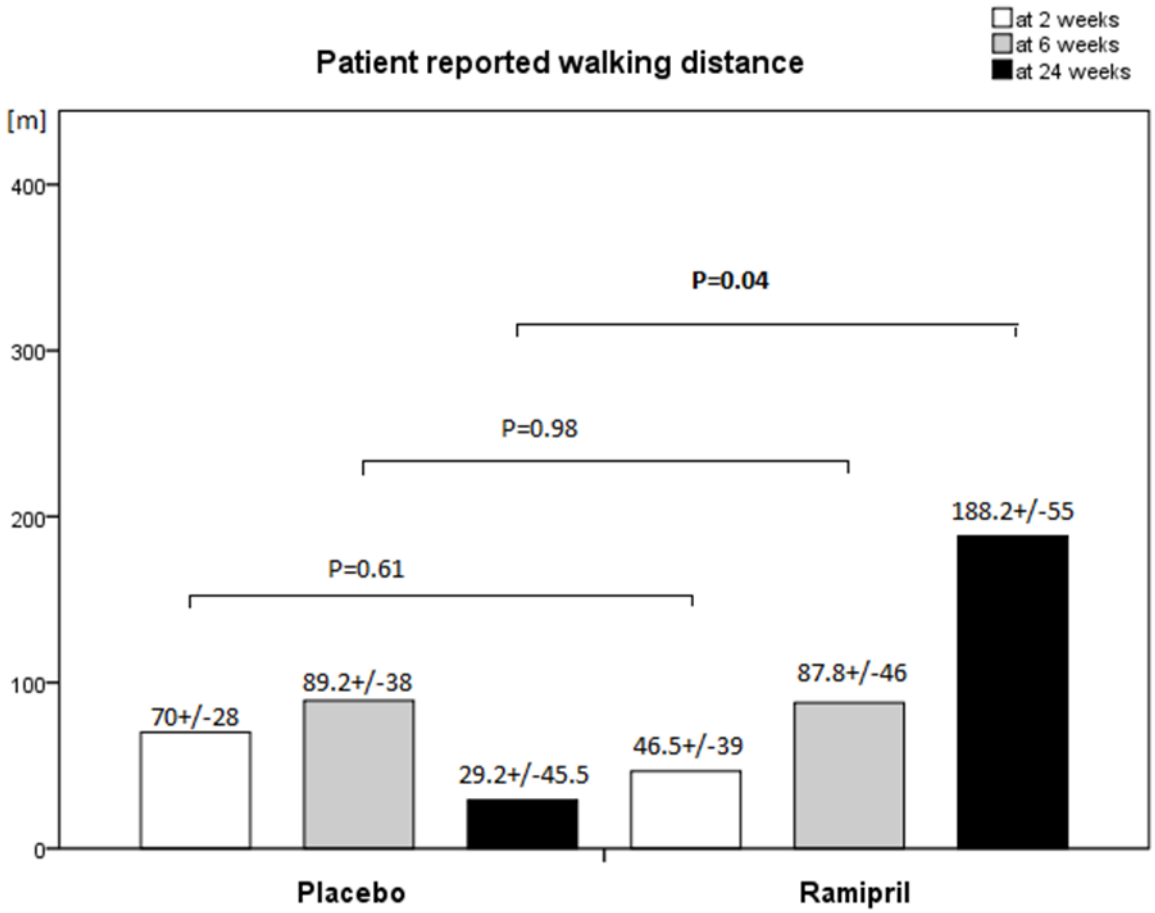

Figure 30c: ***Adjusted mean (SEM) changes in patient reported walking distance in the ramipril and placebo groups at 2, 6 and 24 weeks. *P = 0.043 (1-way ANCOVA)***

Ramipril versus Placebo Effect on Ankle Brachial Pressure Index

Ankle Brachial Pressure Index at Rest

Baseline mean (SD) r-ABPI was comparable between the ramipril and placebo groups; 0.59(0.19) and 0.66(0.16) respectively, P = 0.250.

Ramipril significantly increased r-ABPI by 0.03(0.08) at 24 weeks compared with 2 weeks (–0.04(0.09); P = 0.007) and non-significantly compared with 6 weeks (–0.02(0.15); P = 0.590) (P = 0.560 for comparison between 2 and 6 weeks).

In the placebo group, there was no significant change in r-ABPI at 24 weeks (0.02(0.18)) compared with 2 weeks (0.002(0.11); P = 1.000) and 6 weeks (0.004(0.12); P = 1.000) (P = 1.000 for comparison between 2 and 6 weeks).

There was no significant difference in adjusted mean changes of r-ABPI between the two groups at 2, 6 and 24 weeks (Figure 32). In fact, r-ABPI improved slightly in both ramipril and placebo groups (0.02 (95% CI –0.08 to 0.11) versus 0.03 (–0.05 to 0.10); P = 0.830).

Ankle Brachial Pressure Index Post-exercise

Baseline mean (SD) t-ABPI was comparable between the ramipril and placebo groups; 0.32(0.29) and 0.44(0.21) respectively, P = 0.188.

There was a non-significant increase in the ramipril group at 24 weeks (0.05(0.12)) compared with 2 weeks (0.03(0.07); P = 1.000) and 6 weeks (0.05(0.15); P = 1.000) (P = 1.000 for comparison between 2 and 6 weeks). There was no significant change in t-ABPI in the placebo group at 24 weeks (0.02(0.14) compared with 2 weeks (0.00(0.10; P = 1.000) and 6 weeks (0.04(0.10); P = 0.960) (P = 0.110 for comparisons between 2 and 6 weeks). There was no significant difference in adjusted mean (95% CI) changes in t-ABPI between the ramipril and placebo groups at 2 weeks (0.03 (–0.03 to 0.09) versus 0.005 (–0.04 to 0.05) respectively; P = 0.490), 6 weeks (0.05 (–0.03 to 0.13) versus 0.05 (–0.02 to 0.11); P = 0.950) and 24 weeks (ramipril 0.04 (–0.04 to 0.12) versus 0.02 (–0.04 to 0.09); P = 0.720).

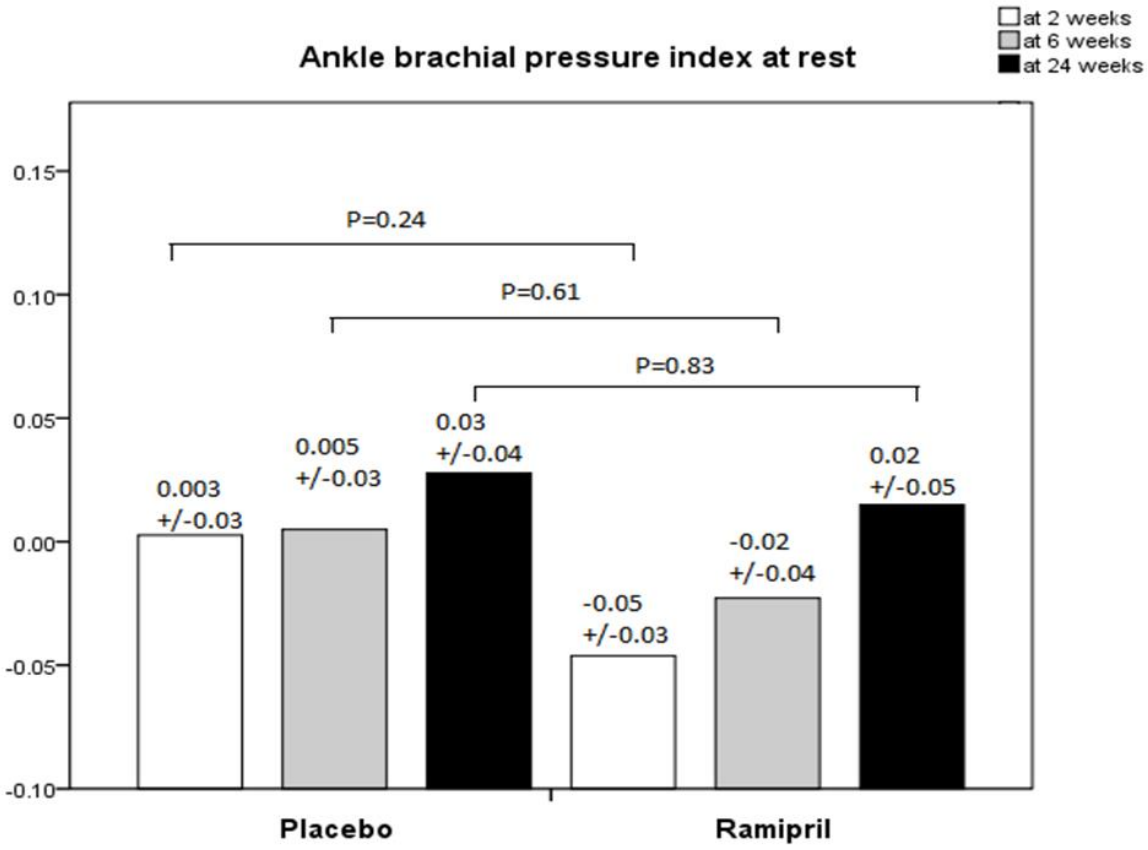

Figure 31: ***Adjusted mean (SEM) changes in ankle brachial pressure index at rest in the ramipril and placebo groups at 2, 6 and 24 weeks***

Ramipril versus Placebo Effect on Arterial Stiffness and Haemodynamic Measurements

There was no significant difference in baseline PWVcf and indices of PWA between the two groups. However, there was a significant difference in baseline diastolic bBP between the groups (P = 0.021). Results of arterial stiffness and haemodynamic measurements throughout the trial are shown in Table 9.

Ramipril decreased AIx after 24 weeks of treatment compared with 2 weeks (P = 0.016); however, AIx non-significantly increased in the placebo group at 24 weeks compared with 2 and 6 weeks (P = 0.012, P = 0.002 and P < 0.001 for comparison between groups at 2, 6 and 24 weeks).

Ramipril decreased AP at 24 weeks compared with 2 weeks (P < 0.001) and 6 weeks (P < 0.001). Similarly, AP decreased in the placebo group at 24 weeks compared with 2 weeks (P < 0.001) and 6 weeks (P < 0.001) (P = 0.026 at 6 weeks and P = 0.080 at 24 weeks for comparison between the groups).

By 24 weeks, compared with placebo, ramipril significantly reduced PWVcf (adjusted mean change –1.47 (95% CI –2.40 to –0.57) m/s; P = 0.002), systolic AoBP (P < 0.001) (P = 0.008 for comparison between the groups at 6 weeks), diastolic AoBP (P = 0.020) (P = 0.005 for comparison between the groups at 2 weeks), aortic PP (P = 0.001), MAP (P < 0.001) (P = 0.021 for comparison between the groups at 6 weeks) and AIx@HR75 (P < 0.001)

(P = 0.004 and P = 0.001 for comparison between the groups at 2 and 6 weeks respectively).

There was a non-significant change in systolic bBP (P = 0.092), diastolic bBP (P = 0.183) (P = 0.022 and P = 0.005 at 2 and 6 weeks respectively) and brachial PP (P = 0.342).

Ramipril significantly increased SEVR at 6 weeks (P = 0.011) and non-significantly at 2 weeks (P = 0.281) and 24 weeks (P = 0.473) compared with placebo. No significant change in ED% was found between the groups at 2, 6 or 24 weeks.

	Baseline		Change at 2 weeks		Change at 6 weeks		Change at 24 weeks	
	Placebo	Ramipril	Placebo	Ramipril	Placebo	Ramipril	Placebo	Ramipril
Brachial systolic blood pressure (mmHg)	146(4)	139(5)	–3(3)	–7(4)	–4(4)	–17(4)*	–2(3)	–13(4)
Brachial diastolic blood pressure (mmHg)	82(1)	76(3)§	0.5(2.0)	–6(2)*	–1(1)	–7(2)*	–0.2(1.0)	–3(2)
Brachial pulse pressure (mmHg)	64(4)	63(5)	–2(3)	–3(3)	–3(3)	–10(4)	–2(3)	–6(3)
Aortic systolic blood pressure (mmHg)	139(6)	130(4)	–3(3)	–10(4)	–1(4)	–14(4)*	4(3)	–16(3)*
Aortic diastolic blood pressure (mmHg)	83(2)	78(3)	2(2)	–5(2)*	–3(3)	–9(3)	–0.03(2.00)	–6(2)*
Aortic pulse pressure (mmHg)	55(6)	52(4)	–4(3)	–7(3)	3(5)	–11(5)	5(2)†	–10(3)*
Mean arterial pressure (mmHg)	105(3)	99(3)	–0.5(2.0)	–7(2)	–3(2)	–10(3)*	2(2)	–9(2)*
Heart rate (beats/min)	70(2)	68(3)	–0.2(1.0)	0.4(2)	–0.3(1.0)	–0.2(1.0)	–2(2)	5(2)*‡
Augmentation pressure (mmHg)	19(2)	18(2)	–1(1)	–4(1)	2(2)	–5(3)*	3(1)†‡	-7(2)†‡
Subendocardial viability ratio (%)	154(8)	144(7)	0.2(4.0)	8(5)	–4(4)	15(5)*	–1(4)	3(5)
Ejection duration index (%)	35(1)	36(1)	–0.03(1.00)	–0.4(1.0)	–0.1(1.0)	–1(1)	0.3(1.0)	0.04(1.00)
Augmentation index (%)	33(2)	34(3)	0.2(1.0)	–4(1)*	2(1)	–5(1)*	3(1)	–8(1)*†
Augmentation index adjusted to 75 beats/min (%)	31(1)	31(2)	0.6(1.0)	–4(1)*	2(1)	–5(1)*	2(1)	–6(1)*
Carotid femoral pulse wave velocity (m/s)	10.7(0.6)	11.2(0.7)	–0.2(0.3)	–0.3(0.3)	–0.9(0.3)	–0.9(0.4)	0.6(0.3)‡	–0.9(0.3)*

Table 9: ***Arterial stiffness indices at baseline, 2, 6 and 24 weeks in both groups. Values are mean (SEM). *P < 0.050, adjusted mean changes at 2, 6 and 24 weeks between groups (1-way ANCOVA); P < 0.050, within-group post hoc comparison between †weeks 2 and 24 and ‡weeks 6 and 24 (1-way repeated-measures ANOVA); §P < 0.050, intergroup analysis at baseline (unpaired t test)***

Ramipril versus Placebo Effect on Biomarkers of Inflammation, Ischaemia Reperfusion and Cardiovascular Prognosis

All laboratory measurements were comparable between the groups at baseline. Baseline and changes in laboratory measurements at 2, 6 and 24 weeks between the groups are shown in Table 10.

Intragroup analysis showed no significant change in the ramipril or placebo group in terms of lipid profile, eGFR, urea, creatinine, CRP, fibrinogen, NTproBNP, r-UACR and t-UACR, except for a significant change in total cholesterol in the ramipril group between 2 and 6 weeks (P = 0.020), and in LDL-C between the two groups at week 6 (P = 0.041).

Nonetheless, after 24 weeks of treatment, there was a non-significant decrease in eGFR (P = 0.232) and NTproBNP (P = 0.452) in the ramipril group compared with placebo. Conversely, ramipril non-significantly increased creatinine (P = 0.070) and urea (P = 0.330) levels compared with placebo.

	Baseline		Change at 2 weeks		Change at 6 weeks		Change at 24 weeks	
	Placebo	Ramipril	Placebo	Ramipril	Placebo	Ramipril	Placebo	Ramipril
Total cholesterol (mmol/l)	4.6(0.3)	4.4(0.2)	0.14(0.1)	0.04(0.10)	0.1(0.2)	0.3(0.2)	–0.1(0.2)	0.3(0.2)
LDL-C (mmol/l)	2.7(0.2)	2.2(0.2)	–0.1(0.2)	–0.1(0.2)	–0.1(0.1)	0.6(0.2)†	–0.2(0.2)	0.03(0.30)
HDL-C (mmol/l)	1.2(0.1)	1.3(0.1)	0.02(0.04)	0.1(0.1)	0.02(0.04)	0(0.04)	–0.02(0.04)	0.02(0.10)
Triglycerides (mmol/l)*	1.4 (1.1–1.9)	1.7 (1.3–3.4)	0.3(0.2)	0.2(0.3)	0.4(0.2)	–0.1(0.2)	0.6(0.3)	0.1(0.3)
Total cholesterol/HDL-C	4.1(0.3)	3.9(0.4)	0.1(0.2)	–0.3(0.1)	–0.01(0.10)	0.3(0.2)‡	0.02(0.20)	0.2(0.3)
eGFR (ml/min/1.73 m^2)*	81(73–90)	82 (70–90)	4(2)	1(3)	–2(2)	0.01(2)	–1(2)	–5(3)
Urea (mmol/l)*	4.4 (3.7–5.2)	4.7 (4.2–6.3)	–0.2(0.3)	–0.2(0.3)	–0.2(0.2)	–0.2(0.3)	–0.5(0.3)	0.01(0.41)
Creatinine (µmol/l)	81.6(3.3)	80(4)	–2(2)	–0.3(3.1)	3(2)	0.3(2)	0.8(3.0)	10(4)
CRP (mg/l)*	3.6 (1.6–4.8)	2.7(1.2–4.8)	–0.6(0.5)	–0.5(0.6)	–0.2(0.4)	–0.7(0.5)	0.3(0.5)	0.2(0.5)
Fibrinogen (g/l)	3.3(0.1)	3.5(0.2)	–0.1(0.1)	–0.1(1.0)	–0.2(0.1)	0.01(0.1)	–0.2(0.2)	0.1(0.2)
NTproBNP (pg/ml)*	75 (48–212)	65 (27–161)	18(24)	4(28)	–0.8(21.0)	–6(25)	12(19)	–11(23)
r-UACR (mg/mmol)*	0.8 (0.4–1.5)	0.6 (0.2–2.4)	–0.01(0.3)	0.1(0.3)	0.6(0.7)	0.9(0.8)	–0.05(0.21)	–0.1(0.3)
t-UACR (mg/mmol)*	1.3 (0.7–2.2)	0.7 (0.2–3.2)	–1(0.3)	–1.0(0.3)	–2(1)	0.04(1.00)	–2(1)	–2(1)

Table 10: ***Laboratory measurements at baseline, 2, 6 and 24 weeks in both groups. Values are mean (SEM) unless indicated otherwise; *values at baseline are median (interquartile range). HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, eGFR: Estimated glomerular filtration rate, CRP: C-reactive protein, NTproBNP: N-terminal pro b-type natriuretic peptide, r-UACR: Urine albumin creatinine ratio at rest, t-UACR: Urine albumin creatinine ratio after treadmill exercise test. †P < 0.050, adjusted mean change at 6 weeks between groups (1-way ANCOVA); ‡P < 0.050, within-group post hoc comparison between weeks 2 and 6 (1-way repeated-measures ANOVA)***

Ramipril versus Placebo Effect on Quality of Life

Results from the three questionnaires (EQ-5D™, SF-36® and VascuQol) are summarized in Table 11.

Overall, patients had low scores in most domains at baseline, with the lowest scores in the domains physical function, role physical and bodily pain of the SF-36®, and pain and activities of VascuQol. There was no significant difference between the groups at baseline. In addition, there was no significant difference between the groups in any of the SF-36® and VascuQol domains after 24 weeks of treatment. VascuQol and EQ-5D™ total scores did not differ significantly between the groups after 24 weeks either.

Patients in the ramipril group scored significantly lower in the mental health domain than patients who received placebo after 2 weeks (P = 0.040), but there was no significant difference at 6 and 24 weeks. Patients in the ramipril group had significantly lower scores in the social domain of VascuQol after 24 weeks compared with week 2 (P = 0.040) and week 6 (P = 0.040). However, there was no such change in the placebo group. Notably, ramipril had a slight positive, but non-significant, effect on the domains physical function and bodily pain in the SF-36®, and pain and activities in the VascuQol; this was comparable to changes seen in the placebo group.

	Baseline		Change at 2 weeks		Change at 6 weeks		Change at 24 weeks	
	Placebo	Ramipril	Placebo	Ramipril	Placebo	Ramipril	Placebo	Ramipril
EQ-5D™ score	0.73(0.02)	0.71(0.02)	0.02(0.02)	0.01(0.02)	0.03(0.02)	0.02(0.02)	–0.01(0.02)	0.02(0.02)
SF-36® domains								
Physical function	53(5)	46(7)	3(3)	0.5(3.1)	2(3)	3(4)	–0.9(5.1)	0.4(5.2)
Role physical	65(9)	46(10)	–1(5)	–9(6)	–2(8)	–1(9)	–3(7)	–11(8)
Bodily pain	57(5)	47(6)	7(4)	2(5)	–3(4)	5(4)	1(4)	1(5)
General health	52(5)	48(7)	0.3(2.1)	3(3)	1(3)	–1(4)	2(4)	–2(4)
Vitality	50(5)	54(7)	2(3)	–1(4)	2(3)	3(4)	–2(4)	–2(5)
Social function	74(6)	77(6)	0.6(4.1)	–0.8(4.2)	–2(4)	–10(5)	–7(5)	–13(6)
Role emotional	79(8)	55(11)	–1(6)	2(8)	3(7)	1(8)	–7(8)	–7(10)
Mental health	70(4)	78(6)	3(4)	–9(4)†	4(4)	–7(4)	–3(3)	–8(4)
VascuQol domains								
Pain	4.1(0.3)	4.0(0.4)	0.5(0.2)	0.6(0.2)	0.3(0.4)	0.5(0.4)	0.2(0.3)	0.5(0.4)
Social*	5.5 (3.5–7.0)	5.5 (4.0–6.0)	–0.05(0.30)	0.4(0.3)	–0.2(0.4)	0.4(0.5)	–0.4(0.4)	–0.2(0.5)‡§
Emotional*	5.4 (3.9–6.2)	5.5 (4.1–6.1)	0.6(0.2)	0.5(0.2)	0.3(0.4)	0.5(0.5)	0.4(0.3)	0.2(0.4)
Symptoms*	5.5 (5.1–6.3)	5.4 (4.8–6.0)	0.08(0.20)	–0.01(0.20)	–0.04(0.30)	0.09(0.41)	0.01(0.20)	–0.25(0.30)
Activities	4.0(0.3)	4.0(0.3)	0.5(0.2)	0.7(0.2)	0.4(0.3)	0.6(0.4)	0.3(0.3)	0.3(0.4)
Total score	5.0(0.3)	5.0(0.3)	0.4(0.2)	0.5(0.2)	0.3(0.3)	0.4(0.4)	0.2(0.3)	0.2(0.3)

Table 11: Quality of life indices at baseline, 2, 6 and 24 weeks in both groups. Values are mean (SEM) unless indicated otherwise; *values at baseline are median (interquartile range). EQ-5D™: EuroQol five-domain questionnaire, SF-36®: UK Short Form 36, version 1, VascuQol: King's College Hospital's vascular quality of life questionnaire. †P < 0.050, adjusted mean change at 2 weeks between groups (1-way ANCOVA); P < 0.050, within-group post hoc comparison between ‡weeks 2 and 24 and §weeks 6 and 24 (1-way repeated-measures ANOVA).

6.4 Further Results

Multivariable Regression Analysis

Multivariable linear regression analysis which included change in MWD as a dependent variable and peripheral systolic and diastolic BP and class of drug (ramipril or placebo) as independent variables showed that the ramipril-induced change in MWD was not associated with its effect on systolic bBP (standardized β coefficient –0.03, P = 0.880) or diastolic bBP (standardized β coefficient –0.04, P = 0.871), but was independently associated with class of drug (ramipril or placebo; standardized β coefficient 0.60, P = 0.001) (adjusted R^2 for model = 0.30, P = 0.008).

To further test whether ramipril's induced lowering of PWVcf and AIx are independent from potential confounding effects such as: HR and MAP for PWVcf and HR for AIx, we performed a multivariable regression analysis. Model 1 included PWVcf as the dependent variable and HR, MAP and class of drug (ramipril or placebo) as independent variables. Model 2 included AIx as dependent variable and HR and class of drug as independent variables. The results of the regression analysis for both models are summarized in table 11. These models clearly show that ramipril's ability to reduce arterial stiffness is independent of potential confounding variables such as MAP and HR.

Correlation Analysis

A correlation analysis was performed to investigate the relationship between walking distance and indices of arterial stiffness. Changes in MWD at 24 weeks showed significant inverse correlation with indices of arterial stiffness (PWVcf, r=-0.43, P= 0.021; AIx, r= -0.50, P= 0.006; AIx@75 beats/min, r= -0.50, P= 0.006; central PP, r= -0.38, P=0.039) (Figures 33-36).

It is well known that indices of arterial stiffness are influenced by changes in HR and MAP . To adjust for these covariates, a multivariable regression analysis was conducted, where the relationship between changes in MWD at 24 weeks and indices of arterial stiffness remained statistically significant (Table 12).

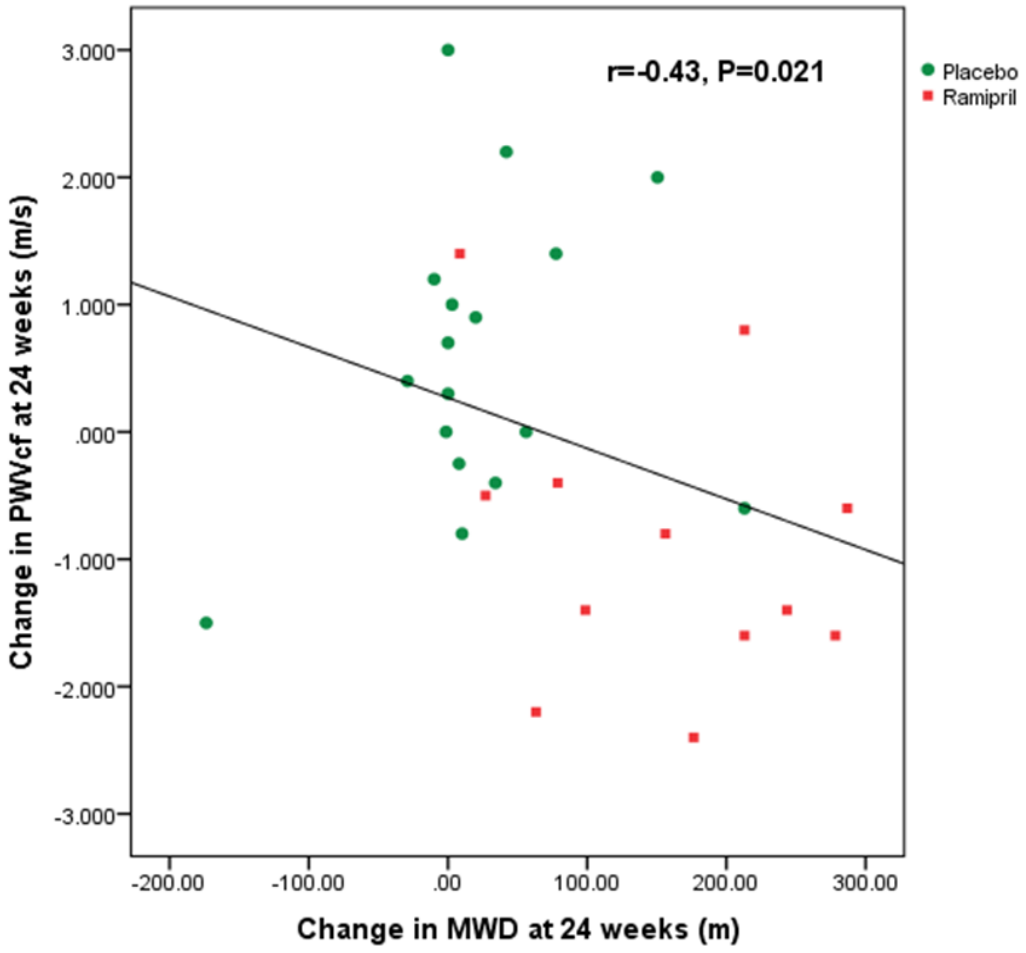

Figure 32: ***Relationship between changes in MWD and PWVcf at 24 weeks. The black line is the regression line***

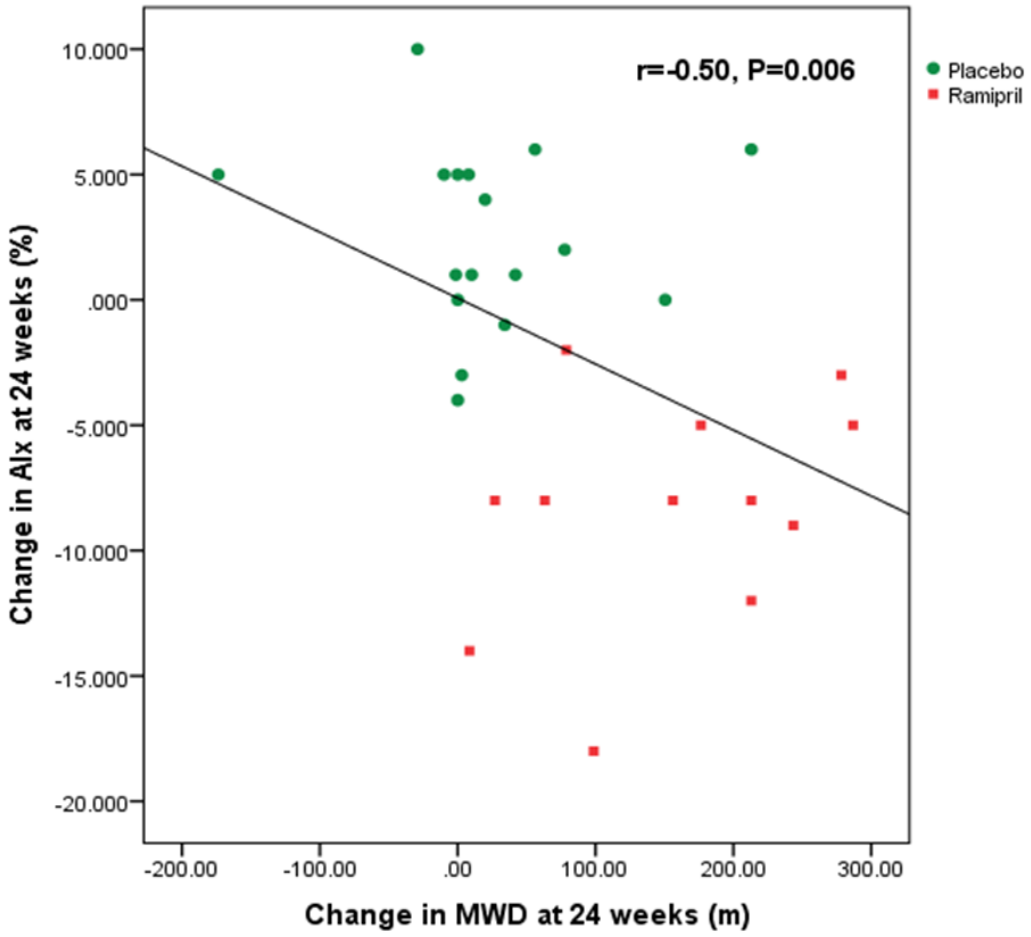

Figure 33: Relationship between changes in MWD and AIx at 24 weeks

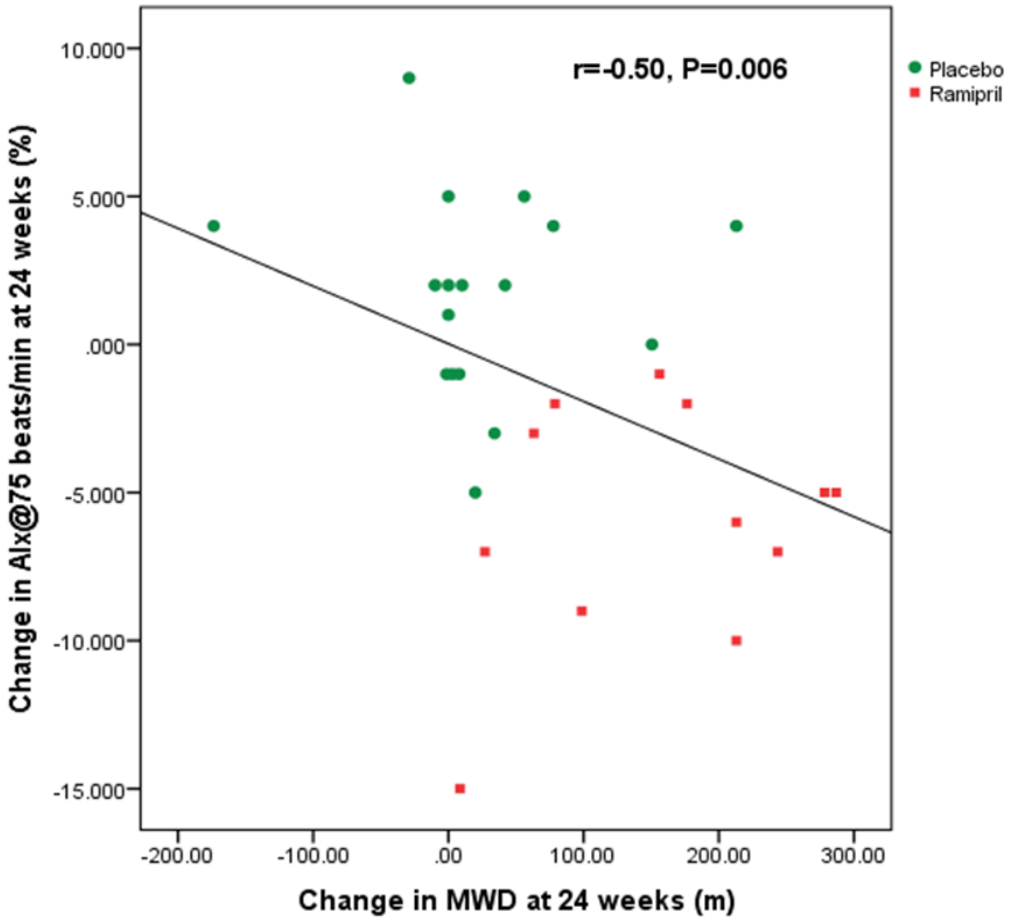

Figure 34: ***Relationship between changes in MWD and AIx@75 beats/min at 24 weeks***

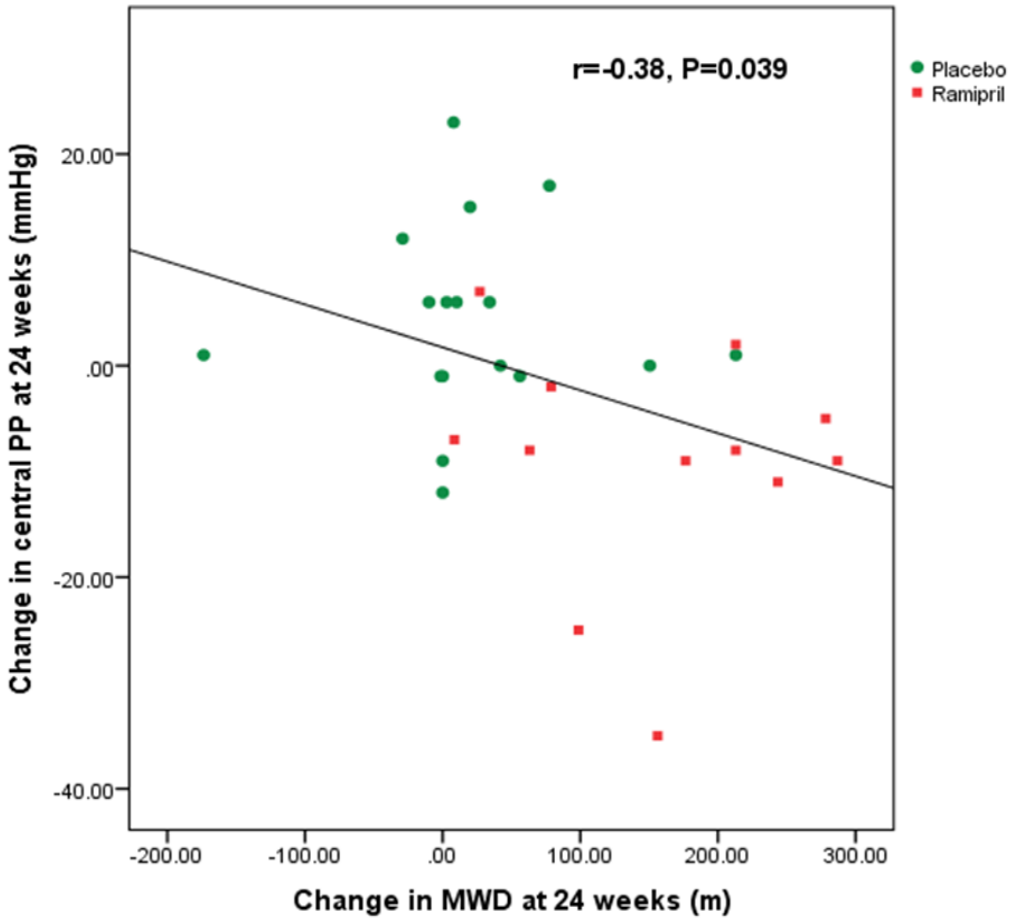

Figure 35: Relationship between changes MWD and central PP at 24 weeks

Dependent Variable	Independent Variables	B- coefficient	P Value
Model 1 :PWVcf	MAP	0.14	0.458
	HR	-0.12	0.502
	Group	-0.44	0.018*
Model 2: AIx	**HR**	-0.39	0.002*
	Group	-0.56	<0.001*

Table 12: ***Multivariable regression analysis of ramipril induced lowering of arterial stiffness after adjustment for HR and MAP for PWVcf and HR for AIx with the group (ramipril or placebo) in both models. * P <0.050. PWVcf: Carotid-femoral pulse wave velocity, AIx: Augmentation index.***

Dependent Variable	Independent Variables	Partial r value	Standardised Coefficient (β)	P value
Δ MWD	**Δ PWVcf**	-0.33	-0.32	0.033*
	Δ HR	-0.08	-0.09	0.651
	Δ MAP	-0.15	-0.15	0.437
Δ MWD	**Δ AIx**	-0.40	-0.35	0.026*
	Δ HR	-0.03	-0.03	0.898
	Δ MAP	-0.34	-0.32	0.099
Δ MWD	**Δ AIx@75**	-0.40	-0.38	0.037*
	Δ MAP	-0.33	-0.31	0.118
Δ MWD	**Δ Central PP**	-0.30	-0.25	0.044*
	Δ HR	0.09	0.14	0.649
	Δ MAP	-0.22	-0.20	0.090

Table 13: ***Multivariable regression analysis of the relationship between MWD and indices of arterial stiffness after adjustment for HR and MAP. * P <0.050. Δ: Change, PWVcf: Carotid-femoral pulse wave velocity, AIx: Augmentation index, AIx@75: Augmentation index adjusted to 75 beats/min, Central PP: Central pulse pressure. AIx@75 beats/min is only adjusted to MAP as it is already adjusted to HR.***

6.5 Adverse Events

Patients underwent clinical examination at each visit, including recording of vital signs, current medications and adverse events. Five of 38 patients enrolled in the run-in phase withdrew because of cough (4) and headache (1). Of 14 patients randomized to receive ramipril, four patients developed cough and one experienced dizziness. Consequently, two patients withdrew from the trial (1 owing to dizziness, 1 because of cough) (Figure 30).

Of 19 patients randomized to placebo, two withdrew without giving a reason. One patient in the ramipril group developed hyperkalaemia by 6 weeks of follow-up, which was resolved without complications and the patients was able to complete the trial. Renal function was monitored closely throughout the trial and no deterioration was observed in any of the trial subjects.

7.1 Overview

This trial was designed to compare the effect of ramipril, an ACE inhibitor, with placebo in patients with IC. Ramipril significantly improved MWD, ICD and PRWD, and decreased indices of arterial stiffness (PWVcf and AIx) compared with placebo. Ramipril had no significant effect on ABPI, biomarkers of inflammation and ischaemia–reperfusion, or QoL scores compared with placebo. Although NTproBNP, a surrogate marker of cardiovascular prognosis, was reduced in ramipril-treated patients, this effect was not statistically significant.

This trial provides a significant contribution to the literature focused upon the clinical effectiveness of ramipril in patients with IC. It is the second randomised controlled trial on the subject in the world and the first in the United Kingdom and in Europe.

Ramipril has been studied previously for PAD; in a double-blind placebo-controlled trial that was limited to non-diabetic patients with infrainguinal disease, Ahimastos and colleagues[109] showed that ramipril improved MWT by 243 per cent, PFWT by 164 per cent, r-ABPI by 0.07 and t-ABPI by 0.08 after 6 months. In contrast, such major improvements were not observed in the present trial, which showed an improvement in MWD by 106 per cent and in ICD by 152 per cent, with non-significant improvements in r-ABPI and t-ABPI. The present trial included patients with diabetes and different levels of

arterial disease (Suprainguinal, infrainguinal and mixed), which makes this cohort more representative of the population with PAD. Nonetheless, at the time of writing this thesis, Ahimastos and colleagues published the results of the largest RCT on the subject[250]. The new trial by the Australian group assessed the effect of ramipril on walking distance and QoL in a cohort of 212 patients with IC. Ramipril improved PFWT and MWT by 75 and 255 seconds, respectively, relative to placebo. Ramipril also improved the physical component of the SF-36 questionnaire by 8.2 (P=0.020) relative to placebo. The latter included patients with infra and suprainguinal disease and patients with diabetes which makes its results generalizable to PAD population.

Our trial was in agreement with Ahimastos et al.[250] in terms of the ability of ramipril to improve walking distance, however, it failed to show a positive effect on QoL or ABPI which contradicted the study by the Australian group.

The effect of other ACE inhibitors (Cilazapril, captopril and perindopril) on walking distance or time has been studied in controlled trials[106-108]. These showed no significant improvement in walking distance, walking time or in ABPI. However, the duration of treatment with ACE inhibitors in these trials was relatively short (4–8 weeks) and two were crossover trials[106,107] with only a small number of patients. The present trial suggests that a longer duration of treatment with an ACE inhibitor (6 months) improves efficacy.

From the clinical perspective, the most important finding of this trial was the improvement observed in ICD and MWD in the ramipril-treated group; however, this was not associated with improvements in QoL. In fact, ramipril had a slight non-significant effect on the domains physical function and pain. This trial may have been too small, as it was not powered to detect a difference in QoL between the two groups.

7.2 Suggested Mechanisms of Action

Improvement in Arterial Stiffness

Arterial stiffness is associated with several pathological conditions including PAD[180,251,252]. In general, there is a lack of evidence regarding ACE inhibitors effect on arterial stiffness in patients with PAD with one study[180] in addition to our study assessing this effect. In Ahimastos et al. study[180] and our study, improvement in arterial stiffness was studied as a potential factor in the improvement in walking ability observed in the two trials. Indeed, significant strong correlations were found between changes in MWD and changes in PWV in the ACE inhibitor-treated groups. This association remained significant after adjustment for HR and MAP. In this trial, ramipril decreased PWVcf and AIx after 24 weeks treatment period which is in agreement with the Australian trial[180]. The mean percentage change in PWVcf after 24 weeks of treatment was -9 +/-10% in the ramipril group compared to an increase of

5+/- 10% in the placebo group (P=0.001). Moreover, in a meta-analysis which included 469 patients with arterial stiffness, ACE inhibitor reduced pulse wave velocity by 1.69 m/s (P<0.00001) (See Chapter 4). Noteworthy, ramipril induced lowering of arterial stiffness in our trial was independent of its effect on potential confounding factors such as HR and MAP.

The mechanism by which ACE inhibitors reduce arterial stiffness has been discussed in detail in the introduction. In brief, ACE inhibitors promote the elastogenic profile of the arterial wall by its inhibitory effect on the ECM and MMPs. In cell culture, ramiprilat decreased collagen deposition by more than 50 per cent and increased elastin and fibrillin-1 deposition by 3-4 folds. It also decreased gene and protein expression of MMP-2 and MMP-3 in arterial wall[21].

The association between improvement in walking ability; observed in patients treated with ramipril; and reduction of arterial stiffness can be explained in relation to PP and calf blood flow. The latter is determined by BP in patients with PAD, although not the case in normal subjects[253]. PP and mean pressure correlate positively with calf blood flow at rest. However, it is during exercise when calf blood flow becomes limited. Interestingly, baseline PP correlates negatively with walking distance[253-255]. Therefore, increased PP is associated with decreased walking ability in patients with IC. This can be attributed to decreased diastolic pressure and its inability to overcome the stiffened arteries found in patients with PAD to achieve sufficient blood flow

to the lower limbs. This mechanism is similar to the one that controls the relationship between arterial stiffness, PP and coronary ischaemic threshold[256-258].

There is substantial evidence that increased PP due to increased arterial stiffness is associated with impaired endothelial function, arterial wall hypertrophy and increased vascular resistance[259-262]. This is associated with further decrease of blood flow to the lower limbs in patients already having stenotic arteries and limited calf blood flow. In a cross sectional study of 2000 patients from the Framingham cohort, increased PP and arterial stiffness were associated with decreased resting and hyperaemic microvascular blood flow independent of Framingham risk factors[263]. Microvascular blood flow is largely dependent on structural changes in arterial wall which confirms the hypothesis that increased PP caused by increased arterial stiffness is associated with structural and functional changes in the arterial wall which decrease calf blood flow during exercise in patients with IC.

Treatment with ramipril results in reduced aortic stiffness and PP which leads to an increase in diastolic pressure sufficient to overcome vascular resistance and improve blood flow to the lower limbs. Reduced arterial stiffness and PP will also result in improved microvascular structure and function which further improves calf blood flow in patients with IC.

Reduced arterial stiffness seem to be the most acceptable mechanism behind the improvements noted in walking ability in patients with IC treated with ramipril.

Maintenance of Collateral Circulation and Angiogenesis

Another potential mechanism of action is that ACE inhibitors increase blood flow to the legs by maintaining collateral circulation through their inhibitory effect on angiotensin II, a potent vasoconstrictor, and by reduction in the breakdown of bradykinin, causing vasodilatation. Roberts et al.[106] have shown that captopril maintained collateral circulation to the lower limbs in patients with IC due to femoral artery stenosis which was explained by the vasodilatory effect of captopril.

Angiogenesis[264,265] is perhaps another suggested mechanism. Animal studies have shown that ACE inhibition with quinaprilat[266] and imidapril [267] promoted angiogenesis in animal models of hind limb ischaemia. In the study by Fabre et al.[266], quinaprilat, an ACE inhibitor with high tissue affinity promoted angiogenesis as potently as recombinant human vascular endothelial growth factor compared to captopril, an ACE inhibitor with low tissue affinity which did not promote angiogenesis. The mechanism of angiogenesis promoted by high tissue affinity ACE inhibitor most likely involves the increased availability of endothelial cell NO. This molecule is critical for the mitogenic effect of vascular endothelial growth factor on endothelial cells[268].

Nonetheless, the improvement in walking distance observed in the ramipril cohort was not accompanied by significant improvements in ABPI; r-ABPI was almost unchanged after 24 weeks of treatment with ramipril and there was only a slight (non-significant) increase in t-ABPI after 24 weeks. This contradicts the theory that ramipril augmented collateral circulation in this cohort of patients through angiogenesis.

Improvement in Endothelial Dysfunction

Endothelial dysfunction is an early step in atherosclerosis that precedes morphological change to the arterial wall and has been linked to increased cardiovascular morbidity and mortality in patients with PAD[269-272]. In addition to its prognostic value, endothelial dysfunction has been linked to the functional status in patients with PAD. In a cross sectional study of 111 patients with PAD[273], greater physical ability was associated with increased brachial flow mediated vasodilatation (FMD) (improved endothelial function). Furthermore, impaired endothelial function will result in a reduction in NO bioavailability in skeletal muscle microcirculation leading to decreased vasodilatation during exercise in patients with PAD which will further worsen IC[274,275].

In a meta-analysis[276], ACE inhibitors improved endothelial function, measured by brachial FMD, by 1.26 per cent (P = 0.002) compared with placebo or no

treatment, and by 0.89 per cent (P = 0.009) compared with other antihypertensive agents in patients with several pathological conditions.

The mechanisms by which ACE inhibitors improve endothelial dysfunction are based on their ability to inhibit angiotensin II production and to increase bradykinin. On one hand, angiotensin II, by binding to angiotensin II type 1 receptor[277], leads to vasoconstriction, endothelial cell migration, proliferation and hypertrophy [278-283], increased uptake and oxidation of LDL by endothelial cells and oxyradical production leading to endothelial dysfunction[277]. Moreover, angiotensin II stimulates the production of superoxide via increasing NADPH oxidase activity in the vascular smooth muscle[284].

ACE inhibitors, via inhibiting angiotensin II, will lead to inhibition of NAPDH oxidase and to a reduction in oxidative stress in the vascular tissue. This is important as increased oxidative stress in the vasculature has shown to predict the risk of future cardiovascular events [285].

On the other hand, bradykinin, by binding to the bradykinin B2 receptor[286], increases production and release of NO, prostacyclin [287,288]and the endothelium-derived hyper- polarizing factor [289] which causes vasodilatation, inhibition of vascular smooth muscle cell proliferation and platelet adhesion[290] and produces tissue plasminogen activator [291] which in turn restores the fibrinolytic balance. Therefore, inhibition of angiotensin II and increasing bradykinin production by inhibition of the angiotensin converting enzyme [292] will result in an improvement in endothelial function.

It is worth mentioning, however, that ACE inhibitors effect on endothelial dysfunction was never studied in the context of PAD.

7.3 Can We Expect Similar Positive Effects From Other ACE Inhibitors?

Most of the trials which showed beneficial effects of ACE inhibitors on walking distance used ramipril as the study treatment and for a long period of time (6 months). However, other studies which used other ACE inhibitors for a short period of time did not show similar benefits. This variability in ACE inhibitors effect on walking ability can be explained by different tissue affinities of ACE inhibitors. Indeed, the degree of inhibition of tissue ACE produced by an ACE inhibitor is directly dependent on the binding affinity of the inhibitor and the concentration of the free inhibitor in the tissue which in turn is dependent on ACE inhibitors dose, bioavailability, blood half-life, tissue penetration and tissue retention. For instance, ramipril has a high tissue affinity and captopril a low tissue affinity. This can explain the positive effect on walking ability and ABPI noted in the trials which used ramipril for patients with IC. It is also worth mentioning that these trials used the maximum dose of ramipril for a relatively long treatment period compared to other trials which will further cause an increase in bioavailability and tissue retention.

In a recent updated systematic review and meta-analysis[293] assessing the effect of ACE inhibitors on walking ability and ABPI which included data from our study[294] and Ahimastos et al.[250] new study in addition to the studies[106-109] which were included in the initial meta-analysis[105] (See chapter 2), the three studies [109,250,294] which used ramipril as the study treatment showed a significant positive effect on walking ability and ABPI compared to studies which used other ACE inhibitors[106-108] which showed worsening of walking ability and ABPI in patients with IC (Figures 37,38 and 39).

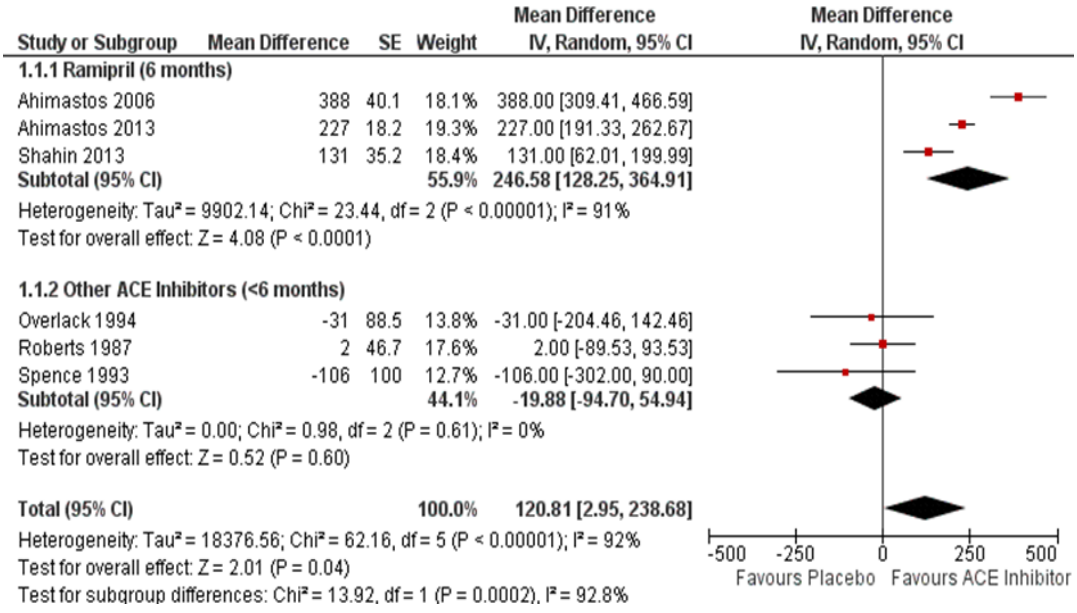

Figure 36: ***Forest plot illustrating ACE inhibitors effect on changes in MWD compared with placebo. The x-axis is in metres. Small squares represent the differences in mean changes in MWD across individual studies between study groups (ACE inhibitor minus placebo). The 95% CI for individual studies are represented by a horizontal line and by a diamond for pooled effect. SE: Standard error, IV: Inverse variance***

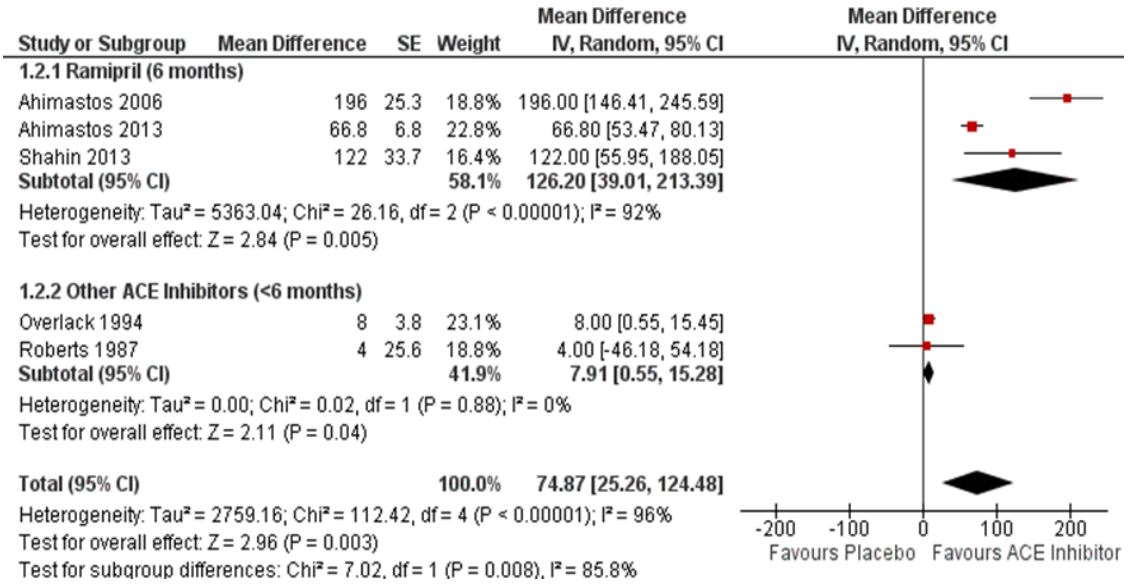

Figure 37: ***Forest plot illustrating ACE inhibitors effect on changes in PFWD compared with placebo. The x-axis is in metres. For further detail see legend for figure 37. SE: standard error, IV: inverse variance***

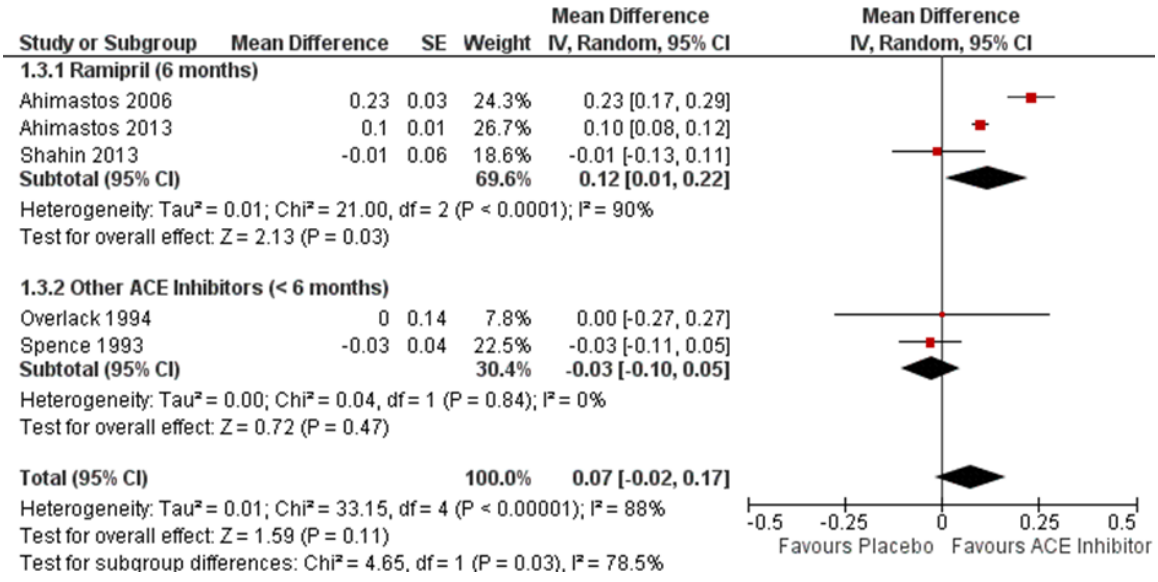

Figure 38: ***Forest plot illustrating ACE inhibitors effect on changes in ABPI compared with placebo. For further detail see legend for figure 37. SE: Standard error, IV: Inverse variance***

7.4 Are the Beneficial Effects of Ramipril on Walking Distance Generalizable to Races Other than the White Population? The Genetic Factor

Although our trial[294] and the two Australian studies[109,250] have shown potential role for ramipril in the treatment of patients with IC, these trials were limited to the White population. It is well known that the ACE genotype plays a role in the variability of ACE activity. Individuals with the DD genotype display twice as high serum ACE concentrations as individuals with the II genotype[295]. Therefore, ACE inhibitors might work better in the I/D and II alleles than in the DD allele. This is true for the Caucasian and Asian populations but might be untrue in the African American population despite having the DD allele.

Furthermore, Brewster et al.[296] have shown that ACE inhibitors work better in the White population than in the Black population. Therefore, the favourable effects of ACE inhibitors noted in the White population with IC might not be observed in the Black population or other populations.

7.5 Study Limitations

Limitations of this study should be acknowledged. This study included a small number of patients due to high withdrawal rate because of cough. Patients included in our trial were offered smoking cessation advice but were not enrolled in a supervised exercise programme as this would have introduced intervention bias into the study. Furthermore, treadmill exercise test is an objective test which largely depends on patients' motivation which might have introduced bias in the trial. Furthermore, the constant-load treadmill exercise test used in this trial does not permit an accurate assessment across the range of functional impairment in the broad population of PAD patients. For example, the incline used in our trial of 10% might have been extreme for patients with severe PAD. On the other hand, some patients with mild PAD might be able to walk for an extended period on a constant-protocol without being limited by claudication which precludes assessment of their maximal limitation. Thus, only a limited spectrum of patients with PAD can be assessed by the constant-load protocol. Although, the graded protocol better reflects the mechanism of the performance limitations in PAD, has the greatest reliability, and allows testing a wide range of functional disease severity in PAD, the constant-load protocol showed high intraclass correlation coefficient of 0.90 with walking time and both protocols are considered acceptable in measurement of walking distance in PAD[297]. Another challenge

or limitation which is worth mentioning is the improvement in walking distance overtime in patients randomised to placebo (Placebo response). The cause of this is likely multifactorial. Patients' comfort and adjustment to walking on the treadmill and optimization of gait are potential factors. Another factor could be the change in walking efficiency. Nevertheless, the observed improvements in MWD, ICD and PRWD in the ramipril-treated group were significantly greater compared to the slight insignificant improvement in the placebo group.

The small number of patients enrolled into the study is a result of the difficulty we encountered during recruitment. The main reason behind this difficulty is that patients with IC would prefer to undergo percutaneous transluminal angioplasty than being managed conservatively. Furthermore, a large number of patients were already on ACE inhibitor or ARB which is considered exclusion criteria.

7.6 Conclusion

In conclusion, this trial provides confirmatory level-1 evidence for using ramipril in the treatment of patients with PAD of the lower limbs. Ramipril improved walking distance and decreased arterial stiffness in patients with PAD. The improvement in walking distance is more than that achieved with other therapeutic agents for PAD such as pentoxifylline or cilostazol (80% improvement)[298,299] but less than that of a supervised exercise programme (120% improvement)[300]. However, whether these favourable effects can be achieved in other ethnicities other than the White ethnicity remains to be seen.

Future trials should focus on investigating the effect of ACE inhibitors on endothelial function in patients with PAD and its relationship to improvements in walking distance and possibly investigating the effect of ARBs on walking distance in this group of patients. However, as level-1 evidence for using ACE inhibitors in patients with PAD is now available, trials investigating the effect of ARB on walking distance in PAD patients might be difficult to be ethically approved. Trials could also investigate the effect of ACE inhibitors in addition to a supervised exercise programme on walking in claudicants.

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Appendix A: Quality of Life Questionnaires

1) In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
○	○	○	○	○

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

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
○	○	○	○	○

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

		Yes, limited a lot	Yes, limited a little	No, not limited at all
a.	Vigorous Activities, such as running, lifting heavy objects, participating in strenuous sports	○	○	○
b.	Moderate Activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○	○	○
c.	Lifting or carrying groceries	○	○	○
d.	Climbing several flights of stairs	○	○	○
e.	Climbing one flight of stairs	○	○	○
f.	Bending, kneeling, or stooping	○	○	○
g.	Walking more than a mile	○	○	○
h.	Walking several hundred yards	○	○	○
i.	Walking one hundred yards	○	○	○
j.	Bathing or dressing yourself	○	○	○

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

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities	○	○	○	○	○
b. Accomplished less than you would like	○	○	○	○	○
c. Were limited in the kind of work or other activities	○	○	○	○	○
d. Had difficulty performing the work or other activities (for example, it took extra effort)	○	○	○	○	○

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

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

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

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

7) How much bodily pain have you had during the past 4 weeks?

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

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

Not at all	A little bit	Moderately	Quite a bit	Extremely
○	○	○	○	○

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

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	○	○	○	○	○
b. Have you been very nervous?	○	○	○	○	○
c. Have you felt so down in the dumps that nothing could cheer you up?	○	○	○	○	○
d. Have you felt calm and peaceful?	○	○	○	○	○
e. Did you have a lot of energy?	○	○	○	○	○
f. Have you felt downhearted and depressed?	○	○	○	○	○
g. Did you feel worn out?	○	○	○	○	○
h. Have you been happy?	○	○	○	○	○
i. Did you feel tired?	○	○	○	○	○

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

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

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

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a. I seem to get sick a little easier than other people	○	○	○	○	○
b. I am as healthy as anybody I know	○	○	○	○	○
c. I expect my health to get worse	○	○	○	○	○
d. My health is excellent	○	○	○	○	○

Figure 26: ***Short Form-36 Questionnaire (UK version 2)***

Mobility

I have no problems in walking about

I have some problems in walking about

I am confined to bed

Self-Care

I have no problems with self-care

I have some problems washing or dressing myself

I am unable to wash or dress myself

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities

I have some problems with performing my usual activities

I am unable to perform my usual activities

Pain/Discomfort

I have no pain or discomfort

I have moderate pain or discomfort

I have extreme pain or discomfort

Anxiety/Depression

I am not anxious or depressed

I am moderately anxious or depressed

I am extremely anxious or depressed

Figure 27: ***EuroQol Questionnaire***

APPENDIX: VASCUQOL QUESTIONS (US ENGLISH VERSION)

The following questions are about how you have been affected by the poor circulation in your legs in the past two weeks. You will be asked about the symptoms you have had, the way that your activities have been affected, and how you have been feeling.

For each question please read all of the answers and then check the one that applies best to you.

For example:

19. In the last two weeks, **problems caused by poor circulation in my legs have made me feel frustrated....**
    - All of the time
    - Most of the time
    - A good bit of the time
    - Some of the time
    - A little of the time
    - Hardly any of the time
    - None of the time

So if you had felt frustrated "hardly any of the time" your answer would be:

- All of the time
- Most of the time
- A good bit of the time
- Some of the time
- A little of the time
- Hardly any of the time
- None of the time

If you are not sure about how to answer a question then please give the best answer you can. There are no right or wrong answers.

Please answer every question.

Thank you

1. During the past two weeks, **I have had pain in my leg (or foot) when walking....**
   - All of the time
   - Most of the time
   - Much of the time
   - Some of the time
   - A little of the time
   - Hardly any of the time
   - None of the time
2. During the past two weeks, **I have been worried that I might injure my leg....**
   - All of the time
   - Most of the time
   - Much of the time
   - Some of the time
   - A little of the time
   - Hardly any of the time
   - None of the time
3. During the past two weeks, **cold feet have given me....**
   - A very great deal of discomfort or distress
   - A great deal of discomfort or distress
   - A good deal of discomfort or distress
   - A moderate amount of discomfort or distress
   - Some discomfort or distress
   - Very little discomfort or distress
   - No discomfort or distress
4. During the past two weeks, because of the poor circulation to my legs, **my ability to exercise or to play sports has been.....**
   - Totally limited, couldn't exercise at all
   - Extremely limited
   - Very limited
   - Moderately limited
   - A little limited
   - Only very slightly limited
   - Not at all limited
5. During the past two weeks, **my legs felt tired or weak....**
   - All of the time
   - Most of the time
   - Much of the time
   - Some of the time
   - A little of the time
   - Hardly any of the time
   - None of the time
6. During the past two weeks, because of the poor circulation in my legs **I have been restricted in spending time with my friends or relatives....**
   - All of the time
   - Most of the time
   - Much of the time
   - Some of the time
   - A little of the time
   - Hardly any of the time
   - None of the time
7. During the past two weeks, **I have had pain in the foot (or leg) after going to bed at night**
   - All of the time
   - Most of the time
   - Much of the time
   - Some of the time
   - A little of the time
   - Hardly any of the time
   - None of the time
8. During the past two weeks, **pins and needles or numbness in my leg (or foot) have caused me....**
   - A very great deal of discomfort or distress
   - A great deal of discomfort or distress
   - A good deal of discomfort or distress
   - A moderate amount of discomfort or distress
   - Some discomfort or distress
   - Very little discomfort or distress
   - No discomfort or distress
9. During the past two weeks, **the distance I can walk has improved....**

   Not at all—check this if the distance is unchanged or has decreased
   - ❑ A little
   - ❑ Somewhat
   - ❑ Moderately
   - ❑ A good deal
   - ❑ A great deal

❑ A very great deal

10. During the past two weeks, because of the poor circulation in my legs, **my ability to walk has been....**
Totally limited, couldn't walk at all
Extremely limited
Very limited
Moderately limited
A little limited
Only very slightly limited
Not at all limited

11. During the past two weeks, **being (or becoming) housebound has concerned me....**
A very great deal
A great deal
A good deal
Moderately
Somewhat
A little
Not at all

12. During the past two weeks, **I have been concerned about having poor circulation in my legs....**
All of the time
Most of the time
Much of the time
Some of the time
A little of the time
Hardly any of the time
None of the time

13. During the past two weeks, **I have had pain in the foot (or leg) when I am resting**
All of the time
Most of the time
Much of the time
Some of the time
A little of the time
Hardly any of the time
None of the time

14. During the past two weeks, because of the poor circulation in my legs, **my ability to climb stairs has been....**
Totally limited, couldn't climb stairs at all
Extremely limited
Very limited
Moderately limited
A little limited
Only very slightly limited
Not at all limited

15. During the past two weeks, because of the poor circulation in my legs, **my ability to participate in social activities has been....**
Totally limited, couldn't socialize at all
Extremely limited
Very limited
Moderately limited
A little limited
Only very slightly limited
Not at all limited

16. During the past two weeks, because of the poor circulation in my legs **my ability to do routine household work has been....**
Totally limited, couldn't perform housework at all
Extremely limited
Very limited
Moderately limited
A little limited
Only very slightly limited
Not at all limited

17. During the past two weeks, **ulcers or sores on my leg (or foot) have caused me pain or distress....**
All of the time
Most of the time
Much of the time
Some of the time
A little of the time
Hardly any of the time
None of the time—(pick this one if you do not have leg ulcers)

18. Because of the poor circulation in my legs, **the range of activities that I would have liked to do in the past two weeks has been....**
Severely limited—most activities not done
Very limited
Moderately limited—several activities not done
Slightly limited
Very slightly limited—very few activities not done
Hardly limited at all
Not limited at all—have done all the activities that I wanted to

19. During the past two weeks, **problems caused by poor circulation in my legs has made me feel frustrated....**
All of the time
Most of the time
Much of the time
Some of the time
A little of the time
Hardly any of the time
None of the time

20. During the past two weeks, **when I have had pain in the leg (or foot) it has given me....**
A very great deal of discomfort or distress
A great deal of discomfort or distress
A good deal of discomfort or distress
A moderate amount of discomfort or distress
Some discomfort or distress
Very little discomfort or distress
No discomfort or distress

21. During the past two weeks, **I have felt guilty about relying on friends or relatives**
All of the time
Most of the time
Much of the time
Some of the time
A little of the time
Hardly any of the time
None of the time

22. During the past two weeks, because of the poor circulation to my legs, **my ability to go shopping or carry bags has been.....**
Totally limited, couldn't go shopping at all
Extremely limited
Very limited
Moderately limited
A little limited
Only very slightly limited
Not at all limited

23. During the past two weeks, **I have worried I might be in danger of losing a part of my leg or foot.....**
All of the time
Most of the time
Much of the time
Some of the time
A little of the time
Hardly any of the time
None of the time

24. During the past two weeks, **the distance I can walk became less**
- ❑ A very great deal
- ❑ A great deal
- ❑ A good deal
- ❑ Moderately
- ❑ Somewhat
- ❑ A little
- ❑ Not at all—check this if the distance is unchanged or has increased

25. During the past two weeks, **I have been depressed about the poor circulation in my legs....**
- ❑ All of the time
- ❑ Most of the time
- ❑ Much of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ Hardly any of the time
- ❑ None of the time

Domains: Activity items—4,9,10,14,16,18,22,24
Symptom items—3,5,8,17
Pain items—1,7,13,20
Emotional items—2,11,12,19,21,23,25
Social items—6,15

Each domain is scored 1-7: = the total of domain item scores divided by the number of questions in the domain.

The total King's College Hospital's VascuQol score is also scored 1-7 = the total of all the item scores divided by 25.

Copies of the translated versions in UK English, Canadian French and English, French, Dutch, Italian, German and Swedish are available from the author.

Figure 28: ***King's College VasculQol Questionnaire***

Appendix B: Patient Information Sheet

ACE Inhibitor Clinical Effectiveness in Intermittent Claudicants

PATIENT INFORMATION SHEET

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being performed and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish.

- Part 1 tells you the purpose of this study and what will happen if you take part
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

What is the purpose of the study?

This study is being conducted for the purpose of obtaining an educational qualification. In this study we will investigate the clinical effectiveness of Angiotensin Converting Enzyme Inhibitor, Ramipril, in patients with intermittent claudication. Ramipril is a drug mainly used to treat hypertension and heart failure but we think that it can be effective in intermittent claudicants in terms of

increasing the distance you can walk and in terms of improving the cardiovascular prognosis.

Why have I been chosen?

You have been approached because you have intermittent claudication.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

The study will start with a baseline assessment where you will be asked to provide the following:

- Your name and address, and, if used by you, mobile telephone number and email address (for the receipt of follow-up questionnaires), date of birth.
- Details of your GP
- Details of your atherosclerosis risk factors
- Weight and height from which the Body Mass Index will be calculated
- Blood pressure will be measured 3 times after 5 minutes rest in the sitting position using semi automated devices. The average of the last 2 readings will be recorded.

Then, the (run in) phase of the study will start. You will have a blood test to check your kidney function, if this is ok; you will be started on a drug called Ramipril 2.5 mg orally once daily for one week. After the one week period you will attend the vascular laboratory to have a 2nd blood test to check your kidney function and if this is ok, Ramipril will be increased to 5mg orally once daily for one week. After that, you will attend the vascular lab and will have a 3rd blood test to make sure your kidney function is still ok. Ramipril will be stopped at this point and you will have a period of two weeks to allow the drug to be washed out of your system. After the run in phase which will last for 4 weeks, if you tolerated the drug and took it regularly, you will be invited back to take part in the reminder of the trial.

The trial phase will start with randomisation. This means that because sometimes we don't know which way treating patients is best, we need to make comparisons. People will be put into two groups and then compared. The groups are selected by a computer which has no information about the individual i.e. by chance. As a result, you will be assigned to either of these two groups: Group one will take ramipril and group two will take placebo which is a dummy drug that has no effect. If you are randomised to receive ramipril, you will be started on 5 mg once daily for 2 weeks and this will be increased to 10 mg once daily for 22 weeks or until the reminder of the study (total of 24 weeks).

If you are randomised to receive placebo, you will also receive placebo until the reminder of the study and will be followed up at baseline, 2 weeks, 6 weeks and 24 weeks. Neither you nor the doctor conducting the study will know which treatment you have been randomised to receive. A simplified diagram at the end of this information sheet summarizes the study phases.

During the trial period which will last for 24 weeks from time of randomisation you will have 4 visits to the vascular laboratory where you will have the following measurements recorded:

1) Treadmill Exercise Test: You will be asked to walk on a treadmill for a maximum of 10 minutes at slow speed and we will record the maximum distance that you can walk. This is the distance that you will be able to walk until you are unable to continue walking any further. This test will be done at baseline, 2 weeks, 6 weeks and 24 weeks.

2) Blood pressure measurement which will happen at baseline, 2 weeks, 6 weeks and 24 weeks. This will be measured using a cuff around your arm attached to a machine which will record your blood pressure automatically.

3) Ankle Brachial Pressure Index measurement: With you lying in a supine position (on your back) we will measure the blood pressure from each arm and from each ankle. This will be done using cuffs around your ankles and arms. It's the same method as recording your blood pressure. This will happen at baseline, 2 weeks, 6 weeks and 24 weeks.

4) Blood and urine samples will be taken at baseline, 2 weeks, 6 weeks and 24 weeks before and after exercise.

5) Pulse Wave Velocity measurement: This is a measurement of the stiffness of the arteries in your body. We will measure this using a machine which uses cuffs to detect pulses. It is a simple and straight forward non-invasive test. The cuff will be applied around 2 arterial sites to measure the velocity between these two points. The two arterial sites we are

planning to use are the carotid artery which is an artery in the neck and the femoral artery which is an artery in the thigh. For the neck pulse we will use a neck collar around the neck which will be able to detect the neck pulse and a cuff around the thigh which will be able to detect the femoral pulse as both the collar and the cuff are attached to a computer which will be able to calculate the velocity automatically. We will record this at baseline, 2 weeks, 6 weeks and 24 weeks.

These investigations are summarized in a flow chart at the end of this information sheet. The flow chart summarizes the tests that you will have in each visit.

After the completion of the 24 weeks trial period, you will be asked to stop ramipril.

It is important that you understand that participating in this study will involve many more visits to the hospital than whilst under normal care. Everything will be explained to you in detail before the study begins. If you wish to participate you will be asked to sign and date the attached consent form before any study related procedures are performed.

The visits will be to the Vascular Laboratory at Hull Royal Infirmary, where you will have the above tests if you agree to take part in this study.

Are there any costs involved?

No cost is involved but patients who will be attending the vascular laboratory will have to make their own travel arrangements

What are the possible disadvantages and risks of taking part in this study?

Appropriate safety measures will be taken at all times. If you develop any severe symptoms because of taking ramipril, we will make sure that you will be treated for that symptom. Furthermore, if you are unable to cope with the drug during or after the run in phase of the study you can leave the study. We will make sure that you don't suffer any severe adverse events because of taking ramipril by applying a protocol of initiating ramipril. You will have a tummy clinical examination and a blood test to assess the kidney function before starting ramipril and you will have follow

up blood tests to monitor your kidney function during the trial period. If you develop a severe complication after starting ramipril you will be taken off the trial and referred to the appropriate medical team at Hull Royal Infirmary to be managed. Application of nitro-glycerine under the tongue can cause a low blood pressure. If your blood pressure becomes very low we will make arrangements for you to be admitted to the hospital.

What are the possible benefits of taking part?

We expect that Ramipril might improve the distance that you can walk and it might relieve any pain you may be experiencing while walking. Ramipril also has shown in other studies that it may prevent you from conditions such as: stroke, heart attack and high blood pressure. This is because Ramipril can reduce the hardening of the arteries, caused by deposits of fat, cholesterol and other substances inside the blood vessel. Your kidneys might be affected if you have diabetes; Ramipril will provide protection for your kidneys from diabetes. You will also benefit from regular clinical examination and blood tests which will monitor your kidney function and lipid levels. Furthermore, information we get might help improve the treatment of people who suffer from pain and cramping that limits walking because of blocked arteries in the legs

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. All information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact details:

Please find below the name, address and telephone number of your study doctor.

Name: Dr Y Shahin, Clinical Research Fellow in Vascular Surgery

Address: Academic Vascular Surgery Unit, Vascular Laboratory, Hull Royal Infirmary, HU3 2JZ
Tel: 01482674643

If you have further questions about this study or your participation, or if during your participation you experience a study related injury, please feel free to contact the person above for further information and/or action to be taken

This completes Part 1 of the information sheet.
If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

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

You are free to withdraw your consent to participate at any time and without giving a reason. This will not affect the standard of care you receive. You are entitled to request identifiable laboratory samples to be destroyed and no further laboratory analysis to be performed.

What happens if I am harmed?

If you are harmed by taking part in this research project, there are no compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action but may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you. If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (see contact details part 1).

Will my taking part in this study be kept confidential?

Information about you will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, your study data will not be made publicly available. Also when the results are published, your identity will be kept confidential. If you consent to take part in this study, your medical records will be inspected will be inspected by the study doctor. Your medical records may also be looked at by people from the national and international regulatory authorities to check that the study is being or has been

conducted properly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site. Blood and urine samples will be analysed by the pathology department in Hull Royal Infirmary

Involvement of the General Practitioner/Family Doctor (GP)

With your permission we will inform your GP and other specialist doctors who are involved in your healthcare about your participation in the study. It may also be necessary to request further information from your GP regarding medical history/medications taken.

What will happen to any samples I give?

Blood and urine samples will be destroyed after the results of all the tests have been reported.

Will any genetic tests be done?

No genetic testing will be performed in this study.

What will happen to the results of the research study?

Once the study is complete, the results will be published and a final report written. You should contact your study doctor if you are interested in receiving copies of any resulting publications. You will not be identified in any reports or publications.

Who is organising and funding this research?

The research is organised by the department of vascular surgery, Hull Royal Infirmary

Who has reviewed the study?

The ethics behind this study has been reviewed and supported by the Leeds West Research Ethics Committee.
If you wish to take part in this study you will be asked to sign a consent form overleaf. A copy of your signed consent form and this patient information sheet will be given to you to keep.

Thank you for taking the time to read this information sheet.

Appendix C: Patient Consent Form

Patient's ID Number:

Patient Consent Form

ACE Inhibitor Clinical Effectiveness in Intermittent Claudicants

Name of researchers: Dr Y Shahin, Mr I Chetter

Please initial

1. I confirm that I have read and understand the patient information sheet Dated 13/05/2010 for the above study and have had the opportunity to consider the information; ask questions and have had these answered satisfactorily. ☐

2. I understand that I am being invited to take part in a research study. I am not taking part in any other research study at this time. I understand risks and benefits, and I freely give my informed consent to participate in the research study described in this form, under the conditions stated in it. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical or legal rights being affected. ☐

4. I understand that relevant sections from any of my medical notes and data collected during the study may be examined by responsible individuals from the NHS trust where the study is being undertaken, local research ethics committee or regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to have direct access to my records. ☐

5. I give consent that my GP and specialist doctors involved in my healthcare may be contacted and access given to my medical notes held by my GP. ☐

6. I agree to take part in the above study. ☐

Name of patient	Date	Signature
Name of person taking consent (if different from PI)	Date	Signature
Name of Principal Investigator	Date	Signature

Copies: 1 for patient, 1 for researcher, 1 to be kept in hospital notes

Version 2-final,01/02/2010, ACE inhibitor clinical effectiveness in intermittent claudicants, consent form

Appendix D: Ethics Committee and Trust Approvals

NHS
National Research Ethics Service

Leeds (West) Research Ethics Committee
Room 22
Floor CD, Block 40
King Edward Home
Leeds General Infirmary
Leeds
LS1 3EX

Telephone: 0113 3923181
Facsimile: 0113 3926799

27 April 2010

Dr Yousef Shahin
Clinical Research Fellow in Vascular Surgery
Academic Vascular Surgery Unit
Vascular Laboratory, Alderson House
Hull Royal Infirmary
HU3 2JZ

Dear Dr Shahin

Study Title:	**A double blind, randomised, placebo controlled trial to study the clinical and cost effectiveness of Angiotensin Converting Enzyme (ACE) inhibitors, ramipril,in patients with intermittent claudication**
REC reference number:	**09/H1307/109**
Protocol number:	**2**
EudraCT number:	**2009-016600-23**

Thank you for your letter of 20 April 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC A list of the sub-committee members is attached.

Confirmation of ethical opinion

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

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

Hull and East Yorkshire Hospitals NHS
NHS Trust

Research & Development Department
Clinical Governance Directorate
2nd floor Daisy Building
Castle Hill Hospital
Cottingham
East Yorkshire
HU16 5JQ

12 May 2010

Dr Yousef Shahin
Clinical Research Fellow in Vascular Surgery
Academic Vascular Surgery Unit
Vascular Laboratory
Alderson House
Hull Royal Infirmary
HU3 2JZ

Dear Dr Yousef Shahin,

Re: R0969 - A double blind, randomised, placebo controlled trial to study the clinical and cost effectiveness of Angiotensin Converting Enzyme (ACE) inhibitors, ramipril, in patients with intermittent claudication.

EudraCT number: (to be received) REC reference Number 09/H1307/109

Subject to full approval being granted by the MHRA, Research Ethics Committee and the Trust, Hull and East Yorkshire Hospitals NHS Trust agrees, in principle, to act as Sponsor for the above study as defined by the UK Clinical Trial Regulations[1] and the Research Governance Framework[2], and agrees to indemnify the aforementioned study accordingly.

Yours sincerely

James Illingworth
Research & Development Manager

[1] UK Clinical Trial Regulations incorporating Statutory Instruments 2004 No.1031, 2005 No. 2754, 2006 No. 2759, 2006 No. 1928, 2006 No. 2984, 2008 No. 941, 2009 No. 1164.
[2] The Research Governance Framework for Health and Social Care (RGFHSC) (2nd Edition, 2005) sets out the broad principle of good research governance.