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The Effects of Xanthine Oxidase Inhibitors on Left Ventricular Mass and Endothelial Function in Patients with Ischaemic Heart Disease

Rekhranj, Sushma

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The Effects of Xanthine Oxidase Inhibitors
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Disease

Sushma Rekhraj

2014

University of Dundee

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**The Effects of Xanthine Oxidase
Inhibitors on Left Ventricular Mass
and Endothelial Function in Patients
with Ischaemic Heart Disease**

By

Dr Sushma Rekhraj

MD Thesis

University of Dundee

April 2014

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INDEX OF ABBREVIATIONS

ACS	Acute coronary syndrome
ACE	Angiotensin converting enzyme
AF	Atrial fibrillation
AGE	Advanced glycation end products
AIx	Augmentation index
AP-1	Activator protein-1
ARB	Angiotensin-II receptor blocker
ASE	American Society of Echocardiography
ASK	Apoptosis signalling kinase
BMI	Body mass index
BNP	B-type natriuretic peptide
BP	Blood pressure
BSA	Body surface area
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CATCH	Candesartan Assessment in Treatment of Cardiac Hypertrophy
CCF	Congestive cardiac failure
CCS	Canadian Cardiovascular Society
CHD	Coronary heart disease
CI	Confidence interval
CKD	Chronic kidney disease
CMR	Cardiac Magnetic Resonance
CT	Computed Tomography
CV	Coefficient of variation
CVD	Cardiovascular disease
DM	Diabetes mellitus
ECG	Electrocardiogram
Echo	Echocardiography
EDV	End-diastolic volume
EndoPAT	Endothelial-Peripheral arterial tonometry
ERK	Extracellular response kinase
ESV	End-systolic volume
FAD	Flavin adenine dinucleotide
FLASH	Fast Low Angle Shot
FMD	Flow mediated dilatation
FVOP	Forearm venous occlusion plethysmography
GTN	Glyceryl trinitrate
HOPE	Heart Outcomes Prevention Evaluation
HR	Hazard ratio
HT	Hypertension
HV	Healthy volunteer
IGF-1	Insulin-like growth factor-1

IHD	Ischaemic heart disease
IL	Interleukin
IVS	Interventricular septum
JNK	Jun-nuclear kinase
LIFE	Losartan Intervention for Endpoint reduction
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMi	Left ventricular mass index
MAPK	Mitogen activated protein kinase
MESA	Multi-Ethnic Study of Atherosclerosis
MHRA	Medicines and Healthcare Products Regulatory Agency
MI	Myocardial infarction
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NFKB	Nuclear Factor KB
NHANES	National Health And Nutrition Examination Survey
NO	Nitric oxide
NOMAS	Northern Manhattan Study
NOS	Nitric oxide synthase
NSTEMI	Non-ST elevation myocardial infarction
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
OPT-CHF	Oxypurinol Therapy for Congestive Heart Failure
OR	Odds Ratio
OS	Oxidative stress
PAT	Peripheral arterial tonometry
PCI	Percutaneous coronary intervention
PIUMA	Progetto Ipertensione Umbria Monitoraggio Ambulatoriale
PKC	Protein kinase C
PW	Posterior wall
PWA	Pulse wave analysis
PWV	Pulse wave velocity
RAAS	Renin-angiotensin-aldosterone syndrome
RCT	Randomized controlled trial
RR	Relative risk
ROS	Reactive oxygen species
RWT	Relative wall thickness
RyR2	Ryanodine receptor
SBP	Systolic blood pressure
SCD	Sudden cardiac death
SD	Standard deviation

SERCA2a	Sarcoplasmic reticulum calcium ATPase
SHS	Strong Heart Study
SLV	Sokolow-Lyon voltage
SNR	Signal-noise ratio
SOD	Superoxide dismutase
SSFP	Steady-State Free Precision
STEMI	ST elevation myocardial infarction
TNF- α	Tumour necrosis factor- α
TOMHS	Treatment of Mild Hypertension
UPCR	Urine protein/creatinine ratio
VT	Ventricular tachycardia
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase

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Finally, I would like to thank my family and friends for their support and encouragement, without whom this thesis would not have been possible.

Above all, praise and thanks to God for all His Blessings and Grace upon my life.

DECLARATION

I hereby declare that the work contained in this thesis was carried out during my appointment as a Clinical Research Fellow in the Centre for Cardiovascular and Lung Biology, Division of Medical Sciences at the University of Dundee (Ninewells hospital and Medical School, Dundee) between February 2009 and February 2011. As the sole author, the content of this thesis is entirely my own work. I was primarily responsible for recruiting, follow-up and analyses of the study in this thesis with the exceptions of the cardiac MRI images that were analysed by Dr Stephen Gandy and laboratory blood tests performed by Lesley McFarlane. All sources of information are acknowledged accordingly. The work described in this thesis has not previously been accepted for a higher degree. The findings of this study have been presented at scientific meetings and published in a peer-reviewed journal.

Signed

Date

SUMMARY OF CONTENTS

Left ventricular hypertrophy is a marker of poor prognosis that commonly affects patients with ischaemic heart disease. It has been associated with an increased risk of all-cause mortality and cardiovascular events including myocardial infarction, heart failure, stroke and arrhythmias. Previous studies of mainly antihypertensive therapies have shown left ventricular hypertrophy to be a reversible risk factor. The LIFE study has shown LVH regression *per se* to be associated with reduced cardiovascular morbidity and mortality, independent of blood pressure reduction. Therefore, novel ways of regressing left ventricular hypertrophy, independent of blood pressure, in patients with IHD could reduce cardiovascular events and mortality in this patient group.

Allopurinol is a xanthine oxidase inhibitor that received FDA approval in 1966 and has been used primarily as a prophylactic treatment for gout. It is a safe, well tolerated drug with minimal side effects.

There are a number of reasons why allopurinol may reduce LV mass in patients with LVH and IHD. Firstly, allopurinol has been shown to regress LV mass in animal studies and more recently in patients with CKD. Secondly, LV afterload is the main determinant of LV mass. Previous studies have shown allopurinol to improve LV afterload by improving arterial stiffness and arterial compliance,

which may result in regression of LV mass. Thirdly, patients with IHD have increased levels of oxidative stress, which contribute to both endothelial dysfunction and left ventricular hypertrophy. Xanthine oxidase is a major producer of reactive oxygen species and allopurinol therapy, being a xanthine oxidase inhibitor, has been shown to reduce vascular oxidative stress.

This was a randomized, double-blinded, placebo controlled study of 66 patients with IHD and LVH, prescribed allopurinol 600mg/day or placebo therapy over a 9 month follow up study period. LV mass was measured at baseline and 9 months using cardiac magnetic resonance imaging. At baseline, 6 months and 9 months, measurements were made of endothelial function using flow-mediated dilatation and assessments of arterial stiffness by measuring augmentation index and pulse wave velocity using applanation tonometry.

In this study, we demonstrated high dose allopurinol therapy over a 9 month treatment period to significantly regress LV mass, improve endothelial dysfunction and measurements of arterial stiffness without a significant reduction in BP.

The improvement in endothelial function and regression of LV mass demonstrated in this study with high dose allopurinol suggests that allopurinol might reduce cardiovascular events and mortality. Future studies should

investigate if this beneficial effect of allopurinol on LV mass, being a surrogate marker, results in a beneficial effect on harder clinical end points such as cardiovascular events and mortality.

1 INTRODUCTION

1.1 Oxidative stress

1.1.1 Background of oxidative stress

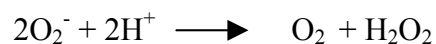
Oxidative stress occurs when there is an accumulation of reactive oxygen species (ROS), which is often due to the inadequacy of the body's intrinsic antioxidant defence system ie. there is an imbalance in the "redox state" of a cell. ROS are chemically reactive molecules derived from oxygen. They include free radicals [superoxide radical (O_2^-), hydroxyl radical (OH)] and non-radical molecules [hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl)].

ROS in low concentration plays a cell protecting role in modulating inflammation and is involved in cellular "redox signalling" pathways. Additionally, transcription factors activated by ROS also stimulate antioxidants such as manganese superoxide dismutase and NOS. When produced in excess, ROS has harmful effects on cell protein (denaturing and inactivating enzymes), lipid (damaging membranes of cells and cellular organelles), carbohydrate and DNA leading to cell damage and death (1).

Gerschman *et al.* first proposed the harmful effects of ROS formed from excessive oxygen. In 1969, McCord and Fridovich supported this theory with the discovery of superoxide dismutase (2). Growing evidence suggests that oxidative stress (OS) is involved in the pathogenesis of various cardiovascular diseases including hypertension, atherosclerosis, endothelial dysfunction, hypercholesterolemia, ventricular hypertrophy, myocardial infarction and heart failure (3). Myocardial ischaemia results in the production of ROS and inflammatory cytokines (such as TNF- α , IL-1 β and IL-6), resulting in cardiac remodelling (4, 5). Additionally, in human studies, administration of antioxidants during an acute MI, angioplasty or heart surgery reduces infarct size and the amount of cardiac dysfunction (3).

1.1.2 Generation of oxidative stress

The superoxide radical (O_2^-) is formed by the addition of an electron to an oxygen molecule leading to a reduced form of oxygen. Hydrogen peroxide (H_2O_2) is produced by the addition of an electron to O_2^- and then undergoing protonation. This reaction is catalysed by superoxide dismutase.



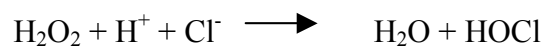
Under normal conditions, H_2O_2 is broken down by catalase and glutathione peroxidase to form H_2O . However, in pathological conditions, H_2O_2 goes on to form highly reactive radicals like OH and HOCl. Highly reactive hydroxyl radicals (OH) can be formed by the Fenton reaction and the Haber-Weiss reaction, which are catalysed most commonly by iron and copper. In the Fenton reaction, the hydroxyl radical is formed when a reduced metal ion donates an electron to H_2O_2 .



In the Haber-Weiss reaction, OH is generated when the superoxide radical interacts with hydrogen peroxide (1).



Hypochlorous acid (HOCl) is formed when H_2O_2 reacts with chloride ion in the presence of myeloperoxidase.



The role of NO on myocardial function has been controversial, partly due to the presence of NOS isoforms (eNOS and nNOS) expressed in cardiomyocytes, resulting in both myocardial relaxation and contraction. NO has been reported to

modulate the function of proteins involved in excitation-contraction coupling such as the L-type calcium channels and ryanodine receptor calcium release channel (RyR2) which causes myocardial calcium uptake and the sarcoplasmic reticulum calcium ATPase (SERCA2a) which causes calcium uptake into the sarcoplasmic reticulum, resulting in reduced myocardial calcium levels. NO causes S-nitrosylation of proteins whereby protein cysteine thiol is modified into S-nitrosothiol (SNO), resulting in changes in protein function (6, 7). However, increased O_2^- levels inhibits nitrosylation and interacts with nitric oxide (NO) to form a peroxynitrite ($ONOO^-$) (1). This highly reactive molecule mediates damaging processes including lipid peroxidation, protein nitration and oxidation of LDL (8) and activation of matrix metalloproteinases (MMPs). Hence, nitroso-redox imbalance may lead to cardiac remodelling and alter excitation-contraction coupling (9). Figure 1 illustrates the ROS production in the heart.

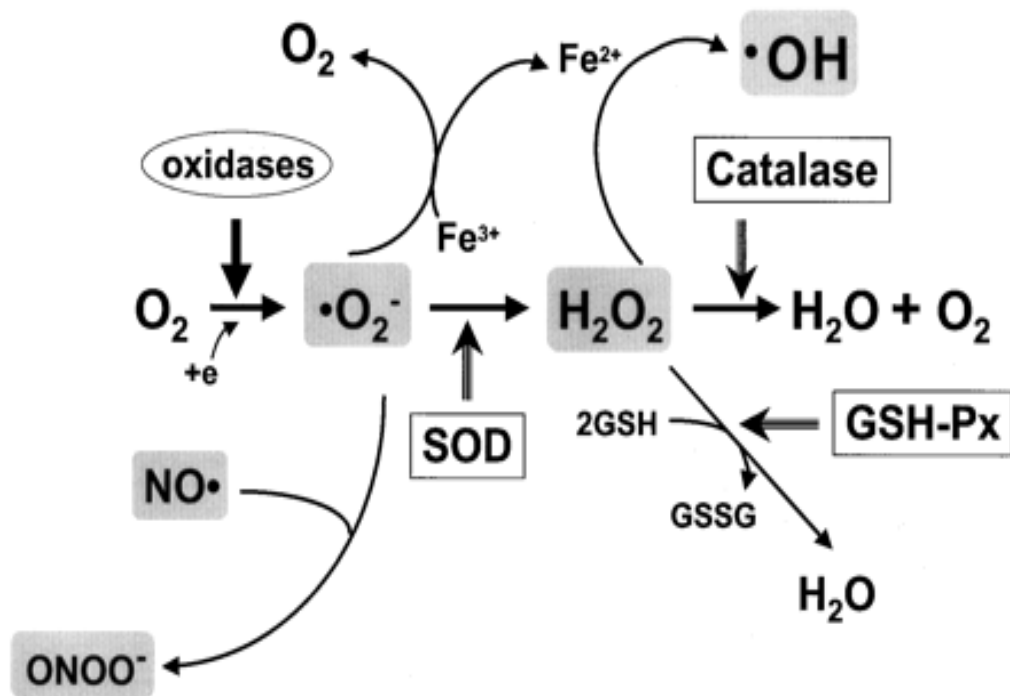


Figure 1. ROS production and antioxidant system in the heart (10)

1.1.3 Sources of ROS

ROS can be generated by neutrophils, vascular endothelial cells and cardiomyocytes by various mechanisms including mitochondria oxidative phosphorylation, NADP/NADPH oxidase, xanthine oxidase and uncoupling of NO synthase (11).

1.1.3.1 Mitochondria

ROS is produced by the mitochondria during cellular aerobic metabolism. “Electron leakage” during oxidative phosphorylation reacts with oxygen molecule to form O_2^- (1). However, normally ROS are controlled by intrinsic antioxidants. During reperfusion in the setting of ischaemia, experimental studies have shown the huge increase in oxygen supply to result in a surge of ROS production by the mitochondria, which can't be adequately managed by the intrinsic antioxidants (12).

1.1.3.2 NADH/NADPH oxidase

NADPH oxidase is an enzyme found in phagocytes, endothelium, vascular smooth muscle cells and cardiomyocytes (13). It is a major source of ROS. All NADPH oxidases have a catalytic Nox subunit, which transfers electrons from NADPH to oxygen leading to the formation of superoxide radical. There are 5 isoforms of Nox identified so far but Nox 2 and 4 are the two main isoforms found in cardiomyocytes and endothelial cells (14).

Increased levels of NADPH oxidase activity has been shown in human subjects with heart failure and experimental models of left ventricular hypertrophy (13). NADPH oxidase activity is stimulated by numerous factors including mechanical stretch, angiotensin II, α -adrenergic agonists, endothelin-1 and TNF-

α (14). ROS generated by NADPH oxidase is the mechanism whereby angiotensin II induces cardiac hypertrophy and remodelling and endothelial dysfunction (14). Hence, inhibitors of the renin-angiotensin system such as ACE inhibitors and angiotensin II receptor blockers have resulted in regression of LVM (independent of BP reduction) (15, 16) and have shown improvement in endothelial dysfunction (17, 18). ROS produced by NADPH oxidase also activates other OS generating systems such as xanthine oxidase and uncoupling NOS (13). O_2^- produced by NADPH oxidase oxidises NOS co-factor BH4 leading to uncoupling of NOS. This will promote further ROS production ie. a vicious cycle may occur (13).

1.1.3.3 Xanthine oxidase (XO)

This enzyme is another important source of ROS and is the primary focus of this research study. Increased enzyme activity has been detected in a range of cardiovascular conditions associated with increased oxidative stress (19-23).

Xanthine oxidoreductase (XOR) is a member of the molybdoenzyme family and was first identified in 1902 by Schardinger in milk. It is a butterfly shaped homodimer that is made of 2 subunits (of approximately 150kDa each) that are independent catalysts. XOR activity requires the presence of 3 cofactors: molybdopterin (Mo-Co), iron-sulphur centres (Fe_2-S_2) and FAD (24). XOR is

present as two interchangeable forms – xanthine oxidase (XO) and xanthine dehydrogenase (XDH). The enzyme tends to be formed as a dehydrogenase in vivo, which is converted to the oxidase form by sulfhydryl oxidation or proteolysis. The XOR gene is located on the short arm of chromosome 2 (24).

Normally XO is found mainly in the liver and small intestine and at very low levels in the heart and endothelium. However, XOR gene transcription is stimulated by numerous factors including hypoxia, lipopolysaccharide, interferon γ , IL-1, IL-6, TNF α and steroids (25). As previously mentioned, xanthine oxidase is also activated by ROS produced by NADPH oxidase (13). NO may also have an effect on XOR activity although there is conflicting evidence (26, 27). There is considerable variation in XO activity in different species. A study by de Jong *et al.* in 1990 found the XO activity (mU/g) to be 33 ± 3 in mice, 28.5 ± 1.4 in rats, 0.59 ± 0.09 in rabbits, <0.1 in pigs and 0.31 ± 0.04 in humans (28). In humans, XO has more than a threefold variation in activity (29).

XOR is a rate-limiting enzyme involved in the final two stages of the purine degradation pathway (see Figure 2). XDH oxidises the conversion of hypoxanthine to xanthine and then xanthine to uric acid whilst reducing NAD⁺ for which it has higher affinity. XO also can oxidise the xanthines whilst reducing oxygen to superoxide radicals (O₂⁻ and H₂O₂) (30).

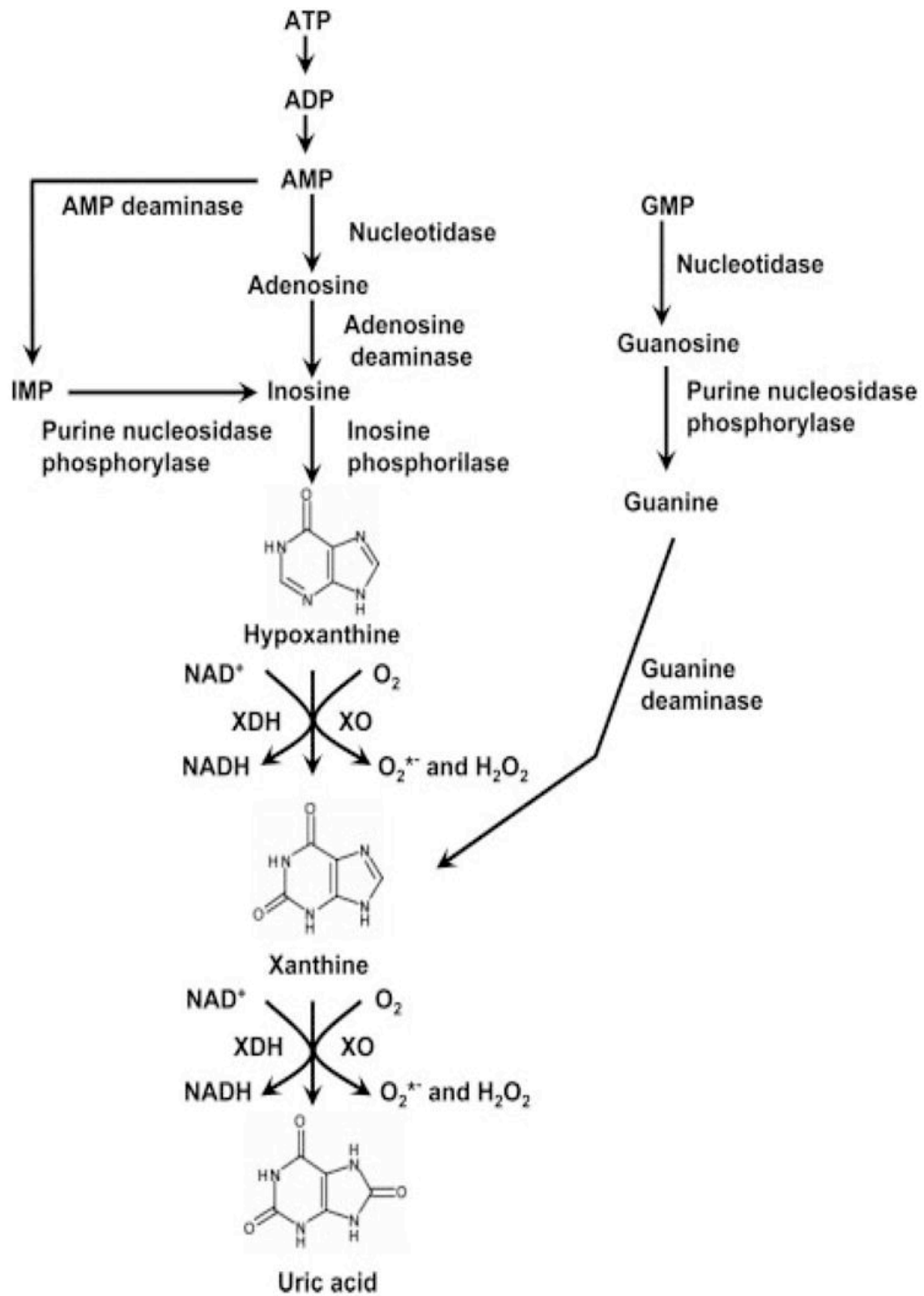


Figure 2. Purine degradation pathway (30)

The conversion of xanthine to uric acid results in the donation of 2 electrons to XOR, which reduces Mo (VI) to Mo (IV). The electrons are then transferred from the Mo-Co to FAD via $\text{Fe}_2\text{-S}_2$. A fully reduced XO has 6 electrons, which is transferred from FAD to oxygen molecules on reoxidation leading to the generation of $2\text{H}_2\text{O}_2$ and 2O_2^- . XDH on the other hand tends to preferentially donate its electrons to NAD^+ instead of O_2 resulting in NADH generation (see Figure 3) (24). The O_2^- generated can combine with NO resulting in cytotoxic peroxynitrite. In the presence of hypoxia, NO is produced by XOR as evidenced by allopurinol therapy inhibiting NO production that is not seen with NOS inhibitors (31).

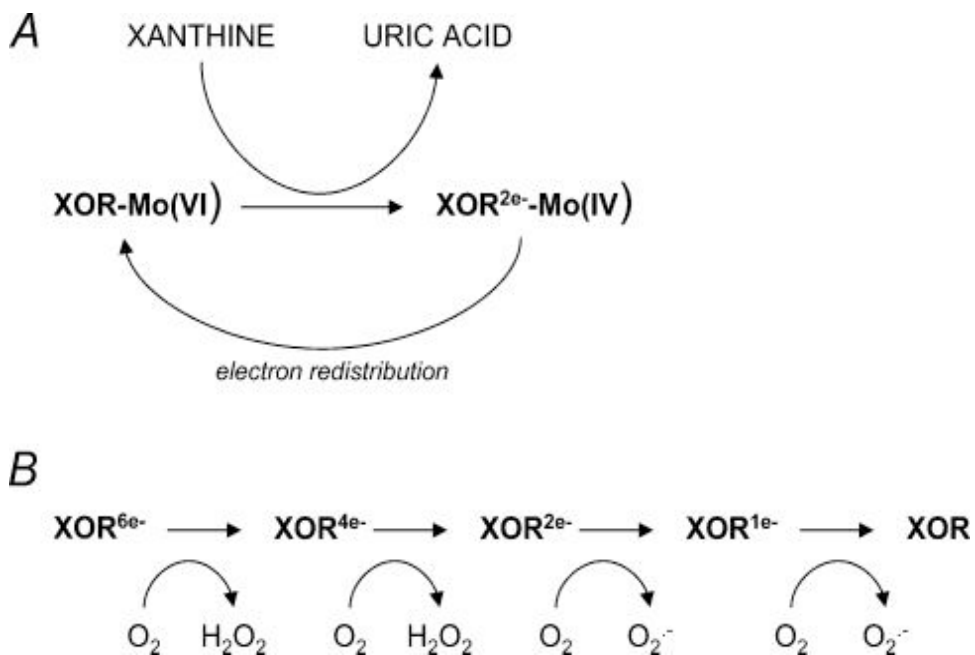


Figure 3. Mechanism of XOR reaction with xanthine (24)

1.1.3.4 Uncoupling NOS3

Normally, NOS3 (an endothelial NOS) reacts with NADPH, L-arginine and O_2 to form NO and L-citrulline. NO inhibits XO and NADPH oxidase activation, cardiac hypertrophy and fibrosis and cardiac dysfunction. Cofactor tetrahydrobiopterin (BH4) is required for NOS3 to remain in a coupled state. When exposed to oxidative stress or to the lack of BH4 or L-arginine, this leads to instability and uncoupling of NOS3 and electrons react with oxygen molecules to form O_2^- . The O_2^- formed then reacts with NO to form peroxynitrite resulting in cytotoxic effects (11). Landmesser *et al.* has reported that O_2^- from NADPH oxidase oxidises BH4, which triggers uncoupling of NOS3 (32).

1.1.4 Antioxidants

There are enzymatic and non-enzymatic pathways that act as antioxidants to degrade and convert ROS into non-toxic molecules. The enzymatic antioxidants include catalase, glutathione peroxidase, superoxide dismutase, thioredoxin and thioredoxin reductase. Superoxide dismutase (SOD) converts O_2^- to H_2O_2 whilst catalase and glutathione peroxidase catalyse the conversion of H_2O_2 to water. Thioredoxin and thioredoxin reductase on the other hand are involved in the production of antioxidants including ubiquinone, lipoic acid and ascorbic acid. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the genes of enzymes involved in the antioxidant pathway such as

NAD(P)H quinone oxidoreductase 1, heme oxygenase 1, glutamate-cysteine ligase and glutathione S transferase (33, 34). Non-enzymatic antioxidants include vitamin E and C, β -carotene, ubiquinone, lipoic acid and uric acid (1).

1.1.5 Oxidative stress in cardiovascular diseases

1.1.5.1 Atherosclerosis

Cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes and smoking are associated with increased levels of oxidative stress. ROS play a role in the development and progression of atherosclerosis by the formation of oxidised LDL, proliferation and migration of vascular smooth muscle cells and endothelial dysfunction (1, 35). Plaque stability is also affected by the activation of MMPs by ROS (2).

In endothelial dysfunction, there is a reduction in vasodilator NO bioavailability. Increased levels of O_2^- interacts with NO, resulting in the formation of toxic peroxynitrite and hence inactivates NO. This will be discussed further in a later section of this thesis on endothelial dysfunction.

1.1.5.2 Myocardial Ischaemia

Myocardial ischaemia especially during reperfusion is associated with the production of ROS and inflammatory cytokines. During an MI, ROS directly causes cell damage and hence affects infarct size. Animal studies have shown that in transgenic mice with overexpression of an antioxidant SOD, there is a reduction in infarct size (36). In the setting of an MI, ROS is also involved in myocardial stunning and LV remodelling. There are numerous ways in which ROS is involved in LV remodelling:

1. Activation of signalling pathways including Apoptosis signalling kinase-1 (Ask-1)
2. Activation of MMPs resulting in changes in extracellular matrix
3. Apoptosis (36)

LV remodelling post MI results in thinning of infarcted areas and a reactive hypertrophy of remote myocardium eventually leading to LV dilatation and dysfunction.

1.1.5.3 Heart failure

To date, animal and human studies have provided evidence of the role of oxidative stress in the setting of heart failure (1). ROS affects cardiac function by inhibiting sarcoplasmic reticulum Ca^{2+} pump SERCA2 (37), suppressing

sarcolemma L-type calcium channels (38) and affecting proteins involved in the excitation-contraction coupling (39).

1.1.5.4 Cardiac hypertrophy

Significant evidence exists for the involvement of oxidative stress in the development of cardiac hypertrophy (1, 11, 13). Induced by neurohumoral stimuli (such as angiotensin II, endothelin I, norepinephrine), cytokines or chronic pressure overload, high levels of ROS lead to cardiac hypertrophy, remodelling, contractile dysfunction, fibrosis and apoptosis (13). This occurs via apoptosis signalling kinases (ASK), transcription factors and matrix metalloproteinases (MMPs). ROS cause cardiac hypertrophy by activating p38 and Jun-nuclear kinase (JNK) MAPKs (mitogen activated protein kinase), ASK-1, extracellular response kinase (ERK) 1/2, protein kinase C (PKC), phosphoinositol 3-kinase (PI3K), tyrosine kinase Src and GTP binding protein Ras (11). Figure 4 shows the molecular signalling pathways in the heart.

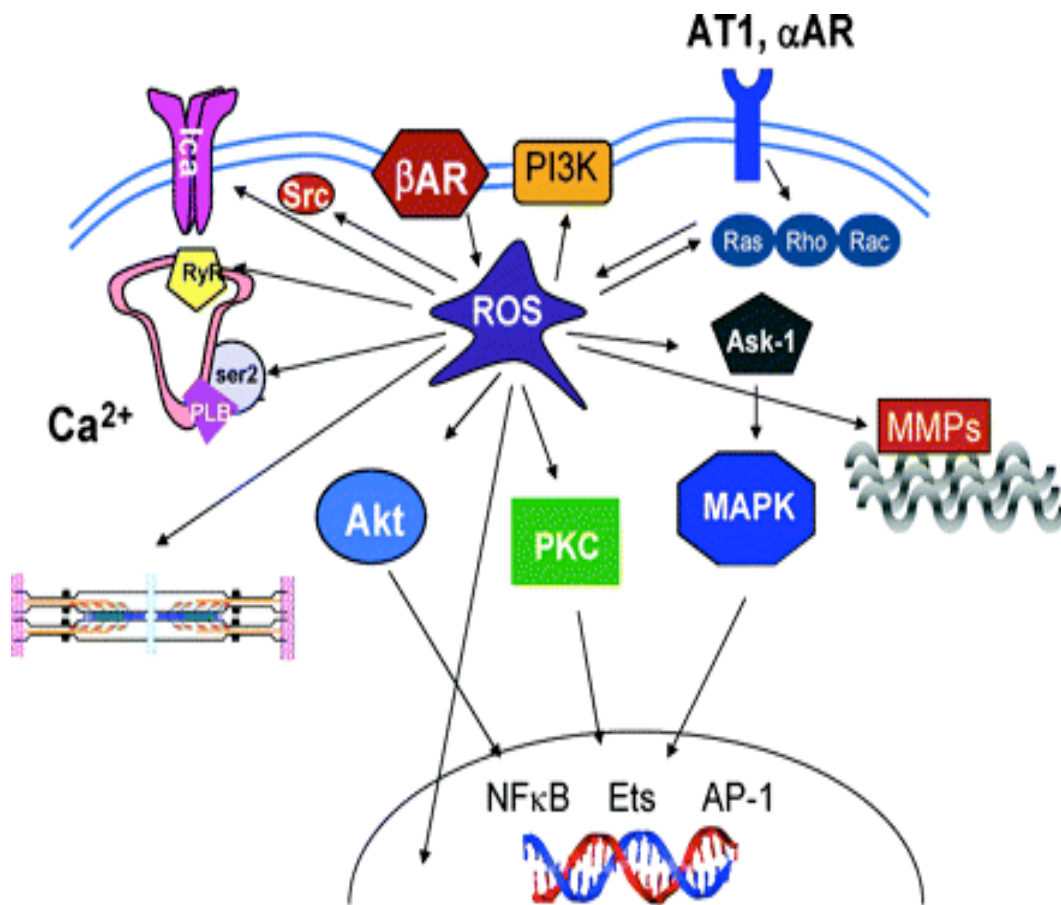


Figure 4. Molecular signalling pathways linking ROS to cardiac hypertrophy and remodelling

Ica indicates calcium channels; α AR, α adrenergic; β AR, β adrenergic; AT1, angiotensin II; MAPK, mitogen-activated protein kinases; PI3K, phosphoinositol 3-kinase; Ask-1, apoptosis signalling kinase-1; PKC, protein kinase C; NF κ B, nuclear factor κ B; AP-1, activator protein-1(11).

Angiotensin II is thought to generate cardiac hypertrophy by ROS generated via NADPH oxidase. It acts via G-protein pathways and involves apoptosis signalling kinase-1 (ASK-1) and mitogen-activated protein kinases (MAPK) signals (1). ROS can also result in cardiac hypertrophy by the action of

transcription factors (NF- κ B and activation protein-1) in altering gene expression (1).

Cardiac fibrosis is involved in both LVH and CCF. ROS causes fibrosis and extracellular matrix remodelling by stimulating cardiac fibroblast proliferation, increasing MMP expression and activating MMP activity via transcription factors NF κ B, Ets and AP-1 (11).

Apoptosis occurs in ischemic, hypertrophied and cardiac dysfunction. Apoptosis occurs at much higher levels of ROS compared to the kind of ROS levels that stimulate hypertrophy. Studies on adult rat cardiac myocytes have shown that stimulation of β adrenergic receptors cause apoptosis within 24 hours by ROS. ROS cause apoptosis by activating p38 and JNK MAPKs, ASK-1 and Akt (1). NOS2 produces high levels of NO that reacts with O_2^- to form peroxynitrite, which causes myocyte apoptosis. In animal MI studies, NOS2 knockout mice had less myocyte apoptosis and better contractile function compared to wild type mice (40).

1.2 Endothelial dysfunction

1.2.1 Background

The endothelium is a single cell layer lining the lumen of blood vessels, which acts as a physical barrier regulating vascular tone, controlling thrombosis, smooth muscle cell proliferation and vascular inflammation in response to various mechanical and chemical stimuli (41). It secretes vasodilating substances such as NO and vasoconstricting substances such as endothelin-1 (see Table 1).

Maintenance of vascular tone

- Nitric oxide
- Prostaglandins (prostacyclin [PGI₂], thromboxane A₂ [TxA₂])
- Endothelial hyperpolarizing factor
- Endothelin-1
- Angiotensin II
- C-type natriuretic peptide

Balancing blood fluidity and thrombosis

- Nitric oxide
- Tissue plasminogen activator
- Heparins
- Thrombomodulin
- Prostaglandins
- Plasminogen activator inhibitor-1 (PAI-1)
- Tissue factor
- Von Willibrand's factor

Control of the vascular inflammatory process

- Monocyte chemoattractant factor-1 (MCP-1)
- Adhesion molecule expression (VCAM-1, ICAM-1, selectins)
- Interleukins 1, 6 and 18
- Tumour necrosis factor

Table 1. Normal functions of the vascular endothelium and partial list of factors elaborated and regulated by endothelium to maintain vascular homeostasis (41)

NO is the main vasodilator secreted by the endothelium. It is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin, NADPH and FAD/FMN and regulated by Ca-calmodulin, resulting in NO and L-citrulline. NO then diffuses into the vascular smooth muscle cells activating guanylate cyclase, by interacting with the iron atom of the heme in guanylate cyclase, thereby increasing cGMP production and reducing intracellular calcium. This results in vasodilatation (42). See Figure 5.

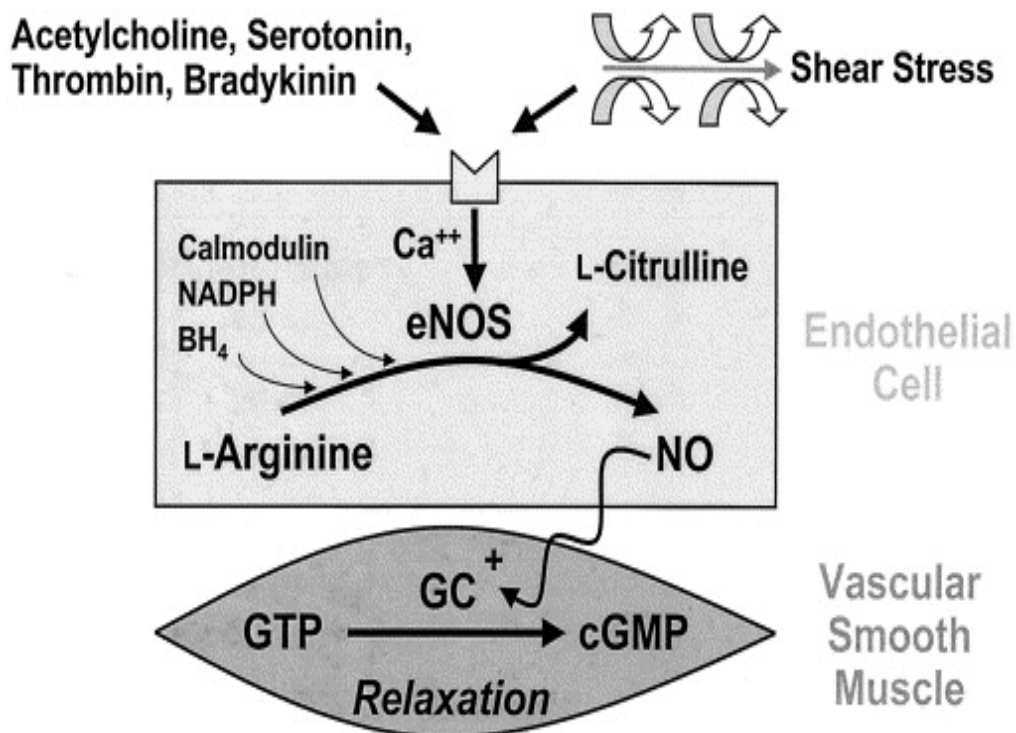


Figure 5. The nitric oxide signalling pathway. BH_4 = tetrahydrobiopterin; Ca^{++} = calcium ion; cGMP = cyclic guanosine monophosphate; eNOS= endothelial nitric oxide synthase; GC = guanylate cyclase; GTP = guanosine triphosphate; NADPH = reduced nicotinamide-adenine dinucleotide phosphate; NO = nitric oxide.

Endothelial dysfunction occurs when the endothelium loses its ability to maintain vascular homeostasis in response to risk factors resulting in vasoconstriction, thrombosis and vascular smooth muscle cell proliferation. Cardiovascular diseases and traditional risk factors result in increased oxidative stress, which inactivates NO by combination with superoxide and other reactive oxygen species. There is reduced local NO levels which normally acts as a vasodilator, inhibits platelet aggregation and monocyte adhesion to endothelium and smooth muscle cell proliferation, thereby increasing the risk of cardiovascular events (43). The risk of an initial or recurrent cardiovascular event correlates with the severity of endothelial dysfunction.

1.2.2 Assessment of endothelial function using FMD

Various invasive methods have been used in the past to assess endothelial function including coronary artery studies and venous occlusion plethysmography. However, a non-invasive technique of assessing endothelial function called flow mediated dilatation (FMD) of the brachial artery was developed and first described by Celermajer *et al.* in 1992 (44). This technique has been widely used in clinical research for assessing endothelium dependent flow mediated dilatation of the brachial artery in response to increased shear wall stress using high resolution ultrasound. A reactive hyperaemia in response to release of the sphygmomanometer cuff after a five minute upper arm occlusion

results in 5 to 7 times increased blood flow and shear stress, resulting in local vasodilator production, predominantly NO. FMD is a measure of the endothelial bioavailability of NO (local production vs. destruction). This vessel response is compared with an endothelium-independent response to GTN.

This technique is an indirect measure of coronary artery endothelial function. Several studies have shown a good correlation between brachial FMD and coronary endothelial function. In 1995, Anderson *et al.* showed that brachial FMD closely related with coronary artery endothelial tests, with a positive predictive value of 95% (45). An advantage of this technique is that it is non-invasive and so can be performed on large number of patients and repeated on the same patient. Additionally, the results are reproducible (46). Limitations of this technique include operator skill as it can be difficult to perform and one needs an adequate training period and it can be time consuming to perform the test and analyze images. Additionally, several factors are known to influence the findings of FMD including high fat meals, vitamin C, caffeine, smoking, menstrual cycle, exercise, temperature and vasodilators.

EndoPAT (Endothelial-Peripheral Arterial Tonometry) is a more recently developed non-invasive method of assessing vasoreactivity. It is a less operator dependent method whereby a plethysmographic probe is placed on the finger index of each hand. It detects the endothelium-mediated changes in the digital

pulse waveform known as the peripheral arterial tone (PAT). However, the EndoPAT method compared to FMD in measuring endothelial function has higher variability in measurements and fewer studies have been performed in assessing the influence of an intervention on PAT ratio (47).

1.2.3 Endothelial dysfunction in IHD

Endothelial dysfunction has been shown to occur in patients with coronary artery disease and in response to traditional risk factors (41). It plays a key role in the pathogenesis of atherosclerosis and may be the earliest manifestation of atherosclerosis, even in absence of angiographic evidence (43). Patients with confirmed CAD on angiogram have worse endothelial function compared to those with cardiovascular risk factors and normal coronary arteries (48). It also plays a role in plaque instability, resulting in plaque rupture and hence acute coronary syndrome (42) (see Figure 6).

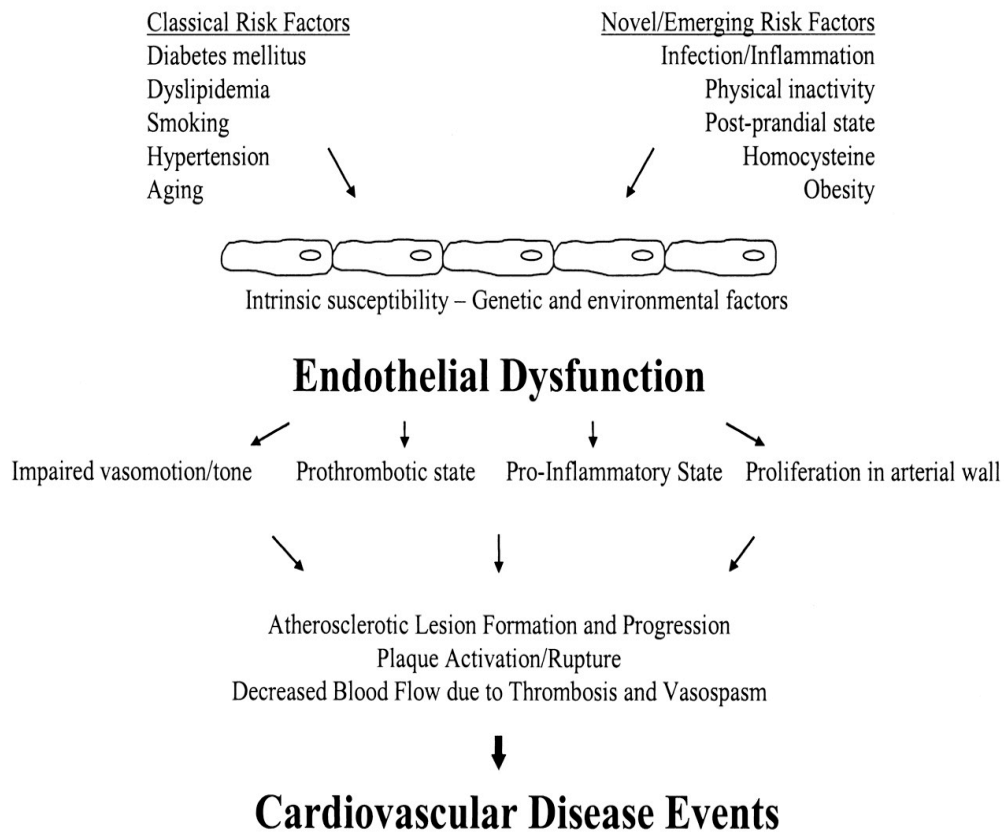


Figure 6. The role of endothelial dysfunction in the pathogenesis of cardiovascular disease events (41)

Patients with stable angina and ACS have similar endothelial dysfunction (endothelium-dependent dilatation in stable angina group: $2.3 \pm 8.1\%$, ACS group: $2.6 \pm 8.9\%$; $p = \text{NS}$ between these two groups, control normal angiogram group: $14.9 \pm 9.1\%$; $p < 0.001$) (49). Endothelial dysfunction is not only related to the presence of CAD but also the number of vessels affected (on univariate analysis $r = -0.67$, $p < 0.01$) and severity of stenosis ($r = -0.52$, $p < 0.01$) (48).

An impaired FMD is associated with an increased risk of cardiovascular events (including angina requiring hospitalization, MI, revascularization, death) (41, 43) and in-stent restenosis (OR 4.5, 95% CI 2.4, 12) (50). It has been shown to have a prognostic role in patients presenting with chest pain (51), ACS without ST elevation (FMD < 1.9% adjusted HR 3.035, 95% CI 1.146, 8.023; p = 0.03) (52) and STEMI patients (53).

1.2.4 Endothelial dysfunction and LVH

Numerous small-scale studies of hypertensive patients have shown endothelial dysfunction to be associated with LVH (54, 55). In a LIFE sub-study of 40 untreated hypertensives, FMD had a negative correlation to LVMI ($r = -0.53$, $p < 0.01$) (54). However, a study by Muiesan *et al.* showed no association between endothelial dysfunction and LVH or LV geometry in hypertensive patients (55). In a sub-study of the Northern Manhattan Study (NOMAS), 2D echo and FMD was undertaken on 867 stroke-free, multiethnic population group. On multiple linear regression, FMD was inversely associated with LVM ($\beta = -1.21 \pm 0.56$, $p = 0.03$). Univariate logistic regression showed each 1% reduction in FMD to be associated with an 8% increased risk of LVH (OR 1.08, 95% CI: 1.03, 1.13 per FMD point; $p < 0.01$) (56). Additionally, in a large population-based study of 6814 cardiovascular disease free adults [Multi-Ethnic Study of Atherosclerosis

(MESA)], endothelial dysfunction was associated with LVM assessed using CMR, independent of cardiovascular risk factors (57).

1.2.5 Treatments shown to improve endothelial dysfunction

As endothelial dysfunction has been associated with an increased risk of cardiovascular events and there is a non-invasive reproducible technique to assess endothelial function, over the years there has been considerable interest to evaluate potential therapeutic options.

Diet high in omega-3 fatty acids and flavonoids such as tea and grape juice have been shown to reduce endothelial dysfunction (58-60). High fat meals on the other hand worsen endothelial function (61). Exercise is very effective in improving endothelial function, likely due to increased NO bioavailability (62). Reduction of cardiovascular risk factors such as reduction of BP, smoking cessation and treatment of diabetes has also been associated with improved endothelial function (41).

Numerous studies have consistently shown statins to improve endothelial function. This is felt likely due to a reduction in serum cholesterol levels and the pleiotropic effects of statins. It reduces expression of inflammatory cytokines

and growth factors, inhibits angiotensin II induced oxidative stress and enhances activity of endothelial NOS, thereby increasing NO production (63-65).

ACE inhibitors and ARBs are also effective in reducing endothelial dysfunction. ACE inhibitors and ARBs reverse endothelial dysfunction by inhibiting angiotensin II induced production of ROS, which usually inactivates NO. ACE inhibitors also inhibit bradykinin breakdown, which increases NO production (66-69). Antioxidants have been investigated extensively as a therapeutic option as oxidative stress is known to play a key role in endothelial dysfunction in atherosclerosis. The evidence for vitamin E is conflicting. The HOPE (Heart Outcomes Prevention Evaluation) study, a large, randomized study, did not show vitamin E to have a beneficial effect on cardiovascular events in high risk patients (70). Vitamin C on the other hand has been shown to have an impact on endothelial function in smokers, heart failure and CAD. However, a combination of antioxidants (beta-carotene, vitamin C, vitamin E) did not demonstrate a beneficial effect on endothelial function or cardiovascular events (71, 72).

1.2.6 Arterial stiffness

1.2.6.1 Background

Arterial stiffness is a measure of the mechanical property of an artery and is an independent risk marker of future cardiovascular events. It tends to affect patients with coronary artery disease due to its association with increased age, presence of cardiovascular risk factors and with atherosclerosis. It is commonly assessed by pulse wave velocity (PWV) directly and pulse wave analysis (PWA) indirectly, which are simple techniques with reproducible results (73). Stiffer the vessels, higher the augmentation index (AIx) and faster the PWV. Both PWA and PWV are inversely related to endothelial function measured with brachial FMD (74).

1.2.6.2 Pulse wave analysis and pulse wave velocity

We routinely assess peripheral blood pressure noninvasively at the brachial artery using a sphygmomanometer. Elevated brachial BP is associated with the development of LVH. However, the central aortic pressure especially in the ascending aorta directly affects the left ventricle and hence is felt to be a better predictor of outcome compared to peripheral BP. The increased LV afterload puts strain on the LV, thereby resulting in LVH to maintain cardiac output. We are now able to assess the central aortic pressure peripherally via the radial artery using applanation tonometry and a validated ‘generalized transfer function’.

The arterial pressure waveform is generated by a forward wave by LV ejection (incident wave) and a reflected wave from arteries in the peripheral circulation. In arterial stiffness, an increased velocity of forward and backward wave results in the wave returning in late systole instead of early diastole, thereby augmenting systolic pressure and reducing diastolic pressure (75) (see Figure 7). The augmented systolic pressure increases cardiac afterload resulting in LVH and increases myocardial oxygen demand whilst a reduction in diastolic pressure reduces coronary perfusion resulting in myocardial ischaemia (76).

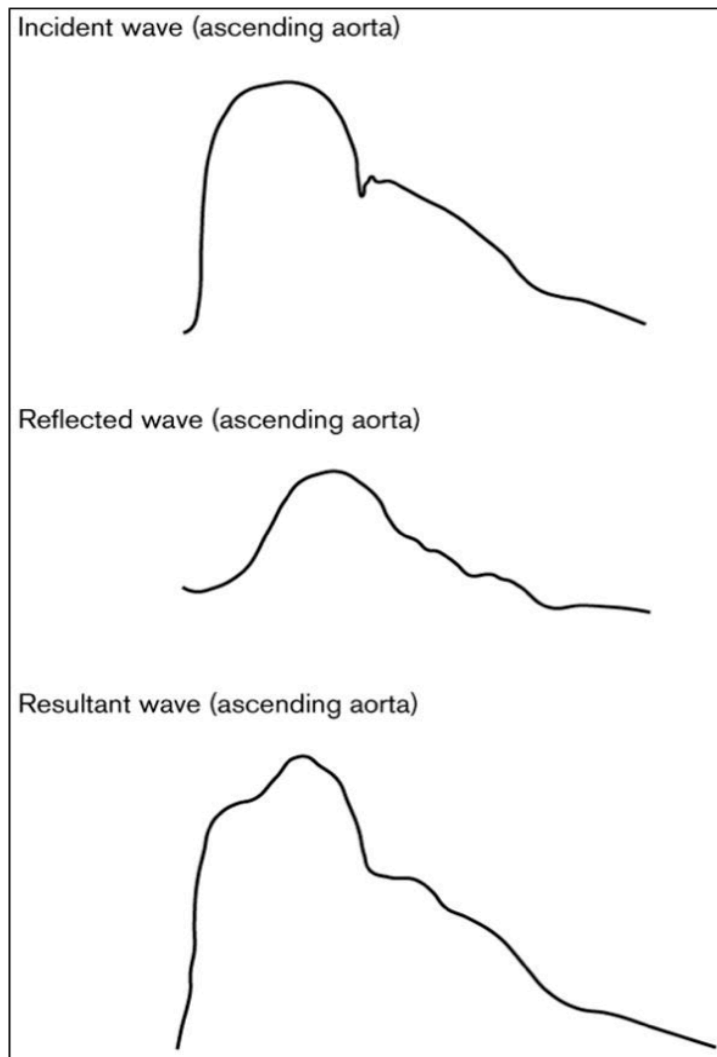


Figure 7. Resultant aortic pressure waveform is a combination of incident wave and reflected wave (260)

Augmentation index measures the effect of the reflected wave on the second systolic peak resulting in increased LV afterload. It is a composite measure including resistance vessel function. This parameter depends on 3 factors: heart rate, PWV and the amplitude of the reflected pulse wave (75).

1.2.6.3 Arterial stiffness studies

Numerous studies have shown arterial stiffness to be a predictor of mortality and cardiovascular events in patients with hypertension, type 2 diabetes and end-stage renal disease (73, 77). Even small changes in PWV of 1m/s have been shown to reduce all cause mortality (adjusted RR 0.71; 95% CI 0.6, 0.86) (78).

A cross sectional study of 465 symptomatic men by Weber *et al.* showed AIx to be a strong risk marker for the presence and extent of coronary artery disease (79). Arterial stiffness is also a predictor of myocardial ischaemic threshold, measured by time to ST depression on the treadmill exercise test for CAD patients (80). This may be partly explained by a reduction in coronary flow reserve seen with large arterial stiffness (81). In patients undergoing PCI, on adjusting for cardiovascular risk factors, angiogram findings and medication, each tertile increase in AIx had a RR of 1.8 for primary endpoint (MI, death and clinical restenosis) (82).

As increased arterial stiffness would mean increased LV workload, Saba *et al.* found a higher AIx to be associated with a higher LVMI. However, Chen *et al.* only found PWV to be associated with LVMI on univariate analysis (75).

1.3 Ischaemic heart disease

1.3.1 Cardiovascular disease and its burden

Cardiovascular disease (CVD) leads to significant morbidity and mortality in the Western and developing world. It is the leading cause of death in the United Kingdom (UK) accounting for 180,000 deaths in 2010, of which 45% were due to coronary heart disease (CHD) (83). In the US, CVD mortality rate has declined by 32.7% from 1999 to 2009 although it still accounted for 787,931 in 2009 (84).

CHD is also the most common cause of death before the age of 75 in the UK. This is despite the CHD mortality rates falling in the UK since the 1970s. The Health Survey for England found that between 1994 and 2006, the prevalence of CHD in men increased from 6% to 6.5% whilst it remained stable for women (4.1% in 1994 to 4.0% in 2006). According to UK health surveys, an estimated 1.2 million men and over 900,000 women suffer from chronic angina. It is also estimated that there are over 20,000 new cases of angina per year (83). In 2009, CHD accounted for 1 in 6 deaths (386,324) in the US. It has been estimated that 635,000 Americans will have their first coronary event each year (84).

Additionally, the total economic cost of CVD in the UK has been estimated to be £19 billion a year of which £6.7 billion is due to coronary artery disease – 27%

due to direct healthcare costs, 47% to loss of productivity and 26% for carers loss of earnings (83). In 2009, the total direct and indirect cost of CVD in US was \$312.6 billion (84). In view of its huge health impact and economic costs, there is significant interest to develop new therapeutic targets to tackle cardiovascular disease.

1.3.2 Chronic stable angina

1.3.2.1 Background of angina

The word ‘angina’ arises from the Latin word ‘angere’ which means ‘to throttle’. This clinical syndrome is characterised by the development of discomfort in chest, jaw, shoulder, arms or back in response to exertion or emotional stress and typically is relieved with rest or glyceryl trinitrate (GTN). Occasionally some patients may not develop chest pain but breathlessness as a symptom of angina. Angina occurs due to the imbalance between myocardial oxygen demand and supply, resulting in myocardial ischaemia. Myocardial oxygen supply depends on oxygen saturation of arterial blood, myocardial oxygen extraction and coronary blood flow (85). Myocardial ischaemia triggers a sequence of events known as the ischaemic cascade (Figure 8). It should be noted that angina only occurs at the final stage of the ischaemic cascade.

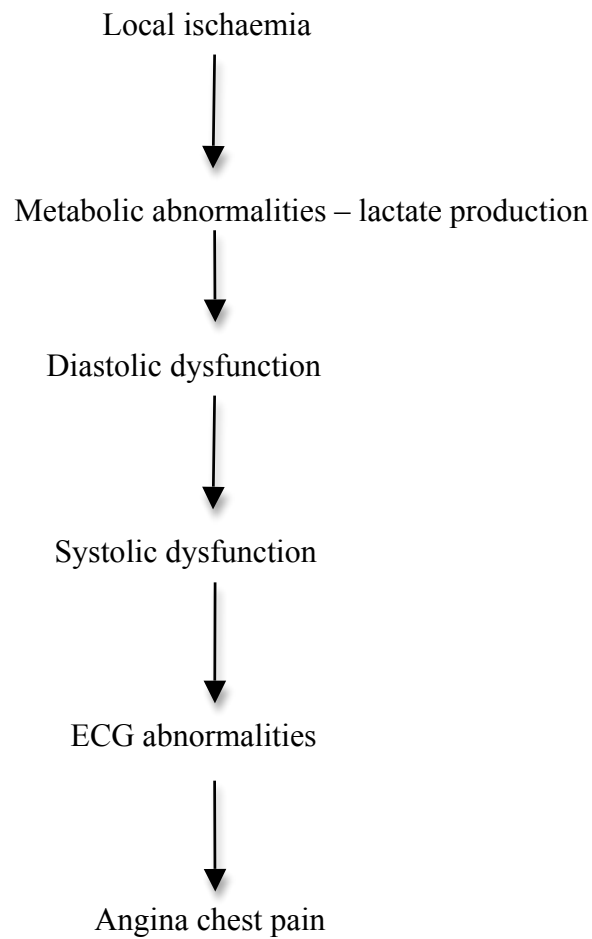


Figure 8. Myocardial ischaemic cascade (86)

The main mediator of angina is adenosine via stimulation of A1 adenosine receptors (87). Atherosclerotic coronary artery disease is the most common cause of myocardial ischaemia. Normally, the coronary blood flow is able to increase 5-6 times during exercise. However in the presence of atherosclerosis, especially >50% narrowing of the coronary arteries, it is unable to reduce vascular resistance and increase coronary flow during exercise, resulting in oxygen demand-supply mismatch (88). Other causes include anaemia, coronary artery

spasm, endothelial dysfunction, aortic stenosis and hypertrophic cardiomyopathy.

Patients with stable angina can go on to develop an acute coronary syndrome (ACS), which includes unstable angina, non-ST elevation MI (NSTEMI) and ST elevation MI (STEMI). In unstable angina, there is worsening of angina symptoms which become more frequent, more prolonged, occur at a lower workload and even at rest. During an MI, there is a prolonged attack of angina usually lasting > 30minutes which is not really helped by GTN and is associated with myocardial damage resulting in an increase in cardiac enzyme levels in the blood.

1.3.2.2 Diagnosis of angina

Taking a careful history is paramount in making a diagnosis of angina. The typical features of angina are discussed in Table 2. Additionally, it is important to ask regarding the presence of exposure to cardiovascular risk factors such as smoking, diabetes (DM), hypertension (HT), family history of ischaemic heart disease (IHD), previous history of peripheral vascular disease or strokes.

Typical angina (definite)	Meets three of the criteria
	<ul style="list-style-type: none"> • Substernal chest discomfort of characteristic quality and duration • Provoked by exertion or emotional stress • Relieved by rest and/or GTN
Atypical angina (probable)	Meets two of these characteristics
Non-cardiac chest pain	Meets one or none of the characteristics

Table 2. Clinical classification of chest pain (85)

A grading system for angina is commonly used to quantify the severity of symptoms and to assess the response to therapy. The Canadian Cardiovascular Society (CCS) classification is commonly used. See Table 3.

Class	Level of symptoms
Class I	<p>‘Ordinary activity does not cause angina’</p> <p>Angina with strenuous or rapid or prolonged exertion only</p>
Class II	<p>‘Slight limitation of ordinary activity’</p> <p>Angina on walking or climbing stairs rapidly, walking uphill or exertion after meals, in cold weather, under emotional stress or only after first few hours after awakening</p>
Class III	<p>‘Marked limitation of ordinary physical activity’</p> <p>Angina on walking one or two blocks on the level or one flight of stairs at a normal pace under normal conditions</p>
Class IV	<p>‘Inability to carry out any physical activity without discomfort’ or ‘angina at rest’</p>

Table 3. Classification of angina severity according to the Canadian Cardiovascular Society (85)

The history taking is complemented with a physical examination, laboratory investigations and cardiac investigations (non-invasive vs. invasive).

1.3.2.2.1 Exercise stress test

This non-invasive test has traditionally been used commonly in patients with suspected coronary artery disease as it is cheap and widely available. It can help in diagnosing coronary artery disease. A positive stress test as defined by ST depression of 1mm has a mean sensitivity of 68% and specificity of 77% in detecting significant coronary artery disease (85). This however depends on the pretest probability, which can be influenced by age and gender. It can also be used for prognostic information and to evaluate response to treatment (85).

Sensitivity is the ability of a test to correctly identify patients with coronary artery disease whilst specificity is the ability of a test to correctly identify patients without coronary artery disease.

Sensitivity = $(\text{true positive} / \text{true positive} + \text{false negative}) \times 100$

Specificity = $(\text{true negative} / \text{true negative} + \text{false positive}) \times 100$

1.3.2.2.2 Myocardial perfusion stress

It is a non-invasive test whereby using exercise or pharmacological agents such as dobutamine or adenosine, radionuclide isotopes such as thallium or technetium-99 labelled agents are taken up by cardiac myocytes and hence provides information on myocardial perfusion. Images are acquired at rest and stress using single-photon emission computed tomography. This technique has a

large body of evidence that it can detect inducible perfusion defects in patients with coronary artery disease and provide prognostic information. It has a sensitivity and specificity of 88% and 77% respectively (89). However, one of the main limitations of this test is the exposure to radiation.

1.3.2.2.3 Stress echocardiogram

Stress echocardiogram is a non-invasive stress test whereby either exercise or a pharmacological agent such as dobutamine or adenosine is used to increase the heart rate and then transthoracic echo is performed to assess for any inducible wall motion abnormalities or viability. Hence it is widely used if the patient is unable to exercise or if the ECG is difficult to interpret for ischaemia such as LVH, left bundle branch block or paced rhythm. Its sensitivity and specificity are 76% and 88% respectively (89).

1.3.2.2.4 Cardiac Magnetic Resonance Imaging (CMR)

CMR can diagnose coronary artery disease by two methods: stress induced wall motion abnormalities (sensitivity 83%, specificity 86%) or perfusion imaging (sensitivity 91%, specificity 81%). It should be noted that CMR is contraindicated in patients with claustrophobia or if they have any implanted metals such as pacemakers and patients should be able to perform breath hold.

1.3.2.2.5 Computed Tomography (CT)

This non-invasive test assesses coronary arteries by the severity of coronary calcification (calcium score) and looking at its anatomy (CT coronary angiogram). It is a good rule out test of significant coronary artery disease in patients with low to intermediate probability. Calcification of coronary arteries is a marker of atherosclerosis that reflects the overall plaque burden and not the severity of specific coronary stenosis (85). However, not all coronary plaques are calcified and not all calcified plaques are severe lesions. The CT calcium score is not very sensitive in detecting plaques without significant calcification (90). The Agatston score is the most commonly used calcium score. A CT calcium scan takes 5 minutes to perform and interpret, is performed without contrast and there is exposure to a lower radiation dose compared to a CT coronary angiogram.

CT coronary angiogram has a sensitivity of 98% and specificity of 88% (91). Its negative predictive value (NPV, proportion of patients with negative test results who are correctly diagnosed) is 96-100% whilst its positive predictive value (PPV, proportion of patients with positive test results who are correctly diagnosed) is 93% (91). However, there is a significant radiation exposure to the patient, involves the use of contrast and it is poor in predicting if a coronary lesion is functionally significant (92). Hence, if a coronary stenosis appears > 50% on CT coronary angiography, further investigations such as a functional test or invasive coronary angiography are undertaken.

1.3.2.2.6 Coronary angiogram

Whilst the non-invasive techniques gives you a likelihood of obstructive coronary artery disease, a coronary angiogram is an invasive technique that gives you a definitive anatomical diagnosis of the presence or absence of coronary artery disease. Additionally, functional severity of the coronary lesions can be assessed by measuring coronary flow velocity or fractional flow reserve (85). However, being invasive there are risks associated in undertaking this test and there is exposure to radiation.

The 2010 National Institute for Health and Care Excellence guidelines suggests that patients with chest pain with an estimated likelihood of coronary artery disease of 10-29% should be offered a CT calcium score. If the calcium score is zero then it is advised to consider other causes of chest pain. If the calcium score is between 1-400, a CT coronary angiogram should be offered. If the calcium score is >400 , then invasive coronary angiography should be offered instead. Intermediate risk patients with an estimated likelihood of coronary artery disease of 30-60% should be offered a non-invasive functional test, which includes myocardial perfusion scan, stress echocardiography or stress CMR. High risk patients, with an estimated likelihood of coronary artery disease of 61-90% should be offered invasive coronary angiography (93).

1.3.3 Treatment of angina

The aim of treating patients with angina is for symptom relief and for prognostic reasons. Management of chronic stable angina includes treating conditions that can exacerbate angina (such as anaemia and thyrotoxicosis), treating traditional cardiovascular risk factors (including smoking, hypertension, hypercholesterolemia, diabetes, obesity), medication (antiplatelets, statins, ACE inhibitors, beta blockers, calcium channel blockers, nitrates, potassium channel activators, *If* channel inhibitors such as ivabradine and ranolazine) and coronary revascularization by percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). These various treatments have improved patients' symptoms and prognosis. However, we seem to have forgotten that LVH itself is an independent predictor of cardiovascular disease and is associated with a poor prognosis. LVH may be considered to be a 'silent killer' as it can remain undetected for many years until finally developing arrhythmias, heart failure or sudden cardiac death. Hence, detecting and treating LVH may yet be another approach of managing these cardiac patients.

1.4 LVH

1.4.1 Definition of LVH

LVH occurs when there is an increase in muscle mass of the left ventricle (LV). An increase in LV wall thickness or LV cavity size or both occurs in response to increased chronic workload and neurohormonal factors. It can occur in response to increased physiological workload (such as anaemia and exercise), pressure overload (such as hypertension and aortic stenosis) and volume overloaded conditions (such as aortic regurgitation and interatrial shunts). According to LaPlace's law, load on the myocardium is equal to $(\text{pressure} \times \text{radius}) / (2 \times \text{wall thickness})$. Therefore in the short term, this increase in muscle mass helps compensate for the increased wall stress but it may be harmful in the long term. Histologically, the cardiomyocytes increase in size (thickness and/or length) and fibroblasts proliferate leading to collagen deposition and fibrosis (94).

1.4.2 Types of LVH

There are four types of geometric LV patterns depending on the LV mass (LVM) and relative wall thickness (RWT). A normal LV has a normal LV mass (LVM) and RWT whilst concentric remodelling has a normal LV mass but increased RWT. Eccentric LVH tends to occur in response to volume overload and has an increased LV mass but normal RWT (95). In this type of hypertrophy,

sarcomeres are added in series leading to lengthening of cardiomyocytes. Concentric LVH tends to occur in response to pressure overload and has an increased LVM and RWT(95). Sarcomeres are added parallel leading to increased cardiomyocyte thickness (See Figure 9) (96).

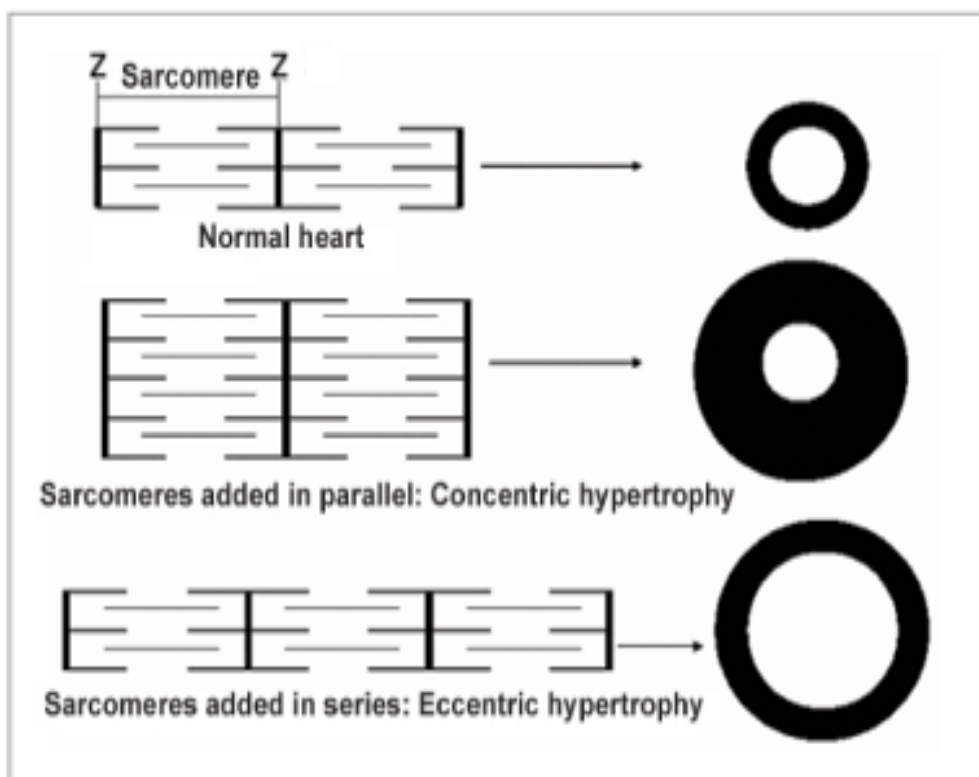


Figure 9. Differentiation between eccentric and concentric LVH (96)

1.4.3 Prevalence

The prevalence of LVH quoted in studies varies depending on the population group being studied and the different LVM cut-offs that have been used to diagnose LVH over the years. The Framingham study was one of the initial big studies to assess the presence of LVH in the general population. In the Framingham study, a prospective epidemiological study of 4976 Framingham residents, LVH was detected in 16% of men and 19% of women using echocardiography (97). Whilst in the Tromso study, which looked at 3287 subjects in Norway, LVH was detected using echo in 14.9% of men and 9.1% of women (98).

LVH occurs commonly in patients with hypertension as a sign of end organ damage in response to the increased cardiac afterload. Its prevalence ranges from 20-61% (99-104) depending on the cut-off values used to diagnose LVH. A study of uncomplicated hypertensives at a workplace treatment programme by Hammond *et al.* found LVH to be present in approximately 12% of subjects with borderline hypertension and 20% of subjects with uncomplicated, mild essential hypertension (99). In the Treatment of Mild Hypertension (TOMHS) study involving 844 mild hypertensives, LVH (indexed to BSA) was detected in 13% of men and 20% of women whilst LVH (indexed to height) was found in 24% of men and 45% of women (100). One of the initial studies of subjects with mild to moderate hypertension found as many as 61% of them to have an abnormally

increased IVS or PW thickness (104). However, BP has not been found to be a reliable marker of the presence of LVH. In the Framingham, LVH was present in 28% normotensive women aged > 65 years (97). The presence of LVH has also been noted in normotensive high risk groups such as stable angina patients (prevalence of 69%) (105) and diabetics (prevalence of 26%) (106).

Various studies have shown LVH to occur commonly in cardiac patients. In a study by East *et al.* of patients with documented coronary artery disease on cardiac catheterization, LVH was detected in 35% (107). Liao *et al.* also undertook a study on angiographically confirmed coronary artery disease but in a black, Chicago population group and found the prevalence of LVH to be 55.6% (108). More recently, a study of stable angina patients in Dundee, Scotland by Ang *et al.* found LVH to be prevalent in 73% (when LVM indexed to BSA) and 75% (when LVM indexed to $ht^{2.7}$) (105). Observational studies have also found LVH to be prevalent in other population groups: 41-72% of diabetics (109), 50% of patients with peripheral artery disease (110) and 74% of chronic kidney disease (CKD) (Table 4) (111, 112).

Studies	Population studied	Echo criteria of LVH	
		Male	Female
Levy (97)	General	$> 150\text{g/m}^2$	$>120\text{g/m}^2$
Schirmer (98)	General	$>145.5\text{g/m}$	$>125.4\text{g/m}$
Hammond (99)	Hypertension	$>134\text{g/m}^2$	$>110\text{g/m}^2$
Liebson (100)	Mild hypertension	$\geq 134\text{g/m}^2$	$\geq 110\text{g/m}^2$
Ang (105)	Angina	$>115\text{g/m}^2$	$>95\text{g/m}^2$
East (107)	Coronary artery disease	$>134\text{g/m}^2$	$>110\text{g/m}^2$
Liao (108)	Coronary artery disease	$>131\text{g/m}^2$	$>100\text{g/m}^2$
Rana (106)	Diabetes	$>134\text{g/m}^2$	$>110\text{g/m}^2$
Dawson (109)	Diabetes	$>134\text{g/m}^2$ $>50\text{g/m}^{2.7}$	$>110\text{g/m}^2$ $>47\text{g/m}^{2.7}$
Wright (110)	Peripheral artery disease	$>134\text{g/m}^2$	$>110\text{g/m}^2$
Foley (111)	Chronic kidney disease	$>131\text{g/m}^2$	$>100\text{g/m}^2$

Table 4. Echo criteria for LVH that have been used in studies of various population groups.

1.4.4 Predictors of LVH

LVH is a disease process that occurs commonly in cardiac patients as a result of haemodynamic and non-haemodynamic factors, including neurohormones.

1.4.4.1 Hypertension

Hypertension is a well-known, extensively studied risk factor for developing LVH. As already discussed, there is a high prevalence of LVH in the hypertensive population and studies have shown a significant positive correlation between office systolic BP (SBP) and LVM ($r=0.29$ to 0.48) (113, 114). High BP results in an increased LVM due to the increased cardiac afterload. It is a physiological adaptation of the heart to reduce wall stress, thereby preserving LV systolic function and preventing cardiac dilatation. In the Framingham Heart Study for every 20mmHg increase in SBP, there was a 43% increase in risk of LVH in men and 25% in women (97).

However, 24 hr ambulatory BP measurements have been found to be more sensitive in indicating those who may have LVH compared to clinic BP measurements (115). Verdecchia *et al.* (1990) found that in the untreated hypertensives, the ambulatory SBP showed a closer correlation ($r= 0.47$) with LVMI than office SBP ($r=0.35$) (116). Higher LVMI has also been detected in patients who have morning blood pressure surges and ‘non-dippers’ (patients

who display little or no night time drop in blood pressure which is part of the normal circadian rhythm) (117, 118).

However, BP is not the only cause of LVH. A study by Chen *et al.* demonstrated that SBP accounted for only 31% of the LVM variance. In fact they found that stroke volume was the most important determinant of LVM (50%) (119). Additionally, LVH has been detected in normotensive subjects.

1.4.4.2 Obesity

Obesity can directly and indirectly have effects on the heart. Increasing body weight is associated with haemodynamic changes resulting in increased blood volume and cardiac output. This volume overload leads to left ventricular dilatation and hypertrophy. Obesity is also associated with arterial hypertension, oxidative stress and insulin resistance which can predispose to LVH (120). Figure 10 shows the mechanisms whereby obesity can result in development of LVH.

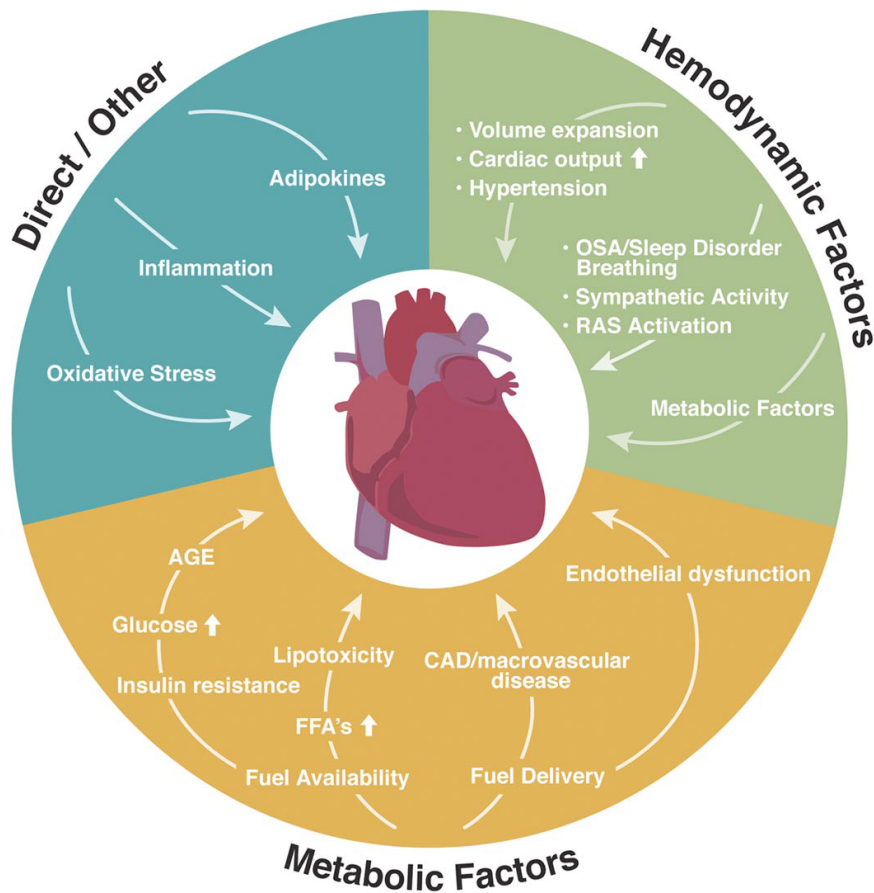


Figure 10. Potential mechanisms via which obesity can influence structure and function of the heart (121)

LVH has a dose response relationship with the severity and duration of obesity. In the Framingham study, for every $2\text{kg}/\text{m}^2$ increase in BMI there was a 47% increased risk of LVH in men and 51% in women. They also demonstrated the additive effects of BP and BMI on LVM resulting in a 17 fold increased risk of LVH compared to the absence of either risk factor (97). In the Tromso study, BMI was the most important variable for having LVH (98). In normotensives, the presence of obesity results in an approximately 18 fold increased risk of

LVH compared to normal-weight individuals (122). In the Strong Heart Study (SHS), LVM was found to correlate better with fat free body mass than with adipose mass, waist/hip ratio, height or height^{2.7} (123). Obstructive sleep apnoea (OSA) is also frequently diagnosed in obese patients. Majority of previous studies have found OSA to be associated with LVH. This maybe due to the associated increased sympathetic tone, hypertension and intermittent hypoxia. A study by Avelar *et al.* looking at severely obese patients showed that the strongest independent predictor of LVH was the average nocturnal oxygen saturation <85% which was followed by BP and BMI (124). This study also suggested that nocturnal oxygen desaturation and not the apnoea-hypopnoea index maybe the true culprit for LVH.

1.4.4.3 Diabetes or insulin resistance

Patients with diabetes are well known to have an increased risk of developing LVH. In the Framingham study, the presence of diabetes or glucose intolerance in women was significantly associated with an increased LV wall thickness and LVM corrected for height (125). Additionally, a study of 173 type 2 diabetics attending a local diabetic clinic in Dundee found LVH (LVMI >134g/m² in men and >110g/m² in women) to occur in 32% of the diabetic patients (106). Following this, Dawson *et al.* carried out an echocardiographic study of 500 type

2 diabetics in Dundee of which 71% had LVH when LVM was indexed to height^{2.7} compared to 43% when LVM was indexed to BSA (109).

This association between diabetes and LVH may be due to a number of factors including insulin resistance, increased oxidative stress, endothelial dysfunction, associated obesity and hypertension. Endothelial dysfunction increases LV afterload, thereby contributing to the development of LVH. The hyperglycemia also leads to non-enzymatic irreversible glycation of proteins resulting in the formation of advanced glycation end products (AGE products). These advanced glycation products activate protein kinase C, which induces proinflammatory cytokines, growth factor release, and fibrosis thereby resulting in endothelial dysfunction and cardiac hypertrophy (126).

A number of large observational studies such as Framingham (127), Strong Heart Study (128, 129) and Whitehall have found a correlation between LVH and insulin resistance although other studies have given conflicting results (130, 131). In animal studies, infusing insulin leads to an increase in LVM (132) whilst inhibiting IGF-1 results in a reduction in LVM (133). In the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale (PIUMA) study of patients with essential hypertension, Verdecchia *et al.* concluded that insulin and insulin-like growth factor-1 (IGF-1) levels independently predicted the LVM (134). Insulin is an anabolic hormone that could result in LVH by a number of

mechanisms. It binds to insulin or IGF-1 receptors resulting in increased DNA and protein synthesis (135). In addition to stimulating myocyte growth, insulin also stimulates collagen synthesis of the vascular smooth muscle cells (136). It also stimulates the sympathetic nervous system (137) and increases renal sodium reabsorption, thereby increasing blood volume (138). Figure 11 shows the potential mechanisms whereby insulin resistance can cause LVH.

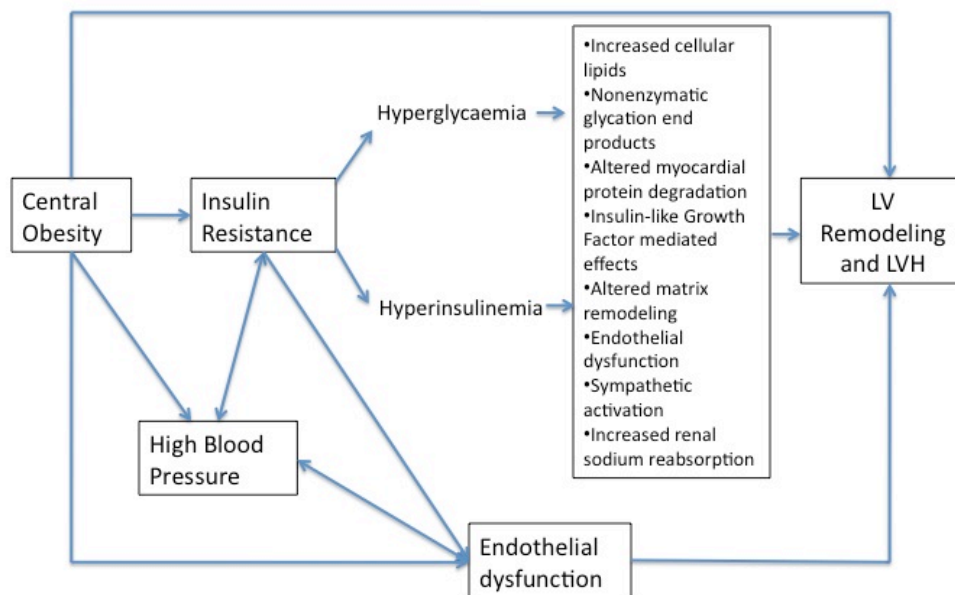


Figure 11. Potential mechanisms by which insulin resistance is associated with LVH (modified from Rutter et al.) (127)

1.4.4.4 Cardiovascular disease

Earlier studies have shown cardiovascular disease to increase the risk of LVH development. In the Tromso study, a history of cardiovascular disease had an OR of 2.22 for the presence of LVH (95% CI 1.63, 3.03) (98) whilst Liao *et al.* noted LVH to be more prevalent in the group with coronary artery disease (55.6%) compared to those without (44.3%) (108). This was again confirmed in the National Health and Nutrition Examination Survey (NHANES) II study where LVH patients were more likely to have had a previous MI (11.3% vs 4.0%; $p < 0.05$) (139).

In the event of an MI, neurohormonal activation (sympathetic nervous system and renin-angiotensin-aldosterone system) and generation of ROS and inflammatory cytokines stimulate intracellular signalling processes that result in cardiac remodelling. There is thinning of the infarcted myocardium with reactive hypertrophic changes of remote myocardium and LV dilatation (140). See Figure 12.

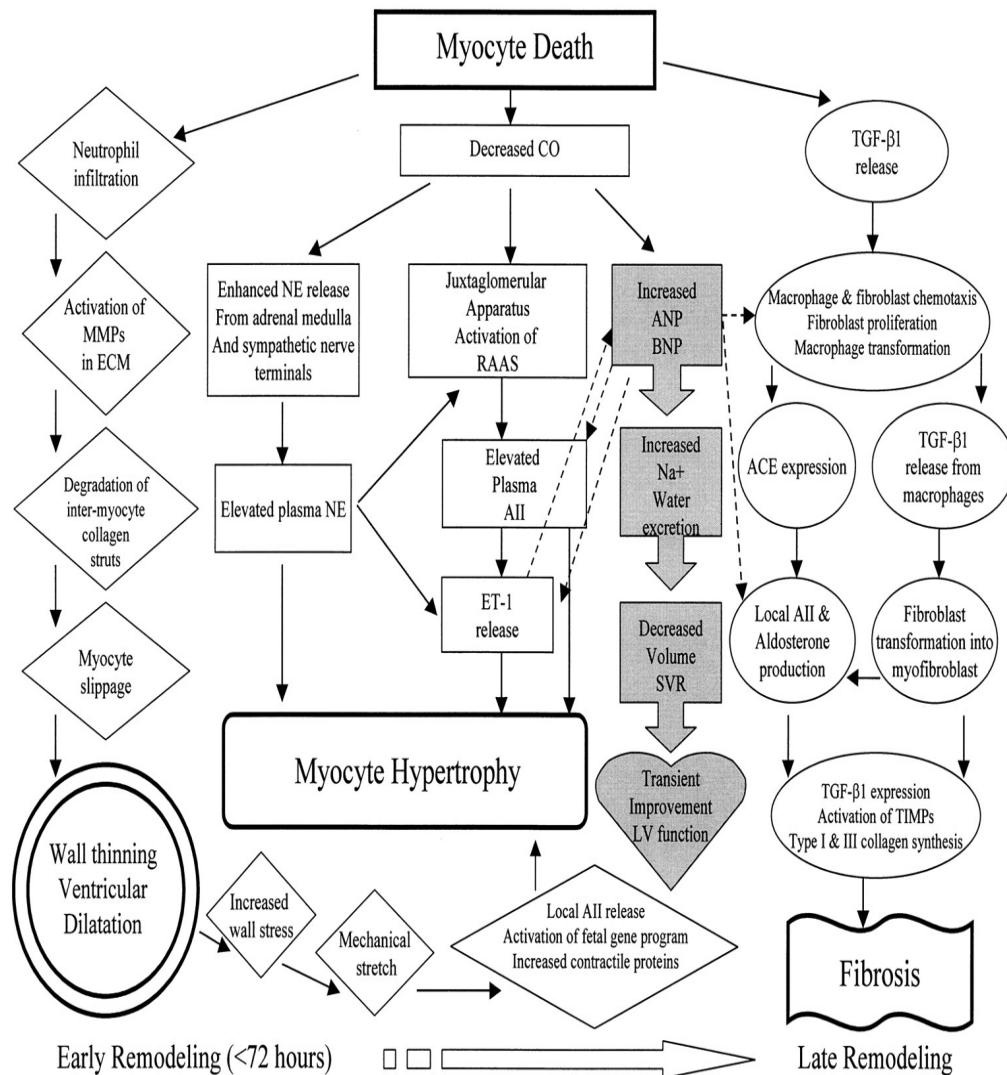


Figure 12. Diagram of the various factors involved in LV remodelling post MI (140)

Subjects with CVD also tend to have risk factors such as DM and HT, which are associated with LVH development as discussed already.

1.4.4.5 Sympathetic Nervous System

Animal studies have shown noradrenaline to stimulate myocardial cell hypertrophy via an $\alpha 1$ adrenergic mechanism (141, 142). Additionally a human study by Schlaich *et al.* showed that in a hypertensive population group, those that developed LVH had a significantly higher cardiac noradrenaline spillover (thereby indicating greater cardiac sympathetic activation) compared to those without LVH. However, the cardiac noradrenaline levels only explained 25% of LVM variance (143).

1.4.4.6 Renin-angiotensin-aldosterone system (RAAS)

The renin-angiotensin-aldosterone system plays a role in the pathogenesis of LVH development. In animal studies, angiotensin II has been demonstrated to induce myocyte hypertrophy (144, 145). Additionally, in patients with essential hypertension, angiotensin II levels positively correlated with LVH (146). In a study of 51 young, male normotensive or mildly hypertensive Caucasians, angiotensin II levels at high salt intake demonstrated a positive correlation with LVM in the hypertensive group which was independent of 24 hour ambulatory BP (147).

Angiotensin II stimulates the development of myocardial hypertrophy by a number of mechanisms. Firstly it is a potent vasoconstrictor and secondly it

potentiates the actions of the sympathetic nervous system, thereby increasing BP. Thirdly it increases oxidative stress by stimulating NADPH oxidase and fourthly it stimulates release of cytokines and growth factors that mediate myocyte hypertrophy. Fifthly angiotensin II stimulates fibroblastic activity (increasing myocardial fibrosis) and finally stimulates production of aldosterone which itself causes cardiac hypertrophy directly by its action on cardiac mineralocorticoid receptors and indirectly through its effects on blood pressure.

1.4.4.7 Age

In the Framingham study, the prevalence of LVH increased with age. LVH affected 6% of those aged less than 30 years compared to 43% of those aged over 69 years old. In fact the risk of LVH was found to increase by 15% in men and 67% in women for every increase in 10 years of age (97). The Tromso study found age to be a weak predictor of LVH, on multivariate analysis (98). On the other hand, the TOMHS study found that after adjusting for SBP and BMI, age was not related to increased LVMI (100).

1.4.4.8 Genes

Studies like the Framingham (148) and twin studies (149) have shown heritability to help explain some of the variance in LVM seen. In the

Framingham, the adjusted intra-class correlation for LVM was 0.15 for parent-child ($p < 0.01$), 0.16 for siblings ($p < 0.01$), 0.06 for second-degree relatives ($p = \text{NS}$) and 0.05 for spouses ($p = \text{NS}$). Heritability of adjusted LVM ranged between 0.24 for second-degree relatives to 0.32 for siblings. However, in a twin study undertaken by Swan *et al*, heritability helped explain 50% of LVM variance ($p < 0.01$) (149). As these studies indicate a genetic role in LVM variance, recently there has been considerable research interest to help identify the responsible genes. In 1994, Schunkert *et al*. found deletion polymorphism of ACE gene to be associated with LVH on ECG especially in normotensive males (150). However in the Framingham, no association was found between the ACE gene and echo LVM (151). As the previous studies had been underpowered, a meta-analysis of 52 studies was performed recently. This meta-analysis demonstrated DD homozygotes to have 1.59 times (95% CI 1.31, 1.92; $p < 0.01$) increased risk of developing LVH compared to II genotypes. This associated risk was found to be more pronounced in males (OR 1.47; 95% CI 1.2, 1.8; $p < 0.01$) and in subjects not on antihypertensives (OR 1.39; 95% CI 1.2, 1.62; $p < 0.01$) (152).

An experimental mouse study has also shown the expression of protein kinase C β to result in cardiac hypertrophy (153). There have also been conflicting results regarding polymorphisms of the aldosterone synthase gene and LVH (150, 154).

The difficulty in identifying the responsible gene may be explained by the fact that multiple genes determine LVM and not just a single gene.

1.4.5 Clinical score to identify LVH in patients with CAD

A validated clinical risk score has been developed by Ang *et al.* to help identify the presence of LVH in cardiovascular patients (see Table 5). Of the risk factors, the presence of bundle branch block on ECG carried the highest adjusted OR of 6.46 in predicting LVH (155).

Risk factor	Adjusted OR (95% CI)	p value	Points
Age (>65 years)	1.94 (1.13-3.35)	0.02	1
BMI (>30kg/m ²)	2.17 (1.24-3.80)	<0.01	1
History of hypertension	2.18 (1.28-3.73)	<0.01	1
Previous MI	1.88 (1.01-3.48)	0.05	1
High BP (>130/80mmHg)	1.73 (0.97-3.07)	0.06	1
Bundle branch block on ECG	6.46 (2.00-20.60)	<0.01	4

Table 5. Risk factors for LVH in CAD patients on multivariable analysis (155)

As the LVH score increased, there was an increase in specificity and PPV but at the great expense of sensitivity and NPV (See Table 6) (155).

LVH score	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≥ 1	98	11	53	88
≥ 2	72	34	57	74
≥ 3	66	70	69	67
≥ 4	33	91	88	57
≥ 5	16	97	84	53

Table 6. Sensitivity, specificity, PPV and NPV of different cutoffs of LVH score in CAD patients (155)

1.4.6 Methods diagnosing LVH

1.4.6.1 ECG

The 12 lead ECG was one of the initial methods of diagnosing LVH. In the Framingham study, LVH on ECG was present in 2.9% men and 1.5% women whilst on echo it was detected in 14.2% men and 17.6% women (156). ECG-LVH has been associated with increased morbidity and mortality (157, 158). It is a cheap, easily performed, widely available test with a high specificity. However, LVH on ECG has a low sensitivity, thereby limiting its ability as a screening tool to identify patients with LVH. The Framingham ECG-LVH criteria had a sensitivity of 6.9% and a specificity of 98.8% (156). A study by Ang *et al.* also found the ECG to be insensitive in detecting LVH in patients with stable angina

(159). Over the years, more than 30 ECG-LVH criteria have developed that have been validated by echo. Despite this, their sensitivities remain between 20-60% (160-162). The sensitivity of the ECG however does depend on the population group it is applied to. Due to differences in sensitivity and specificity of the various ECG criteria, meeting of one set of criteria may not mean meeting other criteria also.

Typical ECG changes found to be associated with the presence of LVH include increased QRS amplitude and duration, left axis deviation, prolonged intrinsicoid deflection and ST-T changes. The Sokolow-Lyon voltage (SLV) criterion, developed in 1949 is one of the most widely used criterion. As the QRS duration is an independent predictor of LVH, the product of Cornell voltage and QRS duration was found to significantly improve the detection of LVH (163). The Cornell product was used in the Losartan Intervention for Endpoint reduction (LIFE) study and is in the European Society of Hypertension guidelines (2007) for assessing LVH on ECG (164) (Table 7).

Name	Criterion	Sensitivity (%) (165)	Specificity (%) (165)
Sokolow-Lyon voltage (166)	$SV1+RV5$ or $V6 \geq 3.5mV$	4-52	53-100
Cornell-voltage (167)	$RaVL + SV3 > 2.8mV$ (men) $RaVL + SV3 > 2mV$ (women)	2-41	89-100
Cornell-voltage duration product (168)	$(SV3+RaVL) \times QRS$ duration $\geq 2440mVms$ (men) $[SV3+(RaVL+8mV)] \times QRS$ duration $> 2440mVms$ (women)	8-32	83-100
Romhilt-Estes point score(169)	1. Any limb lead R wave or S wave $\geq 2mV$ or $SV1$ or $SV2 \geq 3mV$ or $RV5$ to $RV6 \geq 3mV$ (3 points)		
Probable LVH if ≥ 4 points	2. ST-T wave abnormality not on digoxin (3 points) on digoxin (1 point)	7-68	85-99
Definite LVH if ≥ 5 points	3. Left atrial abnormality P terminal force in $V1 > 1mm$ deep with duration $\geq 40msec$ (3 points) 4. L axis deviation $\geq -30^\circ$ (2 points) 5. Intrinsicoid deflection in $V5$ or $V6 \geq 50ms$ (1 point) 6. QRS duration $\geq 90ms$ (1point)	0-41	71-100

Table 7. Various ECG LVH criteria sensitivity and specificity (165-169)

The presence of left bundle branch block on ECG is often seen in cardiac patients post MI or with a conduction abnormality. However, studies of the ability of the traditional ECG criteria to diagnose LVH in the presence of left

bundle branch block have given conflicting results (170-175). ECG diagnosis of LVH in the presence of left bundle branch block can be made upon fulfilling all three criteria (left atrial P wave abnormality, QRS > 155ms and precordial lead voltage criteria $SV_2+RV_6 > 4.5mV$) (170-175). Bundle branch block on an ECG has been shown to be associated with the greatest OR of predicting the presence of LVH in CAD patients (155).

1.4.6.2 Echo

For many years, echo has been the gold standard investigation for LVH and validating ECG-LVH criteria. It is eight times more sensitive in detecting LVH than ECG (156). It is a safe, noninvasive test with widespread availability and low cost, thereby resulting in widespread use in clinical practice and in epidemiological research studies. It has also been validated in necropsy studies (176). 2D M-mode measurement is a commonly used echo method of assessing LVM. In the parasternal long axis view, by aligning the beam perpendicular to the septum and just below the tips of the mitral valve, the M-mode obtained has clear endocardial border definitions to allow measurements to be made. Measurements are then put into a formula (such as Penn or Devereux modified cubed formula) to calculate LVM. Validation studies have shown good accuracy of using the M-mode calculation of LVM based on these cube formulae with necropsy (correlation coefficient > 0.9) (176).

Penn formula: $LV\ mass = 1.04 [(LVIDD+LVPW+IVSd)^3 - (LVIDd)^3] - 13.6$

Devereux modified cubed formula:

$LV\ mass = 0.8 [1.04 [(LVIDd+LVPW+IVSd)^3 - (LVIDd)^3]] + 0.6$

However, echo is limited by poor image quality in up to a third of cases (177-179). It is a technique that is operator dependent in terms of accurate beam alignment and measuring wall thicknesses. Additionally, echo relies on LV wall thickness measurements taken in a 2D plane, a mathematical formula and geometric assumptions of the LV shape (a prolate ellipsoid shape with a 2:1 ratio of long to short axis lengths) to calculate this 3D structure. Small errors in measurements will result in a large effect on LVM due to the cubing in the formulas. Erroneous results can be obtained if M-mode is used in distorted ventricles. M-mode has also been suggested to overestimate LVM by comparison to MRI in studies of patients with hypertension (180) and haemodialysis patients (181). However, this was not found to be the case in necropsy studies. Compared to MRI, 2D echo has been shown to have poor accuracy (SE of the estimate of 29 to 79g; 95% CI 57, 190g) and poor reproducibility (SD of difference between successive measurements of 22 to 40g; 95% CI 45, 78g) (182). Therefore, large patient numbers would need to be recruited in research studies and hence 2D echo isn't the best imaging modality to monitor the effects of medication on LVM.

According to the latest American Society of Echo (ASE) guidelines (183), LVH is defined as LVM indexed to BSA $>115\text{g}/\text{m}^2$ in men and $>95\text{g}/\text{m}^2$ in women. However, in the obese, indexing the LVM to BSA underestimates the presence of LVH whilst indexing the LVM to $\text{ht}^{2.7}$ seems to be more accurate in this population group (184). LVH is defined as $>49\text{g}/\text{m}^{2.7}$ for men and $>45\text{g}/\text{m}^{2.7}$ for women. However, it is important to note that LVM is a normally distributed variable and is in fact quite artificial to have a specific cut-off value to diagnose a person with LVH.

3D modalities such as 3D echo and cardiac MRI provide more robust measurements of LVM than 2D echo as it does not make geometric assumptions. 3D echo has been shown to be comparable to CMR and has a reasonable reproducibility (95% CI $\pm 45\text{g}$) (182). However, this technique requires skill and some patients may not have an adequate acoustic window to obtain images. See Figure 13.

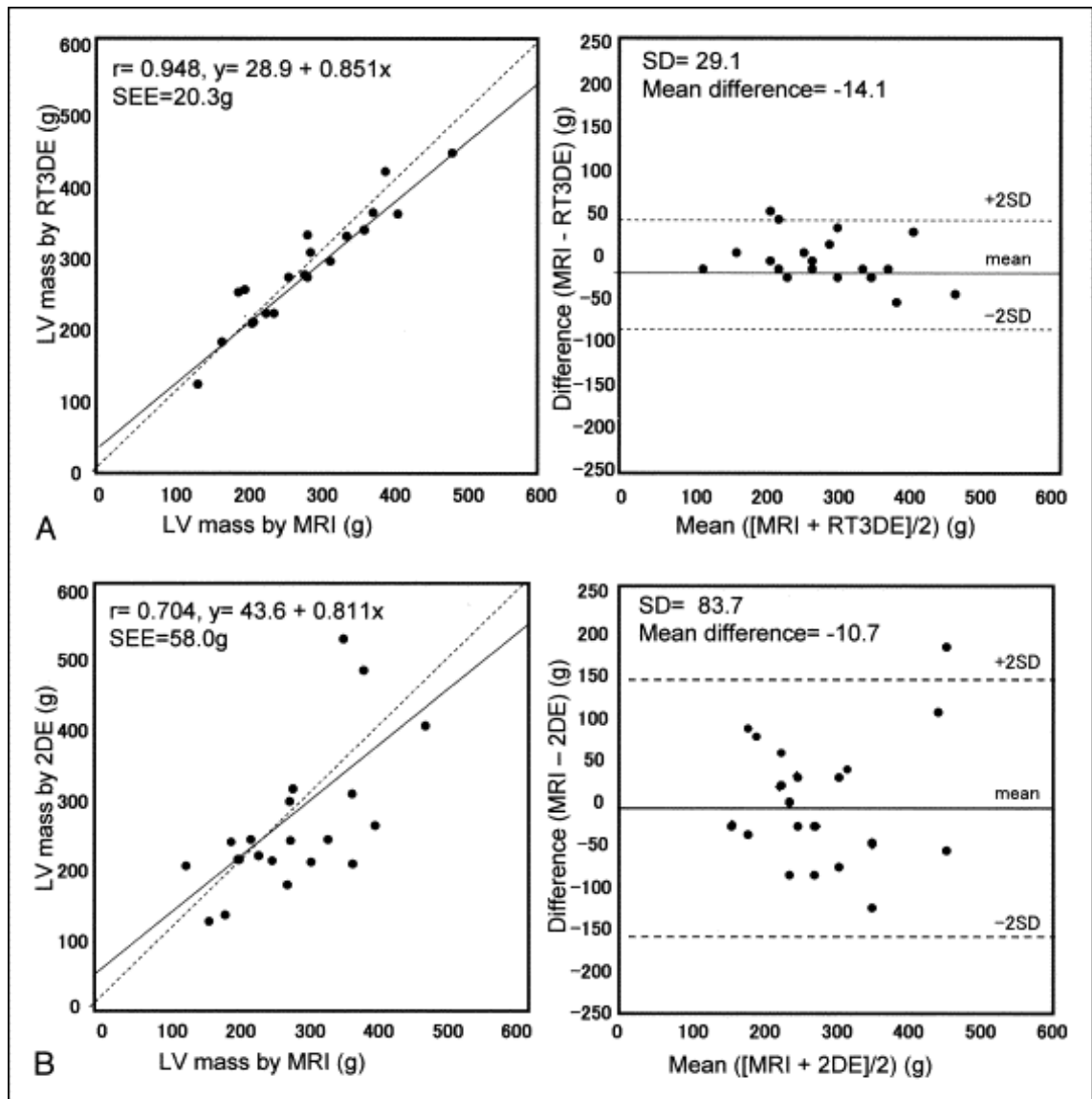


Figure 13. Comparison of 3D echo and 2D echo with MRI for measurement of LVM. A. 3D echo and CMR comparison for LVM measurement - Regression analysis (on left), Bland Altman plot (on right). B. 2D echo and CMR comparison for LVM measurement - Regression analysis (on left) and Bland Altman plot (on right) (185).

1.4.6.3 CMR

There is a significant variation and poor correlation in LVM measurements obtained by M-mode echo and CMR. M-mode echo tends to overestimate LVM (180, 181, 186). In a subgroup study of Prospective Randomized Enalapril Study Evaluating Regression of Ventricular Enlargement (PRESERVE), echo was found on average to have LVM values 27.6g higher at baseline and 37.1g at 1 year compared to MRI (187). In the past decade, CMR has been considered to be the gold standard of measuring LVM. Short axis stack is the most accurate method of assessing LVM. Compared to echo, MRI is more accurate as it does not make geometric assumptions and the myocardial borders are easily identified due to the superior spatial resolution and the contrast between myocardial tissue and blood. In a validation study of human postmortem hearts imaged ex-vivo, there is only a SD of difference of ~ 8g (95% CI ~15g) (188, 189). CMR is accurate even on assessing distorted ventricles such as post myocardial infarction (190) and hypertrophied ventricles (185). CMR is also highly reproducible to quantify LVM. CMR inter-study variability has a mean weighted SD of difference of 7.8g (95% CI 15.3g) (189, 191-193) compared to 27.7g and 19.2g respectively for M-mode and 3D echo (182). It also has a mean weighted intra-observer variability of 4.8g and inter-observer variability of 9g (193, 194). To detect a significant change in LVM of 10g, it is claimed that only 10 to 23 subjects will be needed for CMR (to power the study 80-99%) due to good reproducibility compared to 121 to 283 subjects for M-mode Echo and 58 to 136

for 2D echo (182). Other investigators feel the numbers proposed by these authors are too low. In addition, CMR will be the best modality to undertake serial monitoring to detect changes in LVM, even small ones, as it has a 95% CI of ± 12 to 22g (189, 191-193) compared to ± 45 to 78g for M-mode echo (189, 191), ± 39 g for 2D echo (195) and ± 13 to 45g for 3D echo (196). Other advantages of MRI include no contrast or ionizing radiation is required and there isn't a problem of acoustic windows as with echo.

However, MRI does have some drawbacks. It is not widely available, is expensive, needs specialized expertise and training to perform the scan and interpret images and scan time is at least 45 minutes. Additionally, it is difficult to obtain adequate images in patients with arrhythmias, patients need to be compliant with breath holding and can't be claustrophobic and patients with pacemakers, defibrillators and certain metal implants are contraindicated. Post-processing and interpretation of images can also be more time consuming compared to echo. As discussed already, short axis images are used to determine LVM. However, there is a need for subjective determination of the most basal slice near the mitral valve. As this basal slice has a large cross-sectional area, it will have a significant impact on the measurement of cardiac volume and mass (197).

Although FLASH (Fast Low Angle Shot MRI also known as fast gradient echo cine sequence) has been the traditional method for acquiring cine sequences for assessment of LV dimensions, in current practice SSFP (steady-state free precession or True FISP) cine sequences are used. It allows quicker imaging and improves endocardial and epicardial border definition with less blood flow dependence compared to FLASH, thereby enabling more accurate LVM and function assessment (198). On comparison with FLASH, LV mass measurements at end-systole and end-diastole are 4.8g and 6g respectively lower with SSFP ($p < 0.01$) whilst the mean LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) are higher (5.6mL and 5.3mL respectively; $p < 0.01$) (198).

Most of the CMR studies so far have been undertaken using 1.5T machines. However, 3T machines are now starting to be used increasingly in research. It has a higher field strength and higher signal-noise ratio (SNR), which improves endocardial border definition and image quality compared to 1.5T. Importantly, the increased field strength has not shown to affect the measurements of cardiac mass or volume. Hence normal values for cardiac mass and volume measurements obtained using 1.5T MRI can also be used for 3T scans (199).

1.4.7 Implications/prognosis of LVH

LVH is a strong risk factor for a poor prognosis due to cardiovascular events and all cause mortality in the general population (200), patients with hypertension (201), normotensives (139), coronary disease (202) and renal disease (203). It is also reversible. LVH is said to be the ‘most reliable surrogate marker we have in cardiovascular medicine’ (204, 205). It has been associated with an increased risk of cardiovascular events including coronary heart disease, MI, heart failure, stroke and arrhythmias.

In the Framingham study (a predominantly white general population group), the presence of LVH on the ECG was associated with a threefold risk of coronary heart disease after adjusting for hypertension (158). It also demonstrated LVH on echo to be associated with an adjusted relative risk for cardiovascular events of 1.49 in men and 1.57 in women (200). After adjusting for risk factors, LVH was associated with HR of 2.16 for sudden cardiac death (95% CI 1.22, 3.81, $p < 0.01$) and for each increment of 50g/m in LV mass, there was a 1.45 adjusted HR for sudden cardiac death (95% CI 1.10, 1.92, $p < 0.01$) (206). Additionally, the presence of LVH in middle aged subjects had a six-fold increased risk of stroke (207). Table 8 summarizes the gender-specific adjusted RR of LVH for outcome events for each 50g/m increase in LVM.

Outcome event	Men RR (95% CI)	Women RR (95% CI)
Cardiovascular disease	1.49 (1.20, 1.85)	1.57 (1.20, 2.04)
Cardiovascular mortality	1.73 (1.19, 2.52)	2.12 (1.28, 3.49)
All cause mortality	1.49 (1.14, 1.94)	2.01 (1.44, 2.81)

Table 8. Adjusted relative risk of outcome events for each 50g/m increase (200)

In a 30 year follow up study of the Framingham population, the presence of LVH on ECG had similar risks for clinical presentation and mortality from coronary heart disease as the presence of unrecognized MI on ECG (208). In the MAVI study, an Italian prospective observational multicentre study of 1,033 hypertensive subjects, the cardiovascular event rate was higher in the group with $LVM \geq 125g/m^2$ at 3.2 compared to 1.3 in the normal LVM group ($<125g/m^2$) ($p < 0.01$) (209). After adjusting for risk factors (such as age, smoking, diabetes and creatinine levels), the relative risk of cardiovascular events was 2.08 (95% CI 1.22, 3.57; $p < 0.01$) and there was a 40% increased risk of events associated with an increase in LVM by $39g/m^2$ (95% CI 14, 73; $p < 0.01$) (209) (see Figure 14).

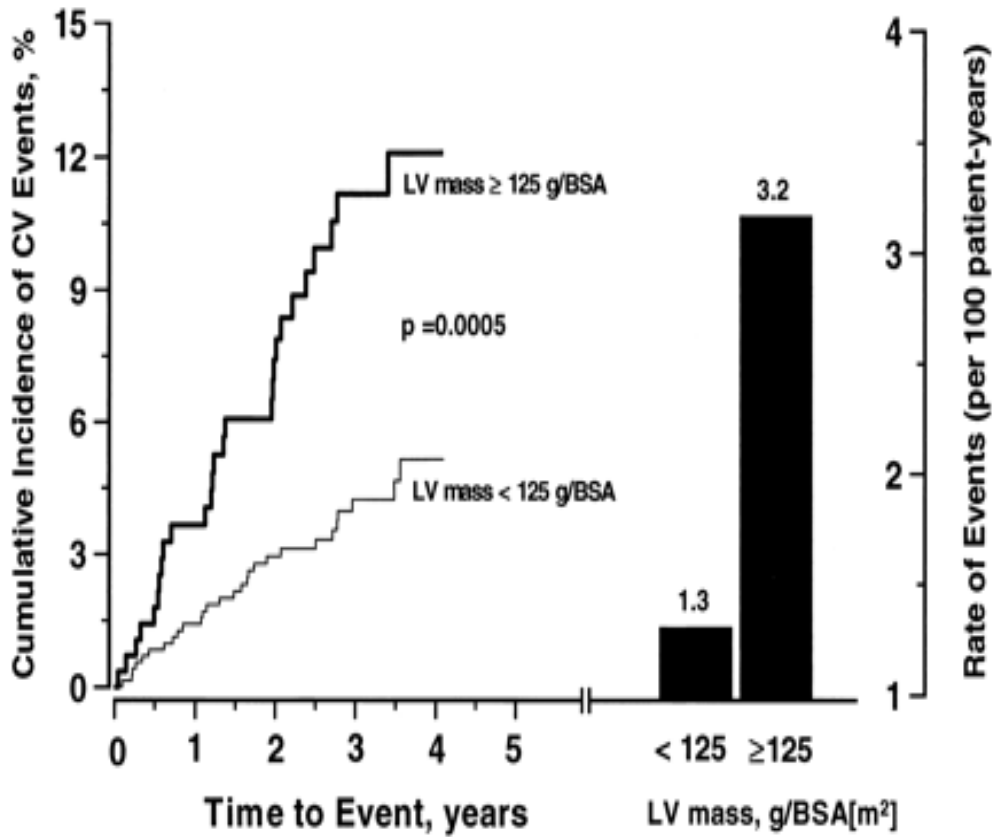


Figure 14. Cumulative incidence (left) and crude rate (right) of cardiovascular (CV) events in subjects with and without LVH on echocardiography (209).

In the PIUMA study of patients with essential hypertension, there was a progressive increase in cardiovascular morbidity and all-cause mortality as the LVMI increased from quintile 1 to 5 (101). The quintile cut-offs for LVMI were gender-specific (men: 92, 105, 120 and 138g/m²; women: 79, 91, 102 and 116g/m²) (see Figure 15).

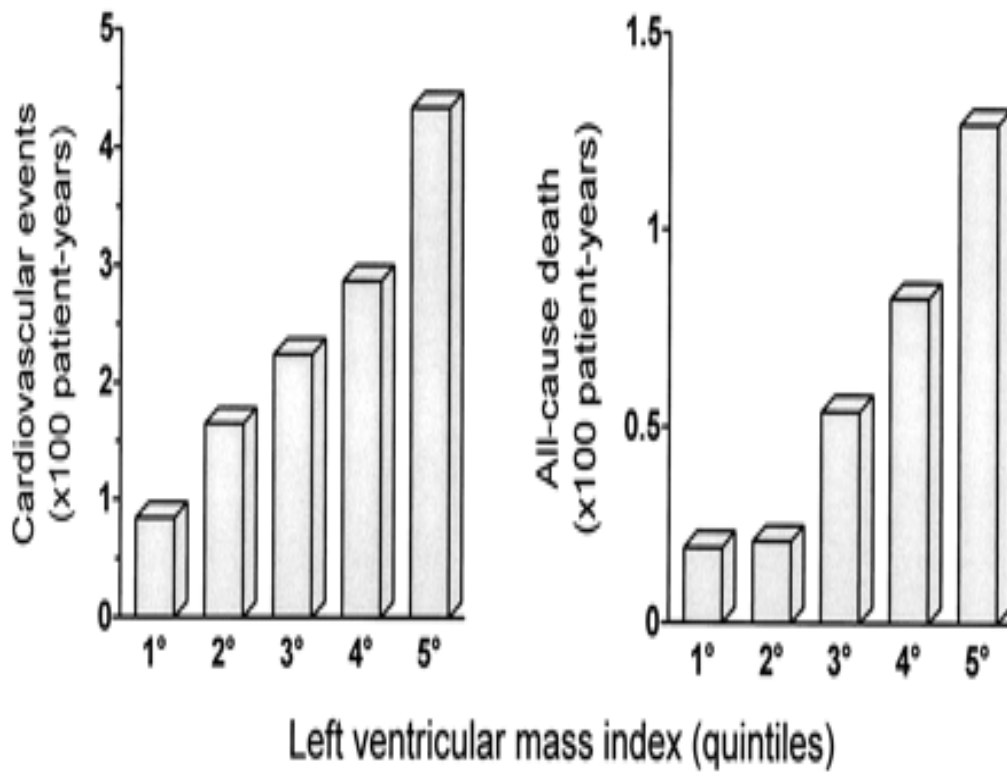


Figure 15. Progressive increase in cardiovascular morbidity and all-cause mortality from first to fifth quintiles of LVMI (101)

On multivariate analysis, patients with an LVMI in the third quintile and above (LVMI > 91.2g/m² in women and >105.4g/m² in men) had a significantly increased risk of cardiovascular events compared to the first quintile. However, in terms of all-cause mortality, only the patients in the fifth quintile of LVMI had a significantly increased risk compared to the first quintile. This is summarized in Table 9.

LVMI	CVS events		All-cause mortality	
	Adjusted HR (95 % CI)	p value	Adjusted HR (95% CI)	p value
Quintile 2 vs 1	1.55 (0.78, 3.08)	0.18	0.94 (0.19, 4.69)	0.94
Quintile 3 vs 1	1.92 (1.01, 3.98)	< 0.05	1.86 (0.48, 7.19)	0.37
Quintile 4 vs 1	2.97 (1.51, 5.84)	< 0.01	2.90 (0.82, 10.48)	0.09
Quintile 5 vs 1	3.51 (1.82, 6.78)	< 0.01	4.30 (1.16, 13.40)	< 0.05

Table 9. Adjusted HR of cardiovascular events and all-cause mortality according to LVMI quintiles (101)

The authors also commented that there was a significantly increased cardiovascular event rate even at LVMI levels $> 105\text{g/m}^2$ in men and $> 91\text{g/m}^2$ in women which are below the current cut-off level to diagnose LVH.

Vakili *et al.* undertook a comprehensive review of 20 studies published between 1960 and 2000 involving 48,545 subjects to assess the prognosis of LVH (210). LVH was diagnosed on ECG or echo using criteria or cut off values that varied between studies. The presence of LVH on ECG was associated with a 1.6 to 4 times increased risk of cardiovascular events and 1.5 to 6.8 times increased risk of all-cause mortality. LVH detected on echo had an adjusted RR for cardiovascular morbidity of 1.5 to 3.5 and 1.0 to 8.0 increased risk of all-cause mortality. Additionally, there was a trend that females with LVH had a worse

outcome than males. In the NHANES II mortality study, the presence of LVH had prognostic implications in both the hypertensives and normotensives (139).

A number of studies have been carried out to assess if whether the type of LV geometry adds any additional prognostic information to LVM. Studies by Koren *et al.* (201) and Ghali *et al.* (95) showed adverse prognostic significance of concentric hypertrophy. However, the Framingham Heart study (211) and a study by Verdecchia *et al.* (212) showed that LVM and not its geometric pattern gave prognostic information.

The prognostic value of LVH is independent of LV function and obstructive coronary artery disease (213). Cooper *et al.* (213) and Ghali *et al.* (202) have documented increased LVM to be associated with a worse prognosis of future mortality in those without CAD than with CAD.

1.4.7.1 Implications of LVH in patients with CAD

The presence and prognostic implications of LVH in CAD patients has been underappreciated despite evidence from a number of studies. A study of a predominantly black population undergoing coronary angiography between 1983 to 1991 found the presence of LVH in patients with CAD to be associated with approximately twofold increased risk of five year mortality, after adjusting for

baseline characteristics and ejection fraction (108). On multivariate analysis, the presence of LVH (RR 2.4) was an even better predictor of mortality than multi-vessel coronary disease (RR 1.6) or impaired LV function (EF <45%) (RR 2.0) (108). East *et al.* showed that the presence of LVH in patients with angiographically confirmed CAD to be the third best prognostic marker after age and coronary disease severity and to have a similar prognostic ability as ejection fraction. LVH was associated with an adjusted RR of 1.56 (95% CI: 1.35, 1.80) for all-cause mortality (107).

In acute MI patients with single vessel disease, LVMI was found to be an independent predictor of cardiac events over a mean follow up period of 32 months (214). The presence of LVH on ECG in acute MI patients is also associated with one and a half times increased one and five year mortality rates (215) and in patients who have undergone primary PCI, detection of LVH on ECG has an increased risk of future cardiovascular events (216). Some studies have found concentric LVH geometry to be associated with the highest risk of cardiovascular events in MI patients (217, 218).

1.4.8 Mechanisms whereby LVH increases CVD risk

1.4.8.1 Myocardial ischaemia

The presence of LVH may result in myocardial ischaemia by a number of possible mechanisms. Firstly, risk factors of LVH such as age, BP and diabetes are also risk factors for the development of atherosclerosis, thereby increasing the risk of cardiovascular events. Secondly, LVH has been associated with changes in blood components such as white cell count, fibrinogen levels and blood viscosity, which increase the risk of cardiac ischaemia (219, 220). Thirdly, in LVH there may be a mismatch in myocardial oxygen demand and supply. The increased muscle mass increases oxygen consumption whilst there is reduced oxygen supply due to impaired coronary flow reserve. Numerous early studies have shown the presence of a reduced coronary reserve in hypertensives with LVH in the absence of obstructive coronary artery disease (54, 221, 222). In a health screening programme of 70 year old males not known to have coronary disease, on exercise test the hypertensives with LVH had a significantly greater ST depression when compared to the healthy without LVH. Additionally, the hypertensive and normotensive groups with LVH were more likely to develop ST depression than the healthy group without LVH (>20% compared with 5%) (223). Hypertensive patients with LVH have also been shown to develop thallium perfusion defects in the absence of obstructive coronary disease (224).

Reduced coronary flow reserve occurs due to the increased resting myocardial blood flow (as a result of increased muscle mass) and blunted coronary vasodilatory capacity. The reduced vasodilatory effect may be due to external compression of blood vessels by the increased left ventricular muscle mass, reduced subendocardial blood flow due to elevated LV end-diastolic pressure, increased coronary vascular resistance, reduced capillaries supplying myocardium, remodelling of coronary vessels and endothelial dysfunction (222).

However, Vogt *et al.* found in a study of hypertensives with normal coronary arteries that there was no significant correlation between the degree of LVH and diminished coronary flow reserve. Instead the authors felt that the reduction in coronary flow reserve was secondary to vascular structural changes due to hypertension such as media hypertrophy, perivascular fibrosis and rarefication of arterioles (225, 226).

1.4.8.2 Heart failure

Studies of the general population (227, 228) and high-risk patients (229) have shown LVH to be a risk marker for the future development of heart failure. In the Cardiovascular Health study of 3,042 participants followed up over 5 years, increased baseline LV mass on echo or ECG was an independent predictor of depressed LV ejection fraction (228). In the Heart Outcomes Prevention

Evaluation (HOPE) trial of 9541 patients with a history of coronary artery disease, stroke, peripheral vascular disease or diabetes with at least another cardiovascular risk factor, 793 (8%) had ECG evidence of LVH. Over a mean follow up period of 4.5 years, 6.1% of those with ECG-LVH developed heart failure compared to 2.9% without ECG-LVH ($p < 0.01$) (229). Heart failure may arise in LVH due to neurohormonal activation, myocardial ischaemia or reduced LV compliance (resulting in diastolic dysfunction).

1.4.8.3 Atrial fibrillation

Traditional risk factors for atrial fibrillation (AF) include age, HT, DM, coronary heart disease and congestive cardiac failure. However, there is also a well-established independent relationship between LVH and AF. In the Framingham study, LVH on ECG was associated with 3-4 times increased risk of AF after adjusting for age which reduced to 1.4 times risk upon adjusting for other risk factors (230). Additionally, every increase of 4mm in LV wall thickness was associated with 28% increased risk of non rheumatic AF (230). In the PIUMA study of 2482 subjects with essential HT, for every 1SD increase in LVM there was 1.2 fold increased risk of AF (95% CI 1.07, 1.34) (231) (see Figure 16).

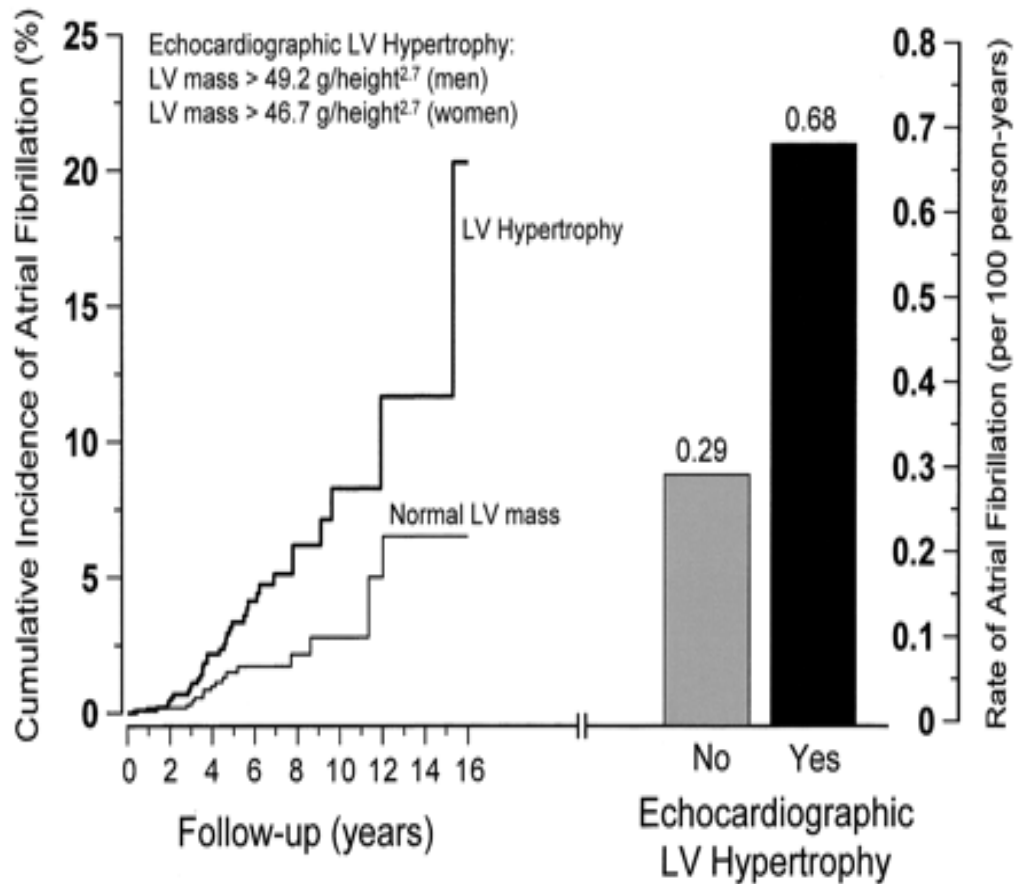


Figure 16. Incidence of at least 1 episode of AF in hypertensive subjects in sinus rhythm with or without LVH on echocardiography (231)

In LVH, the increased LV stiffness results in elevated LV end-diastolic pressure and left atrial pressure eventually leading to left atrial dilatation. In a LIFE sub-study of 941 hypertensives with LVH on ECG, left atrial enlargement was detected in 56% of women and 38% men ($p < 0.01$) (232). Additionally, the persistence or new development of LVH on ECG during a 3 year follow up period was associated with an increased risk of left atrial enlargement with an OR of 1.8 (95% CI 1.1, 3.2) and 3.1 (95% CI 1.3, 7.7) respectively (233). The

Framingham Study (230) and the Cardiovascular Health study (234) have shown left atrial dilatation to herald AF development. In the Framingham, for every 5mm increase in left atrial size, there was a 39% increase risk of AF (230). In the PIUMA study, left atrial diameter lost its ability to significantly predict AF development upon adjusting for LV mass. However, in addition to age and LV mass, left atrial diameter independently predicted chronic AF ($p < 0.01$) (231). Structural remodelling and fibrosis in left atrial dilatation results in electrophysiological changes (influencing atrial depolarisation, shortening of refractoriness and prolongation of conduction time) leading to the formation of multiple re-entrant wavefronts and hence increased risk of atrial arrhythmias (235). LVH is also associated with cardiovascular risk factors that can increase the risk of AF development.

Left atrial size itself is an independent risk factor for major cardiovascular events. In the LIFE study of 881 hypertensives with LVH on ECG, baseline left atrial diameter/ht on Cox regression analysis had a HR of 1.98 (95% CI 1.02, 3.83 per cm/m; $p = 0.04$) for combined cardiovascular death, MI or stroke after adjusting for Framingham risk score and history of AF (236). Additionally in the Framingham study, upon multivariate adjustment every 10mm increase in left atrial size was associated with a RR for stroke of 2.4 in men (95% CI 1.6, 3.7) and 1.4 in women (95% CI 1.1, 1.7). However, this correlation of left atrial size and risk of stroke was attenuated on adjusting for LVM (237).

1.4.8.4 Ventricular arrhythmias

LVH has been associated with an increased risk of ventricular arrhythmias, which may account for the increased risk of SCD seen in this population group. Studies of hypertensive populations have shown those with LVH to have an increased ventricular ectopic activity (238) and episodes of non-sustained VT (239). In a study of 123 men with mild hypertension, Siegel *et al.* showed the presence of echo LVH to have an OR of 2.7 for ≥ 30 ventricular premature complexes per hour (95% CI 0.9, 8.0), OR of 1.7 for multiform extrasystoles (95% CI 0.8, 3.7) and OR of 2.3 for VT (95% CI 0.7, 7.1) (240). Ghali *et al.* showed that in 49 hypertensive patients without coronary artery disease on angiography, the presence of LVH on echo (defined by wall thickness ≥ 1.2 or by LVM indexed to ht ≥ 163 g/m in men and ≥ 121 g/m in women) was significantly associated with an increased frequency and complexity of ventricular arrhythmias (241). Every 1mm increase in wall thickness (IVS or posterior wall) resulted in a twofold increased risk of ventricular arrhythmias and threefold increase in complexity of ventricular arrhythmias (241).

Although not fully understood, there are numerous possible mechanisms to explain the arrhythmogenicity of LVH. Structural changes such as patchy interstitial fibrosis and collagen deposition affects the smooth propagation of electrical impulses through the myocardium. Additionally, there are electrophysiological changes of the hypertrophied myocardial cells that make

them pro-arrhythmic. They include prolongation of the action potential duration (242), slowing of membrane repolarisation (ie. increased QT interval resulting in generation of re-entry mechanisms) (243), repolarisation heterogeneity (resulting in increased incidence of T wave alternans) (244), generation of early and delayed afterdepolarisation (leading to triggered activity) (245) and expression of I_f channels (increasing automaticity) (246). Other possible mechanisms include associated myocardial ischaemia and neuroendocrine factors (sympathetic activity and RAAS) (247).

1.4.9 LVH regression

1.4.9.1 Implications of LVH regression

Numerous studies mainly of the effects of various antihypertensive therapies on hypertensive subjects have shown the beneficial effects of LVM regression independent of BP. These studies have demonstrated LVH to be a poor prognostic risk marker that is reversible. In the PIUMA study of 430 subjects with essential HT treated with antihypertensives and lifestyle measures, there was a reduced rate of cardiovascular events in those with LVM reduction (1.78 per 100 person-years) compared to those with an increase in LVM (3.03 per 100 patient-years) over a 2.8 years follow up period. In fact, a reduction in LVM was associated with a HR of 0.46 (95% CI 0.22, 0.99; $p=0.04$) for cardiovascular

events in a Cox regression model, after adjusting for age and baseline LVH on ECG (248) (see Figure 17).

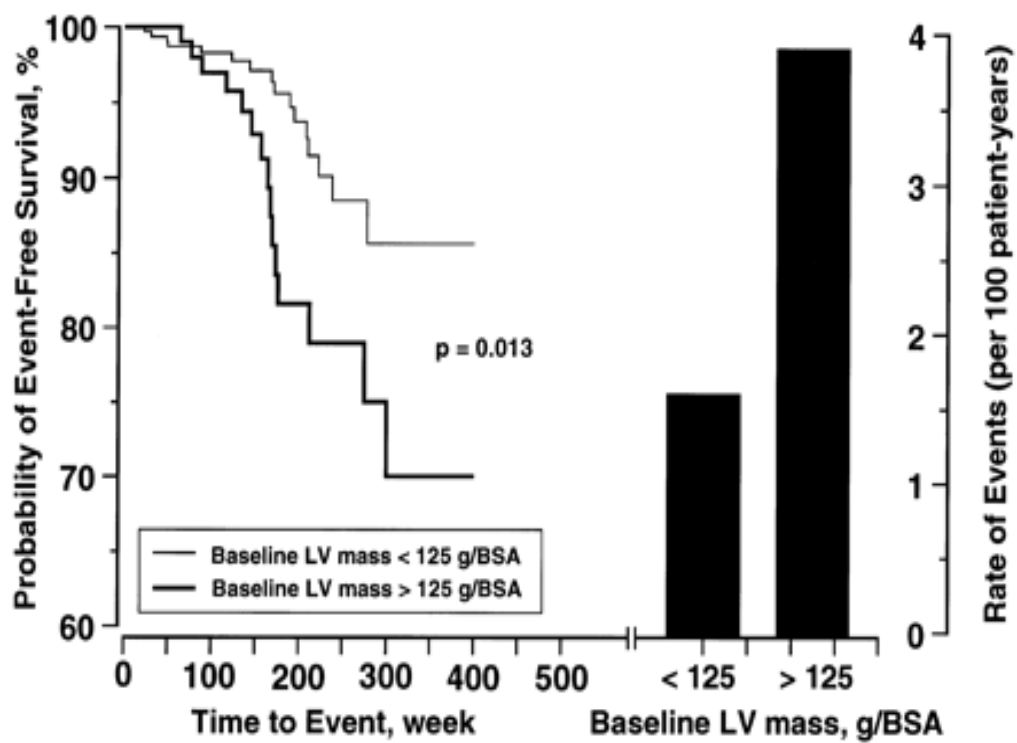


Figure 17. Event free survival in those with LVH (thick line) and without LVH (thin line) on echo at baseline (248)

On a subgroup analysis of subjects with a baseline $LVM > 125 g/m^2$, LVM regression was associated with a significantly reduced event rate of 1.58 events per 100 person-years compared to 6.27 events per 100 person-years in the non-regressors (248) (see Figure 18).

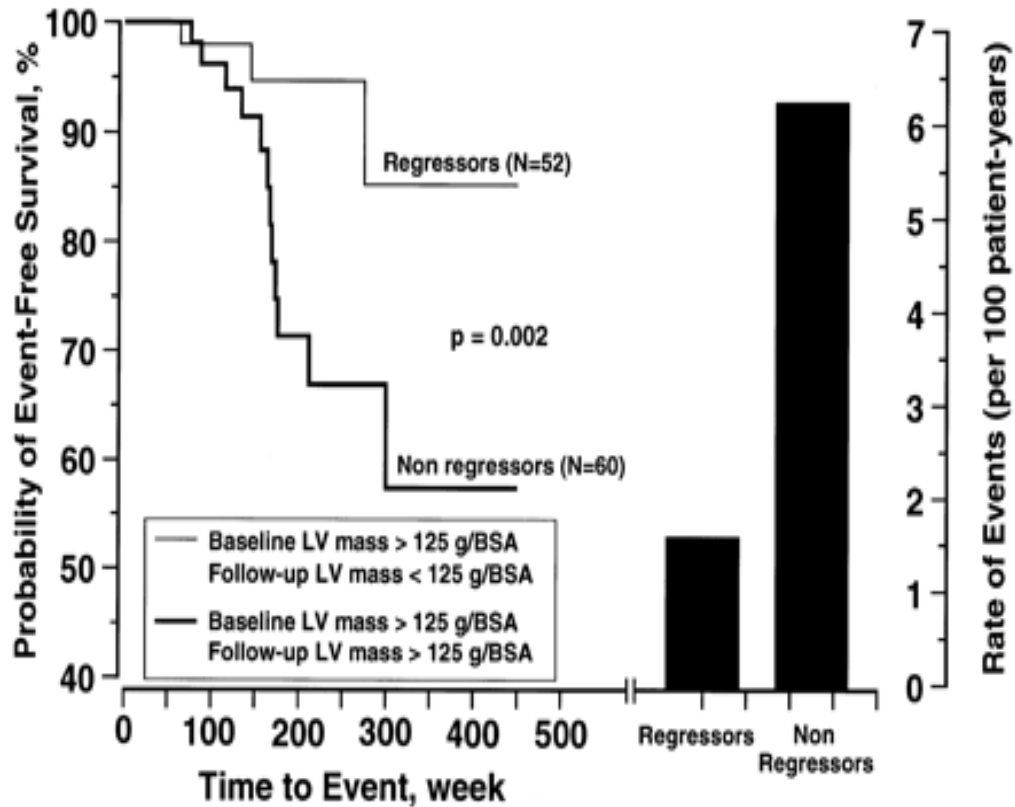


Figure 18. Event rate in subset with echo LVH at baseline visit. Survival curve of those with regression (thin line) or persistence of LVH (thick line) at follow-up (248)

A meta-analysis by Verdecchia *et al.* involving four studies and 1064 hypertensive subjects assessed the impact of LVM reduction on cardiovascular risk in hypertension. The authors noted that regression of LVH was associated with a 59% reduced risk of cardiovascular events compared to those with persistent or new LVH on echo ($p < 0.01$) (249).

The LIFE study has shown LVH regression *per se* to be associated with reduced cardiovascular morbidity (myocardial infarction, stroke, heart failure and AF) and mortality, independent of blood pressure reduction (250-252). A 1-SD (25.3g/m²) reduction in LVMI on echocardiography was associated with a 38% reduction in cardiovascular mortality, a 15% reduction in myocardial infarction and a 24% lower rate of strokes (250). On electrocardiography, a reduction of 1-SD of baseline mean by Cornell voltage-duration product criteria was associated with a 15% reduced risk of developing AF (252) and 28% reduced risk of sudden cardiac death (253). 1-SD of mean reduction on ECG using Cornell product criteria was associated with a 24% reduced risk of heart failure hospitalization (254). Additionally, patients with regression of ECG LVH over a 3 year follow up period was not significantly associated with an increased risk of left atrial enlargement as those with new or persistent ECG LVH changes (233). This may alter these patients' risks of developing AF in the future.

1.4.9.2 Methods of LVH regression

1.4.9.2.1 Pharmacological

In hypertensives, improved BP control has been found to be associated with LVH regression. Previous studies have noted that certain antihypertensives may be better in LVH regression compared to others and these effects may be even independent of blood pressure control. A meta-analysis by Klingbeil and

colleagues in 2003 found that in hypertensives, angiotensin II receptor blockers (ARBs), ACE inhibitors and calcium channel blockers were better at reducing left ventricular mass than beta blockers (255). However, a recent meta-analysis by Fagard *et al.* in 2009 did not show a significant difference in changes in LVM between the various drug treatments except that beta-blockers had less LVM regression compared to ARBs (256).

The HOPE study, assessing the use of ramipril in high-risk patients with controlled BP, found that treatment with ramipril 10mg/day was associated with a significant reduction in LVMI compared to placebo (15). In the LIFE study, losartan was associated with a significantly greater ECG-LVH reduction and LVMI regression compared to atenolol. Importantly both drugs reduced blood pressure equally and hence the superiority of losartan over atenolol at LVH regression was independent of systemic BP (16, 257). In the Candesartan Assessment in the Treatment of Cardiac Hypertrophy (CATCH) study, ARBs were noted to be as effective as ACE inhibitors in reducing LVMI in hypertensives with LVH. In fact, a non-significantly higher proportion of patients on ARB therapy achieved normalisation of LVMI (36.3% vs 28.6%) (258). Reducing systolic blood pressure (SBP) is effective in regressing LVM not only in the hypertensives but also in those patients within the normal BP range (259).

As aldosterone stimulates myocardial fibrosis and LVH, aldosterone antagonists such as spironolactone and eplerenone have been effective in regressing LVM in patients with hypertension and CKD (260-262). Statins, a drug commonly prescribed to patients with IHD, have also been shown in experimental and small scaled human studies to regress LVH by inhibiting neurohumoral activation of myocardium and inhibiting activation of Ras and Rho which play a role in oxidative stress, the production of nitric oxide and the activation of genes involved in myocyte growth (263).

1.4.9.2.2 Non-pharmacological

Non-pharmacological methods of LVH regression include reduction in weight and dietary sodium intake. As discussed already, obesity is a risk factor for the development of LVH. A study by MacMahon and colleagues of 41 young overweight hypertensive patients found that a mean weight reduction of 8.3kg was associated with a 16% LVM reduction which was independent of BP change (264). Additionally, weight loss by gastroplasty in normotensive morbidly obese patients led to a significant reduction in LV mass/ht index and LV dimensions in patients with baseline LVH (265). Sodium restriction over one year in mild-moderate essential hypertension has also been shown to significantly reduce LVM by 5.4% and LVMI by 4.7% (266). Interestingly, the TOMHS study of 844 mild HT found the addition of antihypertensive therapy to be no more

effective in LVM regression than just lifestyle changes to reduce weight, dietary sodium, alcohol consumption and increase physical activity (100).

1.4.9.3 Normotensive LVH and regression

As discussed previously, LVH also occurs in normotensive individuals. This may be because BP only accounts for 32% of variance of LVM. Other contributing factors for LVH development include age, race, obesity, insulin resistance/diabetes and neurohormones. As shown in the NHANES II mortality study, normotensives with LVH have a worse survival prognosis compared to normotensives or hypertensives without LVH (139). Hence, it is important to also treat these patients as aggressively as the HT with LVH (see Figure 19).

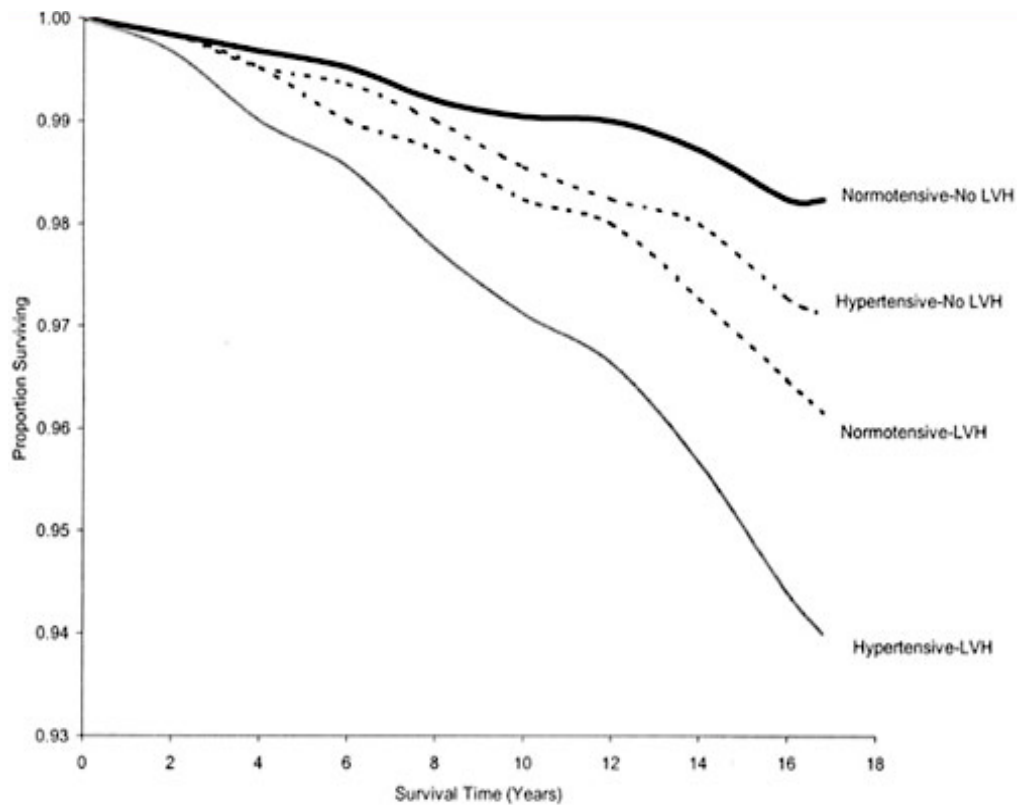


Figure 19. Age adjusted coronary heart disease survival probability based on Cox proportional hazards model (139)

In a HOPE sub-study of high-risk patients with a mean BP of 131/76 and preserved LV ejection fraction, the group treated with 10mg ramipril/day was associated with a significant reduction in LVMI of $2.02 \pm 2.25\text{g/m}^2$ over four years ($p=0.02$) (15). This benefit of ramipril on LVMI remained even after adjusting for age, gender, baseline LVMI and changes in BP. Additionally a CMR study by Simpson *et al.* in Dundee recently showed that even in normotensive patients with LVH, a further significant reduction in LVM was obtained on reducing SBP by ~ 9 mmHg with antihypertensive therapy (259).

1.4.10 CMR and Left atrial volumes

The left atrium is an often forgotten chamber that provides information regarding the left ventricular diastolic function and is a predictor of cardiovascular events such as AF, heart failure, stroke and mortality (267-269). Left atrial volume is superior to area or diameter in predicting adverse cardiovascular outcomes (270). 2D echocardiography has previously been the gold standard for measuring left atrial size but it is reliant on geometric assumptions and is user dependent. Left atrial volumes measured by 3D echo avoids the geometric assumptions and hence has better accuracy than 2D echo. It has been shown to correlate with measurements obtained by multidetector CT and MRI (the current gold standard) (271, 272). However, echocardiography tends to underestimate left atrial volumes compared to MRI, likely due to differences in spatial resolution (272). MRI is the current gold standard for measuring atrial volumes as it does not make geometric assumptions, there is good visualization of endocardial borders and hence there is accuracy and reproducibility.

1.5 B-type natriuretic peptide (BNP)

BNP is one of four natriuretic peptides that share a common 17-peptide ring structure. It is a neurohormone secreted predominantly by the ventricles in response to increased cardiac wall stress and tension. It is synthesized from the ventricular cardiomyocytes as a 132-amino acid peptide pre-pro BNP, which is

cleaved initially into pro-BNP, a 108 peptide, and then further cleaved into the biologically active 32-peptide BNP and the inactive 76-peptide NT-pro BNP (273). BNP causes vasodilatation, natriuresis, diuresis and inhibition of the renin-angiotensin and sympathetic system (273). BNP has a short half-life of 20 minutes compared to 60-120 minutes for NT-pro BNP. The levels of natriuretic peptides are affected by age, gender, BMI and renal function.

High ventricular filling pressures trigger secretion of BNP. Hence, there is considerable evidence using natriuretic peptides for diagnostic and prognostic purposes in patients with heart failure. It can also guide heart failure therapy (273). BNP levels are also elevated in the presence of myocardial ischaemia in patients with stable coronary artery disease and ACS (274-277). Higher BNP levels are associated with increased number of diseased coronary arteries (278, 279). It also has a prognostic role in patients with stable coronary artery disease and ACS (274, 280, 281).

BNP levels are elevated in the presence of LVH. In a study of 320 hypertensive patients, using a BNP cut-off value of 35pg/ml was 73% sensitive and 72% specific in diagnosing echo diagnosed LVH (282). Additionally, in an MRI study of hypertensive patients, a NT-proBNP cut-off value of 35pg/ml was 100% sensitive and 70.6% specific in diagnosing LVH (283). BNP correlates with LVM as pressure and volume overloaded situations stimulate both BNP release

by ventricles and ventricular hypertrophy. In a group of 215 patients referred to the hospital for breathlessness, NT-proBNP levels were significantly elevated patients with LVH whilst the presence of HT did not significantly affect the BNP levels (284). In an MRI study of hypertensives, there was a significant correlation between left ventricular mass and the NT-proBNP level ($r=0.598$; $p<0.01$) (283). In patients with hypertrophic cardiomyopathy, plasma NT-proBNP levels have also shown a positive correlation with LVMI ($r=0.27$; $p=0.05$) (285) and depended on the severity of hypertrophy (286).

BNP also provides prognostic information in patients with LVH. In a LIFE sub-study, Olsen and colleagues showed that in 183 patients with HT and LVH, NT-proBNP above the median value of 21.8 pmol/ml was significantly associated with an increased incidence of the composite endpoint of cardiovascular death, non-fatal stroke or MI in the 123 patients without a history of diabetes or cardiovascular disease. However, there was a non-significant increased risk of the composite endpoint with the 60 patients with a history of diabetes or cardiovascular disease (287).

1.6 Uric acid

As discussed previously, uric acid is an end-product of the purine degradation pathway that is excreted by the kidneys. Humans tend to have higher uric acid levels than other mammals due to inactivation of the uricase enzyme gene during the Miocene period 5-20 million years ago, which tends to breakdown uric acid into allantoin (288). Uric acid levels are higher in men, postmenopausal women, obesity, renal impairment, metabolic syndrome and those taking diuretics such as thiazides (289). Postmenopausal women have raised uric acid levels due to the loss of uricosuric effect of oestrogen (290). Uric acid has both pro-oxidant and anti-oxidant properties (291). Under normal circumstances, uric acid acts as an antioxidant by scavenging reactive oxygen species, chelates transition metals and blocks inactivation of antioxidant superoxide dismutase (289). However, otherwise uric acid becomes pro-oxidant resulting in endothelial dysfunction. In the presence of ruptured atherosclerotic plaques, a antioxidant-prooxidant uric acid redox shuttle occurs whereby the leak of iron and copper metal ions from the plaque undergoes the Fenton and Haber-Weiss reaction (292). See Figure 20.

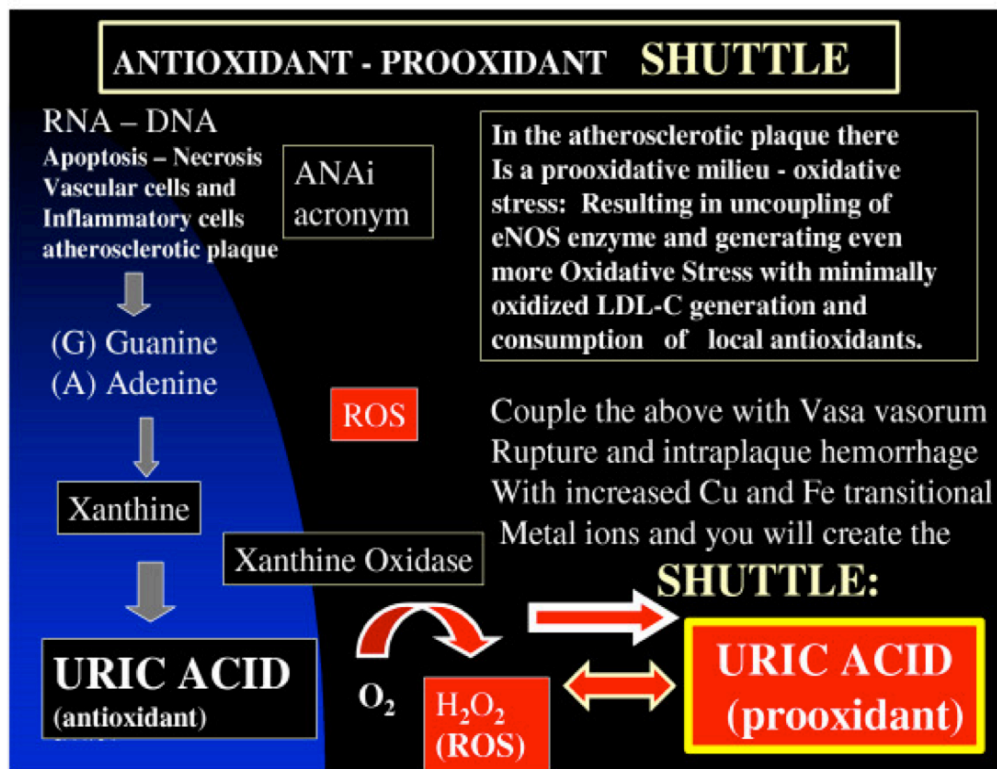


Figure 20. Antioxidant-prooxidant uric acid redox shuttle. It helps explain how the antioxidant uric acid becomes prooxidant in this environment, resulting in damage to endothelium and arterial vessel wall remodelling (292).

Increased serum uric acid levels have been shown to be associated with increased cardiovascular mortality (293, 294) and having increased cardiovascular events (295). Numerous epidemiological studies have shown a relationship between serum uric acid levels and hypertension, metabolic syndrome, coronary artery disease, stroke, heart failure and CKD (296). This association between serum uric acid levels and cardiovascular disease is not only seen in those with significantly raised uric acid levels (>360micromol/l in women and >420micromol/l in men) but also in those in the normal to high

range (310-330 micromol/l) (296). Additionally, the effect of serum uric acid level on cardiovascular events seems greater in women than in men (293, 295).

Figure 21 illustrates possible mechanisms uric acid may mediate cardiovascular disease, hypertension and CKD (291).

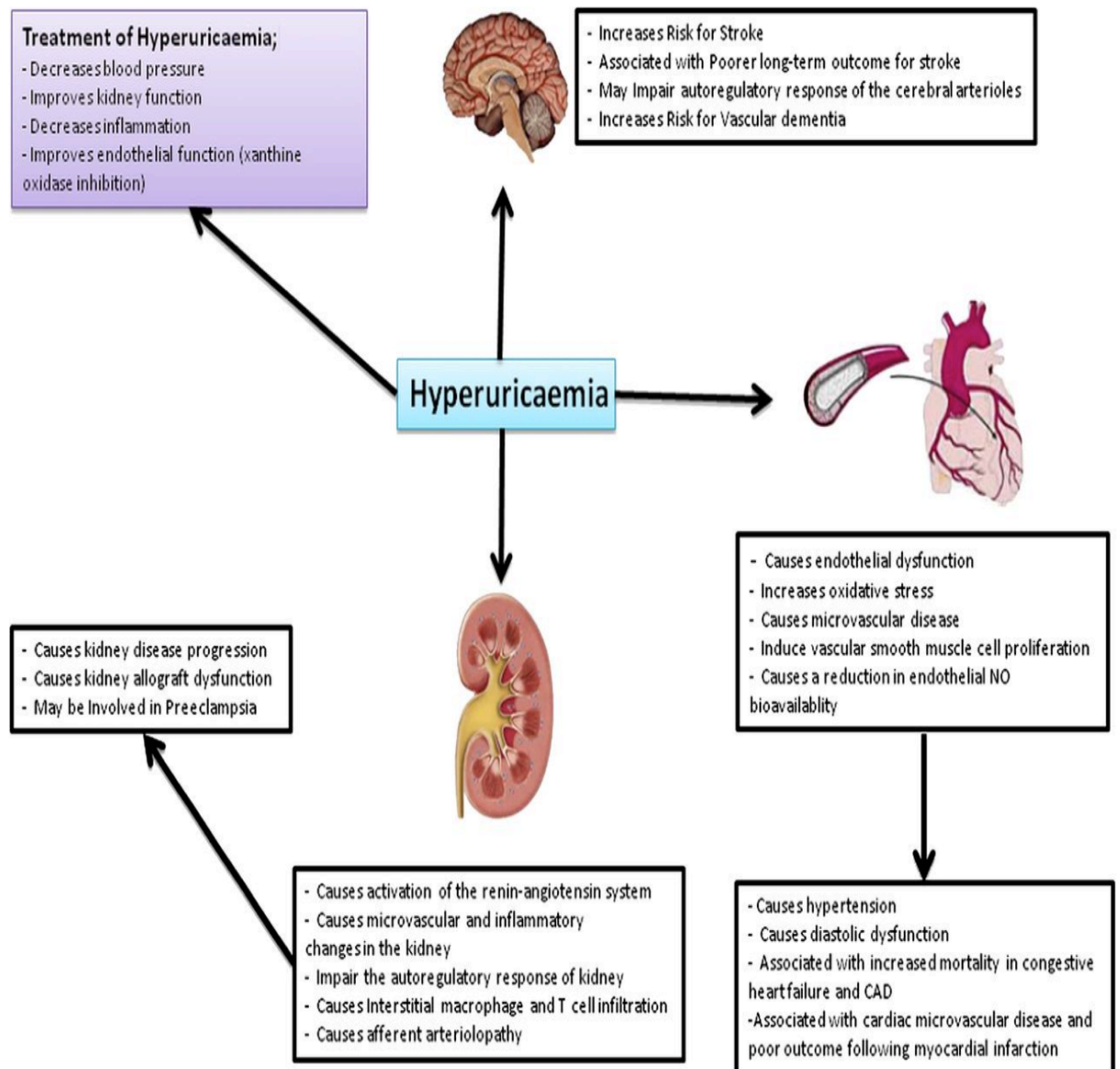


Figure 21. Possible mechanisms uric acid might mediate cardiovascular disease, hypertension and chronic kidney disease (291)

Other studies have suggested that serum uric acid is not a risk factor for cardiovascular disease but is associated with other cardiovascular risk factors such as hypertension, renal disease and increased lipoprotein levels (297).

1.6.1 Uric acid and hypertension

Elevated serum uric acid levels have been shown in numerous studies to be an independent predictor of hypertension (291). A meta-analysis of 18 cohort studies in 2011 showed hyperuricaemia to have an increased risk for hypertension with an adjusted risk ratio of 1.41 (95% CI 1.23, 1.58). Every 1mg/dl increase in uric acid level had an adjusted RR of 1.13 (95% CI 1.06, 1.2). The increased risk seemed greater in the younger subjects ($p=0.02$) and in women ($p=0.06$) (298). However, it is still unclear whether uric acid is the cause or is the consequence of hypertension. Animal studies have shown uric acid to cause hypertension by activating the renin-angiotensin system, downregulating NO, inducing endothelial dysfunction and proliferation of smooth muscle cells in the endothelium (299, 300). Additionally, the development of hypertension was avoided by a XO inhibitor or uricosuric agent (300). However, during the production of uric acid there is also generation of superoxide free radicals and so some of these effects may be actually related to the presence of the free radicals.

1.6.2 Uric acid and LVH

Uric acid has been identified to be independently associated with LVMI (301). After adjustment, each SD increase in uric acid levels has been associated with a 75% increased risk of LVH (302). In hypertensive Japanese patients, Kurata *et al.* found serum uric acid levels to correlate with LVM and LVMI in males but not females (303). More recently, Mitsuhashi *et al.* showed high uric acid levels to be associated with LVH diagnosed on ECG in healthy Japanese men (304).

Uric acid may be associated with LVH due to its effects on smooth muscle cell proliferation, endothelial dysfunction and stimulating inflammatory mediators such as TNF- α and MAPK, which are involved in the hypertrophic signalling pathway (305-307). It may also be due to its correlation with other factors that may be related to developing LVH such as obesity, renal dysfunction, hypertension and insulin resistance (301). However, randomized studies are required to assess if uric acid is a cause of LVH. In fact these randomized trials would need to use probenecid rather than allopurinol since the former reduces uric acid only whereas allopurinol reduces both uric acid and oxidative stress and either of these mechanisms could be involved in producing LVH.

1.6.3 Uric acid and endothelial dysfunction

Some studies have shown uric acid to cause endothelial dysfunction whilst others have not. An infusion of uric acid in healthy volunteers has been shown to worsen endothelial function (289) whilst lowering of uric acid with XO inhibitors improved endothelial function (291). Other studies have shown an infusion of uric acid to improve endothelial function in Type 1 diabetics and smokers (308), raised serum uric acid level not to be associated with endothelial dysfunction in healthy individuals (309) and lowering of serum uric acid in type 2 diabetics did not improve endothelial function in type 2 diabetics (310).

Uric acid can cause endothelial dysfunction by a number of mechanisms. Firstly, uric acid reacts with NO resulting in reduction of NO availability. Secondly, uric acid is pro-inflammatory activating MAPKs and nuclear transcription factors NF κ B, resulting in release of growth factors and vascular smooth muscle cell proliferation (289) (see Figure 22). Thirdly, it activates the renin-angiotensin-aldosterone system (291). However the uric acid molecule is also an antioxidant.

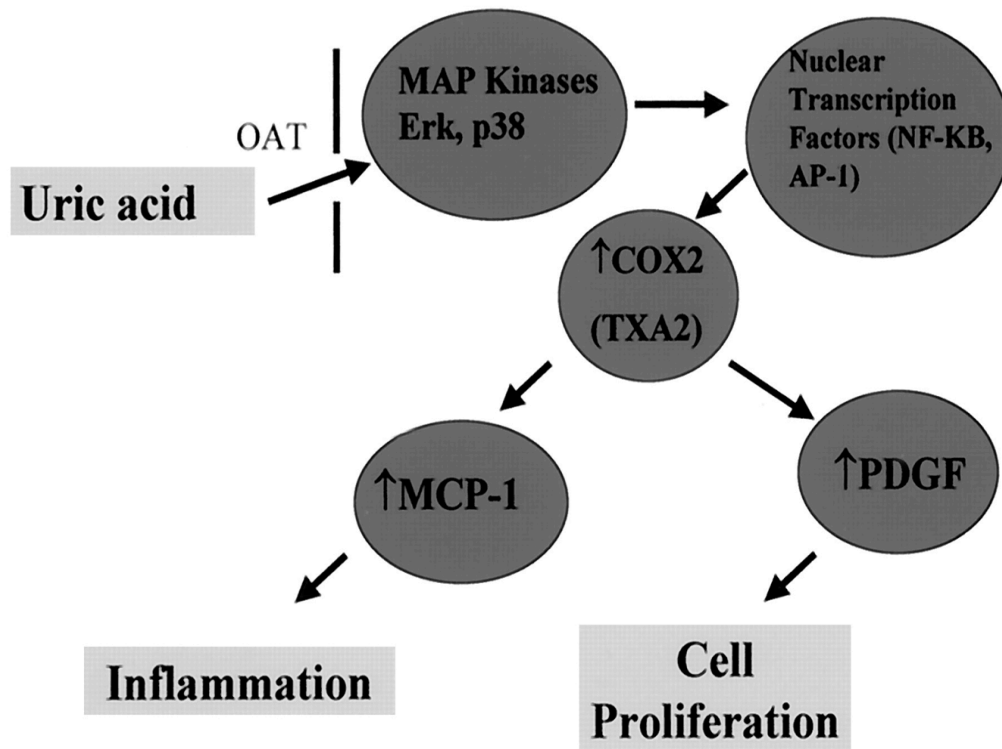


Figure 22. Pathway for uric acid mediated smooth muscle cell proliferation. Uric acid enters via an anion exchanger/transporter (OAT) where it alters intercellular redox, activates mitogen activated protein (MAP) kinases, cyclooxygenase-2 (COX2) and nuclear transcription factors leading to production of thromboxane, platelet derived growth factor (PDGF) and monocyte chemoattractant protein-1 (MCP-1) (289).

1.7 Allopurinol

1.7.1 Background

Allopurinol (1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) is a xanthine oxidase inhibitor that was developed in the 1950s during the search for new cancer treatments. Its discovery resulted in the 1988 Nobel Prize in Physiology and Medicine being awarded to Gertrude Elion and George Hitchings (30). Allopurinol also received Food and Drug Administration (FDA) approval as a prophylactic treatment for gout in 1966.

As already discussed, XO is an enzyme involved in the terminal part of the purine degradation pathway resulting in the formation of uric acid and ROS. As this enzyme has also been shown to play a role in various cardiovascular conditions such as ischaemia, hypertension, heart failure and endothelial dysfunction, XO inhibition has been shown to be beneficial in these conditions (30).

Allopurinol, a weak acid (acid dissociation constant, pKa 9.4), is an analogue of hypoxanthine. It acts as a competitive XO inhibitor at low concentrations but is a non-competitive inhibitor at higher concentrations. It is a prodrug that is oxidized by aldehyde oxidoreductase (AOR) into its active metabolite oxypurinol. For every 100mg of oral allopurinol, ~ 90mg oxypurinol is formed.

Oxypurinol, a stronger acid than allopurinol (pKa 7.7), is an analogue of xanthine and a non-competitive XO inhibitor (30). It is mainly responsible for the pharmacological effects of allopurinol as it has a very long half-life, hence attaining high plasma concentration levels.

There has also been a lot of interest to develop newer, more potent XO inhibitors in the last decade (see Figure 23). Febuxostat is one of the newer drugs that has been approved in the last 5 years as a treatment for gout.

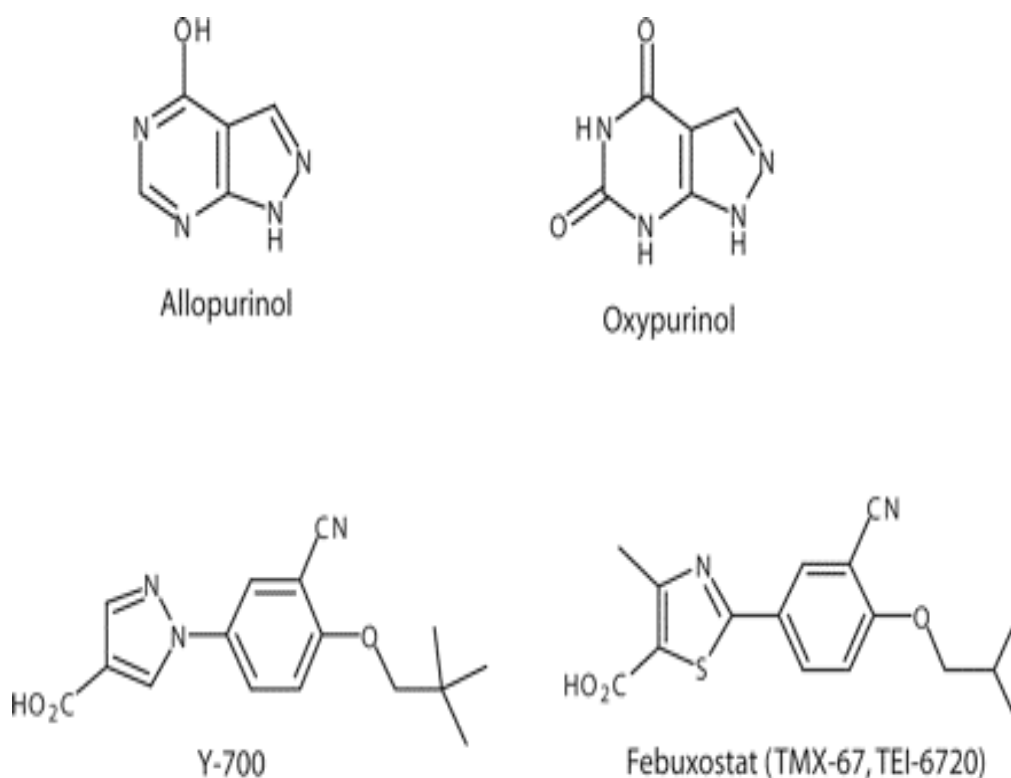


Figure 23. Chemical structures of xanthine oxidase inhibitors (30)

1.7.2 Pharmacokinetics and Pharmacodynamics

Oral allopurinol is absorbed rapidly from the gastrointestinal tract with a good oral bioavailability of $79 \pm 20\%$. 300mg allopurinol reaches peak concentrations of 2mg/L at ~1.5 hours and has a mean $t_{1/2}$ of 1.2 hours. Oxypurinol, on the other hand, takes longer to reach peak levels (~ 4 hours to reach peak concentration of 7mg/L) with a mean $t_{1/2}$ of 23 hours in healthy volunteers. However in patients with normal renal function, there is considerable variability in $t_{1/2}$ of oxypurinol of 9-38 hours (311). The steady state plasma concentration levels of oxypurinol are proportional to allopurinol dosage. After oral intake, the mean volume of distribution (V_d/F) of allopurinol is $1.31 \pm 0.41\text{L/kg}$ compared to $0.59 \pm 0.16\text{L/kg}$ for oxypurinol. A single dosage of allopurinol results in uric acid concentration levels to fall to its minimum over 6 to 24 hours and takes more than 48 hours to recover due to the long $t_{1/2}$ of oxypurinol and uric acid.

Oxidation to oxypurinol is the main route of elimination of allopurinol. The mean renal clearance (CL_R) of allopurinol is 1.54mL/min/kg . Oxypurinol on the other hand is mainly renally excreted with a mean CL_R of 0.34mL/min/kg (311). Therefore, it is important to reduce allopurinol dosage in the presence of renal impairment. In the kidneys, oxypurinol easily undergoes glomerular filtration in view of being unbound and of small molecular size. However, oxypurinol reaches high peak concentrations due to the significant tubular resorption.

Allopurinol's pharmacokinetics and pharmacodynamics *per se* are not affected by ageing. However, oxypurinol is affected by ageing due to the coexisting reduction in renal function. In the elderly, oxypurinol undergoes less clearance (0.24 ± 0.03 in elderly compared to 0.37 ± 0.05 ml/min/kg in the young), has a reduced volume of distribution (0.6 ± 0.09 vs. 0.84 ± 0.07 l/kg), achieves increased peak plasma concentration levels (5.63 ± 0.83 vs. 3.75 ± 0.25 mcg/ml) but has a reduced ability to inhibit xanthine oxidase (time-dependent reduction of plasma uric acid of 83 ± 30 vs. 176 ± 21 mcg/ml/h) (312).

1.7.3 Dosage

The initial dosage is 100-200mg with a maximum single dosage of 300mg. The daily dosage can be adjusted gradually every 2-4 weeks to avoid precipitating acute gout and to avoid side effects. Usual maintenance doses in gout are 100-200mg in mild gout, 300-600mg in moderate gout and 700-900mg in severe gout.

1.7.4 Drug interactions

Alcohol reduces the pharmacological effects of allopurinol by inhibiting its conversion to oxypurinol. Allopurinol increases the risk of rash when given with amoxicillin. Allopurinol also increases anticoagulant effect of warfarin therapy.

It has been shown to increase plasma concentration of cyclosporin and theophylline when given concomitantly and also increases the effects of cytotoxics such as azathioprine and mercaptopurine (313).

1.7.5 Side Effects

Allopurinol is a safe drug that has been used for over 40 years as a treatment for gout. However, there are associated side effects, which tend to be mild. Skin rashes are the most common side effect and tend to be reversible on withdrawing therapy. They may be pruritic, maculopapular or exfoliative. Other side effects include gastrointestinal (nausea and vomiting), malaise, headaches, vertigo, visual and taste disturbances, hepatotoxicity, neuropathy and blood disorders such as leucopenia, thrombocytopenia, haemolytic and aplastic anaemia (313). A rare but potentially fatal reaction associated with allopurinol therapy is allopurinol hypersensitivity syndrome (AHS) or drug reaction with eosinophilia and systemic symptoms (DRESS syndrome). Patients develop a fever, exfoliative rash, lymphadenopathy, arthralgia, eosinophilia, vasculitis, hepatitis and deterioration in renal function. These patients should have their allopurinol stopped immediately and occasionally corticosteroid therapy may even be required. Risk of developing this generalized hypersensitivity reaction is increased in the elderly with reduced renal function where the allopurinol dosage should have been reduced (313). The EuroSCAR study has shown higher doses

of allopurinol (>200mg/day) to be associated with an increased risk of developing Stevens-Johnson syndrome and toxic epidermal necrolysis compared to lower dosages (314). The presence of HLA-B*5801 allele is also an important genetic risk factor for developing severe cutaneous reactions. This genetic allele is more commonly seen in the Han Chinese, Thai and Japanese population than in the Western European population (315). Caution is advised for allopurinol therapy in patients with renal or hepatic impairment, pregnancy and breastfeeding (313).

1.7.6 Allopurinol therapy in various diseases

1.7.6.1 Myocardial ischaemia/ infarction

Ischaemic-reperfusion injury is myocardial damage by ROS that occurs on restoration of blood flow to ischaemic tissue. Increasing evidence supports the role of XO in ischaemia-reperfusion injury that was hypothesized by Granger *et al.* in 1981 and McCord in 1985. During ischaemia, there is increased ATP breakdown leading to the increased hypoxanthine and xanthine levels and increased production of XO. During reperfusion, XO generates superoxide radicals from the oxygen restored to the tissues resulting in tissue damage (24).

The role of XO in the pathogenesis of an MI has been extensively studied with conflicting evidence since the 1980s. Numerous experimental and human studies

have shown allopurinol therapy in the setting of an MI to reduce myocardial infarct size, reduce LV remodelling, improve ventricular function and reduce reperfusion-induced arrhythmias (30). Allopurinol achieves this by inhibiting myocardial XO activity, thereby decreasing myocardial lipid peroxidation and superoxide formation, and its direct free radical scavenging effects. However, other studies have not been able to confirm the beneficial effects of allopurinol therapy on myocardial ischaemia-reperfusion injury (30).

In the event of an acute MI, patients are currently treated with primary coronary intervention. As during reperfusion there is generation of reactive oxygen species, studies have been undertaken assessing the effect of allopurinol in patients undergoing primary PCI. In a randomized controlled trial (RCT) of 38 acute MI patients undergoing primary coronary angioplasty, a single 400mg pretreatment dose of allopurinol inhibited free radical production, resulted in a lower incidence of no-reflow or slow reflow of culprit artery ($p=0.05$), had a significantly higher cardiac index immediately post PTCA ($p=0.02$) and had a higher LVEF at 6 months compared to the control group ($p=0.04$) (316). In another small randomized, controlled study of 40 patients presenting with an acute STEMI undergoing primary PCI, 400mg of allopurinol/ placebo therapy was administered acutely and then 100mg was prescribed for a further month. Allopurinol was associated with greater ST elevation recovery ($p<0.05$), lower troponin level ($p=0.04$) and a lower major adverse cardiac events at one month

follow up ($p < 0.01$). However, there was no significant benefit on LV function (317).

It is important to note that the theory of ischaemia-reperfusion injury is applicable mostly in the setting of an MI resulting in myocardial damage and hence reduction in LV function. In the setting of ischaemia alone, the phenomenon of ischaemia-reperfusion is less relevant than in an MI ie. LV dysfunction is less likely after ischaemia than after infarction.

Allopurinol has been used in the setting of cardiac bypass surgery. Some but not all studies have shown allopurinol to reduce inhospital mortality rate, reduce perioperative MI, reduce cardiac arrhythmias, improve cardiac function and hence reduce the need for inotropic support postoperatively (30).

In a study of ischaemia alone, allopurinol has also been shown to have anti-ischaemic effects in angina patients. In a double-blind cross over study of 65 chronic stable angina patients, 600mg allopurinol for 6 weeks significantly increased median total exercise time (from 301s at baseline to 393s; $p < 0.01$), median time to ST depression (from 232s at baseline to 298s; $p < 0.01$) and time to angina chest pain (from 234s at baseline to 304s; $p < 0.01$) on undertaking an exercise treadmill test. The anti-ischaemic effects of allopurinol might have been due to reduced myocardial oxygen consumption and increased ATP energy

supply to ischaemic tissues as a result of reduced oxidative stress (318). On the other hand, offloading of the LV by allopurinol may have also contributed to this anti-ischaemic effect. It is unlikely that relief of ischaemia-reperfusion injury caused this anti-ischaemic effect of allopurinol as the anti-ischaemic effect was seen at peak exercise before any reperfusion would have occurred.

1.7.6.2 Heart Failure

XO plays a role in heart failure. In animal studies, the myocardial XO activity increases approximately four times with heart failure induced by pacing. In chronic heart failure, increased XO levels and activity have been shown to contribute to the high levels of oxidative stress seen in this condition. Additionally high uric acid levels are frequently seen in heart failure due to its increased production and reduced renal excretion (30). Hence one would expect XO inhibition to be beneficial in this patient group. A retrospective study of 1760 chronic heart failure patients found that long-term high dose allopurinol therapy of $\geq 300\text{mg}$ had a lower long-term mortality than low dose allopurinol therapy of $<300\text{mg}$ (RR 0.59; 95% CI 0.37, 0.95) (319). These findings were confirmed in a more recent population-based cohort study of 4785 heart failure patients (320).

“The failing heart - an engine out of fuel” (321). ATP is required for myocardial contraction, which is depleted in heart failure. In heart failure a phenomenon called “mechanoenergetic uncoupling” occurs which is the reduced efficiency of each myocardial contraction or loss of balance between ventricular work performed and amount of oxygen consumed. One of the possible mechanisms for this phenomenon is the upregulation of XO activity and reduction of NO synthase activity in heart failure (322).

Experimental and human studies have shown XO inhibition to have an inotropic effect and also improve the efficiency of myocardial contraction (19, 20). Cappola *et al.* administered intracoronary allopurinol to nine optimally medically treated heart failure patients with idiopathic dilated cardiomyopathy and found a significant reduction in myocardial oxygen consumption (MVO₂ peak effect, -16 ± 5%; p<0.01) and an improvement in myocardial efficiency (peak effects: dP/dtmax/MVO₂ 22±9%, SW/MVO₂ 40 ± 17%; p<0.05) without affecting cardiac load or systemic haemodynamics (19).

Opie recently illustrated potential sites of action of allopurinol for heart failure as shown in Figure 24.

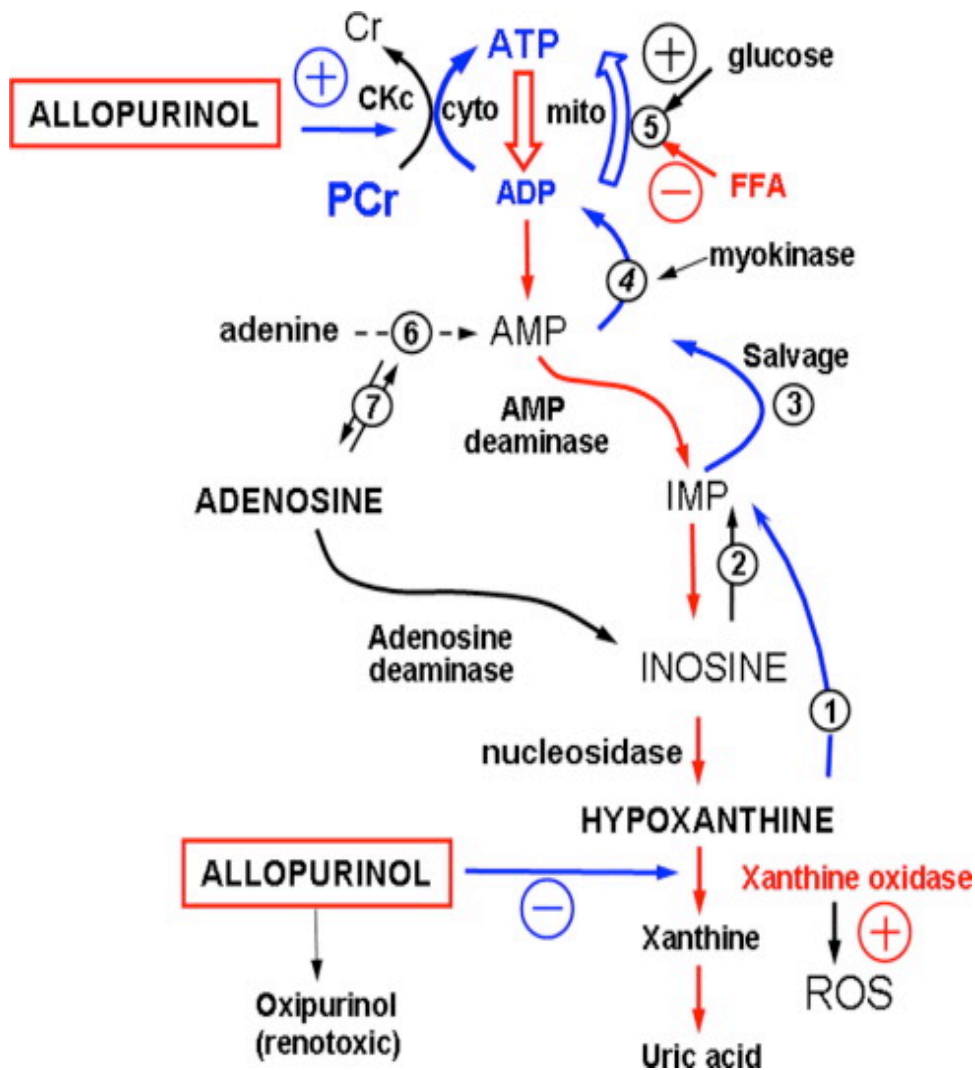


Figure 24. Potential sites of action of allopurinol therapy in heart failure (323)

Pathways indicated by numbers: 1) hypoxanthine phosphoribosyl transferase; 2) inosine kinase activity; 3) low-activity salvage path; 4) myokinase activity; 5) oxidative phosphorylation; 6) adenine phosphoribosyl transferase; 7) adenosine kinase. AMP=adenosine monophosphate; FFA=free fatty acid; IMP=inosine monophosphate.

Hirsch *et al.* showed allopurinol to improve myocardial energy delivery to failing hearts, by increasing high-energy phosphate stores (creatine phosphate,

ATP) and increasing ATP flux via creatine kinase (CK) by attenuating the inhibition of CK by ROS. Allopurinol also increases the amount of free energy released during ATP hydrolysis (324). These mechanisms enable allopurinol to improve mechanoenergetic uncoupling seen in heart failure.

Studies have also shown beneficial effects of allopurinol therapy on endothelial dysfunction in heart failure (will be discussed in further detail in the section on allopurinol therapy in endothelial dysfunction) and significantly reduces BNP levels (325, 326), which is a prognostic marker of heart failure and a measure of intracardiac pressure. However despite this, there has been no beneficial effect of allopurinol therapy noted on the exercise ability in chronic heart failure patients (325).

The OPT-CHF (Oxypurinol Therapy for Congestive Heart Failure) study failed to show any benefit of XO inhibition on the composite endpoint of heart failure morbidity, mortality and quality-of-life. However, a major flaw of the study was the inadequate dosage of XO inhibitor used. 600mg of oxypurinol was prescribed which is bioavailability equivalent to only 81mg allopurinol (327). Currently, the EXACT-HF study is enrolling 250 heart failure patients with high uric acid levels into a randomized controlled study to assess the effects of allopurinol on composite end points, quality of life and six-minute walk test.

1.7.6.3 LVH

Xanthine oxidase inhibitors have been shown to have beneficial effects on LVH in animal studies (328-330), in patients with heart failure (331) and in CKD patients (332). Allopurinol therapy of patients with CKD stage 3 over a 9 month treatment period significantly regressed LVM (332). However, renal patients have very high levels of OS compared to optimally treated patients with IHD which means we cannot be sure that optimally treated IHD patients will have enough OS for allopurinol to alter their LVH. Following on from these studies, the primary objective of this research study was to assess if allopurinol can regress LVM in patients with IHD and LVH.

1.7.6.4 Endothelial dysfunction

Previously published studies have shown allopurinol to reduce endothelial dysfunction associated with various conditions known to have high levels of oxidative stress including type 2 diabetes (333), heart failure (22, 326), CKD (332) and chronic stable angina (334). Allopurinol has also been shown to improve arterial stiffness in patients with chronic stable angina (334), stroke (335) and CKD (332).

2 METHODS

2.1 Study Aims

The main aim of this study was to assess the ability of xanthine oxidase inhibitors (using high dose allopurinol therapy) to regress LVM in patients with LVH and IHD. The secondary aim of this study was to assess the effects of allopurinol on endothelial function in patients with LVH and IHD.

2.2 Study Hypothesis

That nine months of allopurinol therapy will lead to a reduction in LV mass in patients with left ventricular hypertrophy and ischaemic heart disease when compared to placebo therapy.

2.3 Study Overview

This study was conducted between April 2009 and February 2011 at a single centre, Ninewells hospital, Dundee, Scotland. It was approved by the Tayside Research Ethics Committee (reference number 09/S1401/3) and was done in accordance with the Declaration of Helsinki. Approval was also obtained from the Medicines and Healthcare products Regulatory Agency (MHRA) (reference number 21726/0264/001-0001). All participants provided written informed

consent after receiving a patient information sheet at least 24 hours before. This study was registered with the International Standard Randomized Controlled Trial Number register ISRCTN 73579730.

2.4 Trial Design, Randomization and Masking

This study had a randomized, double-blind, placebo controlled, parallel group design which was undertaken over a nine month follow up period. A computer-generated random allocation sequence was employed and a copy was kept in the Clinical Trials Pharmacy department. The participants and the research team directly involved in the study did not have access to this allocation sequence. Additionally, the serum uric acid samples were not analyzed until end of the study.

2.5 Study Participants

Sixty-six adult participants were recruited from Ninewells hospital cardiology databases and local general practices between April 2009 and April 2010.

Inclusion criteria:

1. History of MI or chronic stable angina with a positive stress test (exercise treadmill test or myocardial perfusion scan) or angiographically documented significant coronary artery disease
2. BP < 150/90 mmHg
3. Presence of LVH on echocardiography based on ASE criteria: LVMI >115g/m² for men and > 95g/m² for women (183)

Exclusion criteria:

1. Already taking allopurinol
2. Prescribed azathioprine which interacts with allopurinol
3. Previous adverse reaction to allopurinol
4. Had active gout
5. Renal dysfunction (eGFR < 60ml/min): These patients were excluded as high dose allopurinol will be prescribed in this study and allopurinol is known to have increased side effects in renal impairment
6. Heart failure or LVEF < 45%

7. Contraindications to undergoing an MRI scan such as pacemakers or claustrophobia
8. Malignancy, pregnant, lactating or unable to give informed consent

2.6 Study visits and Investigations

After baseline assessments and investigations [blood tests, flow-mediated dilatation (FMD) of the brachial artery in response to hyperaemia, applanation tonometry and Cardiac Magnetic Resonance Imaging (CMR)], participants were randomly assigned to receive either allopurinol 100mg/day or placebo for two weeks. If this was tolerated, the trial medication dosage was increased to allopurinol 300mg/day for a month. After one month of treatment, the dosage was increased to 300mg twice daily of allopurinol or placebo therapy for a further 7 ½ months.

Patients were followed up at two weeks, six weeks, ten weeks, six months and nine months (see Figure 25). Safety blood tests (FBC, UE, LFT) were performed at each visit. Blood tests were taken at baseline and final visit for full blood count, renal function, liver function, uric acid, glucose, insulin, BNP and oxidized LDL. A urine sample was sent to the laboratory for a urine protein/creatinine ratio (UPCR) at baseline, six months and nine months visit. Office BP was measured for all the patients at each visit. Due to limited number

of ambulatory BP monitors, thirty-two randomly selected patients had a 24 hour BP ambulatory monitor attached at baseline and final visit. All investigations were carried out at the Centre for Cardiovascular and Lung biology, Ninewells hospital, Dundee except the CMR, which was undertaken at the Clinical Research Centre, Ninewells hospital, Dundee. During the study, all patients continued their concomitant medication.

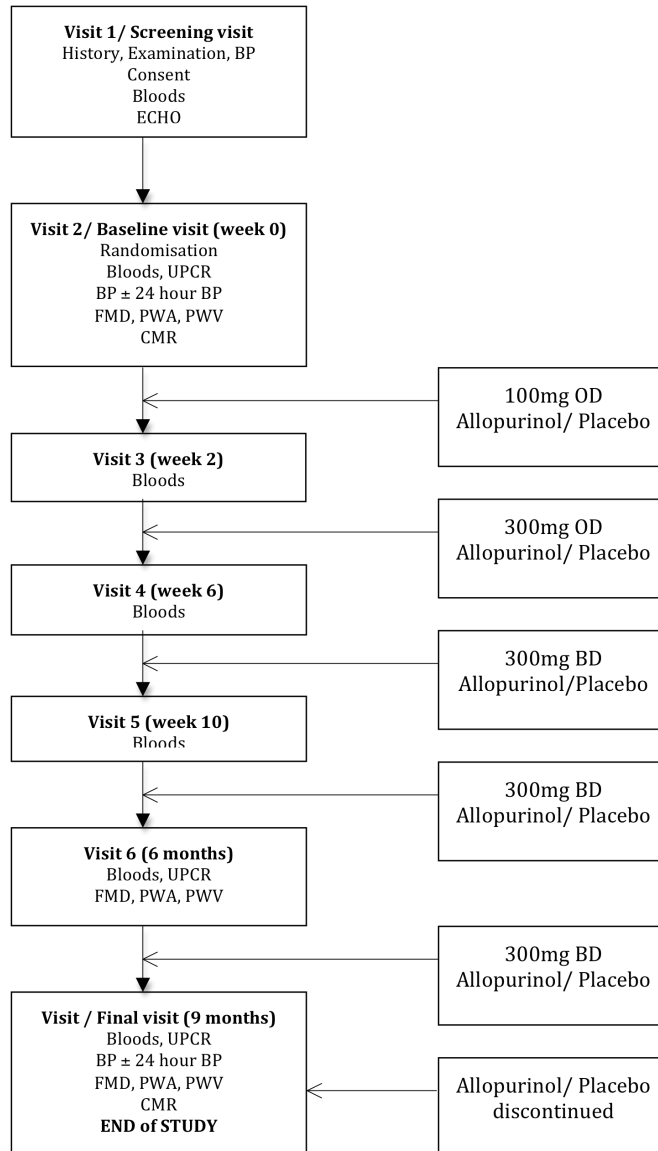


Figure 25. Flowchart of study visits

2.7 Endpoints

The primary endpoint was change in LV mass and LV mass index. Changes in endothelial function and arterial stiffness were secondary endpoints.

2.8 Bloods

Safety blood tests (full blood count, renal function and liver function) were taken at each study visit. Blood tests were performed at baseline and final visit for full blood count, renal function, liver function, uric acid, glucose, insulin, BNP and oxidized LDL.

2.8.1 Haematology and Biochemistry

Blood samples were collected in vacutainer tubes and sent to the Blood Sciences laboratory at Ninewells hospital for measuring full blood count, renal function, liver function and glucose. The samples were analyzed using a Roche Modular Analytics system (COBAS MIRAS) and Roche reagents. The normal reference ranges of these tests are shown in Table 10.

Blood test	Normal range
Haemoglobin (g/dl)	13.0 – 18.0 (men) 12.0 – 16.0 (women)
White cell count (x 10 ⁹ /L)	4.0 – 11.0
Platelets (x 10 ⁹ /L)	150 - 400
Sodium (mmol/L)	135 - 147
Potassium (mmol/L)	3.5 – 5.0
Urea (mmol/L)	4.0 – 12.0 (men) 3.3 – 6.6 (women)
Creatinine (μmol/L)	62 – 106 (men) 44 – 80 (women)
Bilirubin (μmol/L)	0 – 15
Alanine Transaminase (U/L)	13 – 43
Alkaline Phosphatase (U/L)	40 – 150
Albumin (g/L)	36 - 50
Glucose (mmol/L)	3.3 – 5.8

Table 10. Reference ranges of haematology and biochemistry tests

2.8.2 Uric Acid

At the baseline, 6 months and final visit, 5mls of venous blood was drawn into a serum tube and allowed to clot for 10 minutes at room temperature before centrifuging for 10 minutes at 3000 rpm at 4°C. The plasma was aliquoted into

1.5ml plastic containers and stored at -20°C. Uric acid was measured using a colorimetric assay (Alpha sentinel kit). Inter and intra-assay coefficient of variation (CV) was 5% and 5.4% respectively.

2.8.3 BNP

At baseline and final visit, 7mls of venous blood was drawn into EDTA bottles. The blood sample was immediately kept on ice and centrifuged as soon as possible for 10 minutes at 3000rpm at 4°C. The plasma was aliquoted into 5ml plastic containers and stored at -70°C. BNP was extracted from the plasma on C18 columns and measured using a radioimmunoassay (Bachem, St Helens Merseyside, UK). Inter and intra-assay coefficient of variation (CV) was 26% and 14% respectively.

2.8.4 Oxidized LDL

At baseline and final visit, 5ml of venous blood was collected into an EDTA blood bottle. The blood sample was centrifuged for 10 minutes at 3000rpm at 4°C and the plasma was stored at -70°C. Oxidized LDL was analyzed using a Mercodia ELISA kit. Inter and intra- assay coefficient of variation (CV) was 15% and 8.6% respectively.

2.9 BP monitoring

Office BP was measured using an Omron 705IT machine for all the patients at each visit (2, 6, 10, 28 and 39 weeks) by the same blinded investigator after the subject had rested for at least ten minutes. Randomly selected patients had a 24-hour ambulatory BP monitor (Spacelabs Healthcare, Hertford, UK) attached at their baseline and nine month visit.

2.10 Echocardiography

At the screening visit, the participant had a transthoracic echo to assess if they fulfilled the criteria for LVH and to exclude impaired LV function or significant valvular abnormality. The study was carried out using a Sequoia 512 (Siemens, Camberley, UK) ultrasound machine with a 8 MHz linear array probe. With the participant lying on the left decubitus position at a 45° angle, in the parasternal long axis view an M-mode measurement of LVM was obtained by aligning the beam perpendicular to the septum and just below the tips of the mitral valve leaflets. Measurements were made in end-diastole and at the onset of QRS complex as according to the American Society of Echocardiography (ASE) recommendation. Measurements of the IVS, LV internal dimensions and posterior wall thickness were made using the leading edge to leading edge convention and an average of 3 readings were taken (see Figure 26).

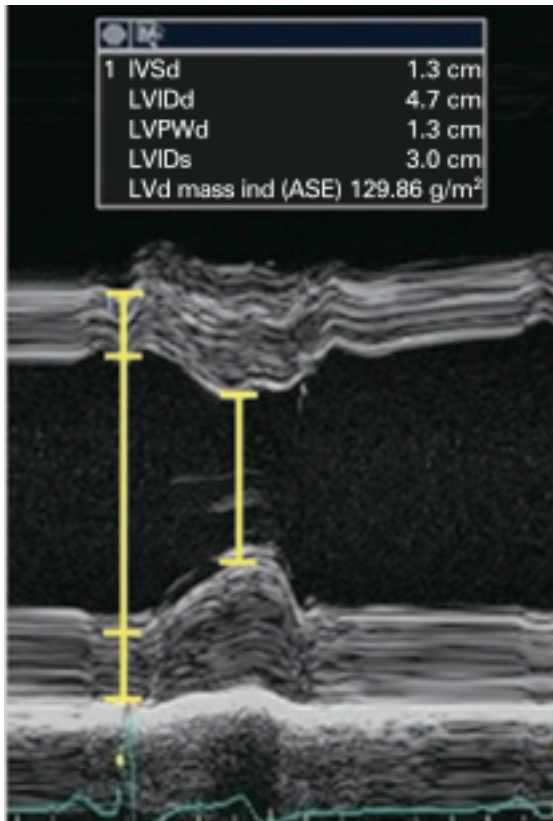


Figure 26. M-mode measurements for calculating LVM

LVM was calculated using the Devereux modified cubed formula: $LV\ mass = 0.8 [1.04 [(LVIDd + LVPW + IVSd)^3 - (LVIDd)^3]] + 0.6g$ (183). LVMI was calculated by indexing the LVM by BSA or $ht^{2.7}$. LVH was defined as LVM indexed by BSA $> 115g/m^2$ in men and $> 95g/m^2$ in women. LVH was also defined as LVMI indexed by $ht^{2.7} > 49 g/m^{2.7}$ in men or $> 45 g/m^{2.7}$ in women (183). If it was not obtainable to get the beam perpendicular to the septum, a 2D measurement of LVM was taken instead of M-mode.

2.11 Cardiac Magnetic Resonance Imaging

Cardiac Magnetic Resonance Imaging (CMR) was performed at baseline and at nine months on a 3T Magnetom Trio scanner (Siemens, Erlangen, Germany) using body array and spine matrix RF coils. The imaging parameters included repetition time TR 3·4ms, echo time of 1·5ms and flip angle 50°. Each slice was 6mm thick. Two slices were taken per breath hold of less than 15 seconds and scan time was minimized using parallel imaging “GRAPPA” factor of two.

2.11.1 Measuring LVM

Following acquisition of localizer scans, a stack of short axis images from the atrioventricular ring to the left ventricular apex were acquired using 2D ECG-gated breath-hold segmented CINE TrueFISP sequence with retrospective gating. CMR images were analysed offline by an independent, blinded MRI Physicist (SG) using commercial software (‘Argus’, Siemens Multi-modality Work Platform, version VB 15). Electronic ‘region of interest’ (ROI) contours were placed around endocardial and epicardial left ventricular borders on all MR image slices at end-diastole and end-systole that were identified to contain 50% or more full-thickness myocardium. From these contours, quantitative measurement of LVM, ejection fraction (EF), end-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV) were derived (Figure 27). Papillary muscles were included in the LVM if the muscle structure was

indistinguishable from the myocardial wall, but otherwise assigned to the LV blood pool. The process of contour placement was repeated such that every patient dataset at both time-points was analyzed twice in order to optimize the measurement precision.

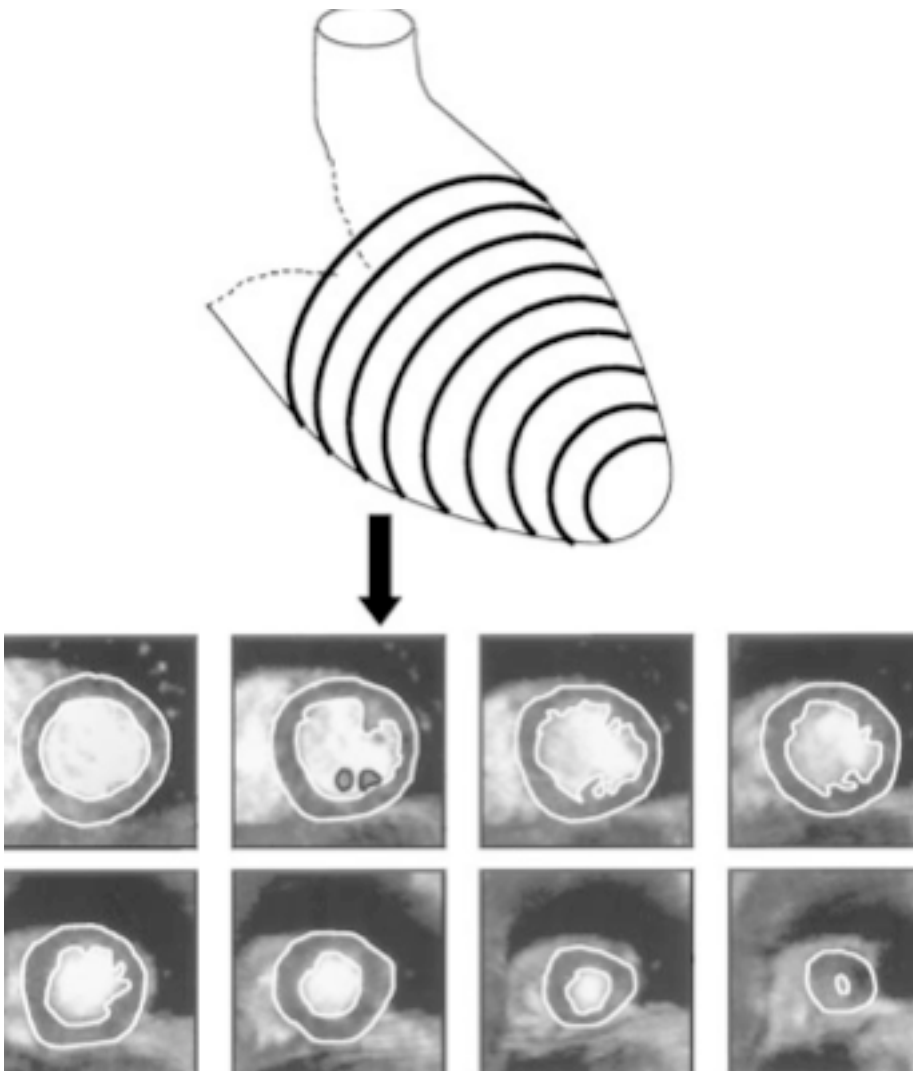


Figure 27. CMR acquisition of short axis images for measuring LVM and LV volumes (182)

Based on our recent departmental 3T CMR study on healthy volunteers, normal ranges of LVM and LV volumes are shown in Table 11.

	Mean	SD
LVEF (%)	68.2	5.2
EDV (ml)	150.8	27.6
ESV (ml)	47.7	10.8
SV (ml)	103.1	21.1
CO (L/min)	6.9	1.6
LVM (g)	112.2	26.0
LVMI (g/m ²)	60.0	8.6

Table 11. Departmental 3T CMR normal values for LVM and LV volumes.

Alfakih *et al.* reported the normal LVM/BSA using 1.5T CMR SSFP sequences was $64 \pm 9.3\text{g/m}^2$ in males and $52 \pm 7.4\text{g/m}^2$ in females (336). Studies have shown that normal values of LVM at 1.5T can also be applied to 3T scans (199).

2.11.2 Measuring left atrial volumes

Following acquisition of localizer scans, a stack of multi-slice 2 chamber images from the lateral side of left atrium (LA) to the atrial septum perpendicular to the plane of the mitral valve were acquired using 2D ECG-gated breath-hold segmented CINE TrueFISP sequence with retrospective gating (see Figure 28). We elected to use the 2 chamber orientation as recommended by Jarvinen et al since the mitral valve plane separating the LA from the LV can be clearly identified (337, 338).

CMR images were analysed offline by an independent, blinded MRI Physicist (SG) using commercial software ('Argus', Siemens Multi-modality Work Platform, version VB 15). Image segmentation of the left atrium was performed twice at atrial diastole and systole. Contours for all image slices across the left atrium were defined at diastole and systole by measuring the blood volume contained by the atrial wall and the mitral valve. The LA appendage and pulmonary veins were excluded wherever possible. From these contours, LA ejection fraction (LA-EF), end-diastolic volume (LA-EDV), end-systolic volume (LA-ESV) and stroke volume (LA-SV) were all derived and indexed to BSA. Repeated segmentations were completed over two time-points in order to optimize the measurement precision.

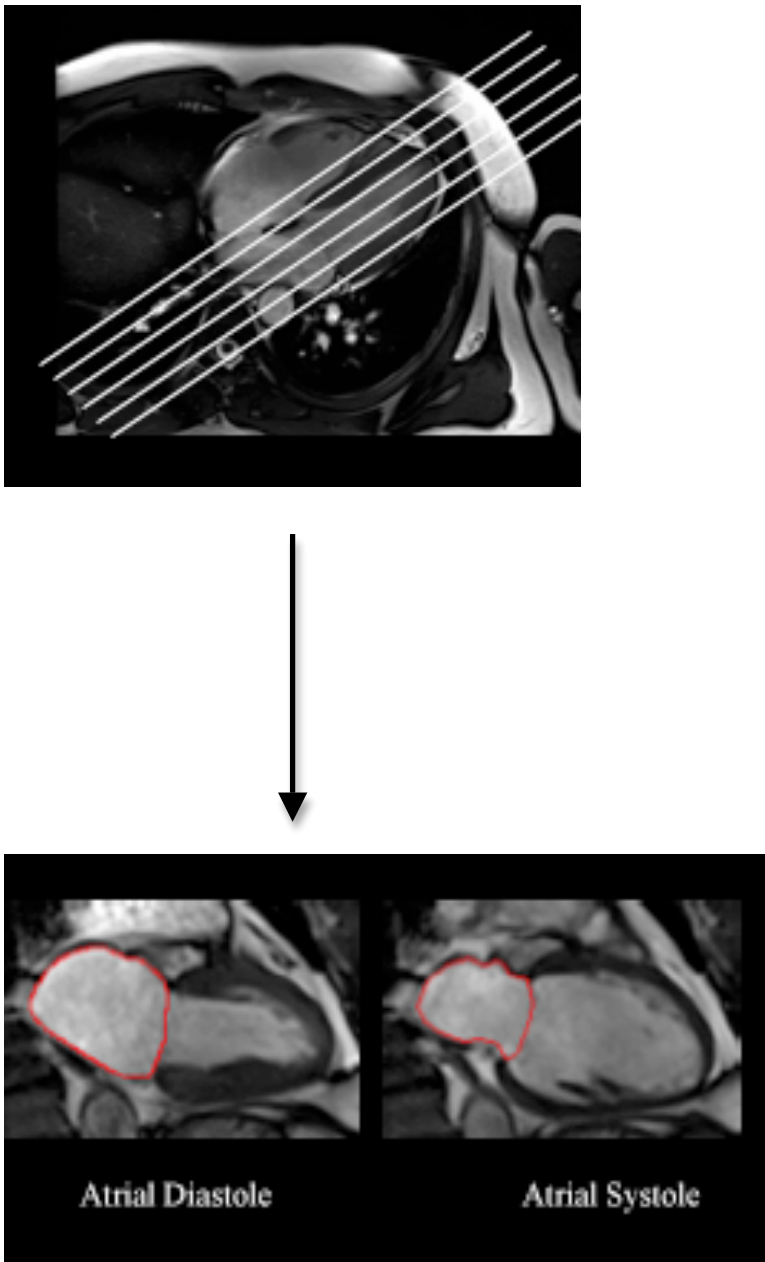


Figure 28. Measurement of LA volumes using CMR

2.12 FMD

Flow-mediated dilatation (FMD) of the brachial artery was performed as set out in the International Brachial Artery Reactivity Task Force guidelines (339). It was performed at the baseline visit, six months and nine months using a Sequoia 512 (Siemens, Camberley, UK) ultrasound machine with a 8 MHz linear array probe.

Patients were asked to fast for 8 hours and not to take exercise, smoke, consume caffeine, high fat food or vitamin C for 4 hours prior to test. If they were on nitrate tablets, they were advised to hold it off the morning of the test until after the study visit. The study was undertaken in a quiet, temperature controlled room (22-24°C) with the patient lying supine on an examination couch with their right arm outstretched on a table. The images of the brachial artery were acquired using ECG gating with the image acquired at the peak of the R wave. A baseline image of the brachial artery was acquired over a minute. Then a cuff was inflated distal to the elbow to 200mmHg for five minutes. On deflation of the cuff, the brachial arterial diameter was recorded for two minutes. After a 10 minute rest period, endothelial independent dilatation was assessed by the response of brachial artery size to 400 micrograms of sublingual glyceryl trinitrate (GTN) spray. FMD was the percentage change in maximum diameter achieved after

cuff deflation relative to the baseline mean artery diameter (see Figure 29). The FMD was analyzed using Vascular Research Tools software (Medical Imaging Applications LLC, Coralville, IA, USA). The acquisition and analysis of the FMD images were performed by a single trained investigator (SR), who was blinded to the allocated treatment.

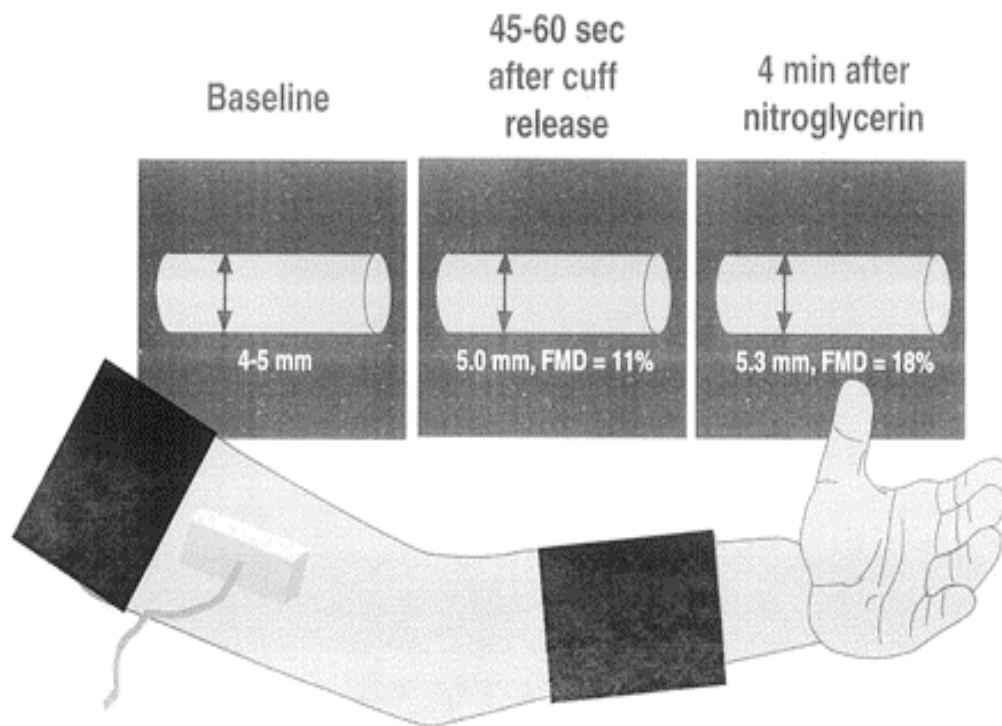


Figure 29. Brachial artery FMD (339)

2.13 Pulse Wave Analysis and Pulse Wave Velocity

2.13.1 Applanation Tonometry method

Pulse wave analysis (PWA) and pulse wave velocity (PWV) were each measured three times at each study visit (baseline, six months and nine months). An average of these three measurements was taken as the value for each study visit. This was performed using the validated Sphygmocor (AtCor, Sydney, Australia) system by a single trained investigator (SR) who was blinded to the allocated treatment. This technique is in widespread use in our institute. Patients were rested in a supine position for at least ten minutes prior to taking measurements. For pulse wave analysis, peripheral pressure waveforms were obtained at the radial artery by applanation tonometry using a high fidelity micromanometer. The central aortic pressure waveform was generated from the radial artery waveform by using a validated, generalized transfer function. From the aortic pulse wave, Augmentation index (AIx) was calculated as the difference between the first and second systolic peak expressed as a percentage of the pulse pressure (see Figure 30). As AIx is affected by heart rate, AIx was normalized for a heart rate of 75 beats per minute (AIx@75).

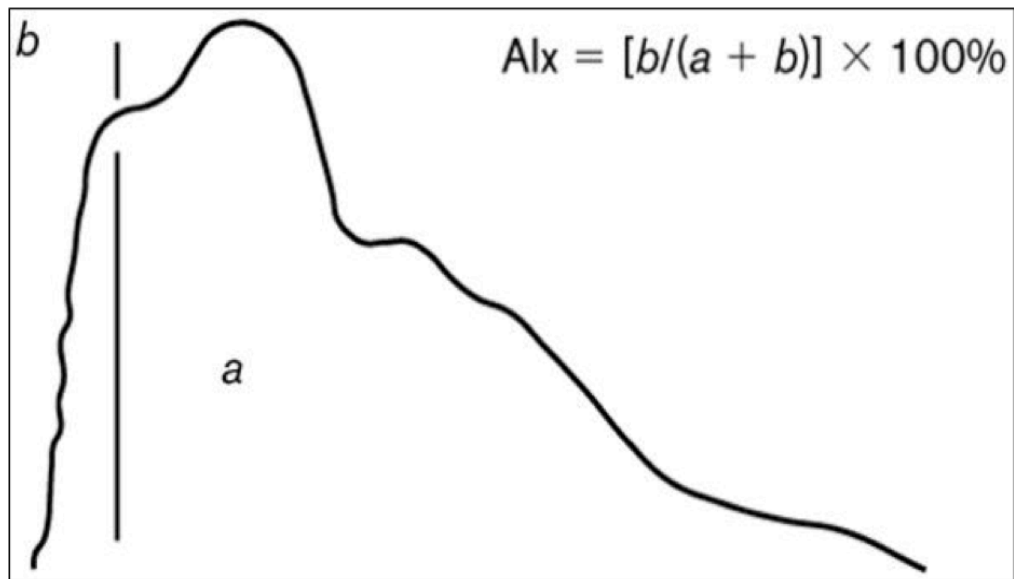


Figure 30. Calculation of Augmentation Index (AIx) (75)

For PWV, radial-carotid waveforms were obtained with ECG gating. Velocity was calculated measuring the time interval between ECG R-wave and the recorded waveforms at each site whilst the distance between the sites was measured manually with a tape measure.

$$\text{Velocity} = \text{distance}/\text{time}$$

2.14 Healthy Volunteers

Thirty age and sex matched healthy volunteers (patients without angina or diabetes) were recruited as a separate group to the main intervention trial to enable comparison of results with the group treated with allopurinol. These volunteers were identified from databases of healthy volunteers who have participated in previous studies in the department. This sub-study was to test if allopurinol improved endothelial function to such an extent that their post treatment endothelial function became as good as a healthy person of the same age.

The inclusion criteria were healthy volunteers aged between 50 to 80 years old. The exclusion criteria included volunteers with diabetes, ischaemic heart disease, heart failure or LVEF <45%, previous stroke, renal impairment (eGFR < 60), history of gout, on allopurinol therapy, pregnant or breastfeeding or unable to consent. These healthy volunteers attended a single study visit when they will undergo a blood test (FBC, UE, LFT and uric acid), applanation tonometry and FMD of the brachial artery.

2.15 Safety

Participants were informed of potential side effects at the screening visit and this was also clearly stated in the Patient Information Sheet. They were also questioned at each study visit for side effects or adverse events. This was documented in the participant's case report form (CRF) and the sponsor was informed of any serious adverse events. Safety blood tests (FBC, UE, LFT) were performed at each study visit.

2.16 Statistical Analysis

Data for continuous variables are presented as mean \pm SD for normally distributed data and median and interquartile range (IQR) for non-normally distributed data. Categorical data are expressed as numbers (%). Comparison between continuous variables were analyzed using the Student t-test or Mann-Whitney U test whilst categorical variables were analyzed using χ^2 test. Correlation was performed using Pearson's or Spearman's. Multivariable regression was performed using the Enter method to correct for the effect of confounding variables on the change in LVM.

All statistical analyses were undertaken blinded as completed case analysis using SPSS version 18.0 (SPSS, Chicago, IL). A two-sided p value < 0.05 was considered statistically significant.

2.17 Sample Size Calculations

In order to detect a 10g change in LVM at 90% power, Myerson *et al.* recommended a total sample size of 26 individuals (182) whilst Grothues *et al.* recommended a total sample size of 30 (192). However, we felt that these recommendations were unrealistic. Based on a previous study by Kao *et al.*, we calculated that we would need 49 patients to detect a 5% change in LVM with 90% power (332). With regards to FMD, a 2% change in FMD is widely regarded as being clinically significant. As our departmental coefficient of variation for FMD measurements was 3%, we calculated that we would need 27 patients per arm to detect a 2.3% change in FMD with 80% power. Hence, 66 patients were required to achieve 90% power to detect a clinically significant change in LVM and 80% power to detect a clinically significant change in FMD at a significance level of $p < 0.05$, allowing for a 20% dropout rate.

3 RESULTS

3.1 Screened Participants

A total of 209 participants were screened of which 143 were excluded due to the following reasons:

- 124 participants did not meet the echo criteria for LVH
- 8 participants had contraindications to undergo 3T CMR
 - Hip replacement (n=2)
 - Valve replacement (n=1)
 - Uncontrolled AF (n=2)
 - Claustrophobia (n=2)
 - Information not available on back surgery metal implant (n=1)
- 11 participants had other reasons
 - Hypertensive (n=4)
 - Social reasons (n=1)
 - LV impairment (n=2)
 - Abnormal liver function tests (n=2)
 - Renal impairment (n=2)

3.2 Randomized Participants

Of the 66 participants recruited, six completely withdrew from the study (allopurinol group n=2; placebo group n=4). Reasons for withdrawal are shown in the flowchart (Figure 31). Other than the patient who developed recurrent gout on allopurinol therapy, the other five study withdrawals were not felt to be related to the study medication. Five of the remaining participants were unable to undergo a CMR (allopurinol group n=4; placebo group n=1) but remained in the study as they underwent the other investigations including bloods, FMD and applanation tonometry.

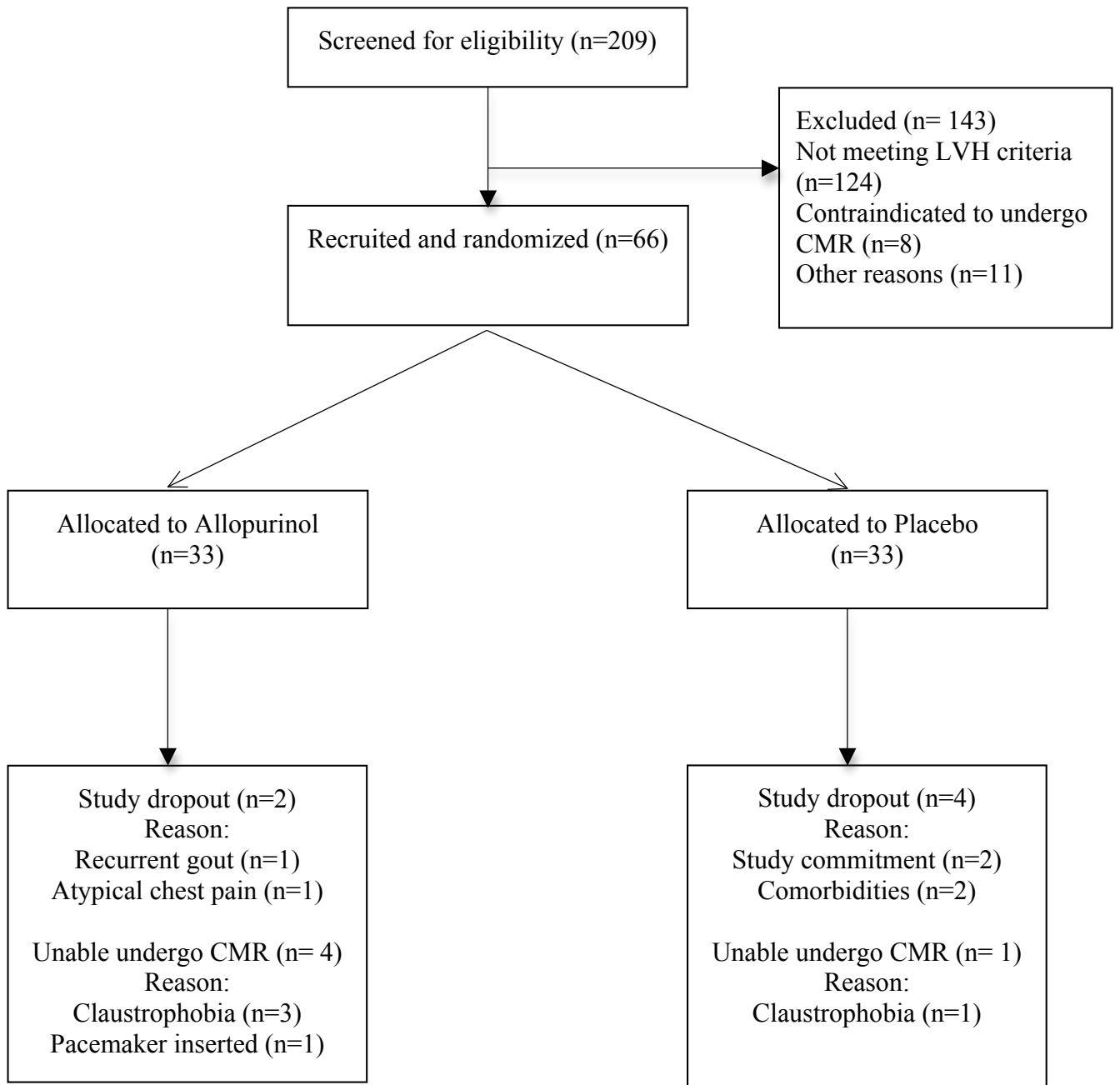


Figure 31. Study Flow Diagram

3.3 Baseline Characteristics

Baseline characteristics of the recruited patients are shown in Table 12. The study participants had a mean age of 65 years, well controlled BP (mean office BP 135/77, mean 24 hr BP 123/72) and were predominantly male. Majority of the baseline demographics was similar between the treatment groups.

	Allopurinol (n=31)	Placebo (n=29)	p value
Age, years	65 ± 6.7	64 ± 7.2	0.64
Male (%)	26 (84)	28 (97)	0.10
BSA, g/m ²	2.1 ± 0.2	2.0 ± 0.2	0.26
Office SBP, mmHg	135 ± 9	134 ± 10	0.87
24hr SBP, mmHg	124 ± 11	121 ± 9	0.42
Office DBP, mmHg	78 ± 7	76 ± 7	0.26
24hr DBP, mmHg	70 ± 5	74 ± 5	0.06
Past MI (%)	13 (42)	17 (59)	0.20
Hypertension (%)	23 (74)	16 (55)	0.12
Diabetes (%)	4 (13)	3 (10)	0.80
Cerebrovascular disease (%)	4 (13)	2 (7)	0.40
Peripheral vascular disease (%)	3 (10)	2 (7)	0.70
Smoking (%)			0.24
Non	7 (23)	12 (41)	
Ex	19 (61)	12 (41)	
Current	5 (16)	5 (18)	
CCS classification			0.88
1	23 (74)	21 (72)	
2	8 (26)	8 (28)	
ETT/MPS positive (%)	26 (84)	19 (66)	0.15
No of vessel disease (%)			0.05
1	2 (6)	12 (41)	
2	11 (35)	8 (28)	
3	9 (29)	6 (21)	
PCI (%)	6 (19)	15 (52)	0.01
CABG (%)	15 (48)	8 (28)	0.1

Table 12. Baseline characteristics of study participants.

Abbreviations: BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; MI, myocardial infarction; CCS, Canadian Cardiovascular Society; ETT, exercise treadmill test; MPS, myocardial perfusion scan; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting. Data presented as mean ± SD. Rest of data presented as number (%).

Table 13 shows that the participants were on optimum medical therapy. All participants were on antiplatelet therapy (aspirin or clopidogrel), 93% were on statins, 70 % were on betablockers whilst 75% were on an ACE inhibitor/ARB.

	Allopurinol (n=31)	Placebo (n=29)	p value
Aspirin (%)	26 (84)	29 (100)	0.02
Clopidogrel (%)	4 (13)	4 (14)	0.94
Statin (%)	29 (94)	27 (93)	0.92
Ezetimibe (%)	3 (10)	2 (7)	0.70
Beta blocker (%)	24 (77)	18 (62)	0.20
Calcium channel blocker (%)	10 (32)	13 (45)	0.30
Nicorandil (%)	2 (7)	6 (21)	0.10
Oral nitrate (%)	9 (29)	11 (38)	0.47
ACE inhibitor (%)	20 (65)	15 (52)	0.32
ARB (%)	7 (23)	3 (10)	0.20

Table 13. Table of study medication

Abbreviations: ACE inhibitor, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Data presented as number (%).

Table 14 shows the baseline study investigations that were undertaken. There was no significant difference in baseline uric acid level, augmentation index or LVM/LVMI measured using CMR. However, the allopurinol group had a significantly higher BNP level and echo LVM measurement, greater endothelial

dysfunction as indicated by a smaller FMD response of brachial artery at baseline.

	Allopurinol	Placebo	p value
Uric acid, mmol/L	0.6 ± 0.1	0.6 ± 0.1	0.33
BNP, pg/ml	31 (21 to 60)	21 (13 to 35)	0.03
FMD, %	4.1 ± 2.1	5.7 ± 2.4	0.01
AIx, %	20 ± 7	19 ± 9	0.73
PWV, m/s	7.6 (6.9 to 8.7)	8.5 (7.4 to 9)	0.02
Echo LVM, g	273 ± 35	251 ± 32	0.02
Echo LVMI, g/m ²	132 ± 14	125 ± 12	0.05
CMR LVM, g	145.7 ± 23.4	136.4 ± 26	0.17
CMR LVMI, g/m ²	71.0 ± 9.0	68.4 ± 11.1	0.35

Table 14. Table of baseline investigations.

Abbreviations: BNP, brain natriuretic peptide; FMD, flow-mediated dilatation; AIx, augmentation index; PWV, pulse wave velocity; LVM, left ventricular mass; LVMI, left ventricular mass index.

Data presented as mean ± SD or median (IQR).

3.4 CMR

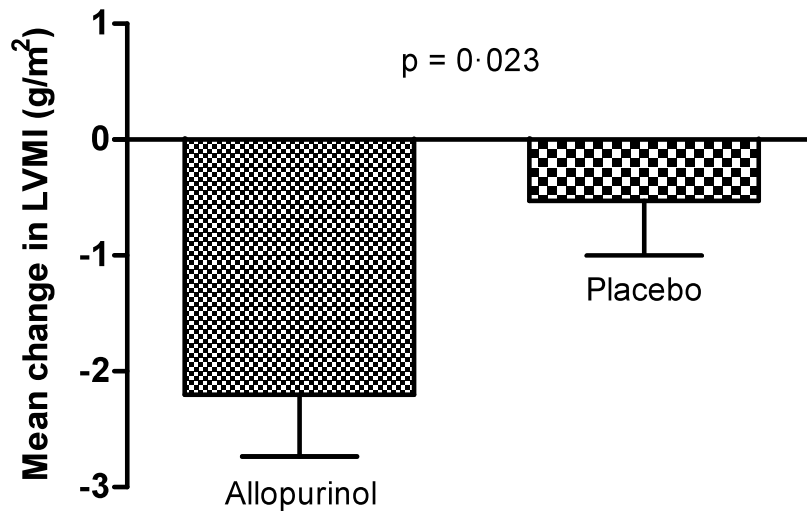
3.4.1 Effect of Allopurinol on LVM

55 patients underwent CMR (allopurinol, n=27; placebo, n=28). At baseline, there was no significant difference in LVM or LVMI. After 9 months of allopurinol therapy, there was a significant reduction in LVM (allopurinol: -5.2 ± 5.8 g, placebo: -1.3 ± 4.5 g; $p = 0.01$) and LVMI (allopurinol: -2.2 ± 2.8 g/m², placebo: -0.5 ± 2.5 g/m²; $p = 0.02$). See Table 15 and Figure 32. Furthermore, the within group changes in LVM and LVMI were significant in the allopurinol group ($p < 0.01$ for both) but not in the placebo group.

	Allopurinol (n=27)	Placebo (n=28)	p value
Baseline LVM, g	145.7 ± 23.4	136.4 ± 26	0.17
Final visit LVM, g	140.5 ± 23.0	135.1 ± 26.2	0.42
Change in LVM, g	-5.2 ± 5.8	-1.3 ± 4.5	0.01
% Change in LVM, %	-3.6 ± 4.0	-1.0 ± 3.3	0.01
Baseline LVMI, g/m ²	71.0 ± 9.0	68.4 ± 11.1	0.35
Final visit LVMI, g/m ²	68.8 ± 8.7	67.9 ± 11.1	0.73
Change in LVMI, g/m ²	-2.2 ± 2.8	-0.5 ± 2.5	0.02
% Change in LVMI, %	-3.3 ± 4.3	-0.9 ± 3.7	0.03

Table 15. The effect of Allopurinol on LVM and LVMI using CMR.

(A)



(B)

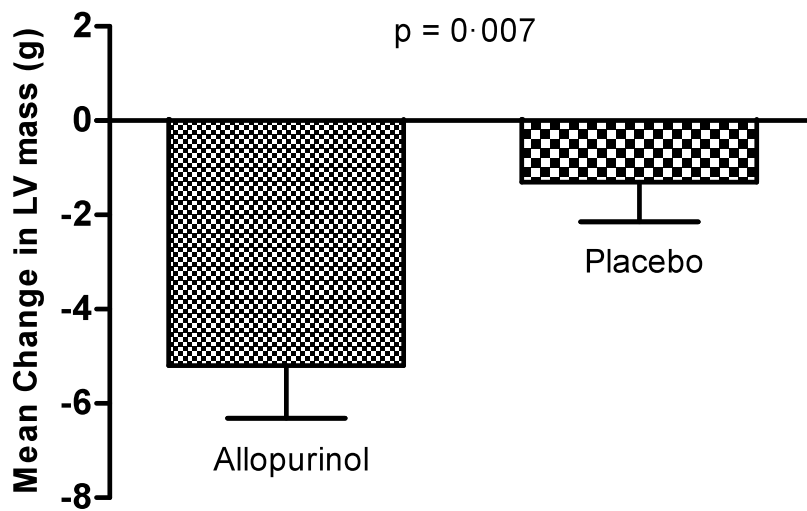


Figure 32. (A) Mean change in LVMI (B) Mean change in LVM
Data are presented as mean \pm SEM.

Allopurinol regressed LVH more in the above median LVM group compared to the below median LVM group. The lack of significant change in LVM in the placebo group in those with an above median LVM at baseline indicates that it was not simple regression to the mean that caused higher LV masses to fall more with allopurinol. See Table 16.

	Allopurinol		Placebo		Between treatment groups	
	Mean change \pm SD	p value	Mean change \pm SD	p value	Mean change \pm SD	p value
Above median LVM group	-5.7 \pm 6.2	< 0.01	-0.9 \pm 5.0	0.54	-4.8 \pm 2.2	0.04
Below median LVM group	-4.4 \pm 5.2	0.03	-1.7 \pm 4.1	0.13	-2.7 \pm 1.8	0.15
Above median LVMI group	-2.9 \pm 2.5	< 0.01	-0.9 \pm 3.1	0.32	-2.0 \pm 1.1	0.07
Below median LVMI group	-1.1 \pm 2.9	0.23	-0.2 \pm 2.0	0.64	-0.9 \pm 0.9	0.35

Table 16. Change in LVM and LVMI in the above or below median group with allopurinol and placebo therapy and between treatment groups

Data are presented as mean \pm SD.

The effect of allopurinol on LVM did not significantly differ between the two gender groups (change in LVM in males $-6.0 \pm 5.9\text{g}$, females $-1.7 \pm 4.2\text{g}$; $p=0.1$) (Figure 33) or if they had a past history of MI or not (change in LVM with previous MI $-6.6 \pm 5.5\text{g}$, no previous MI $-3.3 \pm 6\text{g}$; $p=0.15$).

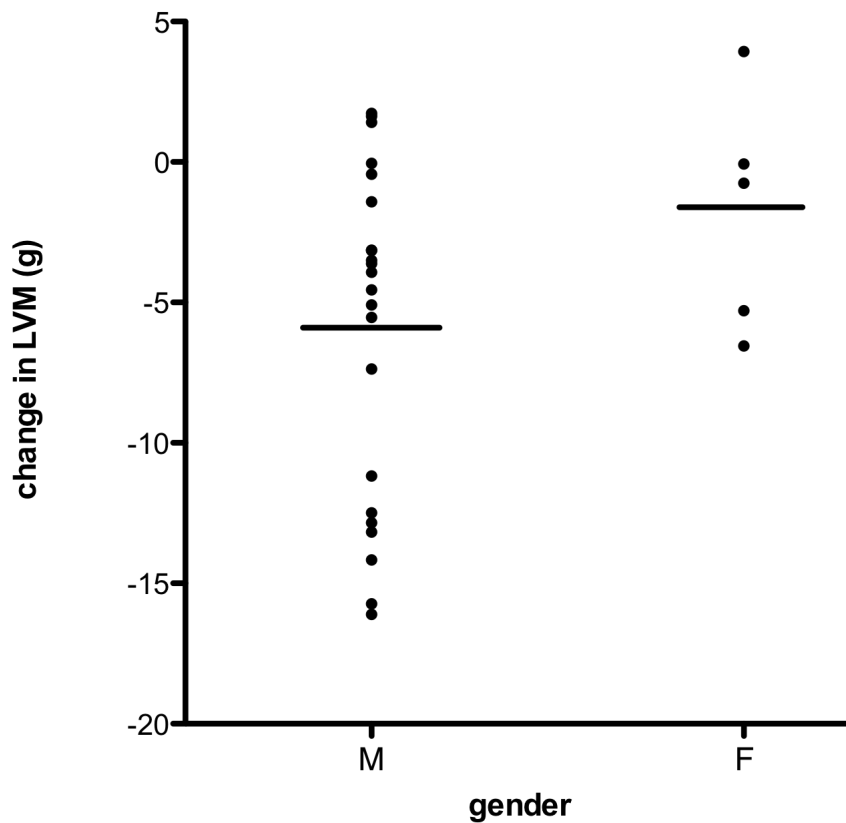


Figure 33. Change in LVM with allopurinol therapy according to gender. Horizontal line indicates mean.

Upon correcting for age, gender, change in office SBP, change in office DBP and baseline LVM, the effect of allopurinol therapy on change in LVM remained significant ($p < 0.01$). Allopurinol also significantly affected the change in LVMI after adjusting for age, gender, change in office SBP, change in office DBP and baseline LVMI ($p = 0.03$).

No significant correlation was seen between the allopurinol-induced change in LVM and either change in FMD ($r = 0.06$, $p = 0.76$), change in PWA ($r = -0.12$, $p = 0.55$), baseline uric acid ($r = 0.25$, $p = 0.22$) (Figure 34) or change in uric acid ($r = -0.18$, $p = 0.38$).

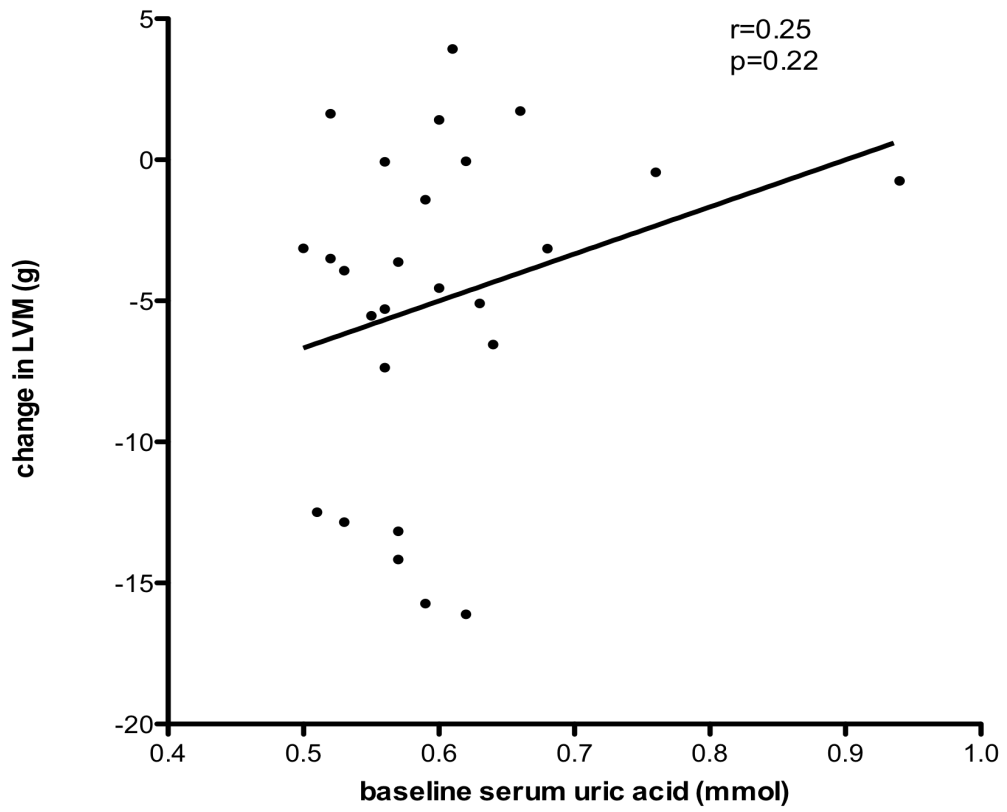


Figure 34. Correlation between allopurinol-induced change in LVM and baseline serum uric acid level.

3.4.2 Effect of Allopurinol on LV volumes

At baseline, there were no significant differences between the treatment arms.

The allopurinol group had a significant reduction in LV ESV ($p < 0.05$) and a non-statistically significant reduction in LV EDV ($p = 0.15$). See Table 17.

	Allopurinol (n=27)	Placebo (n=28)	p value
Baseline EF, %	73.9 ± 8.6	74.2 ± 6.4	0.91
Final visit EF, %	74.5 ± 8.4	73.5 ± 6.3	0.64
Change in EF, %	0.6 ± 3.7	-0.6 ± 3.7	0.24
Baseline EDV, ml	149.2 ± 28.3	136.3 ± 24.3	0.08
Final visit EDV, ml	143.1 ± 21.4	136.8 ± 23.9	0.31
Change in EDV, ml	-6.1 ± 16.4	0.5 ± 16.9	0.15
Baseline ESV, ml	39.6 ± 16.3	35.3 ± 10.4	0.25
Final visit ESV, ml	36.8 ± 13.6	36.6 ± 11.4	0.96
Change in ESV, ml	-2.8 ± 7.8	1.3 ± 7.2	< 0.05
Baseline SV, ml	109.7 ± 22.2	101 ± 20.4	0.14
Final visit SV, ml	106.4 ± 19.3	100.2 ± 17.5	0.22
Change in SV, ml	-3.3 ± 12.8	-0.8 ± 13.3	0.49
Baseline CO, L/min	6.7 ± 1.3	6.1 ± 1.2	0.09
Final visit CO, L/min	6.6 ± 1.3	6.2 ± 1.3	0.28
Change in CO, L/min	-0.1 ± 0.9	0.1 ± 0.9	0.44

Table 17. The effect of Allopurinol on LV volumes.

No correlation was seen between the allopurinol-induced change in ESV and baseline uric acid ($r = -0.1$, $p = 0.6$) or change in uric acid ($r = 0.09$, $p = 0.66$).

No correlation was also seen between the allopurinol-induced change in EDV and baseline uric acid ($r = -0.24$, $p=0.24$) or change in uric acid ($r = 0.2$, $p = 0.3$).

3.4.3 Effect of Allopurinol on LA volumes

At baseline, the allopurinol group unfortunately had a significantly greater LA EDV and LA ESV compared to the placebo group. After 9 months of allopurinol therapy, although there was reduction in LA EDV of 5.1 ± 8.9 ml this was not statistically significant compared to the placebo group ($p = 0.4$). There was also a similar reduction in LA ESV in both treatment groups. See Table 18.

	Allopurinol (n=24)	Placebo (n=25)	p value
Baseline LA EDV, ml	103.1 \pm 24.1	86.7 \pm 16.2	0.01
Baseline LA ESV, ml	58.2 \pm 18.3	46.9 \pm 10.3	0.01
Final LA EDV, ml	98.0 \pm 24.1	83.9 \pm 17.9	0.02
Final LA ESV, ml	56.5 \pm 17.1	45.2 \pm 11.9	0.01
Change in LA EDV, ml	-5.1 \pm 8.9	-2.9 \pm 9.6	0.40
Change in LA ESV, ml	-1.7 \pm 5.1	-1.7 \pm 5.8	0.90

Table 18. The effect of Allopurinol on LA volumes.

No significant correlation was seen between allopurinol-induced change in LA EDV and the change in LVM ($r = 0.17$, $p=0.4$) or change in uric acid ($r = -0.017$, $p=0.94$).

3.5 FMD

The baseline artery size was similar in both treatment groups. There was a statistically significant increase in FMD response to hyperaemia after nine months of allopurinol therapy (change in FMD response to hyperaemia in allopurinol group: $+0.8 \pm 1.8\%$, placebo group: $-0.7 \pm 2.8\%$; $p = 0.02$). See Table 19 and Figure 35.

	Allopurinol (n=31)	Placebo (n=29)	p value
Baseline artery size, mm	4.1 ± 0.6	4.2 ± 0.5	0.56
FMD response to hyperaemia at baseline, %	4.1 ± 2.1	5.7 ± 2.4	0.01
FMD response to hyperaemia at 6 months, %	4.7 ± 2.2	5.4 ± 2.5	0.22
Change in FMD response to hyperaemia at 6 months, %	0.6 ± 2.0	-0.3 ± 2.0	0.10
FMD response to hyperaemia at 9 months, %	4.9 ± 2.2	5.0 ± 2.5	0.93
Change in FMD response to hyperaemia at 9 months, %	0.8 ± 1.8	-0.7 ± 2.8	0.02
FMD response to GTN at baseline, %	11.2 ± 4.8	10.3 ± 4.9	0.51
FMD response to GTN at 6 months, %	10.4 ± 5.3	11.4 ± 5.2	0.45
Change in response to GTN at 6 months, %	-0.8 ± 3.0	1.1 ± 3.1	0.04
FMD response to GTN at 9 months, %	9.7 ± 5.0	11.6 ± 5.0	0.17
Change in response to GTN at 9 months, %	-1.5 ± 2.8	1.3 ± 3.3	< 0.01

Table 19. The effect of Allopurinol on FMD

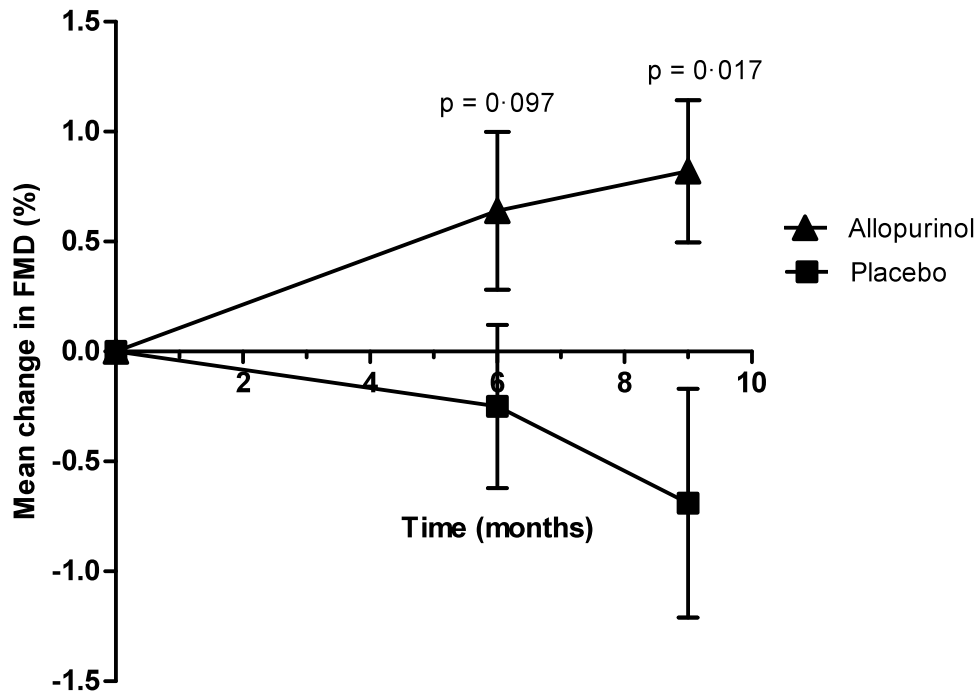


Figure 35. Change in FMD in both treatment arms.

Data presented as mean \pm SEM.

FMD response to GTN measures endothelium independent dilatation. There was a statistically significant reduction in FMD response to GTN after 6 and 9 months of allopurinol therapy (see Table 20). This was assessed in more detail depending on whether the participants were on regular oral nitrates. The 18 patients on regular oral nitrates had their dosage omitted the morning of FMD test. In this subgroup of patients on oral nitrates, the allopurinol treatment arm had a $2.2 \pm 2.9\%$ reduction in FMD response to GTN after 9 months compared to

placebo group who had a $0.8 \pm 4.2\%$ increase in FMD response although this failed to reach statistical significance ($p=0.097$).

	Allopurinol (n=8)	Placebo (n=10)	p value
FMD response to GTN at baseline, %	10.9 ± 5.7	8.8 ± 4.7	0.43
FMD response to GTN at 6 months, %	10.5 ± 6.9	9.0 ± 2.8	0.56
Change in response to GTN at 6 months, %	-0.4 ± 2.1	0.2 ± 4.3	0.70
FMD response to GTN at 9 months, %	8.7 ± 5.2	9.6 ± 3.2	0.52
Change in response to GTN at 9 months, %	-2.2 ± 2.9	0.8 ± 4.2	0.10

Table 20. FMD response to GTN in participants on regular oral nitrates.

However, there was a significant and unexpected difference in FMD response to GTN in the participants who were not on regular oral nitrates. See Table 21.

	Allopurinol (n=22)	Placebo (n=16)	p value
FMD response to GTN at baseline, %	11.5 ± 4.6	11.7 ± 5.0	0.98
FMD response to GTN at 6 months, %	10.3 ± 4.8	12.9 ± 5.9	0.14
Change in response to GTN at 6 months, %	-1.2 ± 3.3	1.2 ± 2.2	0.02
FMD response to GTN at 9 months, %	10.3 ± 5.1	13.2 ± 5.5	0.16
Change in response to GTN at 9 months, %	-1.2 ± 2.8	1.5 ± 2.9	0.01

Table 21. FMD response to GTN in participants not on regular oral nitrates.

A negative correlation was seen between baseline FMD response to hyperaemia and baseline LVM ($r = -0.33$, $p = 0.01$) (Figure 36) and EDV ($r = -0.34$, $p = 0.01$). However, no correlation was seen between the change in FMD response to hyperaemia with allopurinol therapy and baseline uric acid ($r = 0.07$, $p = 0.72$), change in uric acid ($r = 0.03$, $p = 0.87$), change in LVM ($r = 0.06$, $p = 0.76$) (Figure 37) or change in EDV ($r = -0.27$, $p = 0.17$).

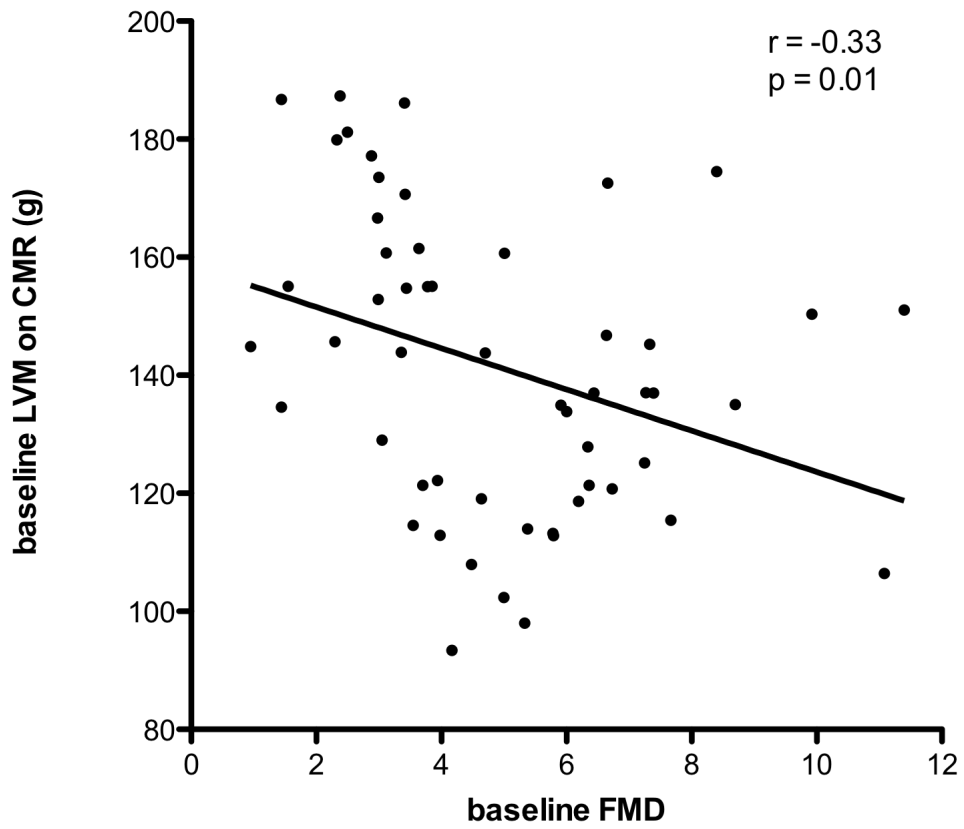


Figure 36. Correlation between baseline FMD and baseline LVM on CMR

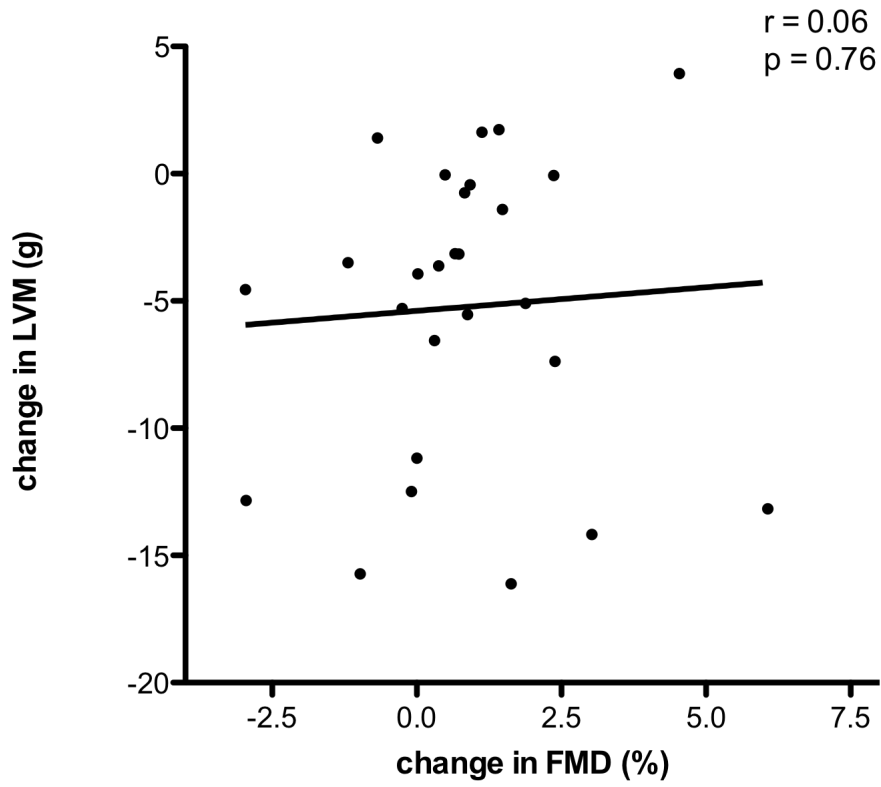


Figure 37. Correlation between change in FMD and change in LVM

3.6 Applanation Tonometry

3.6.1 PWA

At baseline, there was no significant difference in AIx between treatment groups. After 6 months of allopurinol treatment, there was a reduction in AIx of $3 \pm 5\%$ compared to a reduction of $1 \pm 6\%$ for placebo group, although this was not statistically significant. After 9 months of therapy, the allopurinol group had a $3 \pm 5\%$ reduction in AIx compared to $1 \pm 7\%$ for the placebo group. This difference was statistically significant. See Table 22 and Figure 38.

	Allopurinol (n=31)	Placebo (n=29)	p value
Baseline visit AIx, %	20 ± 7	19 ± 9	0.73
6 months visit AIx, %	17 ± 9	18 ± 11	0.73
Change in AIx baseline to 6 months, %	-3 ± 5	-1 ± 6	0.14
Final visit AIx, %	17 ± 9	20 ± 12	0.30
Change in AIx baseline to final visit, %	-3 ± 5	1 ± 7	0.02

Table 22. Effect of allopurinol and placebo therapy on PWA.

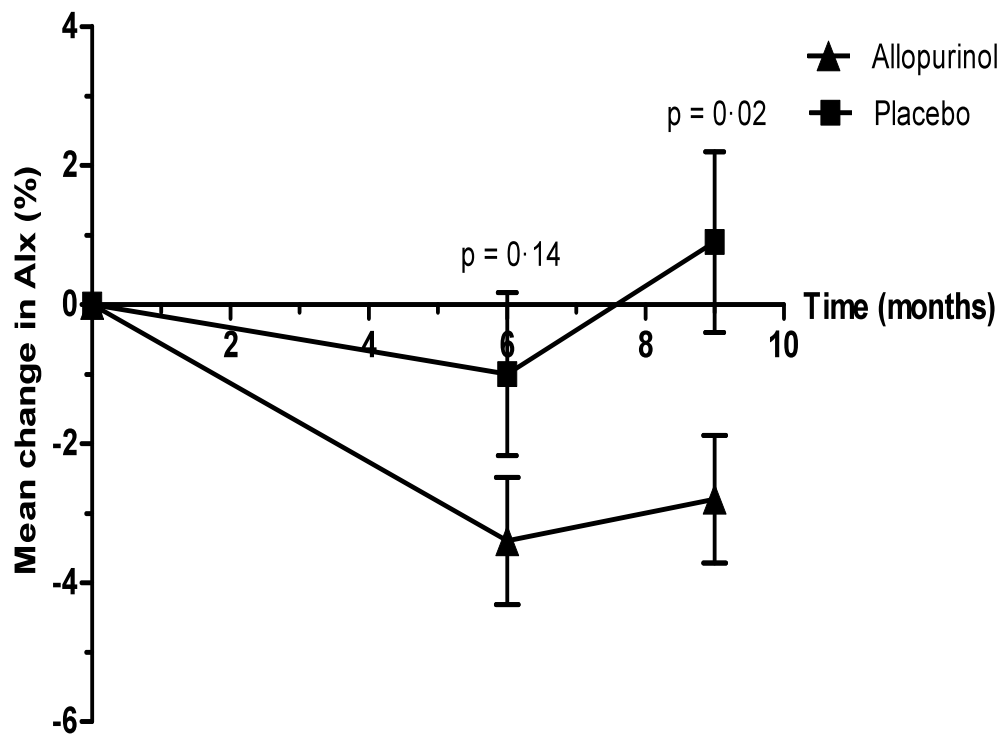


Figure 38. Change in Augmentation index in both treatment arms.

Data presented as mean \pm SEM.

A negative correlation was seen between baseline uric acid and change in AIx, that just missed statistical significance ($r = -0.34$, $p = 0.07$). However, there was no significant correlation between change in uric acid and change in AIx ($r = 0.22$, $p = 0.24$).

3.6.2 PWV

At baseline, the allopurinol group had a significantly slower PWV compared to the placebo group. However, allopurinol therapy for up to 9 months did not have significant effect on PWV. See Table 23.

	Allopurinol (n=31)	Placebo (n=29)	p value
Baseline visit, m/s	7.6 (6.9 to 8.7)	8.5 (7.4 to 9.0)	0.02
6 months visit, m/s	7.7 (6.7 to 7.9)	7.9 (7.3 to 8.8)	0.05
Change in PWV baseline to 6 months visit, m/s	0 (-0.6 to 0.3)	0 (-0.8 to 0.4)	0.85
Final visit, m/s	7.5 (6.9 to 8.2)	8 (7.0 to 8.6)	0.19
Change in PWV baseline to final visit, m/s	-0.14 (-0.8 to 0.3)	-0.2 (-1.3 to 0.4)	0.66

Table 23. Effect of Allopurinol on PWV

Data presented as median (IQR).

3.7 Effect of Allopurinol on BP

There was no significant change in office BP (measured on all the participants) or 24-hour ambulatory BP (measured on a subset of 32 randomly selected participants) in the allopurinol group compared to placebo group. See Table 24 and Table 25.

	Allopurinol (n=31)	Placebo (n=29)	p value
Office SBP (mmHg)			
Baseline visit	135 ± 9	134 ± 10	0.87
6 months visit	134 ± 12	134 ± 12	0.91
Final visit	130 ± 14	131 ± 13	0.65
Change in office SBP (baseline to final visit)	-5 ± 13	-3 ± 15	0.59
Office DBP (mmHg)			
Baseline visit	78 ± 7	76 ± 7	0.26
6 months visit	76 ± 7	78 ± 8	0.15
Final visit	74 ± 8	75 ± 8	0.72
Change in office DBP (baseline to final visit)	-4 ± 8	-1 ± 8	0.20
Office MAP (mmHg)			
Baseline visit	97 ± 6	96 ± 7	0.38
Final visit	92 ± 8	93 ± 8	0.64
Change in MAP (baseline to final visit)	-5 ± 8	-3 ± 9	0.27

Table 24. Effect of Allopurinol and Placebo therapy on office BP

	Allopurinol (n=17)	Placebo (n=15)	p value
24-hr Ambulatory SBP (mmHg)			
Baseline visit	124 ± 11	121 ± 9	0.42
Final visit	126 ± 11	118 ± 12	0.05
Change in 24-hr SBP	2 ± 11	-3 ± 9	0.17
24-hr Ambulatory DBP (mmHg)			
Baseline visit	70 ± 5	74 ± 5	0.06
Final visit	71 ± 7	71 ± 4	0.89
Change in 24hr DBP	1 ± 5	-3 ± 4	0.61
24-hr Ambulatory MAP (mmHg)			
Baseline visit	89 ± 5	90 ± 6	0.64
Final visit	89 ± 6	88 ± 7	0.42
Change in 24-hr MAP	0 ± 7	-2 ± 6	0.20

Table 25. Effect of Allopurinol and Placebo therapy on ambulatory BP

3.8 Effect of Allopurinol on Serum Uric Acid

Allopurinol significantly reduced serum uric acid level (allopurinol group: 0.6 ± 0.1 mmol/L at baseline to 0.3 ± 0.1 mmol/L at nine months; placebo group: 0.6 ± 0.1 mmol/L at baseline to 0.5 ± 0.1 mmol/L at nine months; $p < 0.01$). Hence, allopurinol therapy for nine months lowered serum uric acid level by 46%.

3.9 Effect of Allopurinol on Oxidized LDL

Oxidized LDL is one of many markers of oxidative stress. Allopurinol did not seem to have a significant effect on oxidized LDL levels (change in oxidized LDL in allopurinol group: 1.8 ± 8.9 pg/ml, placebo group 0.6 ± 10.7 pg/ml; $p = 0.65$).

3.10 Effect of Allopurinol on BNP

At baseline, BNP levels did not correlate with the baseline LVM (Spearman's $\rho = -0.20$, $p = 0.89$). Additionally the allopurinol group had a significantly higher BNP level compared to placebo [allopurinol group median (IQR): 31 (21 to 60) pg/ml vs. 21 (13 to 35) pg/ml placebo group; $p = 0.03$]. After 9 months of allopurinol therapy, BNP levels were reduced by -6 (-19 to 5) pg/ml compared to placebo -1 (-7 to 5) pg/ml, which just failed to reach statistical significance ($p = 0.08$). This change in BNP did not correlate with change in LVM (Spearman's $\rho = -0.12$, $p = 0.40$), EDV (Spearman's $\rho = 0.08$, $p = 0.59$), ESV (Spearman's $\rho = -0.09$, $p = 0.53$) or FMD (Spearman's $\rho = 0.02$, $p = 0.90$).

3.11 Effect of Allopurinol on UPCR

Allopurinol did not have a significant effect on UPCR [change in UPCR for allopurinol group: 1.5 (-1.5 to 4.3) mg/mmol, placebo group: 0 (-2 to 4.5) mg/mmol; $p = 0.67$].

3.12 Correlation between Echo and CMR

As shown in Figure 39 there was a strong positive correlation seen between the baseline LVM measured using echo and CMR ($r = 0.5$, $p = 0.01$).

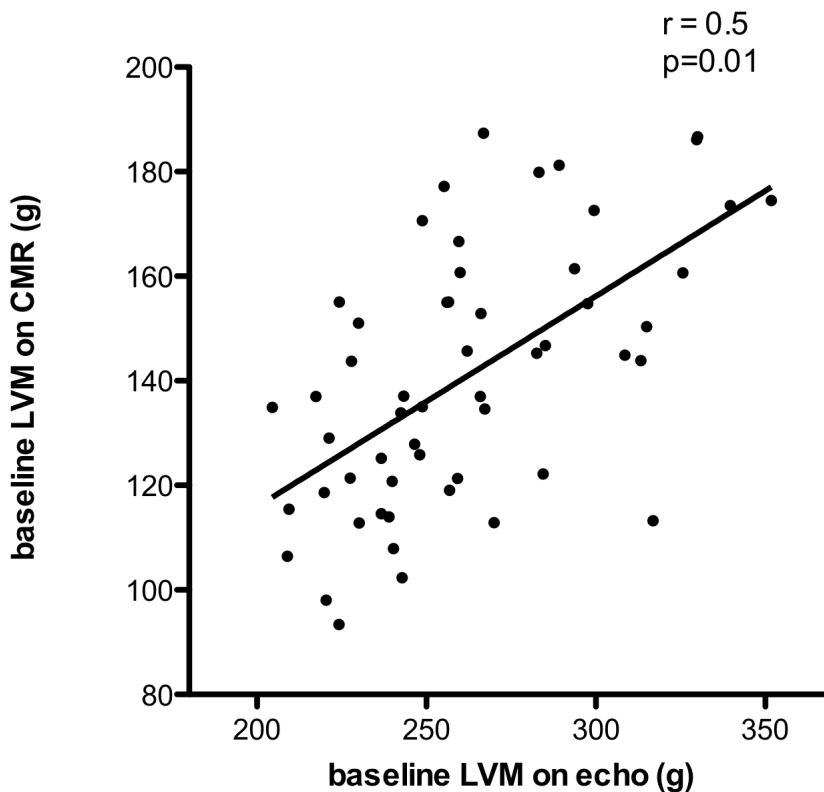


Figure 39. Correlation between baseline LVM measured using echo and CMR

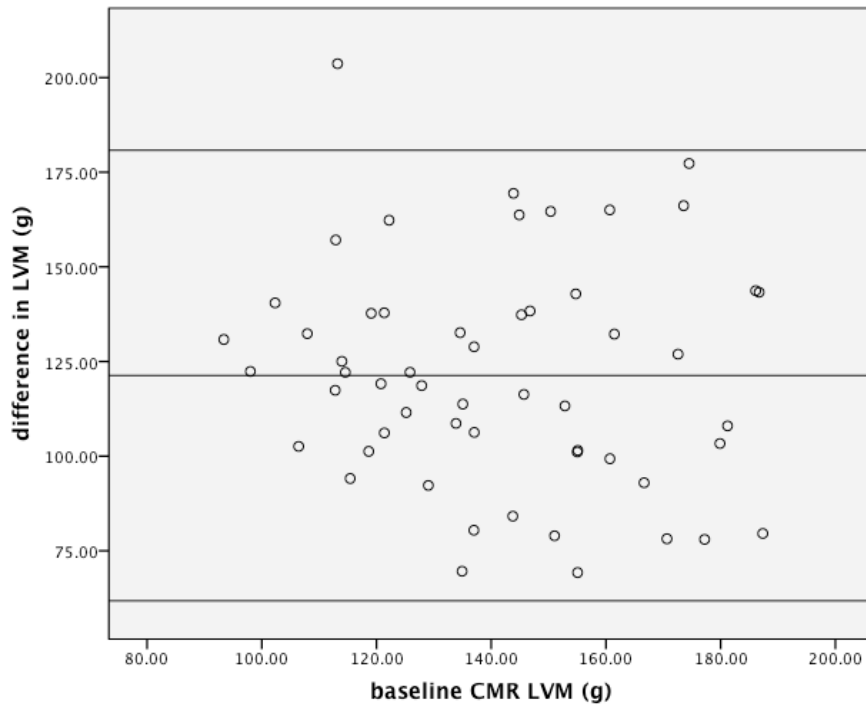


Figure 40. Bland Altman plot comparing LVM measurement by CMR and echo
Middle horizontal line is the mean difference in LVM measurements. Outer two horizontal lines indicate the mean \pm SD difference in LVM measurements.

3.13 Safety

3.13.1 Safety Bloods

Table 26 shows the safety bloods at baseline and after 9 months of study drug. There was a small, statistically significant reduction in white cell count, platelets, urea and creatinine and increase in bilirubin and alkaline phosphatase with allopurinol therapy. However, none of the changes in safety blood tests were clinically significant requiring withdrawal of study drug. The effect of allopurinol on full blood count and liver enzymes are well documented.

	Baseline	Allopurinol	p value	Baseline	Placebo	p-value
Haemoglobin	14.5 ± 1.4	14.2 ± 1.5	0.10	14.8 ± 0.9	14.7 ± 1.2	0.52
WCC	7.5 ± 2.3	7.0 ± 1.8	0.03	7.2 ± 1.6	6.9 ± 1.4	0.22
Platelet	209 ± 53.3	187 ± 46.8	< 0.01	224.6 ± 56.9	218 ± 52.8	0.16
Sodium	140.4 ± 2.2	141 ± 2.7	0.08	141.2 ± 1.9	141.2 ± 1.9	0.95
Potassium	4.4 ± 0.3	4.5 ± 0.4	0.30	4.4 ± 0.3	4.5 ± 0.3	0.03
Urea	6.5 ± 1.5	6.1 ± 1.1	0.03	6.5 ± 1.9	5.8 ± 1.7	0.01
Creatinine	81.8 ± 12.2	75.2 ± 11.6	< 0.01	80.6 ± 13.1	78.1 ± 13.3	0.07
Bilirubin	8.2 ± 2.7	10.2 ± 3.1	< 0.01	11.3 ± 5.5	11.9 ± 5.1	0.51
ALP	60.9 ± 18.2	64.7 ± 19.3	0.01	70.5 ± 18.1	68.8 ± 18.6	0.31
ALT	25.6 ± 8.0	26.9 ± 12	0.31	24.2 ± 7.6	23.6 ± 8.3	0.45
Albumin	44.8 ± 2.1	44.1 ± 2.5	0.10	45.4 ± 2.3	44.6 ± 2.1	0.07

Table 26. Safety bloods at baseline and after allopurinol and placebo therapy

3.13.2 Adverse Events

As allopurinol is a drug that has been prescribed for over 40 years, its side effects are well recognized. Table 27 shows the adverse events reported during this study. Except for one patient who developed gout, all the other adverse events were not felt to be related to the study medication. Allopurinol can exacerbate an acute attack of gout due to a sudden reduction in serum uric acid level. Rashes can affect up to 2% of those taking allopurinol. However, the rash developed by a participant whilst on 300mg study medication was felt not to be related to the study medication as the participant had no problems until commencing an oral antibiotic and the rash resolved once the antibiotic was stopped. The patient with pleurisy had suffered previous episodes prior to commencing study drug. The participant who had a permanent pacemaker inserted during the study period had a longstanding left bundle branch block pattern on 12 lead ECG and he had been admitted with a syncopal episode without a documented bradycardic episode. The participant diagnosed with renal malignancy and another with recurrent episodes of gout whilst on allopurinol 600mg daily was withdrawn from the study. Breaking of the randomization code was not required for any of these participants.

	Allopurinol	Placebo
Hospital admissions for < 24 hours with angina	1	2
Rash	1	0
Pleurisy	1	0
Abdominal pain	0	1
Insertion of pacemaker	1	0
Ankle fracture	0	1
Recurrent gout	1	0
Renal malignancy	0	1

Table 27. Adverse events

3.14 Study drug compliance

This was assessed by measuring the change in serum uric acid levels. As previously mentioned, the allopurinol group significantly reduced uric acid levels by 46% after 9 months of therapy whilst there was no significant change in the placebo arm. All the participants in the allopurinol arm had a reduction in uric acid levels except for one who had a 13% increase in uric acid level. This suggests that this participant was not compliant in taking his study drug.

3.15 Studies of Agreement

A single observer who was blinded to treatment allocation undertook the CMR LVM measurements two months apart. Intra-observer (SJG) measurement of LVM on CMR images had an intraclass correlation coefficient (ICC) of 0.989 (95% limits of agreement 0.982 - 0.993, $p < 0.01$). Figure 41 shows the Bland Altman plot of CMR LVM measurements.

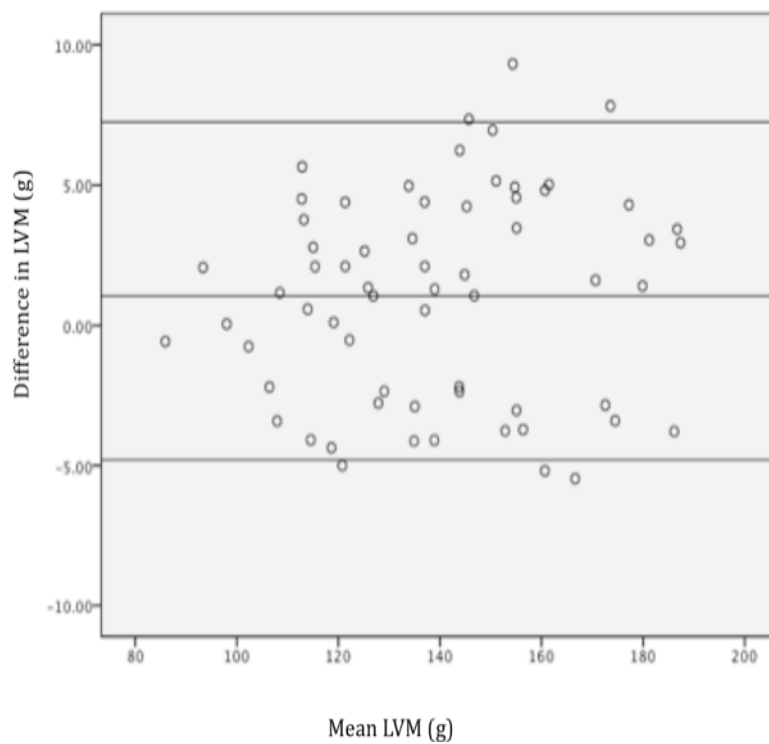


Figure 41. Bland Altman plot of CMR LVM measurement

Middle horizontal line is the mean difference in LVM measurements. Outer two horizontal lines indicate the mean \pm 2SD difference in LVM measurements.

A single observer who was blinded to treatment allocation undertook the FMD measurements one month apart. Intra-observer (SR) FMD measurements had an ICC of 0.991 (95% limits of agreement 0.978 - 0.997; $p < 0.01$). Figure 42 shows the Bland Altman plot of FMD measurements.

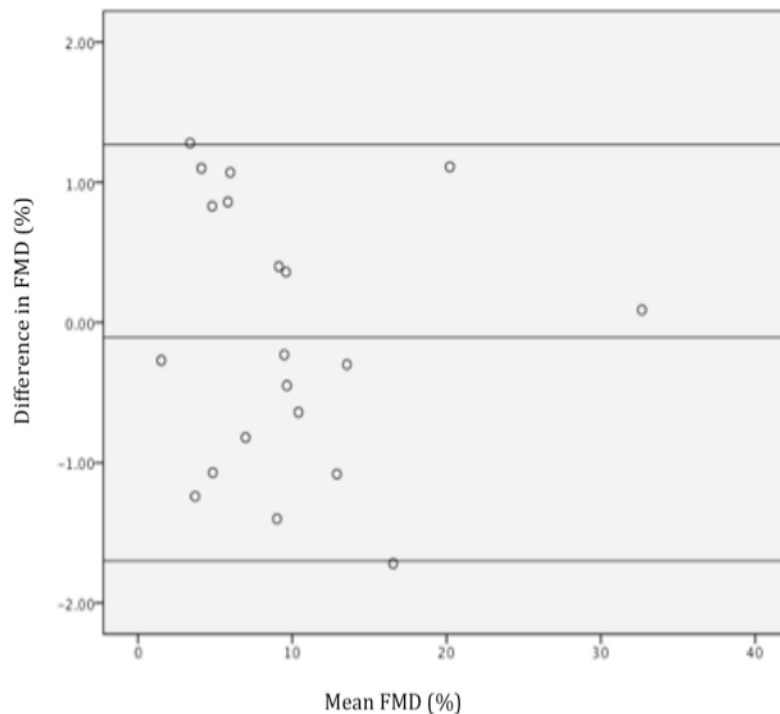


Figure 42. Bland Altman plot of FMD measurements

Middle horizontal line is the mean difference in LVM measurements. Outer two horizontal lines indicate the mean \pm 2SD difference in LVM measurements.

3.16 Medication Changes during Study Period

During the study period, a small number of participants (allopurinol group n=2, placebo group, n=3) had changes made to their medication due to clinical reasons. Three patients had changes made to antianginals (commenced on an antianginal: n=1 allopurinol group, n=1 placebo group; an antianginal stopped: n=1 placebo group). One participant in the allopurinol group had an ACE inhibitor changed to a beta-blocker whilst a participant in the placebo group was commenced on an ACE inhibitor.

3.17 Healthy Volunteers

Table 28 shows the baseline characteristics of the healthy volunteers (HV) compared to the two treatment groups. The HV were age and gender matched to the treatment groups. There was no significant difference in SBP albeit the HV had a lower DBP than the allopurinol group, which just failed to reach statistical significance ($p=0.07$). The HV had a lower baseline uric acid level compared to allopurinol group but similar to the placebo group. Upon performing the baseline endothelial function testing, the HV had a significantly lower AIx, a greater FMD response to GTN and similar PWV compared to patients with IHD and LVH. There was also a greater FMD response to hyperaemia in the HV compared to the allopurinol treatment group but not the placebo group.

	HV (n=30)	Allopurinol (n=31)	Placebo (n=29)	p value *	p value #
Age	65.2 ± 3.8	65 ± 6.7	64 ± 7.2	0.997	0.57
Male (%)	27 (90)	26 (84)	28 (97)	0.26	0.26
BSA, g/m ²	1.9 ± 0.2	2.1 ± 0.2	2 ± 0.2	<0.01	< 0.01
Smoking (%)				0.03	0.36
No	16 (53)	7 (21)	14 (42)		
Ex	12 (40)	20 (61)	13 (40)		
Current	2 (7)	6 (18)	6 (18)		
Office SBP, mmHg	129 ± 17	135 ± 9	134 ± 10	0.11	0.16
Office DBP, mmHg	74 ± 11	78 ± 7	76 ± 7	0.07	0.37
Uric acid, mmol/L	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.04	0.52
AIx, %	13 ± 11	20 ± 7	20 ± 9	< 0.01	0.01
PWV, m/s	7.9 (7.4 to 8.6)	7.6 (6.9 to 8.7)	8.5 (7.4 to 9)	0.36	0.17
FMD, %	6.3 ± 3.7	4.1 ± 2.1	5.7 ± 2.4	<0.01	0.46
FMD GTN, %	17.2 ± 6.1	11.2 ± 4.8	10.3 ± 4.9	<0.01	<0.01

Table 28. Baseline characteristics of HV and the two treatment groups

Data presented as mean ± SD or median (IQR)

*** comparison between HV and allopurinol group**

comparison between HV and placebo group

After nine months of treatment, there was an improvement in FMD and reduction in AIX in the allopurinol group although it did not reach the extent of the HV (see Table 29).

	HV (n=30)	Allopurinol (n=31)	Placebo (n=29)	p value *	p value #
Baseline FMD, %	6.3 ± 3.7	4.1 ± 2.1	5.7 ± 2.4	< 0.01	0.46
9 months FMD, %	6.3 ± 3.7	4.9 ± 2.2	5.0 ± 2.5	0.09	0.12
Baseline FMD GTN, %	17.2 ± 6.1	11.2 ± 4.8	10.3 ± 4.9	<0.01	<0.01
9 months FMD GTN, %	17.2 ± 6.1	9.7 ± 5	11.6 ± 5	<0.01	<0.01
Baseline AIX, %	13 ± 11	20 ± 7	20 ± 9	< 0.01	0.01
9 months AIX, %	13 ± 11	17 ± 9	20 ± 12	0.07	0.02
Baseline PWV, m/s	7.9 (7.4 to 8.6)	7.6 (6.9 to 8.7)	8.5 (7.4 to 9)	0.36	0.17
9 months PWV, m/s	7.9 (7.4 to 8.6)	7.5 (6.9 to 8.2)	8.0 (7.0 to 8.6)	0.13	0.85

Table 29. FMD of the brachial artery and applanation tonometry results of the HV and the treatment groups

Data presented as mean ± SD or median (IQR)

*** p value comparing HV and allopurinol group**

p value comparing HV and placebo group

4 DISCUSSION

To summarize, this study showed high dose allopurinol to regress LVM and improve arterial stiffness and endothelial dysfunction in patients with IHD. In this section of my thesis, I will discuss my study findings in further detail in the context of previous scientific evidence, explore potential mechanisms to explain my findings, highlight limitations of this study and to complete I will suggest potential future studies.

4.1 Effect of Allopurinol on LVM

The main finding of this study is that high dose allopurinol regresses LVM in patients with IHD and LVH who are on current evidence-based optimal medical therapy (allopurinol: -5.2 ± 5.8 g, placebo: -1.3 ± 4.5 g; $p < 0.01$). Allopurinol regressed LVM more in the higher baseline LVM group as might be expected. There is no clear explanation as to why there was a slight reduction in LVM in the placebo group over the 9 month period as there was no significant change in BP or BMI.

Regression in LVM observed in this study with allopurinol therapy is consistent with previous experimental and human studies. Laakso *et al.* found allopurinol to prevent LVH development in spontaneously hypertensive rats (SHR) with negligible BP lowering effects (328). Additionally, in L-NAME exposed rats,

allopurinol prevented an increase in LVMI, which was comparable to an ARB (329). Febuxostat, a non-purine XO inhibitor, has also been shown to be beneficial in attenuating LVH induction by systolic overload but only if commenced early and not delayed until after the development of cardiac hypertrophy (330). In the La Plata study, a randomized, double-blind placebo controlled study of 60 NYHA class II-III heart failure patients, administration of 600mg oxypurinol/day for only a month showed some reduction in LVM although this just failed to reach statistical significance ($p=0.11$) (331). The lack of significant LVM reduction with oxypurinol therapy may be explained by the very short treatment period of only one month and also to the low dosage of XO inhibition therapy used (600mg oxypurinol is equivalent to only 83mg allopurinol). Recently, a RCT undertaken by Kao *et al.* in Dundee found allopurinol 300mg/day prescribed to 67 patients with CKD stage 3 and LVH for a period of 9 months to significantly regress LVMI by 5% ($1.4 \pm 4.7 \text{ g/m}^2$) (332) compared to 3.29% ($2.2 \pm 2.8 \text{ g/m}^2$) in my study. The regression of LVM by high dose allopurinol in this CKD cohort was felt likely due to the improvement in arterial compliance and arterial wave reflection resulting in reduction in LV afterload (332). This is because the fall in AIx correlated with the fall in LVM. It should be highlighted that CKD patients are likely to have much higher levels of OS compared to my study population with optimally treated IHD. Kao *et al.* used a lower dose of allopurinol (300mg/day) compared to our study due to the increased risk of allopurinol toxicity with impaired renal function (332).

The reduction in LVH in percentage terms shown in our study is surprisingly similar to that seen in the LIFE study despite our follow up period being only 9 months compared to 4.8 years in the LIFE study. In our study, allopurinol produced a mean reduction in LVM of 3.57% and of 3.3% in LVMI. In the echo sub-study of the LIFE study, both losartan and atenolol reduced LVMI by 17.4% and 14.4% respectively i.e. the differential effect between losartan and atenolol in regressing LVH was only 3% (250).

The next most relevant study is the 4E-LVH study by Pitt *et al.* because it used MRI to measure changes in LVM as in our study (262). In the 4E-LVH study, adding eplerenone to enalapril produced an extra 7.5g reduction in LVM but also a 4 mmHg fall in SBP. In our study, allopurinol therapy resulted in a 5.2g reduction in LVM with no associated significant BP effect. The percent change in LVM in Pitt *et al.* (2003) cannot be ascertained as surprisingly they did not provide baseline LVM in their paper but their patients were, unlike our study, markedly hypertensive (BP 163/97 mmHg) which means their baseline LVM are likely to have been much greater than ours so that the percent change in LVM seen in our study and this 4E-LVH study might well have been similar, despite the extra BP fall in the 4E-LVH study.

More recently, a CMR study of hypertensive patients with LVH by Burns *et al.* reported 6 months treatment with a combination of valsartan and moxonidine to regress LVM on cardiac MRI by 15% and LVMI by 15.4% whilst a combination of bendroflumethiazide and amlodipine therapy reduced LVM by 10.9% and LVMI by 14% (340). In this CMR study, the observed regression of LVM and LVMI was much greater compared to our study of 3.57% and 3.3% respectively. This is most likely due to the much greater observed mean SBP reduction associated with the antihypertensive therapy prescribed in this study (combination of valsartan and moxonidine: 37mmHg, combination of bendroflumethiazide and amlodipine: 38mmHg) compared to allopurinol therapy in my study (at most 5mmHg depending on which BP parameter is chosen).

Klingbeil *et al.* performed a meta-analysis comparing the effect of various antihypertensives on LVM using echocardiography in patients with essential hypertension (255). They reported a percent reduction in LVMI of 6% for beta blockers, 8% for diuretics and 10% for ACE inhibitors. For ARBs, the fall in LVMI was 13% over 1 year, which meant probably around 9.7% in a 9 month treatment period. These results are higher than ours but differences in ultimate blood pressure (-12mmHg SBP) would be a big contributor, which did not occur in our study or in the LIFE study. A more recent meta-analysis in 2009 by Fagard *et al.* did not show a significant difference in changes in LVM between

the various drug treatments except that beta-blockers had less LVM regression compared to ARBs (256).

The majority of previous studies, as already discussed, involved BP dependent changes in LVM by using antihypertensives. However, SBP accounts for only 31% of LVM variance (119), the LIFE study demonstrated changes in LVM independent of BP (16, 341) and Pitt *et al.* concluded from the 4E-LVH study that “other factors may be as important or more important for reducing LV mass than BP reduction *per se*” (262). OS is increasingly being appreciated to cause cardiac hypertrophy. In our study, we focused on BP independent changes in LVM, which are bound to be less than using drugs that lower BP.

4.2 Effect of Allopurinol on LV volumes

In this study, allopurinol significantly reduced the LVESV ($p < 0.05$) whilst there was a non-statistically significant reduction in EDV ($p = 0.15$). The effect of allopurinol on LV volumes has previously been shown in animal studies. After an experimental MI, allopurinol therapy reduced the LV cavity size (LVEDD) (21) whilst in another animal model XOI attenuated the increase in LVESV and LVEDV post MI by 50%, improved energetics and function without any change in LVM (342). Another animal study found short term allopurinol therapy of 5 days to have a transient reduction in myocardial ROS associated

with a reduction in LV systolic diameter. However, longer periods of allopurinol therapy of 10 weeks resulted in a reduction in LV end-diastolic pressure, LV systolic and diastolic diameters (343). A recent human study of patients with CKD stage 3 and LVH found allopurinol to reduce LVEDV by a mean of 9.6mls although it did not reach statistical significance ($p=0.08$) whilst there was no change in ESV (332).

4.3 Effect of Allopurinol on Endothelial function (FMD)

In this study, 9 months of allopurinol therapy improved the FMD response to hyperaemia by $0.8 \pm 1.8\%$ compared to a reduction of $0.7 \pm 2.8\%$ in the placebo group ($p = 0.02$) although it did not reach the extent of the healthy volunteers. Interestingly, allopurinol significantly reduced the FMD response to GTN compared to placebo group.

The findings of this study are in keeping with previous studies that have shown xanthine oxidase inhibitors to improve endothelial function in numerous study populations including those with diabetes, ischaemic heart disease, congestive heart failure, CKD and smokers (22, 326, 332-334, 344, 345).

Butler *et al.* administered 300mg allopurinol for one month duration to 11 patients with type 2 DM and mild hypertension and 12 healthy age-matched

controls. Allopurinol had a 30% relative improvement in endothelial function to near normal levels with an associated significant reduction in malondialdehyde, a marker of oxidative stress in the diabetic group but not in the healthy controls (333). Farquharson *et al.* and George *et al.* have shown allopurinol to relatively improve endothelial function of NYHA class II-III chronic heart failure patients by 50% and 30% respectively (22, 326). In a study of chronic stable angina patients, 600mg of allopurinol for 2 months improved the FMD from $4.2 \pm 1.8\%$ at baseline to $5.4 \pm 1.7\%$ ($p < 0.01$) with complete abolishment of vascular oxidative stress (334). Additionally in a recent study from our department of patients with chronic kidney disease and LVH, 300mg of allopurinol for 9 months resulted in a 30% improvement in FMD (332). George *et al.* concluded that the improvement in endothelial function resulting from allopurinol therapy is not related to the reduction of uric acid but to the almost complete mopping up of vascular oxidative stress (326).

The beneficial effect of allopurinol on endothelial function is likely to be at the level of the resistance vessels as opposed to the capacitance arteries. The increase in the brachial artery FMD with allopurinol therapy is likely due to the improvement in the hyperaemic response in the small arteries, which increases blood flow and greater shear stress resulting in greater dilatation of the brachial artery.

Although the improvement in FMD with allopurinol was not to the level of healthy volunteers, the healthy volunteers only had a single FMD measurement undertaken and they did not have a screening echocardiogram to exclude cardiac hypertrophy, which all the main study participants had.

As endothelial dysfunction is associated with a poor prognosis, improving endothelial function in my study population (chronic stable angina and LVH) should reduce cardiovascular event rates and will be a mechanism by which allopurinol regressed LV mass. Medication commonly prescribed to patients with IHD such as ACE inhibitors, ARBs and statins can also improve endothelial function (41).

An unexpected finding of this study was that allopurinol reduced the FMD response to GTN compared to the placebo group and this was statistically significant in those not on regular nitrates. Previous studies using allopurinol therapy have not detected such an effect but this was not the primary aim of my study. Studies of subjects with atherosclerosis have shown either a trend or a significantly reduced arterial vasodilator response to GTN which was felt to be explained by smooth muscle cell dysfunction and nitrate tolerance in patients with IHD on regular nitrates (346).

4.4 Effect of Allopurinol on Arterial stiffness

Arterial stiffness occurs not only in the presence of coronary artery disease but increases with the number of diseased coronary arteries (79). In this study, 9 months of treatment with allopurinol significantly reduced AIx without any significant change in brachial BP and this was independent of the change in uric acid.

The beneficial effect of allopurinol on arterial stiffness found in my study is consistent with previous studies of different patient groups including stroke, chronic stable angina and CKD stage 3 with LVH (332, 334, 335). In an RCT of stroke survivors with high uric acid levels, allopurinol 300mg for 2 months reduced AIx from $26 \pm 3\%$ to $20 \pm 2\%$ compared to an increase from $24 \pm 3\%$ to $28 \pm 3\%$ in placebo group ($p=0.03$) and it did not correlate with change in uric acid level (335). In another study of patients with stable angina, 600mg allopurinol for 2 months resulted in a reduction in AIx from $27 \pm 5\%$ to $25 \pm 5\%$ ($p < 0.01$) without a significant change in placebo group (334). In a study of patients with CKD stage 3 and LVH, 300mg allopurinol for 9 months reduced AIx by $5 \pm 9\%$ compared to an increase of $1 \pm 6\%$ in the placebo group ($p=0.02$) (332). In all these studies, there was no significant change in BP associated with allopurinol therapy. As AIx correlates with superoxide anion production (347), allopurinol is likely to have reduced AIx by reducing the production of ROS by XO.

There are other commonly prescribed cardiovascular medication including beta-blockers, ACE inhibitors, ARBs, calcium channel blockers, nitrates and statins that also decrease arterial stiffness and arterial wave reflections (73).

In arterial stiffness, an increased velocity of forward and backward wave results in the wave returning in late systole instead of early diastole, thereby augmenting systolic pressure and reducing diastolic pressure (75). The augmented systolic pressure increases cardiac afterload, which is the main determinant of LVM, resulting in LVH whilst reducing coronary perfusion. Hence, a reduction in afterload by allopurinol should result in regression of LVM. A study by Hashimoto *et al.* found Aix to be better at predicting LVM regression than brachial BP (348). In a study of CKD stage 3 patients with LVH, the authors concluded that the likely explanation for allopurinol to regress LVM in their patients was due to the reduction in LV afterload (332).

As arterial stiffness is associated with a poor prognosis in patients with coronary artery disease (82), the reduction of arterial stiffness with allopurinol therapy should result in a reduction in cardiovascular morbidity and mortality. Noman *et al.* concluded that the reduction in arterial stiffness may have been one of the mechanisms by which allopurinol was anti-ischaemic in angina patients (318).

In this study, allopurinol did not have an effect on PWV. This could be explained by us measuring PWV between the carotid-radial sites instead of carotid-femoral. The carotid-femoral PWV technique has mainly been used in previous epidemiological studies as it measures along the aortic-iliac pathway and so gives information as to what pressures the LV is exposed to. In my study we decided to use the carotid-radial PWV technique as we felt that having to take measurements from the groin of patients may put them off from taking part in the study and this was not a primary study outcome measure. However, as shown by Tillin *et al.* the site of measuring PWV does matter and carotid-femoral was better than carotid-radial or femoral-posterior tibial (349).

4.5 Effect of Allopurinol on left atrial volumes

In this study, although there was a reduction in left atrial diastolic volume after 9 months of allopurinol therapy, this was not statistically significant. There may have not been a significant reduction in left atrial volumes due to the relatively short follow up period. In LVH the increase in LV end-diastolic pressure results in left atrial dilatation. As the LVM regresses, the reduction in LV end-diastolic pressure will then indirectly result in reduction in left atrial size, which will likely to need a longer follow up to see a change. Previous studies of LVM regression have not assessed its effects on left atrial size. However, LVM regression has been shown to reduce the incidence of AF in the LIFE study,

which has been postulated to be due to reduction in left atrial volumes, a risk marker of AF.

4.6 Effect of Allopurinol on BP

In this study, allopurinol did not significantly reduce office BP compared to placebo therapy (mean change in office SBP: allopurinol group -5mmHg vs. placebo group -3mmHg, $p=0.59$; mean change in office DBP: allopurinol group -4mmHg vs. placebo group -1mmHg, $p=0.2$). Additionally, a subgroup of patients also had a 24-hour BP monitor, a more reliable measure of BP control, which did not show a significant difference between the two treatment groups (mean change in 24hr SBP: allopurinol group +2mmHg vs. placebo group -3mmHg, $p=0.17$; mean change in 24hr DBP: allopurinol group +1mmHg vs. placebo group -3mmHg, $p=0.61$).

Our findings are consistent with the majority of previous published work using allopurinol therapy in patients with IHD (318, 334), heart failure (326), diabetes (333), stroke (335) and CKD (332). However, a RCT with cross-over design of 30 adolescents with hyperuricemia (serum uric acid levels ≥ 0.36 mmol/L) and hypertension found allopurinol 200mg twice daily for 1 month to significantly reduce office SBP (mean change in SBP: allopurinol -7mmHg vs. placebo -2.0mmHg, $p < 0.01$) and office DBP (mean change in DBP: allopurinol -5mmHg vs. placebo -2mmHg, $p=0.05$). Allopurinol also significantly reduced

24-hour ambulatory BP (mean change in 24-hour SBP: allopurinol -6mmHg vs. placebo 1mmHg, $p < 0.01$; mean change in 24-hour DBP: allopurinol -5mmHg vs. placebo 0mmHg, $p < 0.01$). Allopurinol therapy resulted in a mean reduction in serum uric acid levels from 0.41 mmol/L to 0.25 mmol/L (350). Raised uric acid levels have been associated with the presence of hypertension (291). However, it is still unclear whether uric acid is the cause or is the consequence of hypertension. Uric acid has been shown to stimulate renin release and in this adolescent study, there was an associated reduction in plasma renin activity with allopurinol therapy which may help explain the reduction in BP (350).

A study of patients with chronic stable angina by Noman *et al.* found high dose allopurinol for 6 weeks to not significantly affect office BP but there was a significant reduction in SBP and DBP at stage 1 of exercise testing (318).

4.7 Effect of Allopurinol on oxidative stress

There is overwhelming evidence that oxidative stress mediates cardiac hypertrophy (1, 11, 13) and inactivates NO resulting in endothelial dysfunction (2, 351). A number of experimental and human studies have shown allopurinol to reduce oxidative stress (24, 326, 334, 343).

In this study, allopurinol regressed LVM and improved endothelial function but it did not seem to have an effect on oxidized LDL, a marker of oxidative stress. There may be a number of reasons why? Firstly oxidative stress is the oxidation of proteins, nucleic acids and lipids by ROS. There are many available urine and plasma OS biomarkers that have been used in studies including oxidized LDL, isoprostanes and malondialdehyde. Oxidized LDL is only one of the many available biomarkers and it was used in my study as we have had some experience measuring it in our department. Oxidized LDL is not a single, chemically homogenous entity (352) and so there a number of oxidized LDL assays that detected different epitopes. The assay used in my study measures oxidized apolipoprotein-B. Secondly, OS biomarkers have limitations and for example studies using statins have also not always been able to show a reduction in OS biomarkers (353, 354). Thirdly, assessment of vascular OS may be better than measuring plasma or urine OS biomarkers as it measures vascular function instead of blood or urine levels and it is measured in vivo (334). The gold standard method of measuring vascular OS is by forearm venous occlusion plethysmography (FVOP) and infusing intra-arterial high dose vitamin C, which is a scavenger of free radicals. A study by Rajendra *et al.* found allopurinol to have a greater effect on vascular OS than on plasma OS biomarkers (334). FVOP was not undertaken in my study as it is an invasive procedure that is quite time consuming. Finally, the study was not powered to assess for changes in oxidized LDL levels.

4.8 Effect of Allopurinol on BNP

In this study, there was a poor correlation between baseline BNP and baseline LVM. This is unlike some previous studies that have shown a positive correlation between BNP and LVM in the general population (355), in hypertensives (283, 356, 357) and patients with hypertrophic cardiomyopathy (285, 286). A possible explanation for poor correlation seen in my study is that we have not accounted for the degree of myocardial ischaemia in the study patients. As previously discussed, BNP levels are also elevated in the presence and degree of myocardial ischaemia (274-276, 278, 279). Hence, the BNP levels measured at baseline will reflect not only the LVM but also myocardial ischaemia.

In my study, allopurinol numerically reduced BNP (median BNP reduction 6 pg/ml), which just failed to reach statistical significance ($p=0.08$). However, baseline BNP levels between the active and placebo groups were rather different making the change data hard to interpret. As already mentioned, BNP secretion is triggered by increased ventricular filling pressures. Hence, the reduction of BNP in my study is likely related to the significant reduction in LVESV and arterial compliance and LV afterload and non-significant reduction in LVEDV. The other theory is whether the reduction in LVM resulted in the reduction in BNP. Studies have shown regression of LVM with ACE inhibitors, ARBs and

spironolactone to be associated with a reduction in BNP (257, 358, 359). In other studies, regression in LVM in patients with CKD stage 3 and LVH with allopurinol therapy (332) and normotensive patients treated with antihypertensives (259) were not associated with a significant change in BNP. My study did not see a correlation between the change in BNP and change in LVM but it was not powered to assess this.

Previous studies have been conflicting in the effect of allopurinol on BNP levels (318, 325, 326, 332, 334). In a study of patients with CKD stage 3 and LVH (332) and another study of patients with chronic heart failure NYHA class II-III (326), allopurinol did not affect BNP levels. However, in other studies allopurinol significantly reduced BNP in patients with chronic stable angina (318) and heart failure (325) whilst non-significantly in another study of patients with angina (334).

4.9 Possible mechanisms Allopurinol reduced LVM

There are numerous possible mechanisms by which allopurinol regressed LVM. Firstly, patients with IHD tend to develop endothelial dysfunction and increased arterial stiffness. As allopurinol improved endothelial dysfunction and arterial stiffness without a significant reduction in BP, the reduction in LV afterload is likely to result in regression in LVM, as LV afterload is known to be a better predictor of LVM than brachial BP (348). An effect on LV afterload is supported

by our significant reduction in end-systolic volume and non-significant reductions in both end-diastolic volume and BNP. Secondly allopurinol reduced uric acid levels which has been associated with LVH due to its effects on endothelial function, stimulation of hypertrophic signalling pathway and its correlation with other factors such as obesity, hypertension and renal impairment (301, 305-307). However, it is still unclear if uric acid is a cause of hypertrophy. Finally, allopurinol being a XO inhibitor is likely to have reduced oxidative stress, which plays a role in the development of LVH. We did not detect a reduction in oxidized LDL but this is only one of the numerous OS biomarkers and all assays have limitations. Previous studies have shown an improvement in vascular tissue OS with allopurinol (24, 326, 334, 343) and it would have been better if instead we measured vascular OS using FVOP. Findings of this study should be confirmed in larger scaled studies.

4.10 Prognostic benefit of LVM regression

Patients with IHD commonly develop LVH, which has been said to be the ‘most reliable surrogate marker we have in cardiovascular medicine’ (204, 205). LVH has been associated with an increased risk of cardiovascular events including coronary heart disease, MI, heart failure, stroke and arrhythmias. It is a reversible, poor prognostic marker. Numerous landmark studies including HOPE and LIFE have shown LVM regression to be associated with an improved

prognosis. Majority of the previous studies assessing LVM regression have been on hypertensive populations treated with antihypertensive therapy although the prognostic benefit of LVM regression was independent of BP reduction.

In the PIUMA study, Verdecchia *et al.* demonstrated the group with regression of LVM to be associated with 1.2 times lower cardiovascular event rate per 100 person-years compared to the group of non-regressors ($p=0.03$, log-rank test) (248). The reduction in cardiovascular event rate was even greater in the LVM regressors (4.5 times) compared to the non-regressors in the subgroup with a higher baseline LVMI ($\text{LVMI} > 125 \text{g/m}^2$) ($p < 0.01$, log-rank test) (248). Koren *et al.* also showed that hypertensive patients with unchanged or regression of LVM had a lower cardiovascular event rate compared to those with an increase in LVM (360). In the HOPE substudy, patients with regression or absence of LVH on ECG compared to those with development or persistence of LVH had a lower risk of primary composite endpoint of cardiovascular death, MI or stroke (12.3% vs. 15.8% respectively, $p < 0.01$) and of congestive heart failure (9.3% vs. 15.4% respectively, $p < 0.01$) (361). In 2003, a meta-analysis by Verdecchia *et al.* which included 4 studies of hypertensive patients, regression of LVH on echo was associated with a 59% reduced risk of cardiovascular events compared to persistence or new LVH ($p < 0.01$) (249).

The reduction in LVH shown in our study is similar in percent terms to that seen in the echo substudy of the LIFE study. In the LIFE study, the differential effect between losartan and atenolol in regressing LVH was only 3% over 4.8 years (250). This was associated with a reduction in all-cause mortality of 28%, cardiovascular mortality of 38%, sudden cardiac death of 19%, myocardial infarction of 15%, new congestive heart failure of 36%, new onset atrial fibrillation of 12% and stroke of 24% in favour of losartan over atenolol and were independent of any change in BP (250, 252-254). The LIFE study is the best guide as to what effect allopurinol might have on future cardiovascular events because it is only in the LIFE study that the effect of LVH changes on cardiovascular events can be ascertained in the absence of any differential effect on blood pressure. It remains to be seen whether allopurinol induced LVH regression can deliver anything like these effects that were seen between the groups in the LIFE.

4.11 Prognostic benefit of Allopurinol on future cardiovascular events

The LIFE study is the best possible guide as the effect we saw with allopurinol on LVM was comparable to the differential effect seen between losartan and atenolol in LIFE. Hence it is feasible that allopurinol might reduce cardiovascular events. Four factors also amplify the chance that allopurinol will

reduce future cardiovascular events. Firstly, we only gave allopurinol for 9 months in our study (LIFE was 4.8 years) and allopurinol's effect on LVM is likely to be greater the longer it is given. It is clear from the echo LIFE sub-study that this is likely the case ie. LVH regression occurs gradually over 2 years and then plateaus. Secondly, allopurinol also has anti-ischaemic activity (318) and this extra effect should also help it to reduce cardiovascular events. Thirdly, the beneficial effect of allopurinol on vascular function, seen in this study, should also enhance the chance that allopurinol will reduce cardiovascular events. Fourthly, we also found LV ESV to fall significantly after allopurinol in this study. LV ESV has been shown to be a predictor of survival post MI (362) and heart failure hospitalizations in patients with stable coronary artery disease (363). Overall our suggestion that allopurinol might reduce future cardiovascular events is now not just based on its effect on LVM here but also on its anti-ischaemic effect, its effects on endothelial function and on its effects on LV end systolic volume and LV afterload (Aix). Together they represent a group of fairly reliable surrogates which all change in the correct direction.

4.12 Choice of dosage

In this study we chose to use 600mg/day allopurinol, which was gradually uptitrated over 6 weeks. Previous studies have shown 600mg/day allopurinol to be well tolerated and have a greater beneficial effect than lower doses (319, 320, 326). George *et al.* found that allopurinol had a steep dose response on endothelial function. High dose allopurinol (600mg) had a greater effect on endothelial function compared to 300mg allopurinol [mean % change in forearm blood flow \pm SEM on forearm venous occlusion plethysmography (FVOP): 240.3 \pm 38.2% for 600mg allopurinol vs. 152.1 \pm 18.2% for 300mg allopurinol vs. 74 \pm 10.3% for placebo; $p < 0.01$] (326).

A couple of observational studies have also found higher dose allopurinol (\geq 300mg) to be associated with a lower cardiovascular event rate and mortality compared to lower dose allopurinol ($<$ 300mg) (319, 320). Dosage of allopurinol is very important as highlighted in the OPT-CHF (Oxypurinol Therapy for Congestive Heart Failure) study. The study failed to show any benefit of XOI on the composite endpoint of heart failure morbidity, mortality and quality-of-life most likely due to the inadequate dosage of XOI used. 600mg of oxypurinol prescribed in the study had a bioavailability equivalent to only 81mg allopurinol and reduce serum uric acid levels by 26% compared to 46% in this study (327).

5 STUDY LIMITATIONS

5.1 Study design and population

This was a randomized controlled study with a relatively small sample size undertaken at a single centre. However, this was a proof of concept study that was adequately powered for the study primary outcome measure. As cardiac MRI has a greater accuracy and reproducibility in measuring LVM compared to echo, we were able to reliably detect changes in LVM using smaller sample sizes. However, my study findings need to be confirmed in larger scale RCT studies.

The follow up period of this study was amended early from 1 year to 9 months as we were unable to commence the study during my first 6 months due to difficulty obtaining study medication. The recent changes in MHRA regulations resulted in less pharmaceuticals companies being able to supply the study medication at a reasonable cost. In order to complete recruitment and the study within my 2 years at University of Dundee, we had to reduce the follow up period. In the 4E-LVH study, the effects of eplerenone, enalapril and eplerenone/enalapril combination on LVM were measured using CMR over a 9 month follow up period (262). Additionally, Kao *et al.* found allopurinol to regress LVM in CKD patients over 9 months follow up period (332). Hence, we felt that 9 months should be a sufficient follow up period for our study.

However, it is possible that if we had given high dose allopurinol over a longer treatment period, the treatment effect would have been larger.

In this study, only 10% of the recruited study participants were female. Females tend to be under-represented in cardiovascular research despite the increasing burden of cardiovascular disease in females. Since 2006, only 30% of study participants in cardiovascular clinical trials have been female (364). This has implications as the current cardiovascular evidence for females has been extrapolated from research studies with predominantly male participants. In view of the differences in biological, hormonal and psychosocial factors and the implications of cardiovascular risk factors, there are gender differences in the presentation and progression of cardiovascular disease and their response to treatments (364).

5.2 24-hour ambulatory BP monitoring and central BP

In this study, only around 50% randomly selected participants had a 24-hour BP monitor due to the limited availability of 24-hour BP monitors in our department. All participants had an office BP measurement at each clinic visit. A 24-hour BP monitor more accurately reflects BP control compared to office BP but this was not a primary outcome measure. However, it is reassuring to see that

there was no significant change in BP with allopurinol therapy in both the office BP and the subgroup that had a 24-hour BP monitor.

We did not measure central BP noninvasively using Sphygmocor but relied on measuring the brachial BP. In some studies, measuring brachial BP seemed just as good an estimate of central BP as other noninvasive devices (365, 366). Brachial BP is generally used to calibrate the Sphygmocor system but its measurement has been shown to be the cause of inaccuracy and underestimation of central BP (367).

5.3 Assessment of ongoing cardiac ischaemia

75% of the recruited study patients had previously had a positive exercise stress test or MPS. However, as part of this study we did not assess for the ongoing ischaemic burden. This is important as in theory myocardial ischaemia is a mechanism by which there is a production of ROS resulting in cardiac hypertrophy and possibly the reduction in LVM seen in this study with allopurinol therapy may be due to the reduction in myocardial ischaemia as shown by Noman *et al* (318).

5.4 Primary endpoint

The primary endpoint was the change in LVM, which is a surrogate marker of poor prognosis instead of being a hard clinical endpoint such as morbidity or mortality. This should be addressed in future studies.

5.5 Effect of Allopurinol on LVM

In this study the effect of allopurinol on LVM, although significant, is modest. The reduction in LVM may have been larger if the treatment period was longer. However, it is similar in percentage terms to what was seen in the change in LVM between atenolol and losartan therapy groups, in the echo sub-study of the LIFE trial (16).

5.6 Safety of long term high dose Allopurinol therapy

The safety profile of allopurinol is well established as it has been used for 40 years as a treatment for gout. However, the safety of high dose (600mg/day) allopurinol for more than 9 months is unknown.

5.7 Healthy volunteers subgroup

Unlike the main study, the healthy volunteers in the sub-study did not undergo a screening echocardiogram to exclude any structural cardiac disease such as left ventricular hypertrophy. However, this was not part of the main study.

6 FUTURE STUDIES

6.1 Longer follow up studies

In this study we have seen a modest reduction in LVM over a 9 month follow up period. An important question remains - if we gave allopurinol for a longer study period, does it have a sustained effect on LVM regression and result in a greater overall reduction or do we lose the initial beneficial effect we have seen of LVM regression? In the LIFE study, most of the LVM reduction occurred within 2 years of treatment (16). This should be explored in future studies but would require greater time commitment from study patients to take the study drug for a longer study period and a more costly study to fund the additional study visits.

6.2 Greater baseline LVM

A number of studies have shown baseline LVM to significantly correlate with the change in LVM seen with allopurinol and antihypertensive therapy (255, 332). My study group had relatively mild LVH and allopurinol regressed LVM more in the higher baseline LVM group. Hence, repeating the study with a greater baseline LVM is likely to result in greater reduction in LVM with allopurinol therapy. As myocardial ischaemia is one of the mechanisms of developing LVH, patients with more severe angina may have greater degrees of LVH.

6.3 Hard clinical end points

This study looked at the effect of allopurinol on LVM, which is a surrogate marker of poor cardiovascular outcomes. However, it would be more clinically important to address hard cardiovascular clinical endpoints. High dose allopurinol has been shown in 'real world' population-based cohort studies to reduce cardiovascular events and mortality in heart failure patients and those taking allopurinol therapy (320, 368). Two small RCTs have also shown allopurinol to reduce cardiovascular events in acute STEMI patients undergoing primary PCI and CKD patients (317, 369). However, it has not been assessed in patients with chronic stable angina. Due to the relatively low event rate in chronic stable angina patients with an annual non-fatal MI rate of 0.5-2.6% and

annual mortality rate of 0.9-1.4% (85), such a study would require a very large sample size.

6.4 CMR perfusion with late GAD

Allopurinol regressed LVM in my study. However, it is unknown if this was partly due to its anti-ischaemic effect. This could be assessed further by undertaking a CMR perfusion study. Additionally, myocardial fibrosis is a key component of LVH especially due to LV remodelling. Fibrosis and scar can be detected with CMR using late gadolinium (GAD) enhancement imaging. Hence, it would be interesting to see if when allopurinol regressed LVM it also reduces the degree of myocardial fibrosis.

6.5 CMR spectroscopy

In ischaemia, the imbalance in ATP demand vs. supply leads to a reduction in phosphocreatine levels thereby resulting in a fall in PCr to ATP ratio. Allopurinol has been shown to improve cardiac energetics in heart failure patients by increasing ATP synthesis and efficiency (324). Hence, it would be worthwhile exploring whether the anti-ischaemic effect of allopurinol therapy on IHD patients is associated with improved cardiac energetics. CMR spectroscopy is a non-invasive technique that assesses cardiac metabolism without use of an

external tracer. Cardiac ^{31}P -MRS gives us information regarding the energy state of the heart by the PCr to ATP ratio.

6.6 Acute coronary syndrome

Allopurinol has been shown to be anti-ischaemic in chronic stable angina patients (318). The next step would be to assess allopurinols' anti-ischaemic effect in patients having an ACS or STEMI. As in these situations there would also be increased levels of oxidative stress, the effect of allopurinol on the degree of myocardial damage should be assessed by measuring troponin levels, LV function using echocardiography and infarct size using CMR with late gadolinium enhancement. Small scaled RCTs have assessed the use of oral allopurinol on STEMI patients (316, 317) which will need to be confirmed in larger scaled RCTs. Additionally, instead of giving allopurinol orally other options include intravenous or intracoronary if the patient is undergoing coronary angiography.

6.7 Other patient groups

Similar to my patient cohort, patients with peripheral artery disease have increased level of oxidative stress and commonly develop LVH (50%) and endothelial dysfunction (110, 370). As shown by the beneficial effects of

allopurinol in my study, it is likely that allopurinol will have a similar effect on endothelial function and LVM in the patients with peripheral artery disease.

The other group worth exploring is the effect of allopurinol on LVM in patients with LVH and resistant hypertension despite numerous antihypertensive therapies. As allopurinol does not significantly reduce BP it may be able to regress LVH, which is commonly seen in this patient group, by reducing oxidative stress and improving endothelial function. In this group of patients, allopurinol might protect the heart (LVH) against the continuing high BP.

6.8 Other inhibitors of Xanthine oxidase

As allopurinol has been shown to be effective in regressing LVM, other XO inhibitors such as febuxostat and oxypurinol should also be assessed in future human studies. Febuxostat is a non-purine, selective XO inhibitor that has been approved in the last 5 years for the treatment of gout, especially for those intolerant to allopurinol. It is an orally administered drug that is more effective in reducing uric acid levels in gout patients compared to allopurinol with similar adverse event rates (371). Febuxostat has been shown in experimental studies to reduce oxidative stress and inhibit the development of LVH in a pressure overloaded ventricle (330). As it is a more potent XO inhibitor, future studies

should examine if febuxostat is more effective than allopurinol in regressing LVM.

Oxypurinol is another XO inhibitor that is the active metabolite of allopurinol and has a longer half-life. In the La-PLATA study, oxypurinol regressed LVM just short of statistical significance despite having a short follow up period of only one month and being prescribed at a fairly low dose of 600mg/day, which has a relative bioavailability that is equivalent to only 81mg of allopurinol (327, 331). Hence, it would be worthwhile repeating the study to assess the effects of oxypurinol on LVM using a higher dose over a longer follow up period.

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8 PUBLICATIONS AND PRESENTATIONS

8.1 Original articles

High dose Allopurinol reduces left ventricular mass in patients with ischemic heart disease. JACC 2013 March 5; 61(9): 926-32.

8.2 Abstracts and Presentations at Scientific Meetings

8.2.1 Abstracts

High dose Allopurinol improves endothelial dysfunction in patients with ischemic heart disease and left ventricular hypertrophy but does not match age and gender matched controls. Circulation 2012; 126: A13446

8.2.2 Presentations

8.2.2.1 Oral

European Society of Cardiology congress 2012, Munich – High dose allopurinol regresses LVH and improves endothelial function in patients with chronic stable angina.

8.2.2.2 Moderator Poster

British Cardiovascular Society Annual Conference 2013, London. **High dose Allopurinol improves endothelial dysfunction in patients with Ischemic heart disease and left ventricular hypertrophy but does not match age and gender matched controls.**

8.2.2.3 Poster

American Heart Association congress 2012, Los Angeles, US. **High dose Allopurinol improves endothelial dysfunction in patients with Ischemic heart disease and left ventricular hypertrophy but does not match age and gender matched controls.**