DOCTOR OF MEDICINE

The Effect of Allopurinol on Left Ventricular Mass in Patients with Treated Essential Hypertension

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University of Dundee

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ABPM	Ambulatory Blood Pressure Monitoring
ACE	Angiotensin Converting Enzyme
ACEI	Angiotensin Converting Enzyme Inhibitor
AE	Adverse Event
AIx	Augmentation Index
ANP	Atrial Natriuretic Peptide
AP-1	Activator Protein-1
AR	Adverse Reaction
ARB	Angiotensin Receptor Blocker
ASE	American Society of Echocardiography
AT1R	Angiotensin 1 Receptor
ATII	Angiotensin II
BB	Betablocker
BH4	Tetrahydrobiopterin
BHS	British hypertensive society
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
BPIS	Brief Participant Information Sheet
BSA	Body Surface Area
CAT	Catalase
CCB	Calcium Channel Blocker
cGMP	Cyclic Guanosine Monophosphate
CHF	Congestive Heart Failure
CI	Confidence Interval
CKD	Chronic Kidney Disease
CMRI	Cardiac Magnetic Resonance Imaging
CRF	Case Report Form
CTIMP	Clinical Trial of Investigational Medicinal Product
CV	Cardiovascular
CVA	Cerebrovascular Accident

DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Reporting
ECG	Electrocardiogram
ECM	Extracellular Matrix
EDV	End Diastolic volume
EF	Ejection Fraction
EoSRES	East of Scotland Ethics Service
ERK	Extracellular Signal Regulated Protein Kinase
ESV	End Systolic Volume
ET-1	Endothelin-1
Ets	E26 transformation-specific
FAD	Flavin adenine dinucleotide
FBC	Full Blood Count
Fe2-S2	Iron-sulphur clusters
FMD	Flow Mediated Dilatation
GAGs	Glycosaminoglycans
GCP	Good Clinical Practice
GPCR	G Protein Coupled Receptor
GPX	Glutathione peroxidases
GRE	Gradient-echo
GTN	Glyceryl Tri-nitrate
GWAS	Genome Wide Significance
HBPM	Home Blood Pressure Monitor
HHD	Hypertensive Heart Disease
HR	Hazard ratio
HsCRP	High sensitivity C-reactive Protein
HTN	Hypertension
IHD	Ischaemic Heart Disease
IMP	Investigational Medicinal Product
ISRCTN	International Standard Randomised Controlled Trial Number

LA	Left Atrial
LDL	Low density lipoprotein
LFTs	Liver Function Tests
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
LVIDd	Left Ventricular Internal Dimension Diastole
LVM	Left Ventricular Mass
LVMI	Left Ventricular Mass Index
MAO	Monoamine oxidases
MAP	Mitogen Activated Pathway
MAPK	Mitogen-Activated Protein Kinase
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Authority
MI	Myocardial infarction
MMP	Matrix Metalloproteinase
Mo-Co	Molybdopterin
MRA	Mineralocorticoid Receptor Antagonist
mRNA	Messenger Ribonucleic Acid
MSNA	Muscle Sympathetic Nerve Activity
NAD+	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NFAT	Nuclear Factor Activated T Lymphocytes
NF_kB	Nuclear factor-kappaB
NICE	National Institute for Health and Care Excellence
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NRES	National Research Ethics Service
NT-proBNP	N-terminal Pro Brain Natriuretic Peptide
OS	Oxidative Stress
PAD	Peripheral Arterial Disease
PDE	Phosphodiesterase's
pGC	Particulate Guanylyl Cyclase

PI	Principal Investigator
PICP	Procollagen Type I Carboxyterminal Propeptide
PIS	Participant Information Sheet
PKG	cGMP Dependent Protein Kinases
PT	Preferred Term
PWA	Pulse Wave Analysis
PWTd	Posterior Wall Thickness Diastole
PWV	Pulse Wave Velocity
RAS	Renin Angiotensin System
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
REDOX	Reduction-Oxidation
RHTN	Resistant Hypertension
ROS	Reactive Oxygen Species
RR	Risk Ratio
RWT	Relative Wall Thickness
SAE	Serious Adverse Events
SAR	Serious Adverse Reaction
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEE	Standard Error of Estimate
sGC	Soluble Guanylyl Cyclase
SHARE	Scottish Health Research Register
SMD	Standard Mean Difference
SmPC	Summary of Product Characteristics
SNPs	Single Nucleotide Polymorphisms
SNS	Sympathetic Nervous System
SOC	System Organ Class
SOD	Superoxide Dismutase
SOP	Standard Operating Procedure
SPCRN	Scottish Primary Care Research Network
SSFP	Steady State Free Precession

sST2	Soluble ST2
SUSAR	Serious Unexpected Adverse Reaction
SWTd	Septal Wall Thickness Diastole
TASC	Tayside Medical Sciences Centre
TBARS	Thiobarbituric acid reactive substances
TCTU	Tayside Clinical Trials Unit
TGF-β1	Transforming Growth Factor β1
TIA	Transient Ischaemic Attack
TRUE-FISP	True Fast Imaging with Steady-State Free Precession
TrxR2	Thioredoxin Reductase
U&Es	Urea & Electrolytes
UA	Uric acid
UKCRN	United Kingdom clinical research network
VSMC	Vascular Smooth Muscle Cells
XDH	Xanthine Dehydrogenase
XO	Xanthine Oxidase
XOR	Xanthine Oxidoreductase

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I am very grateful to the British Heart Foundation who funded the study and the donations that have allowed this research to be conducted.

Finally, I dedicate this thesis to my very patient and understanding wife Rachael and three children, Ben, Euan and Eilidh who have supported me throughout my time as a research fellow.

DECLARATION

I declare I am the sole author of this thesis during my time as a Clinical Research Fellow at the University of Dundee. All data was collected and analysed by me, apart from biomarker analysis which was conducted by Leslie MacFarlane. I was employed as British Heart Foundation Clinical Research Fellow within the Department of Molecular and Clinical Medicine at the University of Dundee from August 2014 to August 2017.

I declare this that this thesis has not previously been submitted for a higher degree, all sources of information have been acknowledged accordingly.

Signed …….. Date …….

THESIS SUMMARY

Left ventricular hypertrophy (LVH) confers a high cardiovascular risk independent of blood pressure. It is prevalent in hypertensive patients even when the blood pressure is controlled, and LVH regression has prognostic benefit. Residual risk remains in well controlled hypertension and therefore novel non-blood pressure lowering therapies are required to regress LVH with the aim of improving cardiovascular morbidity and mortality.

Increased activation of redox signalling by oxidative stress (OS) leads to myocyte hypertrophy and fibrosis and is a major non-haemodynamic contributor to LVH. Allopurinol can act as a potent anti-oxidant by inhibiting xanthine oxidase generated reactive oxygen species and has been shown to improve vascular OS and reduce LVH in other conditions such as chronic kidney disease, diabetes mellitus and ischaemic heart disease. The main aim of this thesis is to investigate whether allopurinol regresses LVH in patients with optimally treated, well-controlled hypertension.

The trial design was a double-blind placebo-controlled study of 66 patients with hypertension and echocardiographic LVH. Patients were randomly allocated to allopurinol 600mg daily or placebo for 12 months. The primary outcome was the change in left ventricular mass detected by cardiac magnetic resonance imaging (CMRI) from baseline to the final visit. Secondary end-points assessed change in flow mediated dilation, augmentation index, pulse wave velocity, blood pressure control, biomarkers (Urate, High sensitivity C-Reactive Protein (HsCRP), Thiobarbituric acid reactive substances (TBARs), N-terminal prohormone B-Type Natriuretic Peptide (NT-proBNP), Procollagen type I carboxy-terminal Propeptide (PICP) and soluble ST2 (sST2) and other CMRI parameters.

The two groups were well matched at baseline, importantly there were no statistically significant differences in gender, BMI, blood pressure, number of antihypertensive medications, urate (allopurinol 374.31 ± 85.63 μmol/L, placebo 347.28 ± 108.33μmol/L) and LV mass. Allopurinol failed to regress left ventricular mass (LVM) compared to placebo (indexed LVM -0.18 ± 2.39 $g/m^{1.7}$ vs -1.60 ± 1.60$g/m^{1.7}$; p = 0.009). OS markers (TBARs) increased from baseline in the cohort taking allopurinol compared to placebo (0.26 ± 0.85uM vs -0.34 ± 0.83uM; 0.007). No significant change was seen in FMD, AIx, PWV, BP, other biomarkers or the other CMRI parameters.

Uric acid (UA) is a major antioxidant in human plasma but can become a pro-oxidant in certain conditions. By lowering uric acid with allopurinol, we have increased oxidative stress, altered the redox balance unfavourably and attenuating LVM regression compared to placebo.

In conclusion, allopurinol prevented LVM regression in normo-uricaemic subjects with well controlled hypertension and LVH, potentially from increased oxidative stress secondary to the reduction of urate, an antioxidant. This trial demonstrates that LVM regression with allopurinol is not universal and future trials should carefully select cohorts who are most likely to benefit.

1 INTRODUCTION

1.1 Uric Acid Biology

Uric acid is the end-product of the purine degradation pathway (Figure 1). At physiological pH ninety-eight percent is in the ionised form urate [4]. Extracellularly urate combines with sodium to form monosodium urate which has a solubility limit of 380μmol/L. If this limit is exceeded it may lead to crystal deposition in tissues and joints that causes a profound inflammatory response called gout [4].

During the Miocene epoch mutations occurred in the primate uricase gene rendering it inactive, thus humans cannot metabolise urate [5]. It has been proposed that there may be a genetic advantage of a non-functioning uricase gene due to the protection from oxidative damage or by maintaining blood pressure when dietary ingestion of salt was low [5-7].

Urate levels are dictated by purine ingestion, de-novo synthesis in cells, excretion and the activity of xanthine oxidase [4]. Two thirds of uric acid is excreted by the kidneys however most (90%) is subsequently reabsorbed by the renal tubules, the remainder is eliminated by the gastrointestinal tract [4].

Urate levels vary significantly within humans and tends to be higher in men, postmenopausal women (uricosuric effect of oestrogen), in subjects with reduced glomerular filtration rate (reduced excretion), obesity/insulin resistance (insulin stimulates resorption in the proximal tubule) and dyslipidaemia [5]. URAT1 is an anion transporter in the renal proximal tubule and is important in the resorption of urate, inhibited by probenecid and losartan explaining their uricosuric effects [4]. Loop and thiazide diuretics elevate urate by volume depletion and reduced tubular secretion [8].

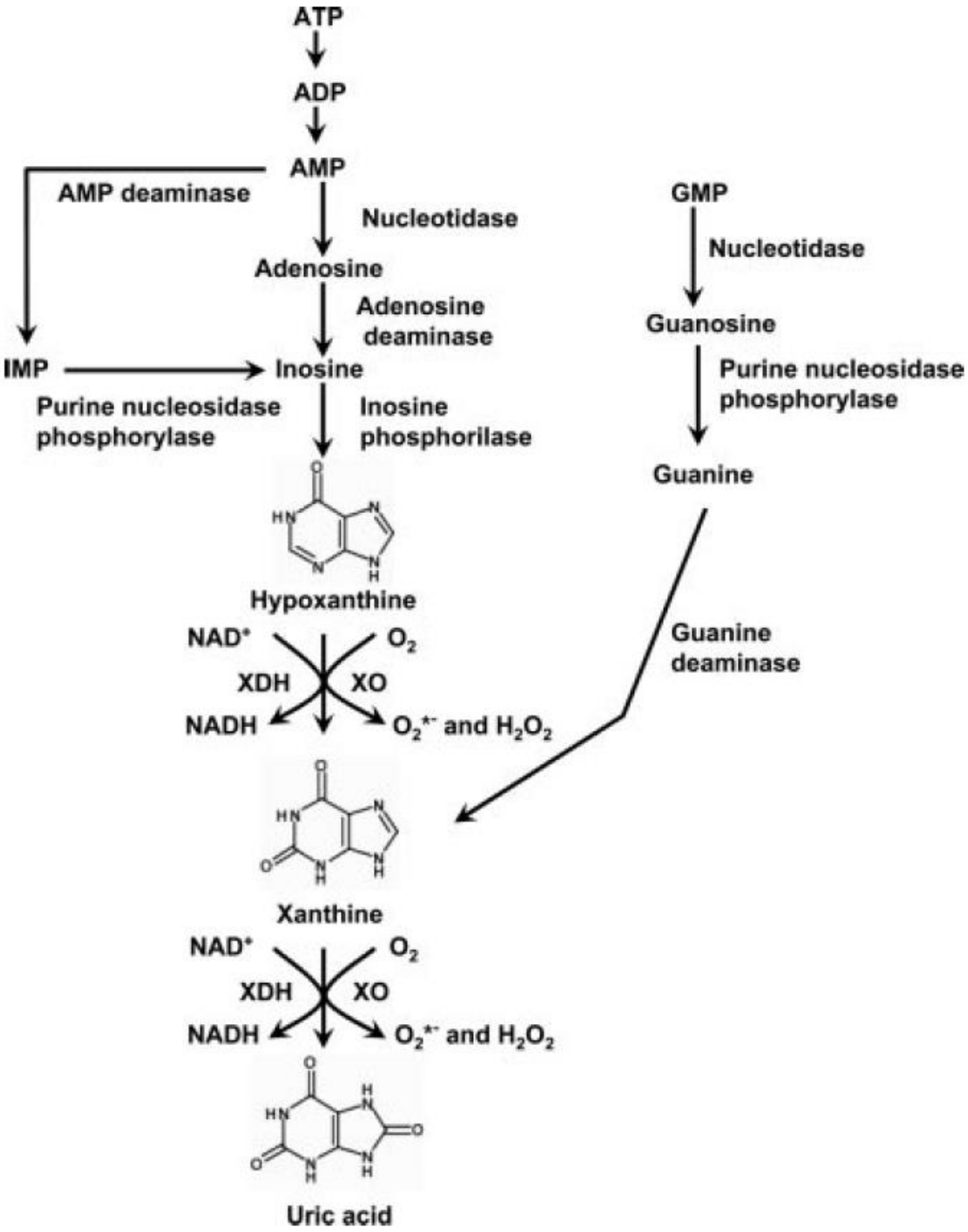


Figure 1 - Purine Degradation Pathway [9]

1.2 Uric Acid as an Antioxidant

Urate has been shown to be a powerful scavenger of singlet oxygen, peroxynitrite, peroxyl and hydroxyl radicals and is the main antioxidant in plasma [5, 6, 10].

Furthermore, it can chelate transition metals, prevent the degradation of superoxide dismutase (SOD3) an enzyme critical for maintaining endothelial and vascular function and prevent nitration of tyrosine residues by peroxynitrite [5, 11]. Although this reaction produces a urate radical it is markedly less reactive and can be regenerated by ascorbate [5]. Urate can also reduce the oxo-heme oxidant formed when peroxide reacts with haemoglobin and protects erythrocytes from peroxidative damage preventing lysis [10]. Animal experiments have demonstrated that acute elevations in UA may provide anti-oxidant protection in the brain, liver and cardiovascular system [6]. Human studies have found that lowering UA with urate oxidase demonstrated no improvement in endothelial function or AIx in either healthy subjects or those with type II diabetes [12] and systemic administration of UA had no detrimental effect on measures of haemodynamics (AIx, BP, systemic vascular resistance index, baroreflex sensitivity and cardiac index) or nitric oxide dependent endothelial function in healthy male adults [13]. In fact intravenous administration of UA has been shown to has been shown to improve antioxidant function in healthy non-smokers at rest and exercise [14] and improve endothelial function in both type I diabetes mellitus and in smokers [15]. Co-infusion of uric acid with alteplase for acute stroke was compared to alteplase alone in the URICO-ICTUS trial [16]. Although statistically non-significant the addition of UA increased the percentage who had an outcome defined as “excellent” compared to the placebo arm (39% vs 33% respectively) with no increase in adverse events, suggesting that UA is a clinically meaningful antioxidant in this context.

1.3 Uric Acid as a Pro-Oxidant

Although urate is an important antioxidant in serum it can have a pro-oxidant effect in certain conditions such as low levels of other anti-oxidants or intra-cellularly, therefore it should be thought of as a conditional pro-oxidant. In-vitro when UA is added to LDL then incubated with Cu^{2+} there is a delay in oxidation however when added later when α-tocopherol (the major antioxidant of LDL) levels were already reduced the oxidation rate was increased, an effect prevented by ascorbate [17]. Uric acid has also been found to stimulate NADPH oxidase activity and ROS generation in mature mouse adipocytes resulting in a decreased NO bioavailability, increased protein nitrosylation and lipid oxidation [18]. UA has also been shown to increase the generation of hydrogen peroxide and 8-isoprostane within rat vascular smooth muscle cells presumed to be mediated by the RAS system as the effect was attenuated by captopril or losartan [19]. UA reacts with peroxynitrite forming urate derived radicals, hence UA can inhibit peroxynitrite mediated effects, but this leads to the formation of the aminocarbonyl radical that can propagate oxidative reactions in particular the peroxynitrite mediated oxidation of liposomes and LDL [20].

1.4 Uric Acid and Cardiovascular Risk

High UA levels are associated with and can predict the development of cardiovascular diseases including hypertension and LVH [5, 6, 21]. Furthermore the first National Health and Nutrition Examination Survey (NHANES I) demonstrated a positive independent association with increasing serum UA and cardiovascular mortality [22]. Two large South-East Asian general population studies have observed a U-shaped curve for all-cause and cardiovascular mortality associated with serum UA levels [23,

24]. The first trial reported serum uric acid levels between 300 – 410umol/L had the lowest risk of events [23]. A more recent study demonstrated the same U shaped risk curve but described gender specific levels of serum UA above and below which risk increased (female 149 – 506umol/L, male 208 – 500umol/L) [24]. The PIUMA study found that the risk was J-shaped in both genders, the optimal urate in males was 309umol/L and 232umol/L in females [25]. Evidence suggests that extremes of UA are detrimental, a mechanistic explanation could be the “urate redox shuttle” (Figure 2) described by Hayden et al where UA could act as an anti or pro-oxidant depending on the environmental milieu within an atherosclerotic plaque [26]. Finally a mendelian randomisation study found that after adjustment an genetically predicted increase in UA of 59umol/L increased the risk of cardiovascular death by 77% (HR, 1.77; 95% confidence interval, 1.12 to 2.81) suggesting causation in adverse events [27].

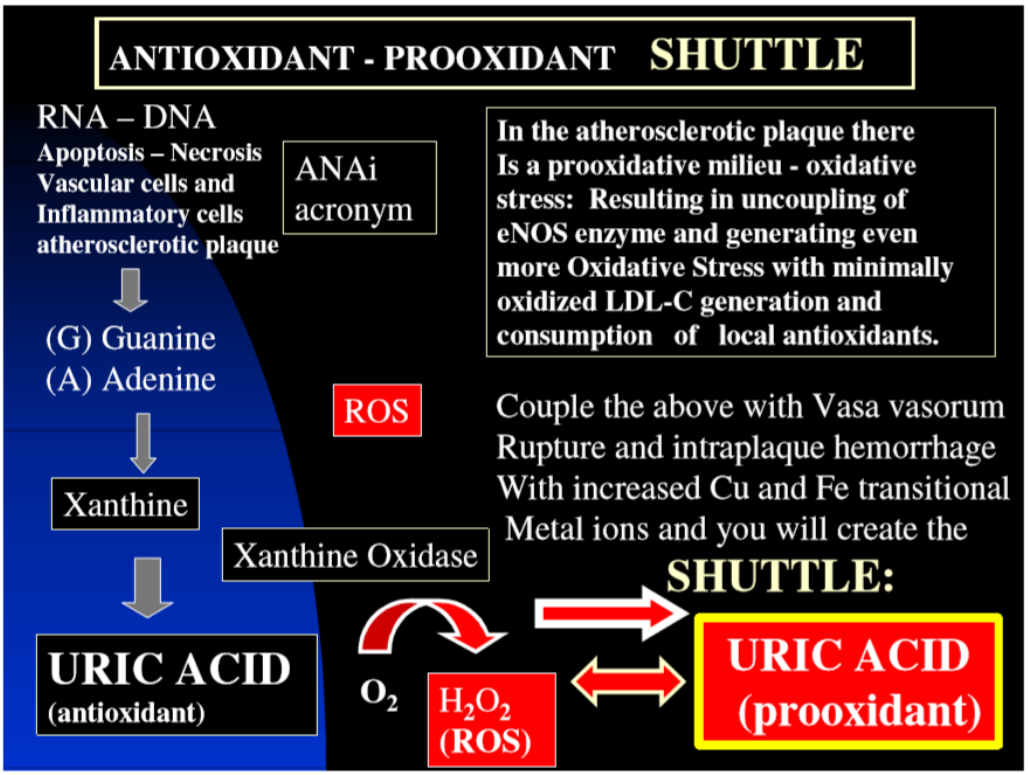


Figure 2 - The Urate Redox Shuttle [26]

1.5 Allopurinol

During their work with purine analogues in the 1950's, Gertrude Elion and George Hitchins developed a hypoxanthine analogue (allopurinol) that blocked xanthine oxidase in the hope that it would improve the anti-cancer effects of 6-mercaptopurine[28]. Realising it would also block the formation of uric acid it was subsequently tested in patients with hyperuricaemia and gout and was found to be clinically effective [28]. A discovery for which they were awarded the Nobel prize in 1988 [29]. The drug was subsequently approved by Food and Drug Administration (FDA) in 1966 for the treatment of gout and remains to this day the first line agent for the treatment for primary and secondary hyperuricaemia [9, 29].

Allopurinol has the chemical structure 1,5-dihydro4H-pyrazolo[3,4-d]pyrimidin-4-one (Figure 3) [9]. It inhibits xanthine oxidase either directly as a competitive or non-competitive inhibitor depending whether the concentrations are low or high respectively [9]. Most of its pharmacological action is via its main metabolite oxypurinol (Figure 3) due to the short half-life of allopurinol [9, 30]. In addition purine biosynthesis is reduced via feedback inhibition of hypoxanthine phosphoribosyl transferase [30].

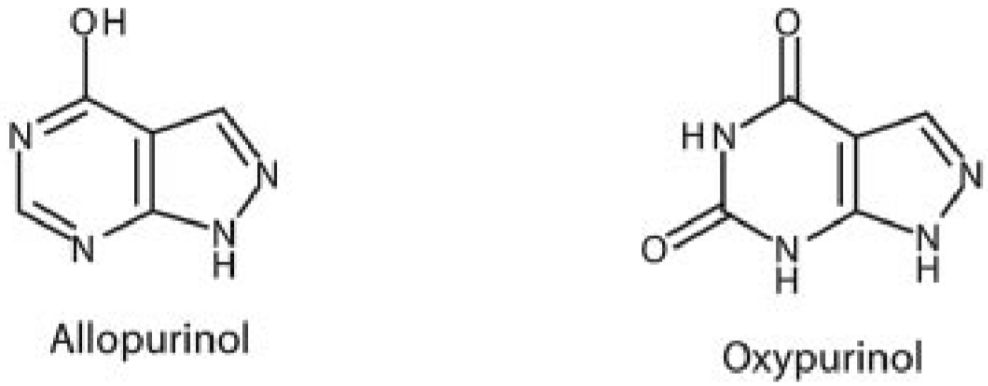


Figure 3 - Chemical structures of allopurinol and oxypurinol [9]

Allopurinol reacts with XOR at Mo-Co to yield oxypurinol which binds to Mo inhibiting enzyme interaction with substrate [31]. Reduction of Mo-Co leads to the electron transfer to FAD and reduction oxygen i.e. enzyme turnover and ROS generation occurs before inhibition [29].

1.5.1 Pharmacokinetics and Pharmacodynamics

After oral ingestion allopurinol is detected in the blood after 30 to 60 minutes with a bioavailability of 67% to 90%. Peak plasma levels are achieved around one and a half hours, the $t_{1/2}$ is 1.2 ± 0.3 hours and hence levels fall to undetectable levels within six hours [30, 32]. Peak oxypurinol levels occur around 3 to 5 hours but are more sustained as the $t_{1/2}$ is 23.3 ± 6.0 hours [30, 32]. Allopurinol is negligibly bound by plasma proteins and the mean volume of distribution of allopurinol is 1.3L/kg and oxypurinol is 0.62 L/kg [30, 32]. 20% of allopurinol excreted in faeces, <10% in urine the remainder is converted to oxypurinol that is in turn excreted in the urine [30]. Mean renal clearance of allopurinol is 1.54mL/min/kg and oxypurinol 0.34mL/min/kg [32]. Patients with chronic kidney disease will consequently have higher plasma levels and will require a reduction in dose to avoid toxicity.

1.5.2 Indication and Dosing

Allopurinol is clinically indicated for lowering urate after episode(s) of gout or nephrolithiasis or in condition where there is a predictable risk such hyperuricaemia after chemotherapy, the so called “tumour lysis syndrome”[30]. The starting dose is 100mg/day increasing up to a maximum of 900mg/day in divided doses, using the lowest dose to achieve satisfactory urate reduction, the usual clinical dosing is 300mg [30, 33]. Inhibition of XOR prevents the formation of ROS (Figure 1) and hence has the potential to reduce oxidative stress and is the hypothesised mechanism for improvements in LVM and vascular function in this study. Additional direct antioxidant effects have been demonstrated in experiments and using animal models within cardiomyocytes, kidney, liver and retinal tissue that may also contribute to improving

oxidative stress overall [34-38]. George et al demonstrated a steep dose response relationship between allopurinol and endothelial function, a dose of 600mg/day of allopurinol was found to completely abolished vascular oxidative stress in subjects with heart failure [39]. Subsequent studies in our institution have used 600mg safely [40-43]. Animal models have found additional free-radical scavenging effects at up to 50mg/kg far beyond the XO inhibiting dose used by George et al[44], so it is unclear whether 600mg is the optimal dose or even higher doses could provide additional benefit.

1.5.3 Side effects and Important Interactions

Adverse reactions caused by allopurinol generally of a minor nature however the incidence is higher with renal or liver dysfunction and hence it should be used with care in these patients [30]. The most common side effect is a drug rash, fortunately hypersensitivity reactions known as DRESS are uncommon as is nausea and vomiting [30]. Stevens-Johnson syndrome or toxic epidermal necrolysis are rare, but serious complications [30]. Severe skin reactions are more frequent in those with a genetic predisposition such as HAN Chinese and those with HLA-B*5801 allele [45]. Important medication interactions include the inhibition of theophylline and the metabolism of azathioprine to 6-mercaptopurine. Both of the later medications are inactivated by the action of xanthine oxidase and hence concurrent use of allopurinol increases their activity [30].

1.5.4 Evidence of use in Cardiovascular Disease

1.5.4.1 Endothelial Dysfunction/Arterial Stiffness

Endothelial dysfunction and increased vascular stiffness are predictors of adverse outcomes in HTN and there is some data that reversal influences prognosis [46, 47]. Improvement in endothelial function with allopurinol has been demonstrated in some pathologies but inconsistently in others, therefore the magnitude of the effect may be explained by the level of OS related to xanthine oxidase activity and/or urate level. Allopurinol improves endothelial function in hyperuricaemic subjects with chronic heart failure (CHF) [39, 48-50]. George et al also demonstrated this effect was due to a reduction in vascular oxidative stress and independent of uric acid lowering as probenecid a uricosuric agent had no effect on endothelial function [39]. Interestingly, Doehner et al found that an intra-arterial infusion of allopurinol had no effect on endothelial function in a small number (n=10) of normo-uricaemic CHF control patients [49]. Two of three randomised controlled trials (RCT) in chronic kidney disease have demonstrated an improvement in endothelial function (FMD) with 300mg allopurinol [51-53], a meta-analysis of these studies concluded that allopurinol significantly improved endothelial function overall [54]. The same meta-analysis has failed to demonstrate an improvement in endothelial function in those with diabetes but cites heterogenicity, dosing and duration could have confounded the result [54]. There are few studies assessing the effect of allopurinol on endothelial function in essential hypertension in the absence of other significant comorbidities. In one such study by Cardillo et al oxypurinol failed to improve forearm blood flow to acetylcholine in subjects with hypertension but did in normotensives with dyslipidaemia, baseline urate level was unfortunately not reported in the study [55]. Mercuro et al found

allopurinol improved FMD in participants with increased uric acid levels and high cardiovascular risk (including a proportion of those with hypertension) [56] and healthy hyperuricaemics [57] taking allopurinol compared to normouricaemic controls. Allopurinol improves augmentation index (AIx) a measure of vascular stiffness in a number of pathologies from stroke [58], IHD [43, 59] and chronic kidney disease [53]. A recent meta-analysis concluded that treatment with allopurinol had a significant and favourable effect on AIx but not on PWV [60].

1.5.4.2 Hypertension

Feig et al (2008) conducted a small (n=30) randomised double blind, placebo-controlled crossover study of untreated, uncomplicated, mildly hypertensive, hyperuricaemic (≥6.0mg/dL) adolescents. Treatment with allopurinol resulted in a significant reduction in both urate, renin, systemic vascular resistance and BP (24-Hr systolic BP -6.3mmHg, diastolic BP -4.6mmHg) [61]. To answer whether reducing urate itself or xanthine oxidase generated ROS with allopurinol explained the results of the trial above, a study using probenecid, allopurinol and placebo in an obese pre-hypertensive adolescent population was completed [62]. A significant and similar reduction in both BP and systemic vascular resistance compared to placebo was found in both treatment groups [62]. Kanbay et al have demonstrated an improvement in BP with allopurinol in a hypertensive hyperuricaemic and healthy adult populations compared to untreated normouricaemic controls [57, 63]. However many studies have not demonstrated an improvement in BP with treatment with allopurinol [64]. A meta-analysis of 738 participants from 10 studies concluded that allopurinol treatment had a small and significant reduction systolic (3.3mmHg; 95% CI, 0.8-5.8mmHg; p = <0.001)

and diastolic (1.4mmHg; 95 CI, 0.1-2.7mmHg; p = < 0.04) blood pressure in hyperuricaemic subjects (Figure 4, Figure 5) [64].

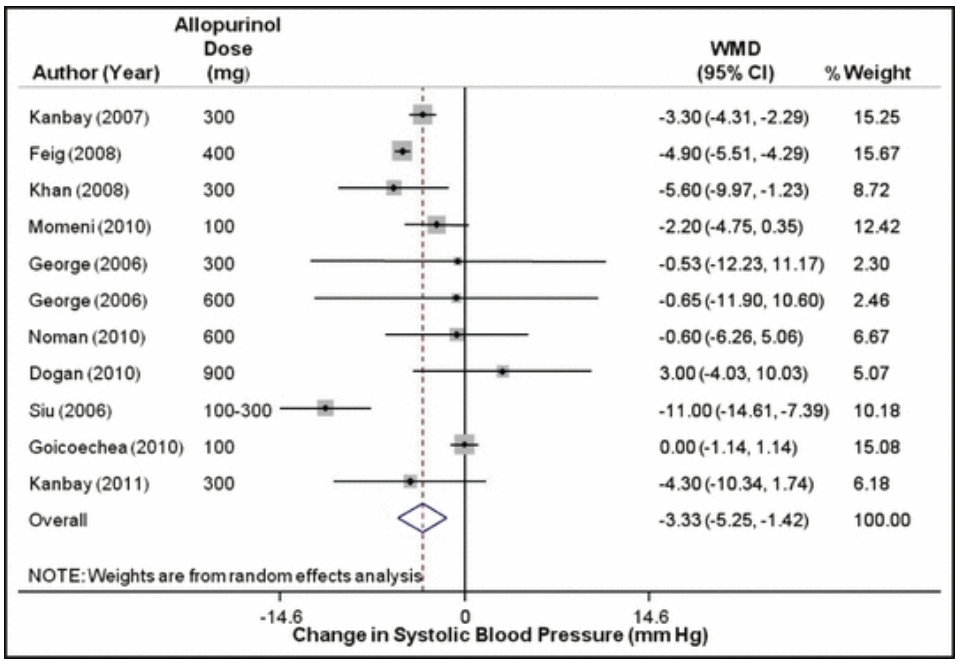


Figure 4 - Forrest Plot of the Effect of Allopurinol on Systolic BP [64]

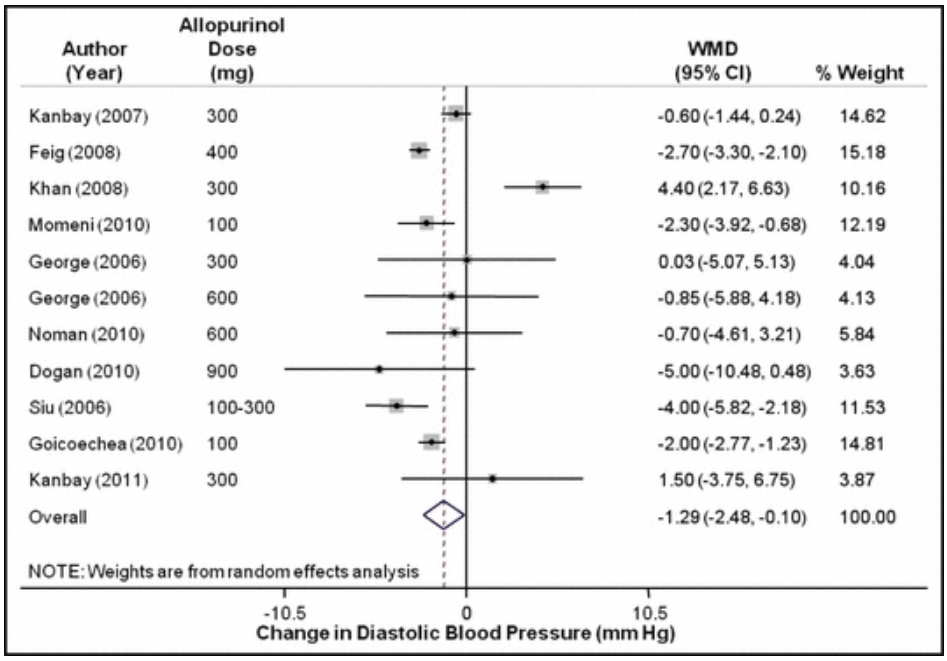


Figure 5 - Forrest Plot of the Effect of Allopurinol on Diastolic BP [64]

1.5.4.3 Left Ventricular Hypertrophy Regression with Allopurinol/Oxypurinol

The La Plata study observed a reduction in echocardiographic LV mass in a cohort with congestive heart failure after one-month treatment with oxypurinol, although non-significant it was suggestive of a potential benefit of xanthine oxidase inhibition on left ventricular mass [65]. Three randomised controlled trials have demonstrated a significant reduction in LV mass measured by cardiac MRI after treatment with allopurinol for nine months. Kao et al demonstrated a significant reduction in indexed LV mass of 1.42 ± 4.67g/m^2 in the intervention arm (allopurinol 300mg/day) versus an increase of 1.28 ± 4.45g/m^2 (p = 0.036), in subjects with stage 3 chronic kidney disease and LVH [53]. Rekhraj et al found high dose allopurinol (600mg/day) regressed LV mass compared to placebo in a cohort with ischaemic heart disease (-2.2 ± 2.78g/m^2 versus -0.53 ± 2.5g/m^2) (p = 0.023) [59]. Finally, Szwejkowski et al also demonstrated a reduction in LVM index with allopurinol (600mg/day) versus placebo (-1.32 ± 2.84g/m^2 versus +0.65 ± 3.07g/m^2) (p = 0.017) [40].

1.6 Alternative Urate Lowering Medications

Febuxostat is a potent, non-purine selective inhibitor of XO, indicated for the treatment of gout and tumour lysis syndrome in those intolerant to allopurinol[66]. The Allopurinol and Placebo-Controlled Efficacy Study of Febuxostat (APEX) and Febuxostat versus Allopurinol Controlled Trial (FACT) trials demonstrated that febuxostat were superior at lowering serum UA than allopurinol [67, 68]. Although more effective at lowering UA, there was no significant difference in gout flares between the allopurinol or febuxostat [67]. Interestingly the APEX trial a numerically higher but statistically non-significant incidence of cardiovascular events in the febuxostat arm. This has been investigated further in the Cardiovascular Safety of Febuxostat and Allopurinol in Patients with Gout and Cardiovascular Morbidities (CARES) trial and found a significantly higher all-cause and cardiovascular mortality in patients with gout and cardiovascular disease treated with febuxostat compared to allopurinol [69]. Hence febuxostat is not recommended for use in ischaemic heart disease or heart failure [66]. Although a mechanism has not be identified by the authors it is possible excessive UA reduction may contribute.

Probenecid and Benzbromarone are potent uricosuric agents, can be used as an alternative to allopurinol for the treatment of gout, but have been replaced by allopurinol and febuxostat [70, 71].

1.7 Oxidative Stress

Reactive oxygen species (ROS) consist of radical and non-radical atoms or molecules derived from oxygen. A radical is characterised by the presence of an unpaired

electron(s) and is unstable and highly reactive. Examples of radicals are superoxide, peroxide and hydroxyl groups. Non-radical ROS such as hydrogen peroxide and peroxynitrite are also powerful oxidants and react readily with surrounding molecules [3, 72]. ROS are produced in small amounts during the normal biochemical processes of the body and are required for normal "redox signalling" pathways, host immune response [3, 73], and at normal levels are non-pathogenic [74]. Oxidative stress (OS) is the term used when an excess of ROS are generated and/or antioxidant capacity is decreased [73] and has important pathological consequences (Figure 6).

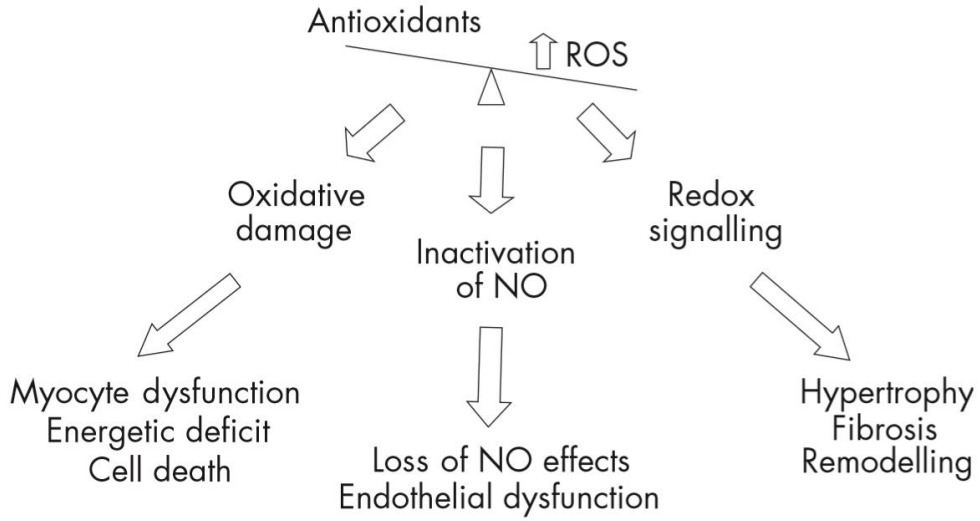


Figure 6 - Oxidative Stress Effects [74]

Important endogenous sources of ROS include xanthine oxidase (XO), NADPH oxidases, uncoupling of nitric oxide synthase (NOS) and from mitochondria during oxidative phosphorylation [75]. Homeostasis is maintained by antioxidant defences balancing the endogenous/exogenous sources of ROS and are broadly divided into enzymatic and non-enzymatic antioxidants. Oxidative stress is implicated in a number of

cardiovascular conditions such as atherosclerosis [76], heart failure [77], myocardial infarction, hypertension [75] and left ventricular hypertrophy [73].

1.7.1 Xanthine Oxidoreductase

Xanthine oxidoreductase (XOR) is a complex molybdoflavoenzyme [78] that consists of two interchangeable forms, xanthine oxidase (XO) and xanthine dehydrogenase (XDH) [31], the former is inhibited by allopurinol and is the primary focus of this thesis. Both forms of XOR catalyse the terminal steps of the purine degradation pathway (Figure 1).

The XOR enzyme is a homodimer consisting of catalytically independent subunits, each subunit contains three domains each containing a specific cofactor(s), molybdopterin (Mo-Co), flavin adenine dinucleotide (FAD) and two iron-sulphur (Fe_2-S_2) clusters [31]. Mo-Co is the site of purine oxidation, FAD enables NAD^+ and O_2 reduction, and Fe_2-S_2 facilitates electron flow between Mo-Co and FAD (Figure 7) [29].

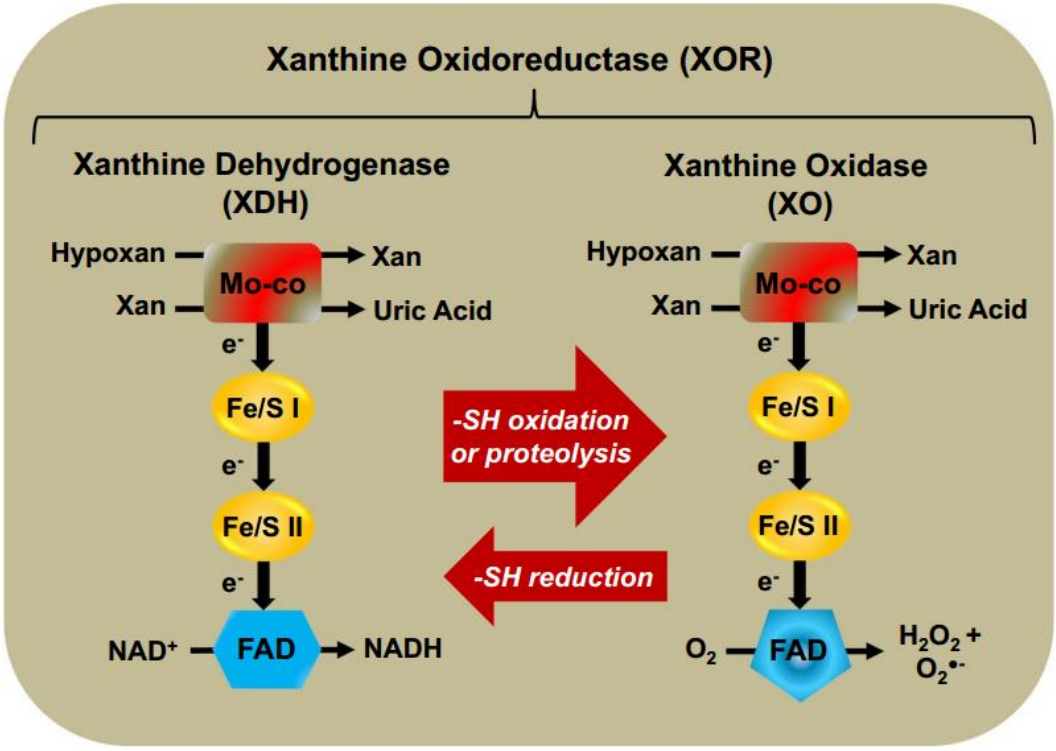


Figure 7 - Actions of XOR enzymes [29]

XO exhibits little reactivity with NAD^+ and hence transfers purine derived electrons only to oxygen generating superoxide and hydrogen peroxide [29]. XDH in contrast can reduce NAD^+ or oxygen but has a higher affinity for the former due to rapid and tight binding, but only when NAD^+ is available [31]. When NAD^+ is in short supply XDH acts as a NADH oxidase subsequently reducing oxygen to superoxide, however XDH reacts slowly with oxygen so the rate of superoxide generation is 25% that of XO [31]. ROS generated by purine metabolism can be converted to other ROS such as the peroxynitrite or the highly reactive hydroxyl radical formed by the Haber-Weiss reaction (Figure 8) [3, 31, 72]

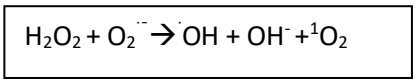


Figure 8 - Haber-Weiss Reaction [3]

Compared to other mammals, XOR is in relatively low abundance and activity in humans, even between individuals there can be as much as a threefold variation of activity [31, 54]. The highest levels of XOR activity have been identified in human liver, small intestine and mammary gland [79]. Low levels of XOR protein/activity have been demonstrated in the human heart and endothelium [31]. Although basal gene expression in humans is low a number factors that regulate transcription have been identified (Figure 9), and increased XO activity has been demonstrated in a number of cardiovascular conditions including dilated cardiomyopathy [80], heart failure [81], pressure overload hypertrophy [73] and hypertension [82].

Positive regulators	Negative regulators
Hypoxia	Hyperoxia
Lipopolysaccharide	
Interferon γ	
Interleukin-1	
Interleukin-6	
Tumor necrosis factor α	
Dexamethasone	
Cortisol	
Prolactin	

Figure 9 - Regulators of XOR gene expression [31]

Under certain conditions such as inflammation/hypoxia reversible conversion of XDH to XO by oxidation of thiol groups (Cys535, Cys992) and/or irreversible conversion by proteolysis occurs (Figure 7) enhancing ROS generation [31]. When oxygen tension and pH are normal the predominant ROS generated by XO is hydrogen peroxide, furthermore there is an inverse relationship between oxygen tension and XO generated hydrogen peroxide further enhanced by a reduction in pH [29]. The relationship between oxygen tension and XOR activity is clearly established but the

mechanism is poorly understood but thought to involve both post-translational modification, transcriptional regulation or both [29]. XOR is released into the circulation in response to hypoxia/ischaemia of endothelium where it is irreversibly changed to XO [29]. Plasma XO has an affinity for vascular endothelial glycosaminoglycans (GAGs) and provides a mechanism for XO generated ROS in vascular beds [29]. Peroxynitrite a potent oxidant, is formed by the reaction of superoxide with NO, in hypoxic and acidic conditions XO can generate both molecules and therefore potentiate the formation of peroxynitrite (Figure 10) [29, 31]. As discussed previously NADPH oxidase generated ROS can further amplify ROS generation by the activation of XO [81].

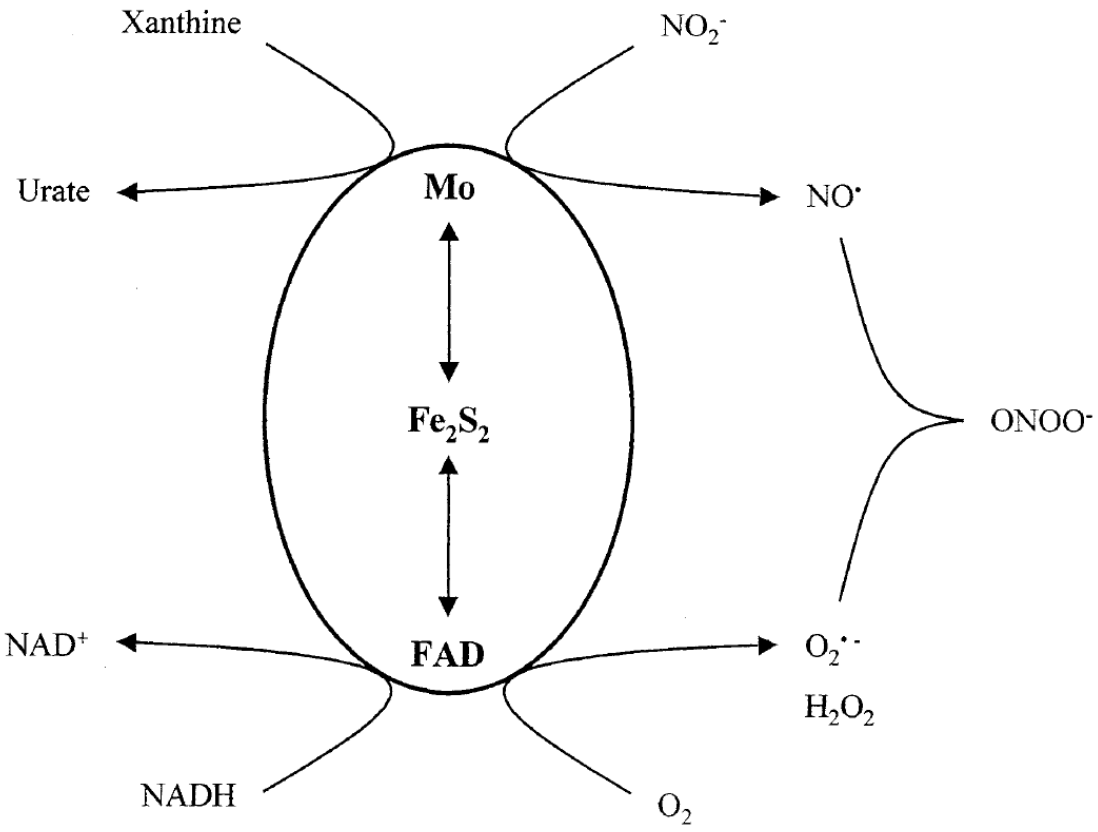


Figure 10 - XOR catalysed production of NO and peroxynitrite [78]

1.7.2 NADPH Oxidases

NADPH oxidases catalyse electron transfer from NADPH to molecular oxygen, resulting in superoxide [81] a reaction that has been found in a variety of cells including vascular smooth muscle and endothelial cells, fibroblasts and cardiomyocytes [81]. Enzyme activity is stimulated by angiotensin II (ATII), endothelin-1 (ET-1), cytokines, growth factors, oxidised low density lipoprotein (LDL), sheer stress and mechanical stretch [81]. ROS generated can lead to further increase in reactive oxygen species by activation of xanthine oxidase and NOS uncoupling [81]. NADPH oxidase is a major source of ROS involved in redox signalling and is important factor in the pathophysiology of hypertension, atherosclerosis, heart failure and left ventricular hypertrophy [81, 83]. ACE-I and ARBs are highly effective inhibitors of ATII dependent NADPH oxidase activation, have been shown to improve endothelial function and regress arterial remodelling and LVH, and are used routinely in heart failure and hypertension [81, 84-87].

1.7.3 Nitric Oxidase Uncoupling

Nitric oxide synthase (NOS) catalyses the reaction of L-arginine, O_2 and NADPH forming L-citrulline and nitric oxide (NO). It exists in three isoforms all of which have been detected in cardiomyocytes, vascular smooth muscle and vascular endothelial cells [88]. A number of cofactors are required for the reaction including tetrahydrobiopterin (BH4), calmodulin, flavin mononucleotide and flavin adenine dinucleotide [88]. Under normal physiological conditions NO acts as a signalling molecule activating soluble guanylyl cyclase (sGC) then cyclic guanosine monophosphate (cGMP) [89]. cGMP exerts its actions on the cardiovascular system via cGMP dependent protein kinases

(PKG) and cGMP regulated phosphodiesterase's (PDE) [88]. cGMP-PKG signalling stimulates cell proliferation and increases permeability in the vascular endothelium, inhibits cell proliferation and mediates vasorelaxation in vascular smooth muscle and in the cardiomyocyte inhibits hypertrophy, modulates contractility and mediates apoptosis in all (Figure 11)[88].

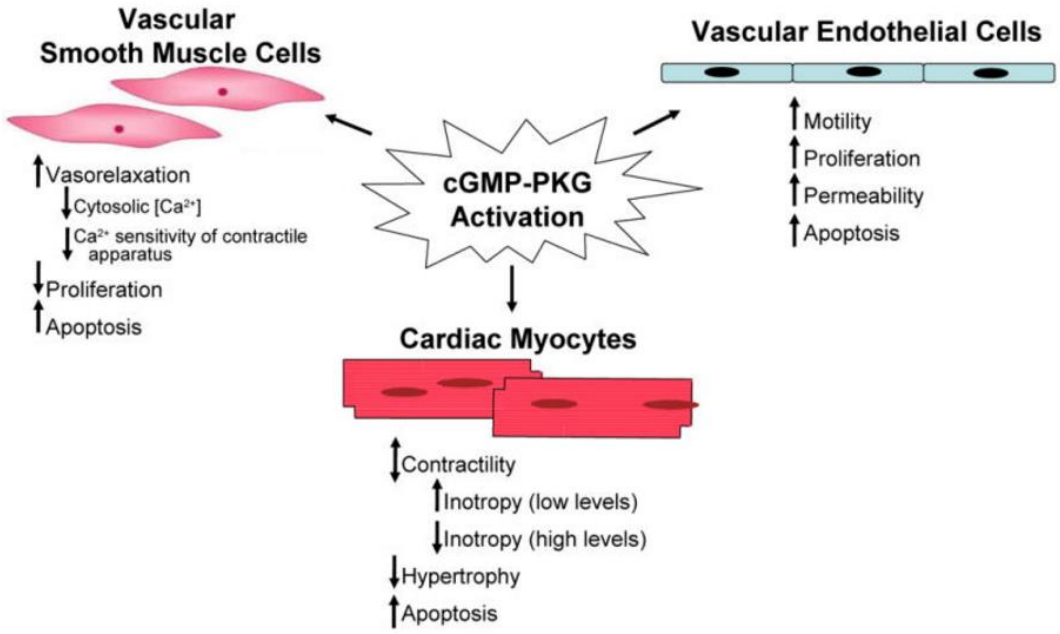


Figure 11 - cGMP-PKG actions [88]

[90]. cGMP-PDEs are regulated by and catabolise cyclic nucleotides [88]. There are 11 isoenzymes of which a number are expressed in cardiomyocytes, vascular endothelial and vascular smooth muscle cells [88]. PDE5 has been implicated as an important regulator of cGMP in cardiac myocyte hypertrophic response to pressure-overload [88]When BH4 or L-arginine is deficient, or BH4 is oxidised NOS becomes "uncoupled" and generates superoxide[81].

1.7.4 Oxidative Phosphorylation

A small amount of superoxide is generated during oxidative phosphorylation in the mitochondria [81]. Usually converted to water by the action of superoxide dismutase, glutathione peroxidase and catalase, under pathological conditions the hydroxyl radical can be formed [81].

1.7.5 Enzymatic/Non-Enzymatic Antioxidant defences

Superoxide dismutase (SOD) is known to exist in three forms [91], manganese (MnSOD), copper (CuSOD) and zinc superoxide dismutase (ZnSOD) [92]. MnSOD activity accounts for the majority of the SOD activity in the heart [91]. Present in the mitochondria it converts superoxide generated from oxidative phosphorylation to hydrogen peroxide (Figure 12) [91]. Genetically modified mice without MnSOD activity die from a dilated cardiomyopathy soon after birth and no inherited diseases have been found lacking MnSOD suggesting it is a critical enzyme [91]. Expression of MnSOD is induced by oxidative stress [93]. CuSOD and ZnSOD are extracellular and are less important as transgenic mice lacking this activity develop normally but appear to have increased susceptibility to central nervous system injury [93].

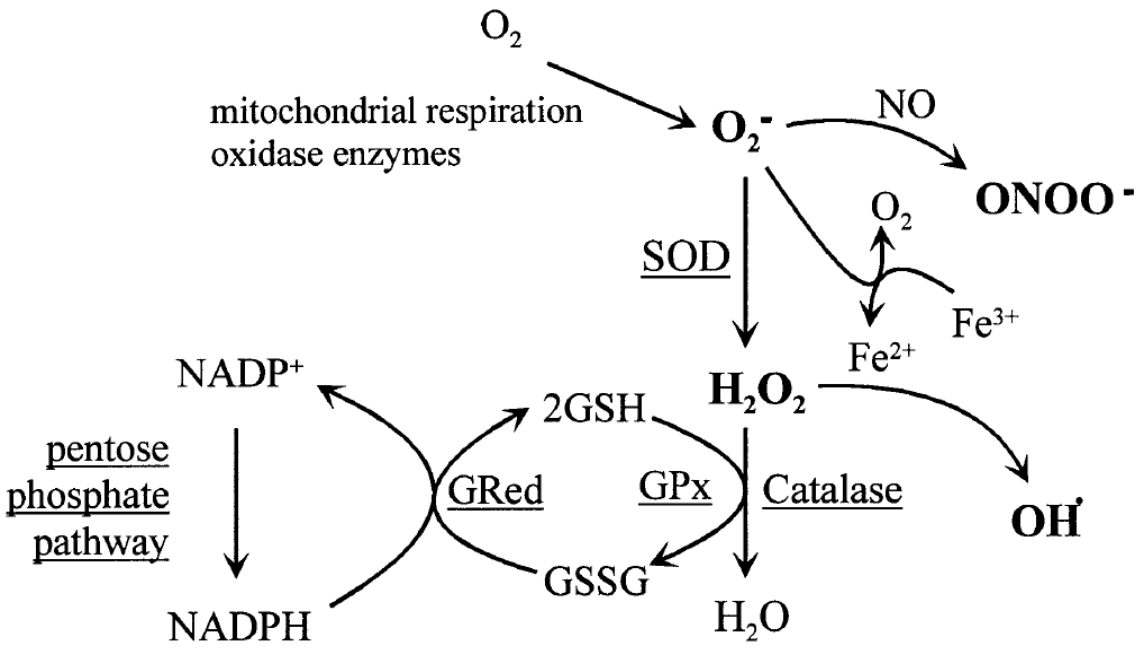


Figure 12 - Enzymatic Antioxidant Defences [91]

Glutathione peroxidases (GPX) and catalase (CAT) are the enzymes responsible for the degradation of hydrogen peroxide generated by SOD to water (Figure 12). Reduced glutathione is oxidised [91] then recycled by glutathione reductase by the conversion of NADPH to $NADP^+$ in the pentose phosphate pathway [91]. Catalase also detoxifies phenols and alcohols via coupled reaction with hydrogen peroxide [93].

Thioredoxin in mammalian cells reduce peroxides directly [93] and thioredoxin reductase is involved in the regeneration of ubiquinone, lipoic acid and ascorbic acid which are important antioxidants [3]. Complete deletion of the thioredoxin reductase gene (TrxR2) caused foetal death in mice and TrxR2- deficient mice died shortly after birth from dilated cardiomyopathy [94].

Important non-enzymatic antioxidants include vitamins C and E, B-carotene, ubiquinone, lipoic acid, glutathione and urate[3].

1.8 Hypertension

Primary (essential) hypertension, hereafter referred to as hypertension (HTN) develops as a consequence of the interaction between environmental and genetic factors and accounts for most patients with an elevated blood pressure. Secondary hypertension has recognised aetiology and is diagnosed in the remaining 5-10% [95]. Current NICE guidelines definitions of the stages of hypertension are listed below [96].

- Stage 1 HTN - Clinic BP of ≥140/90 and ABPM ≥135/85mmHg.
- Stage 2 HTN - Clinic BP of ≥160/100 and ABPM ≥150/95mmHg.
- Severe HTN - Clinic systolic pressure ≥180mmHg or diastolic BP ≥110mmHg.

Hypertension is a leading preventable risk factor for cardiovascular disease (ischaemic and haemorrhagic stroke, coronary artery disease, heart failure, peripheral arterial disease and renal disease) worldwide[95]. Although a “normal” BP is difficult to define, the observed risk is strong, graded, continuous and positively related to the degree of hypertension [95]. The development of target organ damage e.g. LVH represents a strong and independent predictor of risk and should prompt intensification of treatment [97]. The term resistant hypertension (RHTN) is used when a blood pressure remains above target despite the use of optimal doses of three antihypertensive agents of different classes (ideally one of the agents should be a diuretic) [98]. Post hoc analysis of clinical trials and observational studies have estimated a prevalence of 10-20% of the hypertensive population [99]. A retrospective cohort study of 205,750 subjects with hypertension found a significantly increased cardiovascular risk compared to those with controlled BP on less than three antihypertensive drugs (HR

1.47; 95% CI 1.33-1.62, p = <0.001) [100]. This excess risk could in part be attributed to the high prevalence of LVH in this group, estimated as between 55-81% of patients with RHTN[101]. Alternative therapies are required to address the additional risk as conventional treatments are not fully effective.

1.8.1 Epidemiology

Several factors have been recognised that influence the development of HTN e.g. gender, race, nutrition, alcohol consumption and physical exercise. The influence of age however has been consistently demonstrated to be positively correlated with systolic BP [102]. The Framingham study demonstrated this relationship of age and systolic BP (Figure 13). Diastolic BP rises with age until around the fifth decade and then falls with advancing age[103].

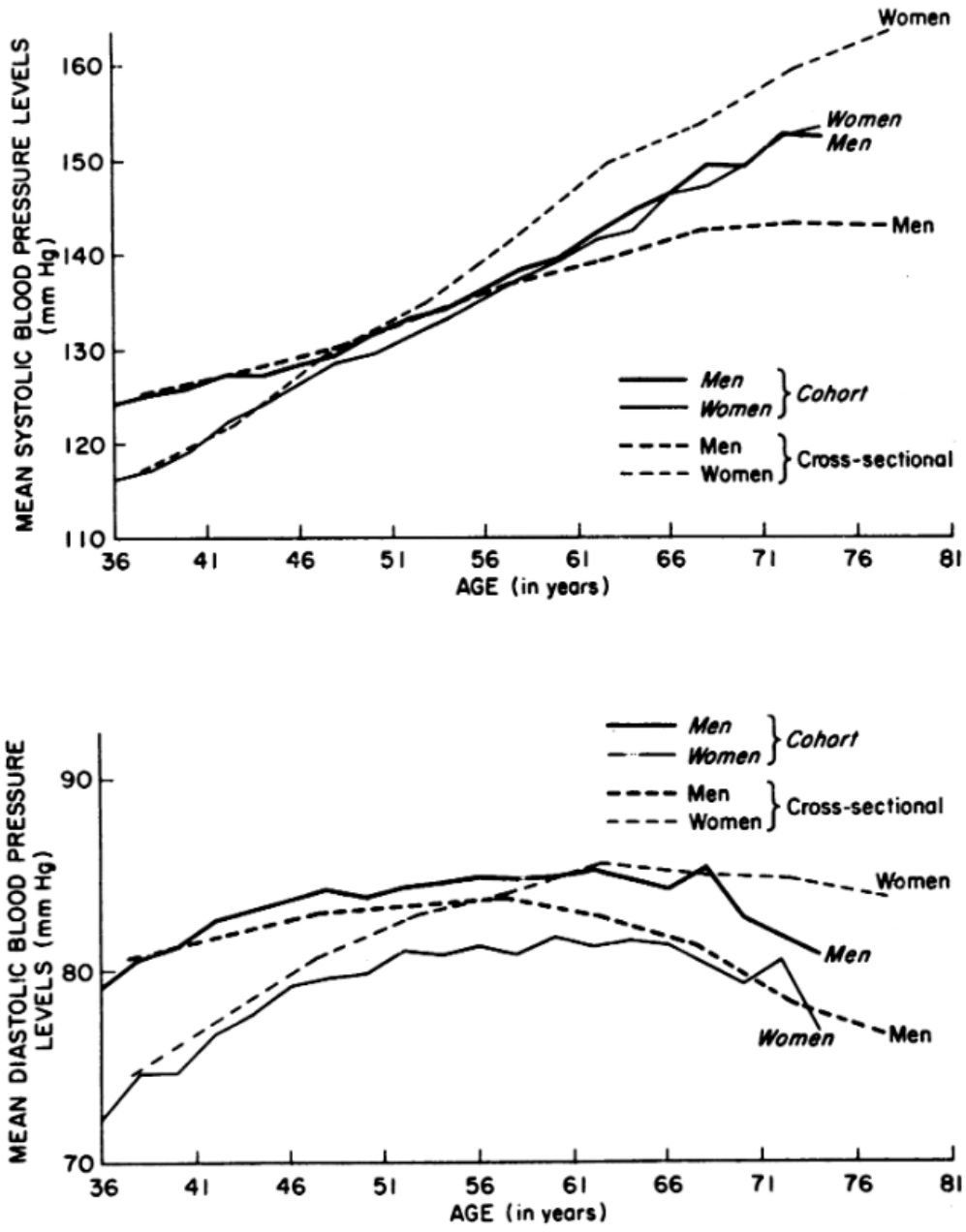


Figure 13- Framingham study trends in BP with age [103]

The prevalence of hypertension in the England was 32% in men and 27% in women in 2014 and relatively static (Figure 14)[104]. Although the percentage well controlled has increased over the last decade to approximately 30% the remainder are either untreated or poorly controlled (Figure 14) [104].

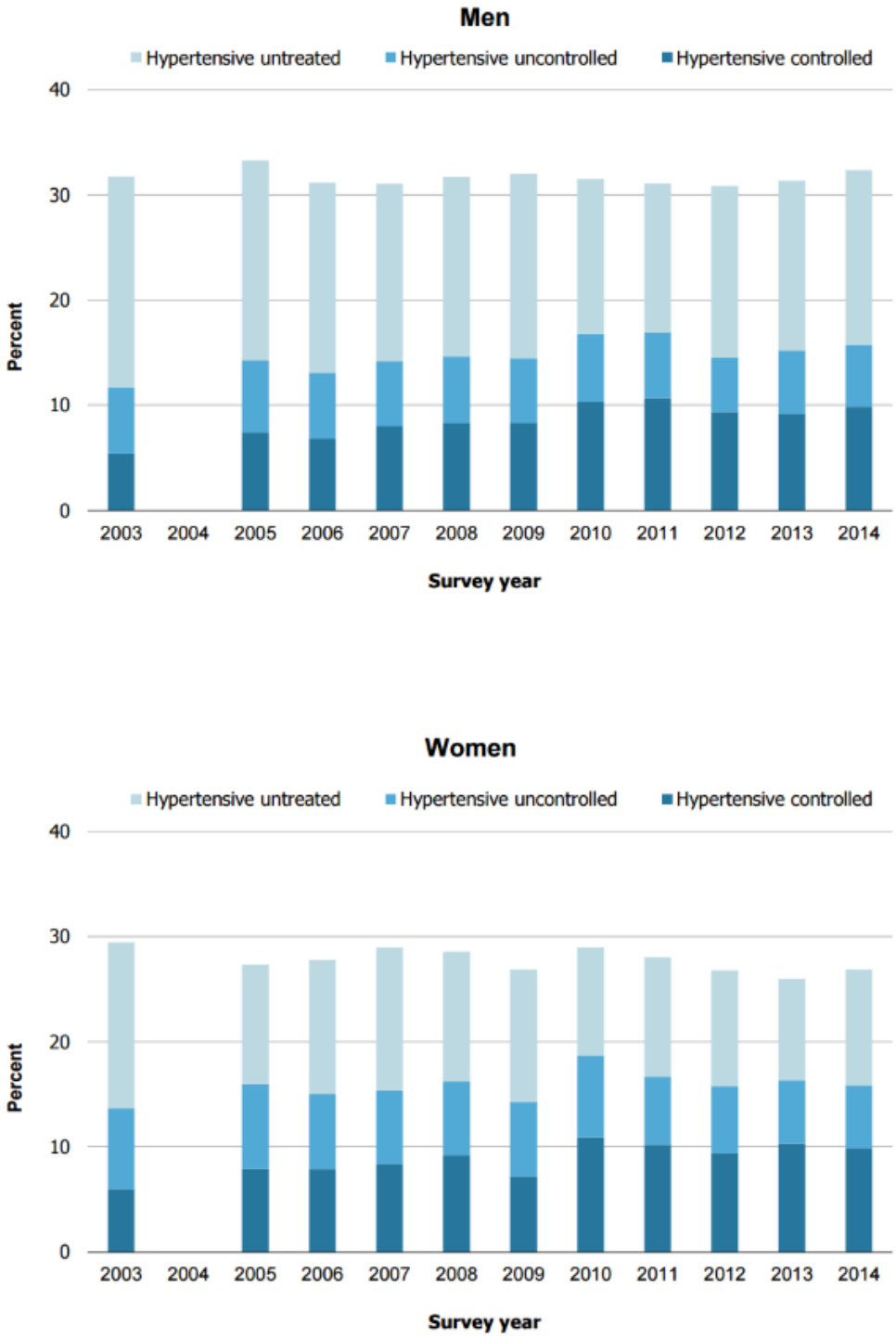


Figure 14 - Prevalence of HTN England [104]

1.8.2 Pathophysiology

The pathophysiology of hypertension is complex, multifactorial and incompletely understood. Broadly there is an interaction between environment and susceptibility

genes leads to an effect on cardiac output, vascular structure and function leading to hypertension.

1.8.2.1 Genetics

Complex genetic traits are defined as those without a simple "Mendelian" one-to-one relationship between genotype and phenotype, and are seen in hypertension and LVH[105]. A study in the 1960's by Mial et al. surmised that in a general population the majority of systolic and diastolic variation was secondary to environmental factors but that a multifactorial pattern of inheritance could explain the remaining variability [106]. Observations that a family history of HTN increases the risk fourfold support a genetic role in its pathophysiology [107]. Over forty single nucleotide polymorphisms (SNPs) that increase systolic and diastolic BP with genome wide significance (GWAS) have been identified [107]. The effect size of this genetic variation is small and doesn't fully explain the heritability of hypertension however it is expected that undiscovered loci with larger effect sizes may do so in the future [107].

1.8.2.2 "Environmental" Triggers

Observational studies have independently associated increased body mass index, elevated salt and alcohol consumption, and reduced physical activity with an increased risk of developing HTN [108]. Interventional studies using weight loss[109], increased exercise [110], diet modification [111], reduced sodium [112] and alcohol intake [113] all lower BP and support causal role of environmental factors in the pathogenesis of hypertension. The expected age related increase in BP in "Westernised" populations

doesn’t occur in primitive isolated societies further supporting a strong environmental influence on BP [114].

1.8.2.3 Renin Angiotensin System

The renin angiotensin system (RAS) is a hormonal system important in the regulation of normal BP and in the pathogenesis of hypertension (Figure 15). Since the initial discovery of a factor (renin) released by the kidneys that controlled BP by Tigerstedt and Bergman in 1898, the knowledge and complexity of this system has increased[115]. The system can be broadly divided into the “classical” systemic and local RAS and both have a role in the pathogenesis of HTN and end organ damage.

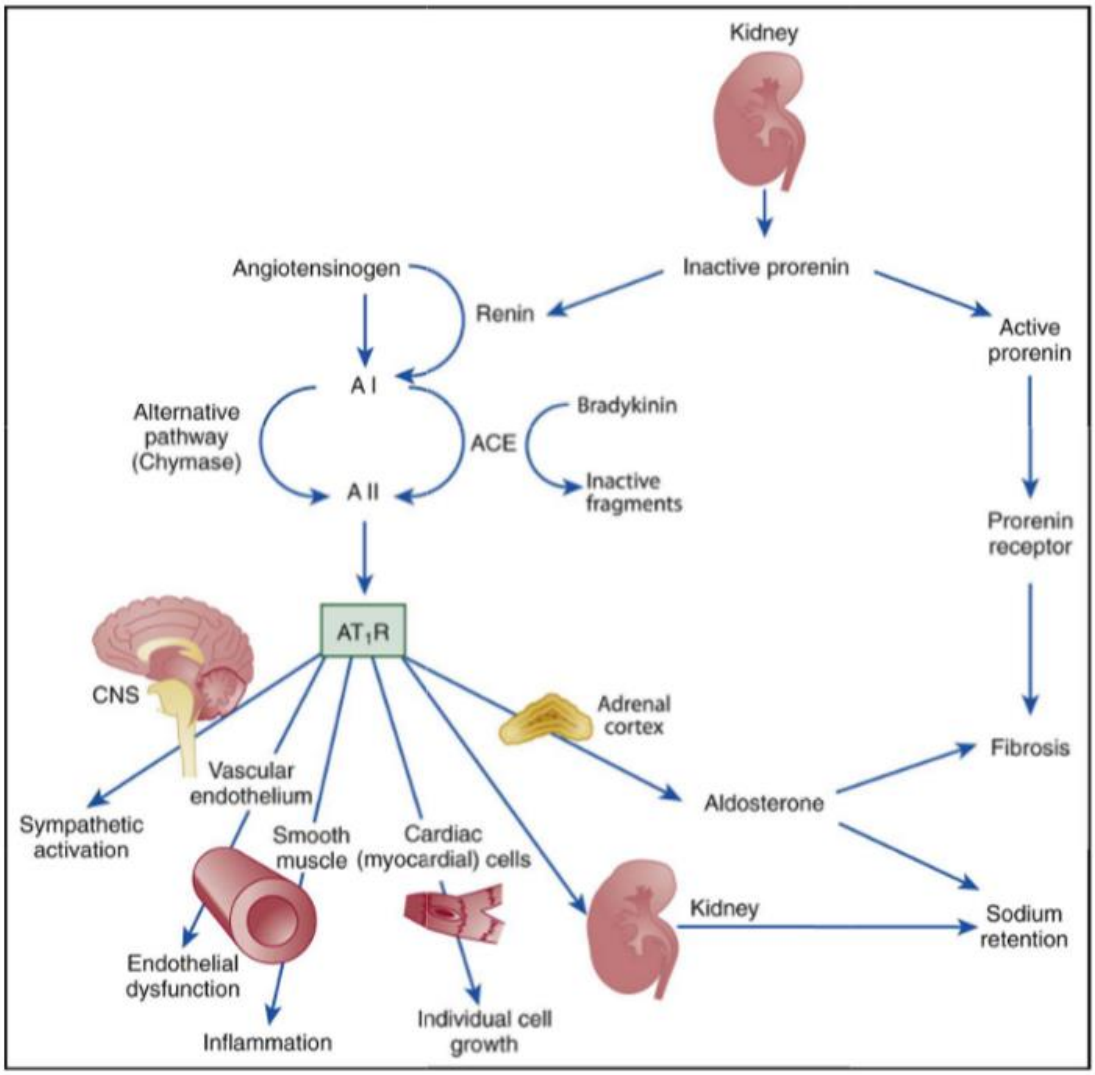


Figure 15 - Renin angiotensin aldosterone system [116]

primary physiological function of the RAS is to control vascular tone and water homeostasis [111]. Renin is released from the juxtaglomerular apparatus after stimulation of the afferent arteriolar baroreceptors by hypoperfusion or detection of reduced sodium chloride in the distal tubule and vice versa [110]. In addition, β2-adrenergic receptor stimulation by norepinephrine released from the renal sympathetic neurones also leads to renin release [110]. Angiotensinogen is cleaved by renin to form angiotensin I, which is then converted to angiotensin II (ATII) by angiotensin converting enzyme (ACE). ACE simultaneously degrades vasodilator

peptides such as bradykinin [110]. ATII is the major effector peptide of the RAS and stimulates G-protein coupled receptors AT1 and AT2 which generally have opposing effects [110], animal models suggest an imbalance of receptor expression that might in part explain why AT1R action dominates. AT1R is widely distributed in the heart, kidneys and vasculature and stimulation has several effects (Table 1) that includes the generation of ROS [114]. Infusions of ATII into rats has been shown to increase both BP and doubled vascular superoxide formation that was associated with impaired vasodilation, that was reversed by the administration of Losartan [117]. Although ACE is predominantly expressed in high concentration on the pulmonary vasculature [118] it can be found in the endothelium of vasculature in all tissues and nonendothelial parenchymal cells in the heart, kidney and inflammatory cells [119].

Table 1 - Effects of ATII via AT_1R (adapted) [114]

Vasoconstriction
Cardiac & Vascular Remodelling
SNS activation
Superoxide Generation
Aldosterone, Vasopressin & Endothelin Secretion
Renin Inhibition
Cardiac Contractility
Thrombosis
Inflammation

Synthesis of ATII within the cell or interstitium with function and regulation independent of or in conjunction with of circulating system has been demonstrated with both autocrine and paracrine effects [115]. Stretching rat cardiomyocytes in vitro causes hypertrophy in the absence of neuronal and hormonal factors [120],[121]. Several candidate factors have been demonstrated to cause hypertrophy in-vitro, but evidence suggests that ATII is a critical mediator for stretch induced response in cardiomyocytes [122]. ACE induction occurs in conditions of increased wall stress (i.e. pressure overloaded) and effects changes in fibroblasts, myocytes and the vasculature [119]. Endothelial ACE is upregulated by vascular injury from a variety of insults including hypertension leading to a reduction in bradykinin and an increase in local ATII [119]. Aldosterone increases resorption of sodium in the cortical collecting duct but also stimulates vascular remodelling by smooth muscle cell proliferation, vascular extracellular matrix deposition and increases OS [95].

1.8.2.4 Autonomic nervous system.

The autonomic nervous system has an important physiological role controlling blood pressure by its effect on cardiac output and peripheral resistance in healthy individuals (Figure 16) [123]. Sympathetic overdrive and impaired vagal response exists in those predisposed to developing hypertension and in those with hypertension, and is thought to have a role in both the pathogenesis and end organ damage associated with hypertension [123]. Furthermore the RAS and SNS have been shown to interact in a positive feedback loop to further augment BP [124]. Norepinephrine has been detected at higher levels in essential hypertensive patients than in healthy controls in most studies [125]. Studies using radiolabelled norepinephrine have demonstrated

that the elevated levels are caused by increased release from nerve terminals and is most pronounced in the brain, kidney and heart [125]. Microneurographic measurement of muscle sympathetic nerve activity (MSNA) provides a valid measure of resting sympathetic nerve activity to muscle vasculature that contributes to peripheral resistance and hence BP [126]. Elevated MSNA has been demonstrated as a generalised phenomenon in hypertension [123], progresses with severity [127], is more pronounced in those with organ damage (i.e. LVH, LV systolic and diastolic dysfunction) and resistance to treatment [123]. MSNA is increased in normotensive obese patients and even more marked in obese hypertensive subjects thought to be explained by elevated insulin, renin, leptin and endothelin secretion that have SNS stimulating effect [128]. In a number of animal experiments renal denervation prevents or reduces the severity of hypertension [129], human trials initially demonstrating promise [130], [131] but may have been biased by trial design and SYMPLICITY HTN-3 trial did not show a reduction of systolic BP [132].

A reduction in vagal tone and elevated SNS activity have been associated with the development of hypertension in animal models [123] and evidence of an impaired vagal response has also been demonstrated in human studies. Heart rate and cardiac output remained significantly higher after intravenous beta-blocker in hyperkinetic (i.e. high resting cardiac output and heart rate) borderline hypertension compared to controls but atropine administration equalised its effect [133]. Another study found atropine had a smaller effect on heart rate in young borderline hypertension than controls [134]. Impairment of baroreflex cardiovascular control has also been demonstrated in lean patients with hypertension, obese normotensives and of greater magnitude obese subjects with hypertension [128]. Further evidence of

parasympathetic dysfunction with hypertension comes from the observation of a gradual reduction of bradycardia/ tachycardia responses to baroreceptor stimulation/deactivation with increasing severity of hypertension compared to normotensives [123]. Baroreceptor activation therapy has been demonstrated to be effective in long term reduction of BP in RHTN patients [135]. Supporting evidence that the SNS has a role in vascular and cardiac remodelling comes from animal models. Stimulation of α_1-adrenoreceptor in rat aorta smooth muscle cells induced hypertrophy [136]. Sympathetic denervation of rabbit arteries decreased weight, wall thickness and contractility [137].

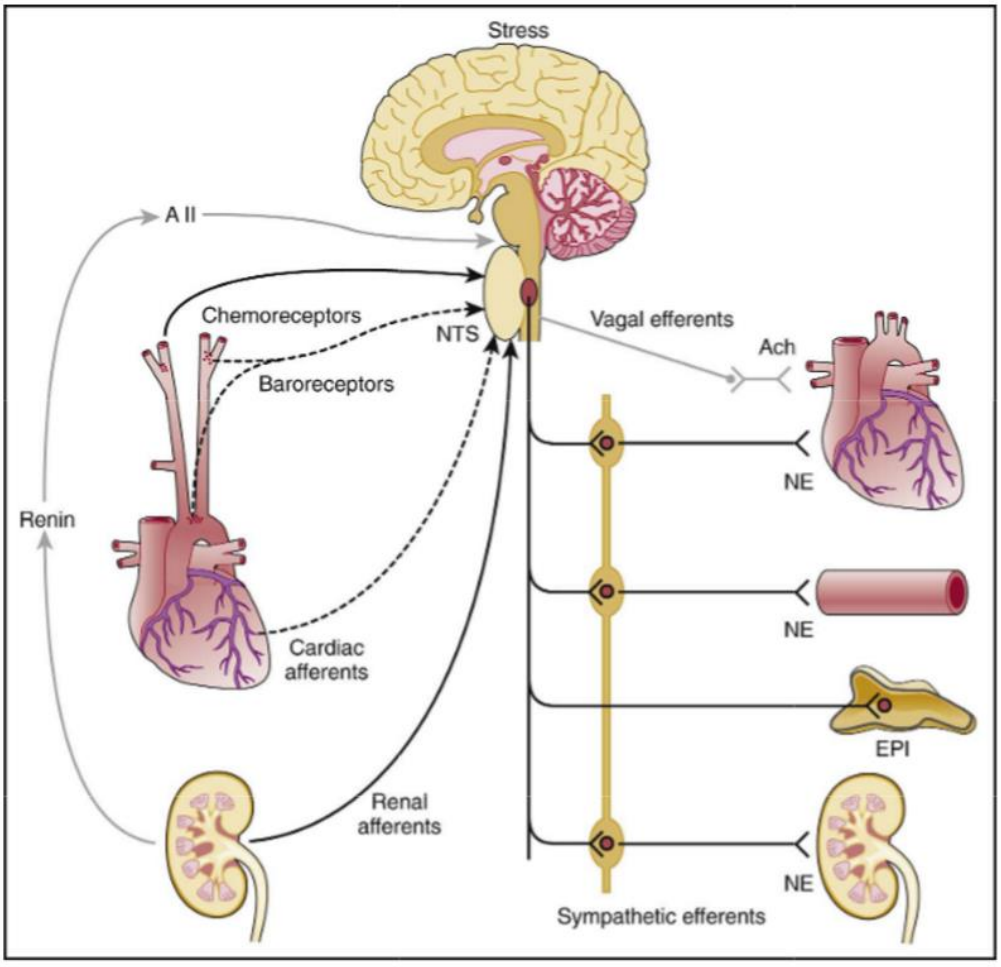


Figure 16 - Sympathetic Nervous System [138]

1.8.2.5 Insulin Resistance

Insulin resistance a cardinal feature of the metabolic syndrome has been demonstrated in untreated hypertensive patients with normal body weight and glucose intolerance [139]. Insulin resistance impairs the protective effects of the insulin stimulated NO pathway instead causing vasoconstriction, vascular smooth muscle cell proliferation, inflammation, sodium resorption and sympathetic nervous system activation via the mitogen-activated protein kinase pathway (MAPK) [140].

1.8.2.6 Endothelial Function

Increased peripheral resistance is a cardinal feature of established hypertension[114], caused by functional (endothelial dysfunction), structural (vascular remodelling) and mechanical alterations (vascular stiffness) of resistance arteries [87].

The endothelium of blood vessels is a paracrine organ that regulates vascular tone via vasodilators (nitric oxide, natriuretic peptides, prostacyclin) and vasoconstrictors (endothelin, angiotensin II)[95, 141]. Nitric oxide (NO) additionally protects the vasculature from atherosclerosis, thrombosis and inflammation by inhibiting platelet adhesion and aggregation, leucocyte adhesion and proliferation of vascular smooth muscle cells [141, 142]. Hypertension induces ROS generation in the vascular wall, predominantly from NADPH oxidases, but also from xanthine oxidase, cyclooxygenases and reduced superoxide dismutase activity (Figure 17)[95, 142]. Excess superoxide oxidises BH4, uncouples endothelial nitric oxide synthase, reacts with NO to form the pro-inflammatory oxidant peroxynitrite with the effects of further ROS generation,

inflammation, reduced NO bioavailability and endothelial dysfunction[95, 124, 141, 142].

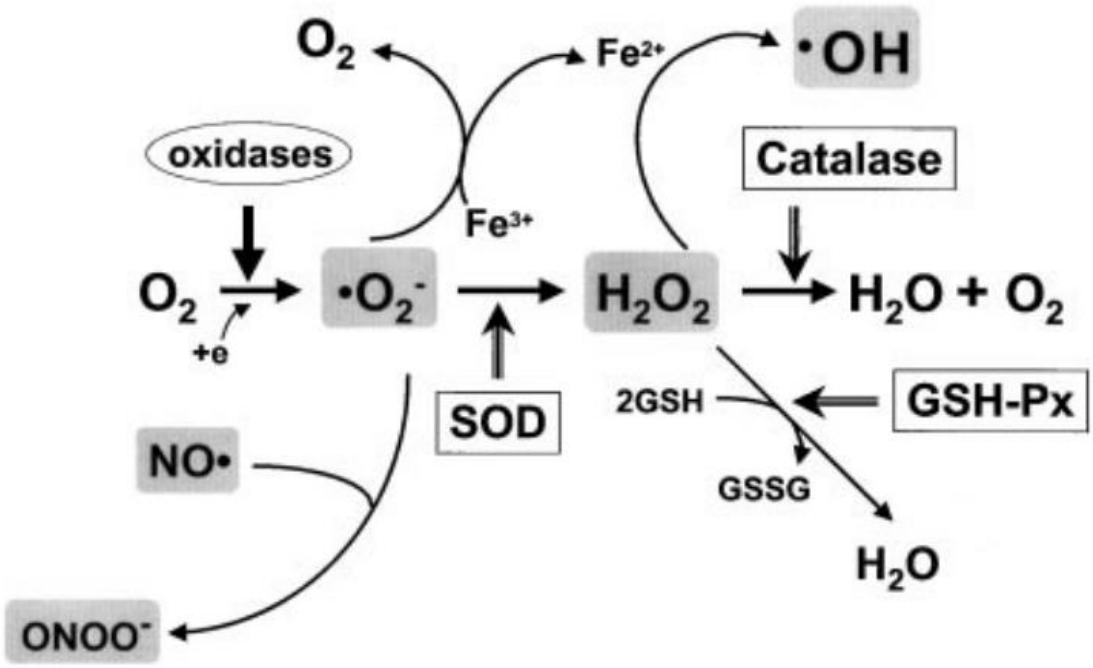


Figure 17 - Vascular ROS [143]

Endothelial dysfunction is an important factor in subjects with hypertension and has been demonstrated in the early stages [142, 144-146]. A number of small trials have demonstrated an inverse relationship with endothelial function and LVM [147-149] but not all [146]. The largest study to support this relationship was a sub-group analysis of 2447 of the Multi-Ethnic Study of Atherosclerosis trial which found that a 0.5g/m^2 reduction in indexed LVM improved flow mediated dilation by 1% after adjustment ($p<0.001$) [150].

1.8.2.7 Vascular Remodelling & Stiffness

Structural remodelling of resistance arteries occurs early and has been demonstrated in patients with only mild hypertension, preceding other clinically detectable target organ damage [144, 151]. Inward eutrophic remodelling is the usual pattern in essential HTN where there is narrowing of the lumen but preservation of the medial cross-sectional area of small arteries [151]. In secondary hypertension and conditions where the endothelin system is activated (salt-dependent/malignant hypertension, diabetes) inward hypertrophic remodelling is found, characterised by an increase in the media to lumen ratio [151]. The cell volume and number of vascular smooth muscle cells (VSMC) are similar to normotensives in eutrophic remodelling but there are changes in the extra cellular matrix [151, 152]. Increased type I and III collagen mRNA and protein synthesis has been detected within fibroblasts and increased collagen has been found within the media of resistance arteries [87]. ROS are of critical importance in vascular remodelling, predominantly from NADPH oxidase, and to a lesser degree xanthine oxidase [153]. ROS activate redox sensitive signalling molecules causing VSMC contraction, cell growth, apoptosis and increased ECM [154].

Angiotensin II is a major stimulant of vascular remodelling (Figure 18), increasing collagen formation by p38 mitogen activated-protein kinase (MAPK) and extracellular signal regulated protein kinase (ERK) pathways[155] and attenuating MMP activity [87]. Furthermore ATII activates redox sensitive genes NF_kB, AP-1 that upregulate adhesion molecules, chemokines and monocyte/macrophage recruitment in the vascular wall causing inflammation [156]. Angiotensin converting enzyme inhibitors and AT_1R antagonists have been demonstrated to regress arterial remodelling,

improve endothelial function, reduce vasomotor tone, lessen inflammation and normalise aberrant signalling in vascular smooth muscle [87].

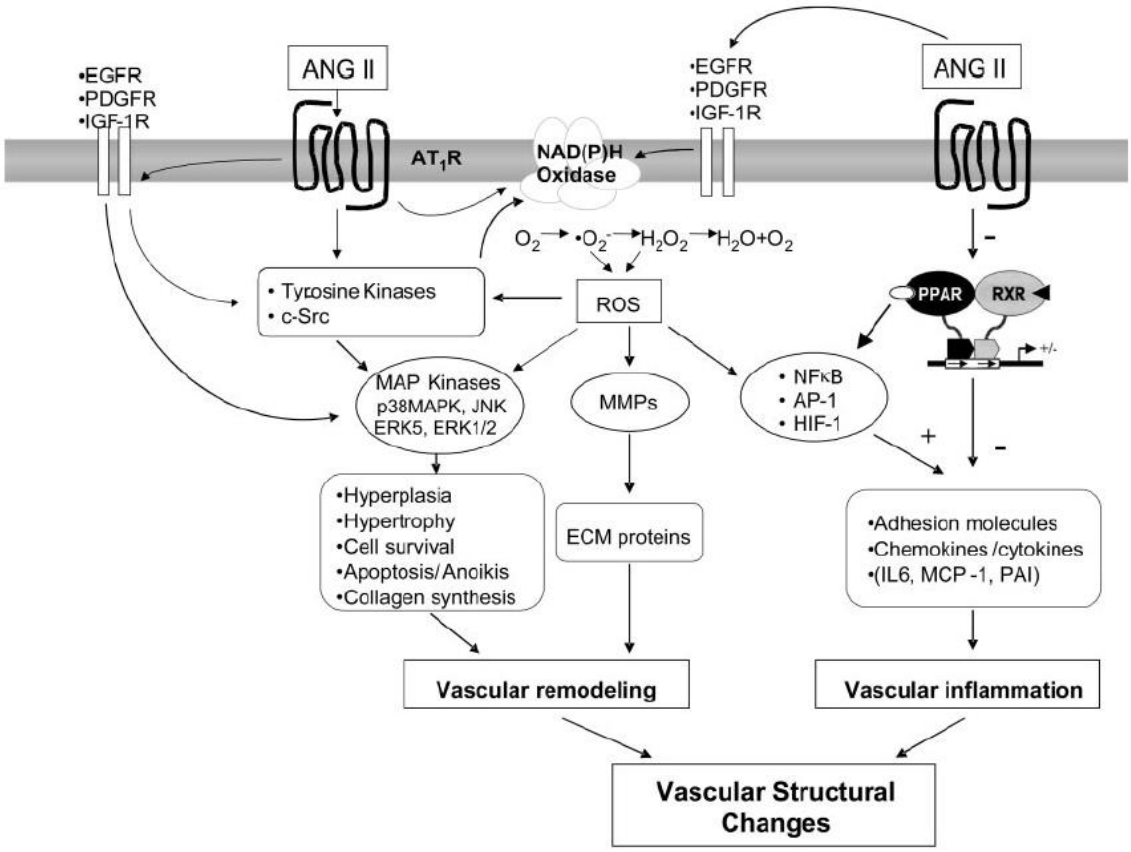


Figure 18 - Vascular remodelling and the RAS [153]

Fragmentation of elastin fibres, accumulation of extracellular collagen, inflammation, calcification and medial smooth muscle necrosis leads to reduced compliance and dispensability of central conduit vessels, termed vascular stiffness [47] and can develop in advanced hypertension [156]. Pulse wave velocity (PWV) is the rate of propagation of a pulse of blood though the arterial circulation and can be used as a direct measure of central vascular stiffness that increases with age and in those with cardiovascular risk factors including hypertension [157]. Increased aortic PWV is a

predictor of adverse outcomes in HTN [47], with an independent association with cardiovascular events [158], all cause and CV mortality [159]. Pulse pressure is a composite of the forward pressure created by ventricular contraction and the reflected retrograde waves (Figure 19)[160]. Reflection occurs at branch points or sites of impedance mismatch, as vessels stiffen the PWV increases and the reflected wave arrives earlier augmenting central systolic and pulse pressure [160].

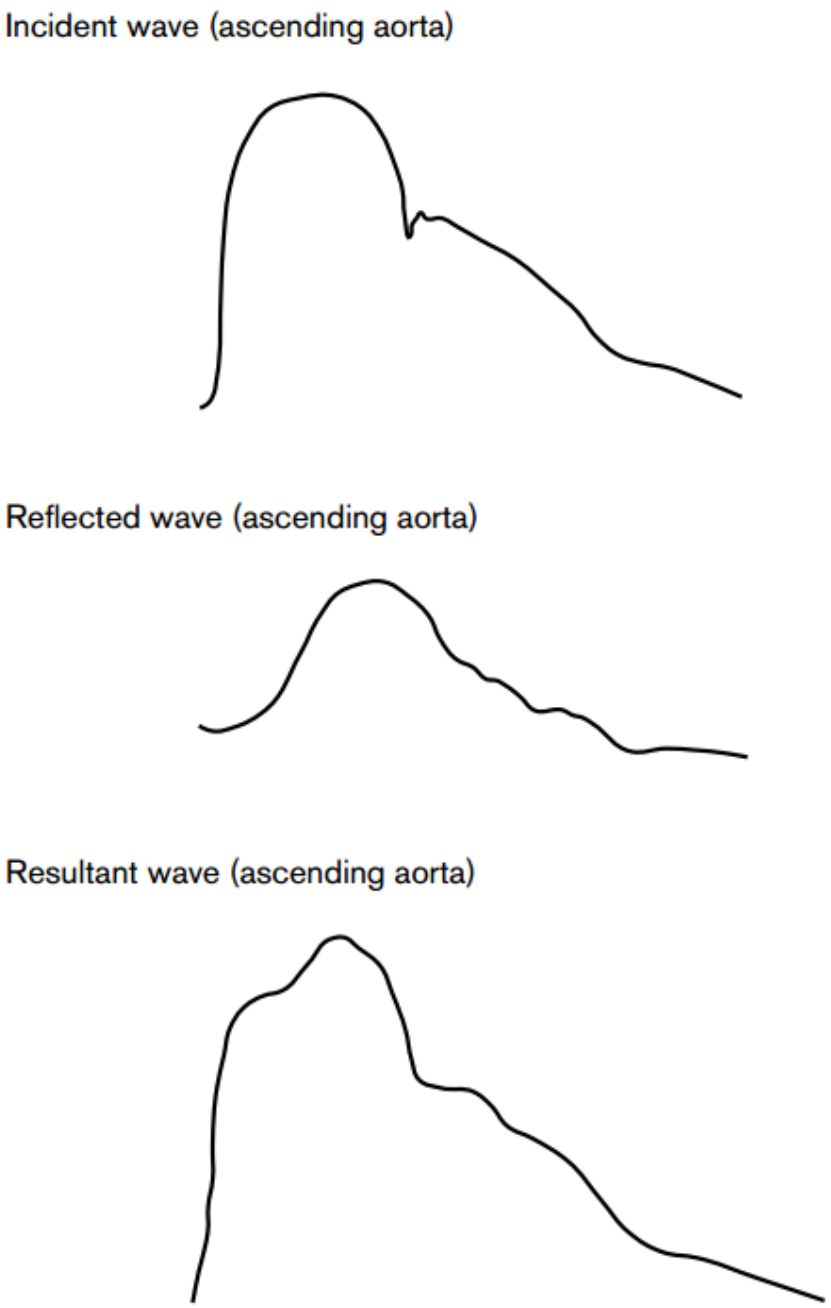


Figure 19 - Incidental, reflected and resultant pressure waveform [161]

Isolated systolic hypertension is the usual form over 55 years of age (Figure 13) [157] and confers greater CV risk than an elevated diastolic pressure [162]. Increased central pulse pressure has the effect of increasing afterload and hence promoting LVH, vascular remodelling and atherosclerosis [47].

Augmentation index (AIx) is the percentage of the pulse pressure caused by the reflected pulse wave and is a surrogate marker of vascular stiffness and a measure of the ventricular load [157]. Although AIx has been demonstrated to correlate with both cardiovascular risk scoring (r= 0.35 – 0.685) [163] and with CV risk factors (r=0.604) [164] it has failed to predict risk in a hypertensive cohort [165]. AIx does however have an independent and positive correlation with left ventricular mass index in hypertensive and normotensive subjects [166, 167]. A twin study using qualitative genetic modelling concluded the inheritability of AIx was 37%, more striking than that for BP (13-25%) with only a small percent attributed to other potential genetic influences of AIx (i.e. height, heart rate, mean arterial pressure) [168]. Although there is a linear correlation between PWV and AIx (r = 0.41), the latter is influenced by BP, heart rate, gender, age, height and vasoactive drugs independent of changes of vascular stiffness [157].

ACE-I, ARB, CCB and MRA all reduce vascular stiffness thought to be either directly by reducing wall stress or reduced impedance in peripheral arteries delaying wave reflection or indirectly by anti-fibrotic actions [151]. A number of RCTs have been conducted to assess the effect of allopurinol on PWV and AIx, with conflicting results [60]. A meta-analysis concluded that Allopurinol improved AIx but not PWV [60].

1.8.2.8 Uric Acid and Hypertension

An elevated uric acid is a strong independent predictor of hypertension in almost every published study [7]. A meta-analysis comprising 55,607 subjects from North America. Asia and Europe, found an adjusted risk ratio of 1.41 (95% CI, 1.23-1.58) for the development of hypertension if hyperuricaemic, a relationship that is more

pronounced in younger individuals [169]. Support of a causal relationship initially came from animal models. When rats were treated with an uricase inhibitor, developed an elevated blood pressure attenuated with xanthine oxidase inhibition or uricosuric agents [7].

Human studies have demonstrated that elevated uric acid after consumption of large quantities of fructose lead to an elevated blood pressure, an effect prevented by treatment with allopurinol [170]. A two-phase mechanism has been proposed (Figure 20). In the first phase uric acid-dependent activation of the rennin-angiotensin system occurs leading to increased oxidative stress, reduced endothelial nitric oxide and salt resistant hypertension [171, 172]. Over time altered renal microvascular changes occur causing uric acid independent, salt sensitive hypertension in the second phase [7, 171, 172].

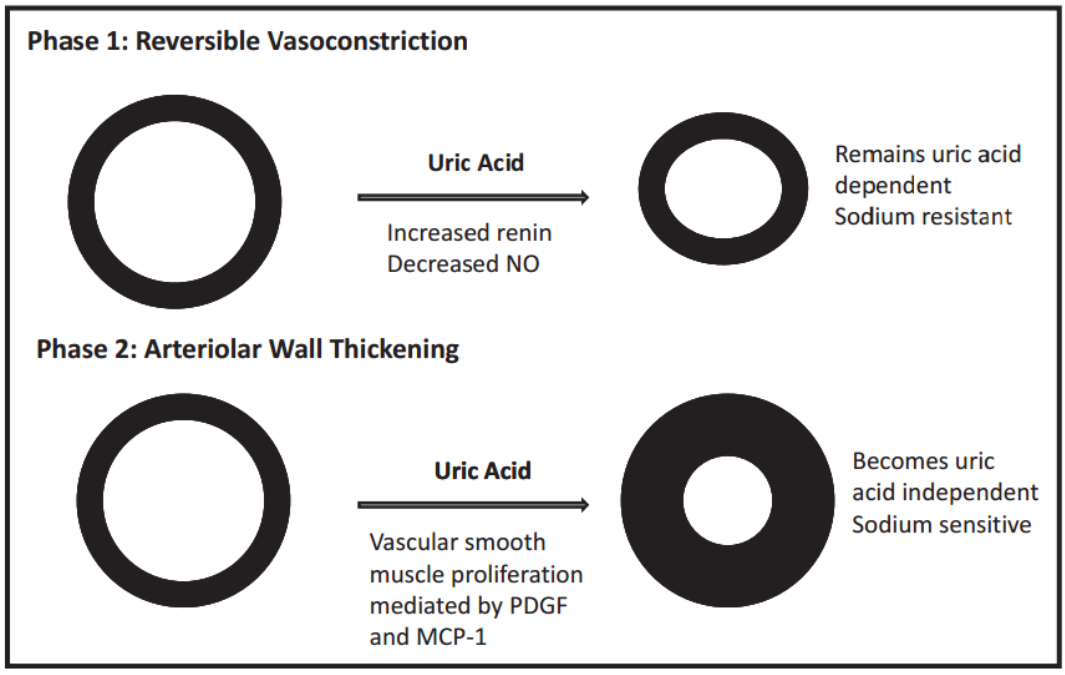


Figure 20 - Model of development of hyperuricaemic induced hypertension [171]

The mechanism is the entry of uric acid via the URAT-1 channel followed by kinase and nuclear transcription factor activation the production of cyclo-oxygenase 2, growth factors, inflammatory proteins (CRP, monocyte chemoattractant protein-1) and ultimately vascular smooth muscle proliferation [171]. Feig et al proposed this model explained the greater association of elevated uric acid with hypertension and youth [171].

1.8.3 Treatment of HTN (NICE Guidelines)

The treatment of hypertension consists of lifestyle measures and/or antihypertensive medications. Lifestyle measures include advice regarding smoking cessation, improving diet, weight loss, increasing exercise, reducing alcohol, salt and caffeine consumption. Pharmacological interventions are indicated in patients with stage I hypertension and target organ damage, cardiovascular or renal disease, those with diabetes or those with a 10-year CV risk >20% and all patients with stage 2 or severe hypertension. Medications are added in a stepwise approach illustrated in Figure 21 aiming for a target office BP of <140/90mmHg or ambulatory (ABPM) or home BP monitoring (HBPM)<135/95mmHg in patients under 80 years of age. An office BP of <150/90mmHg and ABPM/HBPM < 145/85mmHg for those over 80 years of age.

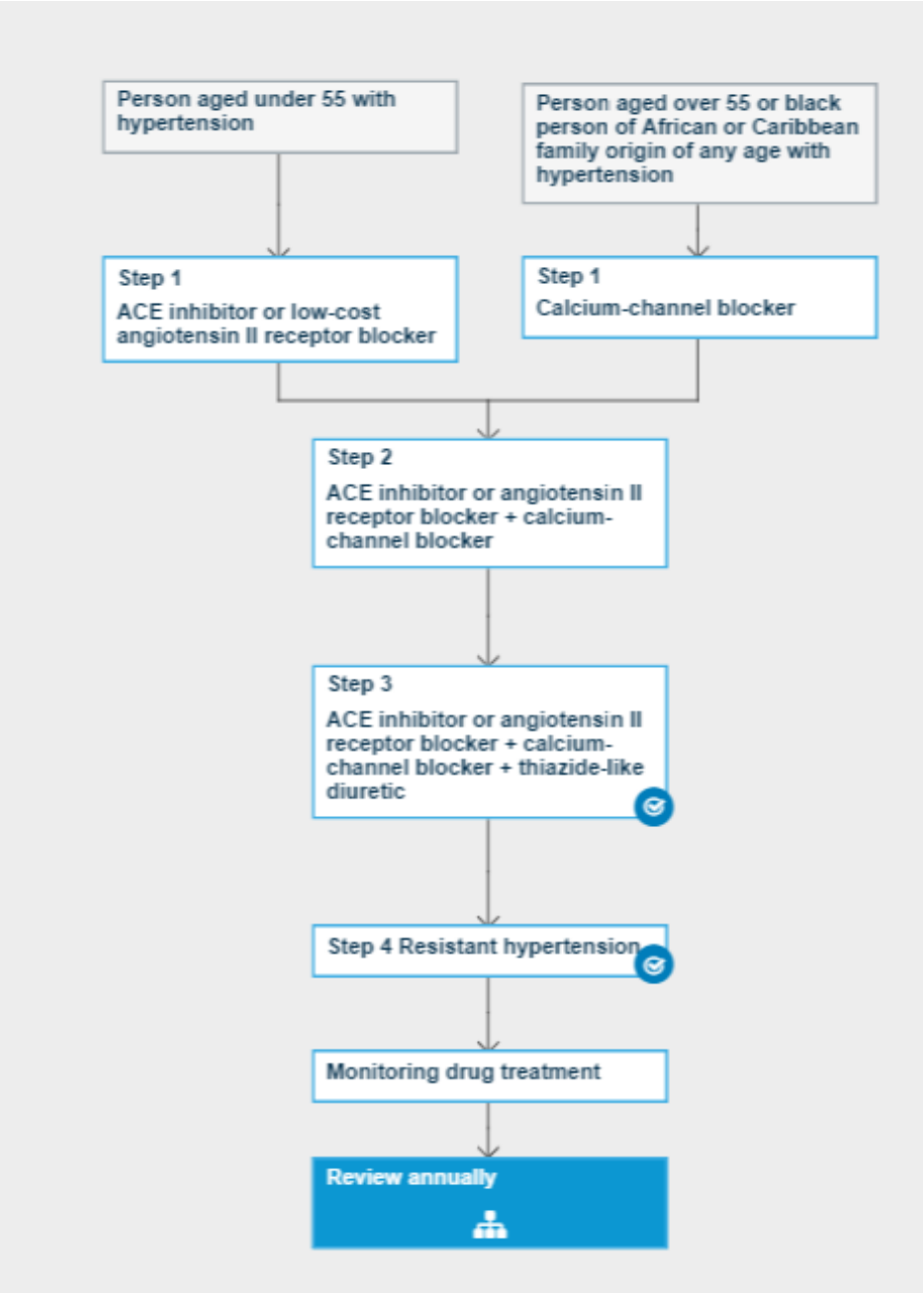


Figure 21- NICE Treatment Steps For HTN [96]

1.9 Left Ventricular Hypertrophy

1.9.1 LVH Prevalence and Risk Factors

1.9.1.1 General Population

The prevalence of LVH depends on the population studied and the criteria by which you define it. Over time the threshold for diagnosing LVH by echo has fallen and hence assessing the change of prevalence is challenging. The Framingham heart study, a prospective epidemiological study of the residents of Framingham, Massachusetts investigated the prevalence of LVH. Initial studies using ECG found 3% of subjects fulfilled criteria for LVH [173]. Echocardiography of 4976 subjects found 16% of men and 19% of women had LVH [174]. A multivariate analysis demonstrated significant independent associations with age, systolic BP, obesity, valve disease, antihypertensive medication, angina and myocardial infarction were demonstrated [174]. A marked increase in the prevalence with age was found (Figure 22), occurring in 6% under 30 to 43% in over 70 years of age [174].

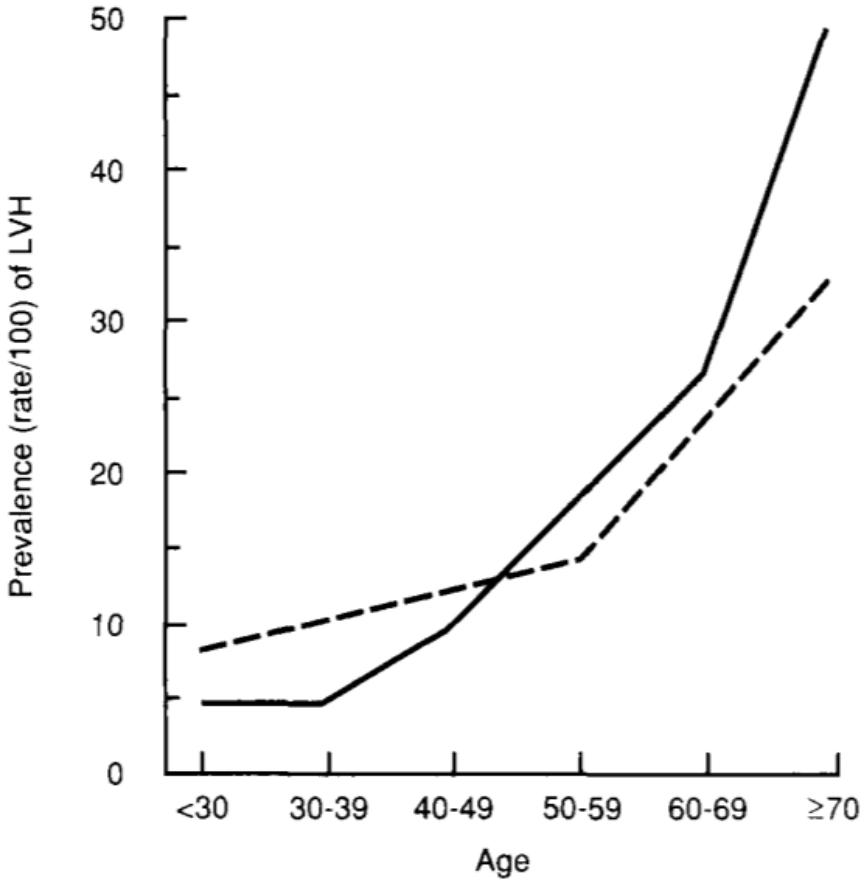


Figure 22 - Prevalence of LVH with Age [174]

(Male dashed line, Female solid line)

A more contemporary prospective observational study 3287 subjects from Norway found 14.9% of men and 9.1% of females has LVH. Independent risk factors and odds ratios are shown in Figure 23[175]. The PAMELA study found that a serum uric acid >5.1mg/dL at baseline predicted the development of LVH over ten years after adjustment for confounders [21].

Variable	Odds ratio (95% CI) for LVH	Wald score
	n=2794 (cases=334)	
Age (10 years)	1·19 (1·02–1·39)	4·94
Gender (male=1, female=0)	2·20 (1·68–2·88)	33·4
Body mass index (3·8 kg . m^{-2})	1·96 (1·72–2·22)	107·9
Valvular heart disease	4·19 (2·79–6·30)	47·7
Systolic blood pressure (20·8 mmHg)	1·46 (1·29–1·67)	33·1
Cardiovascular disease	2·22 (1·63–3·03)	25·3
Antihypertensive medication	1·60 (1·16–2·19)	8·32
ROC area	0·80	

Figure 23 - Independent risk factors for LVH [175]

1.9.1.2 LVH Prevalence in Hypertension

A review of the prevalence of echocardiographic LVH in treated and untreated hypertensive patients was conducted by Cuspidi et al [176]. Thirty studies, totalling 37,770 patients from the first decade of this century were selected. Using lower thresholds for LVH diagnosis the prevalence of LVH in the pooled population was 40.9%. There was significant heterogeneity between studies and criteria by which LVH was diagnosed varied. The prevalence in untreated hypertension was 19-48%, increasing to 58-77% in the “highest risk” populations that included severe/resistant hypertension and those with ECG-LVH. LVH persists even in well treated hypertensive patients, 17% of subjects from the PAMELA population had LVH despite a mean BP of 120.2±8.5/75.7±4.9mmHg [177]. Observational data has found a continuous

relationship between age, systolic BP and LVH (Figure 24) [174]. LVM correlates more closely with 24 hour mean ambulatory BP (ABPM) than clinic BP [178].

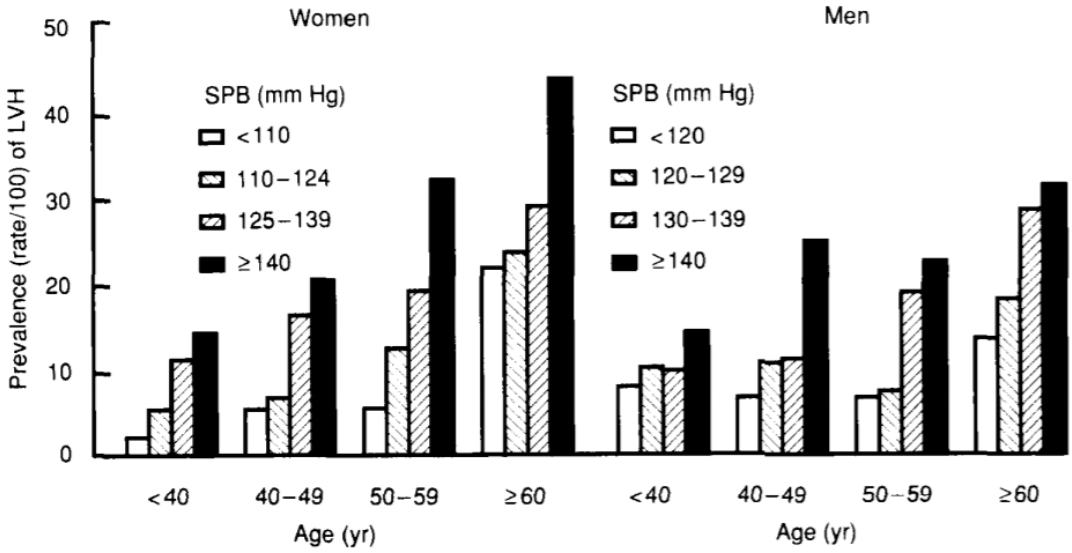


Figure 24 - LVH prevalence with age and BP quartiles [174]

1.9.1.3 Genetic factors

Epidemiological studies have demonstrated that <50% of the variance in left ventricular mass can be explained by conventional risk factors [168]. Heritability of LVH is supported evidence from observational studies in general populations [179], sibling [180] and twin studies [181, 182]. In humans there is evidence of an association of several genes related to hormones (i.e. ATII, ANP, catecholamine's, and mineralocorticoids) or the cardiomyocyte (i.e. myosin, heat shock proteins, growth factors) but a causal relationship has been difficult to establish [183]. Several single nucleotide polymorphisms (SNPs) have been identified with associations to LVH in genome wide association studies (GWAS) but have not been replicated in other populations [184]. It is thought that multiple variants, each with modest effect size are

involved in modulating LVM [185]. Genetic polymorphism of the RAS and other pathways have been implicated in the development of LVH and may account for a significant variance in LV mass independent of BP [186] and response to treatment [187].

1.9.2 Risk of LVH

Echocardiographic LVH was associated with an increased cardiovascular morbidity and mortality even after adjustment for other major risk factors in the Framingham population [188]. Increased risk has also been consistently demonstrated in specific pathologies such as end-stage renal failure [189], hypertension [190], ischaemic heart disease [191] and normotensives [192]. A review of 20 studies assessing the risk of CV events with ECG or echocardiographic LVH (total of 48,545 subjects) calculated an overall adjusted mean risk ratio of CV morbidity of 2.3 and all-cause mortality of 2.5 [193]. A head to head study of 1089 black participants demonstrated that LVH independently conferred a higher risk of death (RR 2.4; 95% CI 1.7 to 3.2) than multi-vessel coronary artery disease (RR 1.6; 95% CI 1.1 to 2.2) or reduced LV function (EF <45%) (RR 2.0; 95% CI, 1.4-2.7) [194]. Schiallaci et al found a positive linear increase in risk of CV events and all-cause mortality across the quintiles of LVM in a cohort of 1925 uncomplicated hypertensive patients (Figure 25). After accounting for baseline differences between quintiles, risk was significantly elevated from quintile 3 i.e. males 119.8g/m^2, females 101.8g/m^2. This roughly corresponds to the values currently used in the ASE guidelines as the cut off for LVH (♂>115g/m^2, ♀>95g/m^2) [1].

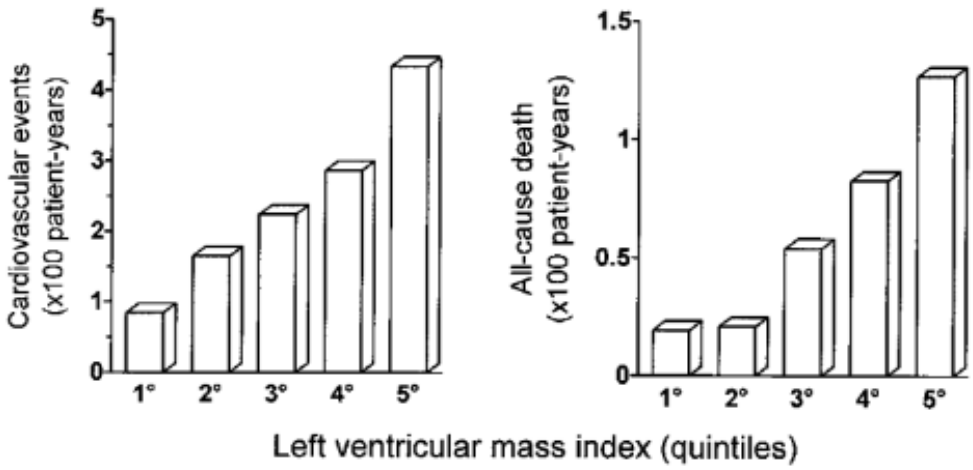


Figure 25 - Relationship of LVM and risk in Essential HTN [195]

Several distinct patterns of abnormal LV geometry have been described (Figure 26) and can provide additional prognostic information. Those with hypertension and normal geometry are at the lowest risk which increases progressively from concentric remodelling through eccentric to concentric hypertrophy [196].

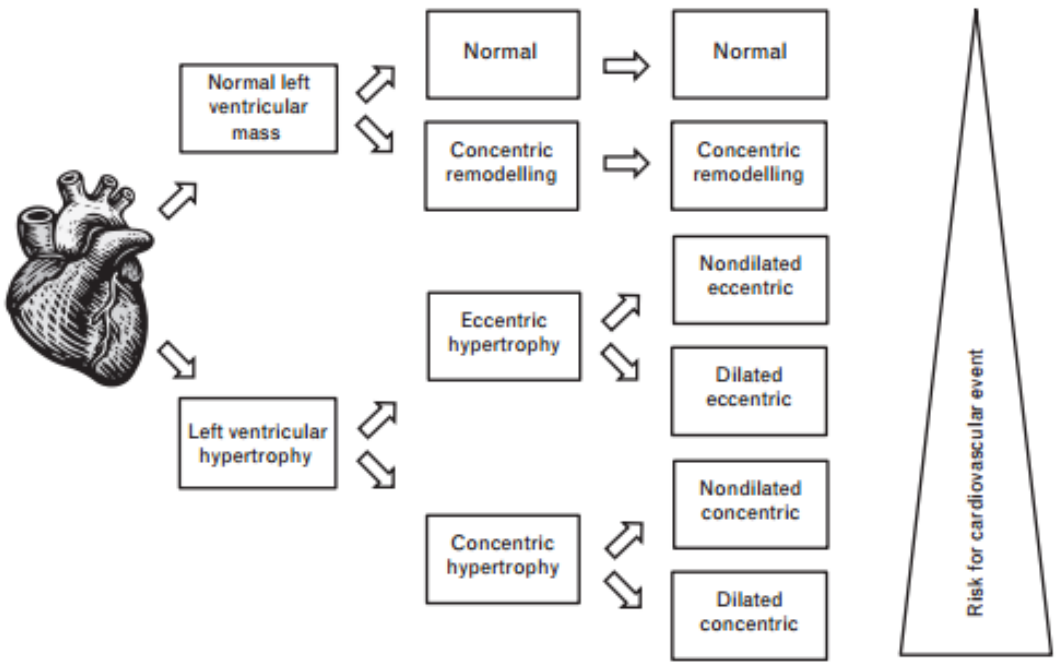


Figure 26 - LV geometry and CV risk [196]

1.9.3 Pathophysiology of Left Ventricular Hypertrophy

LVH is characterised by cardiomyocyte hypertrophy, proliferation of the extracellular matrix and rarefaction of the coronary micro-circulation which may ultimately cause ventricular dysfunction, arrhythmia and ischaemia (Figure 27)[197].

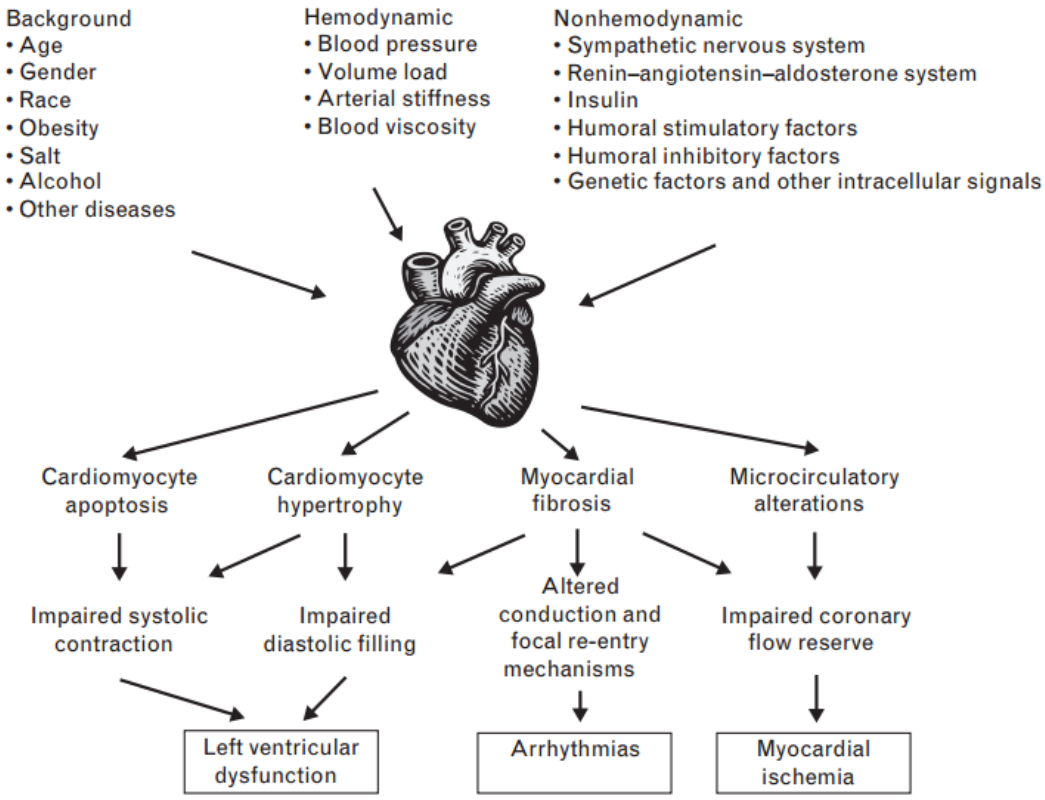


Figure 27- Factors involved in myocardial remodelling in HHD [198]

Two patterns of hypertrophy occur, concentric and eccentric. Left ventricular mass is increased in both but chamber volume is reduced in the former and increased in the latter, expressed in the measurement of relative wall thickness (RWT) i.e. the ratio of the LV wall thickness to the diastolic diameter (Figure 28). Concentric hypertrophy is typical of conditions that increase afterload (e.g. aortic stenosis or hypertension) the

latter usually occurs in response to volume overload (e.g. aortic or mitral regurgitation) [185, 199, 200].

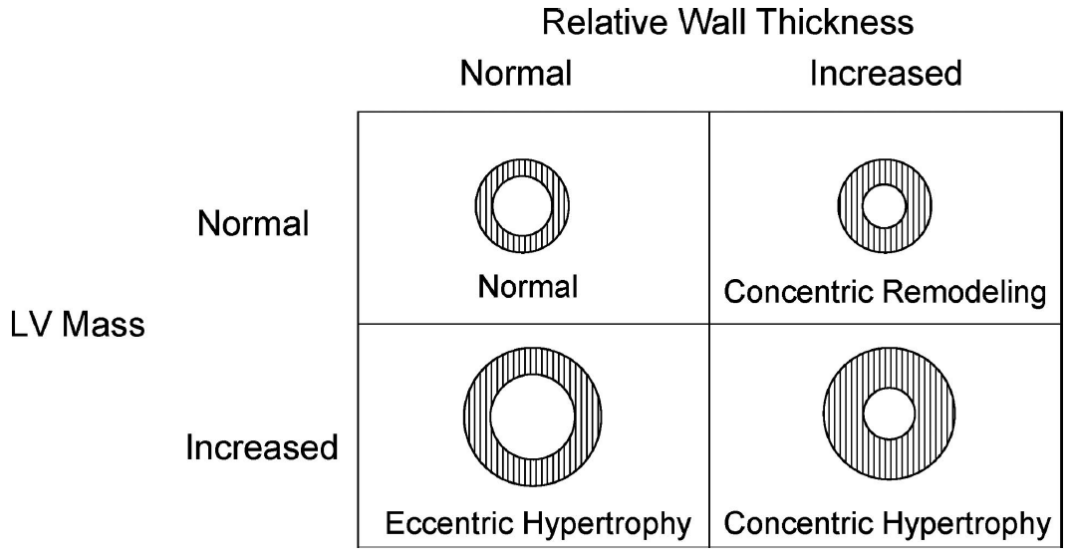


Figure 28 - Classification of LV Geometry [185]

Left ventricular wall stress is inversely proportional to wall thickness in accordance with Laplace's Law (*Figure 29*). LVH therefore compensates for an increased afterload by normalising wall stress to maintaining ejection fraction [199, 200]. There is evidence in some cases with marked hypertrophy wall stress can be sub-normal and ejection fraction supra-normal suggesting an exaggeration response [199].

LV wall stress (σ) = Ventricular Pressure (P) x Radius (r)/Wall thickness (h)

Figure 29 - Laplace's Law

Cardiomyocytes are terminally differentiated after birth, hence hypertrophy not hyperplasia of cardiomyocytes occurs [199]. This is achieved by either the parallel addition of sarcomeres increasing myocyte width or sarcomere replication in series with myocyte lengthening causing concentric or eccentric hypertrophy respectively (Figure 30) [199].

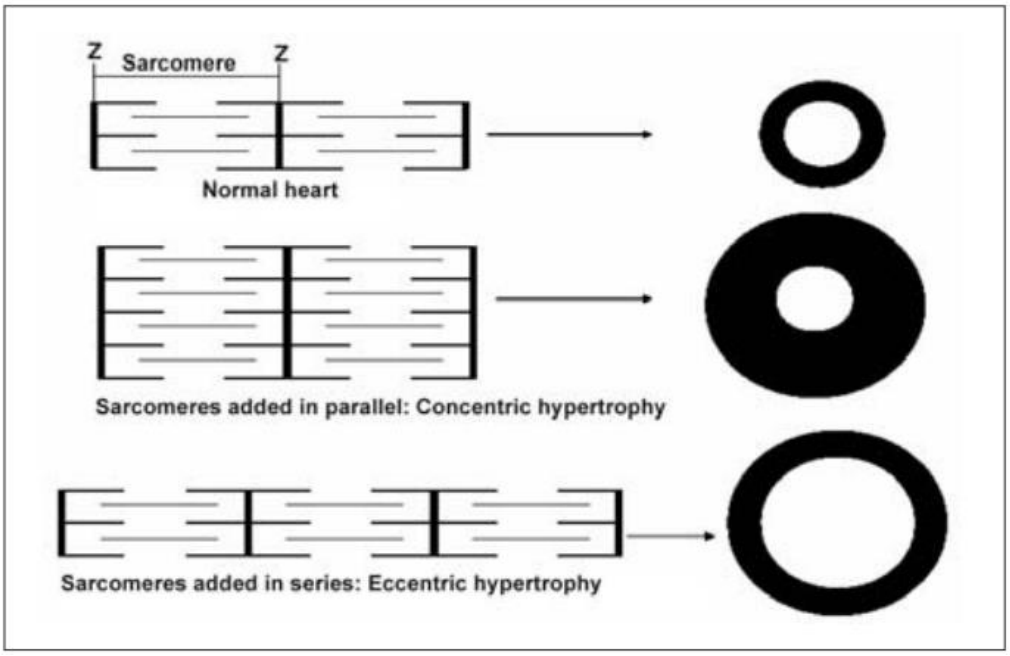


Figure 30- Patterns of LVH and changes in the myocyte [197]

Increased myosin heavy chain synthesis has been demonstrated within hours in dogs with acute pressure overload [201]. This contrasts with volume overload where there is no increase in myosin heavy chain production [201, 202] but a reduction in the degradation rate [202]. Apoptosis is abnormally stimulated in hypertrophied hearts caused by mechanical stress, humoral factors (ATII, aldosterone) and oxidative stress resulting in direct damage to the cell membrane, organelles and DNA shifting the balance towards cell death versus survival[3]. Apoptosis is an important factor in the evolution to decompensated heart failure in hypertensive heart disease (HHD) [203].

The ECM of the myocardium is primary composed of collagen type I but also contains elastin, laminin, fibronectin, collagen type III and V that are responsible for supporting the biomechanical load [199]. Unlike cardiomyocytes fibroblasts retain their mitotic capacity [204]. Disruption of the balance of extra-cellular matrix production and degradation can lead to a disproportional increase in extra-cellular volume that can occupy as much 30% of the myocardium [199]. Post-mortem examination of subjects with hypertensive heart disease found that the collagen volume fraction was significantly increased and proportional to the severity of the hypertrophy [205]. Pro-fibrotic factors are thought to be triggered by defective ECM-cell contact, ischaemia or trophic factors such as catecholamine's, cytokines, ATII, aldosterone and ROS [154, 199]. Elevated procollagen type I (biomarker for type I collagen formation) and impaired diastolic function in mild-to-moderate hypertension and normal LVM suggests that fibrosis is an early feature of hypertensive heart disease [206] .

Patients with hypertension and LVH can have signs and symptoms of myocardial ischaemia without obstructive coronary stenosis [207, 208]. This is due to changes in the vascular compartment such as perivascular fibrosis, endothelial dysfunction and a lower capillary/arteriolar density resulting in an impaired coronary flow reserve [209].

Left ventricular hypertrophy develops when a there is an imbalance of hypertrophic and antihypertrophic signalling that act on the cellular and extra-cellular matrix (Figure 31).

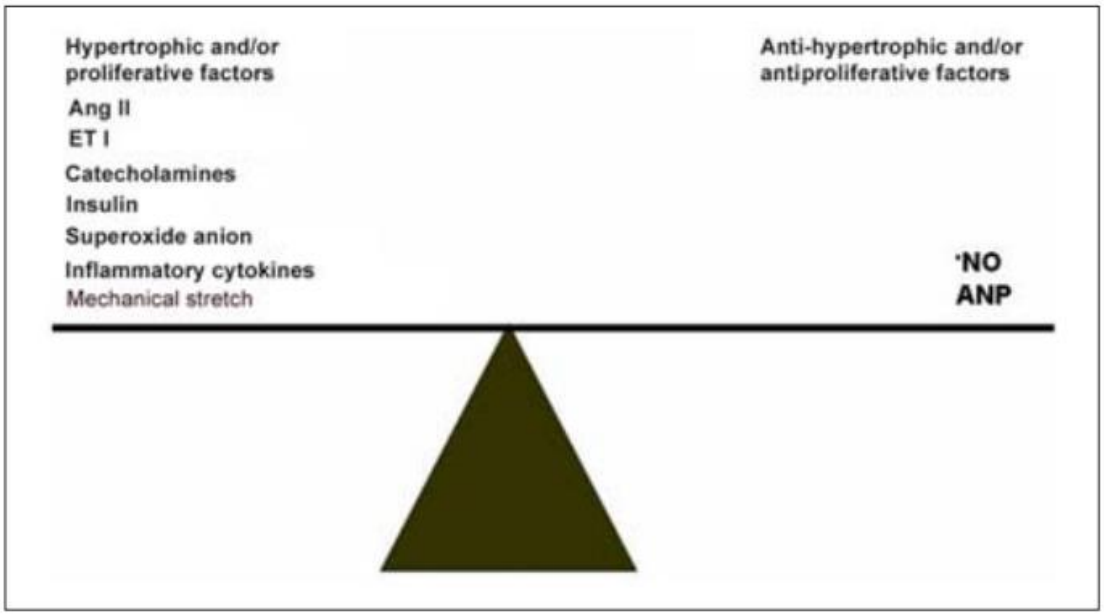


Figure 31 - Hypertrophic versus anti-hypertrophic factors[197]

Unlike the physiological adaptation in athletes the changes are ultimately maladaptive, exaggerated and result in altered perfusion, arrhythmia or ventricular dysfunction [102]. ROS have a role in normal cellular nitrous-redox signalling, and disruption to this balance is an important factor in the pathophysiology of LVH. Seddon et al proposed three broad pathophysiological effects of OS in the heart. Firstly direct cellular oxidative damage leads to dysfunction, energetic deficit and cell death, secondly inactivation of NO causes endothelial dysfunction and lastly activation of redox signalling promotes hypertrophy and fibrosis [81].

The primary stimulus for hypertrophy is via neurohumoral, mechanical, or a combination of both factors [197]. Disruption of cell to cell or cell to ECM contact is thought to be transmitted via focal adhesion complexes (integrins) that connect the cellular cytoskeleton to the ECM [199]. Released in response to pressure overload neurohumoral factors can act in an autocrine, paracrine or neuroendocrine fashion [121, 204] and include vasoactive peptides (ET-1, ATII), catecholamines, direct

activators of protein kinase C, peptide growth factors, cytokines and arachidonate metabolites that act predominantly via G protein coupled receptors (GPCR) (Figure 32) [204].

Agonist Type	Examples	Point of Action
Vasoactive peptides	ET-1,[180,181] Ang II[182]	$G\alpha_q/G\alpha_{11} \rightarrow PtdInsP_2$ hydrolysis → nPKCs
α_1-Adrenergic agonists	Norepinephrine/epinephrine,[183] phenylephrine[184]	$G\alpha_q/G\alpha_{11} \rightarrow PtdInsP_2$ hydrolysis → nPKCs?
Direct activators of PKC	Tumor-promoting phorbol esters[47,48]	nPKCs/cPKCs
Peptide growth factors	Fibroblast growth factors,[185] insulin-like growth factor 1[186]	Receptor protein tyrosine kinases
Cytokines	Cardiotrophin-1[187]	gp130/interleukin-6 receptor
Arachidonate metabolites	Prostaglandin $F_{2\alpha}$[62,63]	JNKs
Mechanical stretch[188–190]	Autocrine/paracrine factors[191] (ET-1,[192,193] Ang II,[193,194] or $PGF_{2\alpha}$)?	$PtdInsP_2$ hydrolysis/PKCs/JNKs?
Cell contact[195]	Not known	Not known

nPKCs indicate novel isoforms of PKC; cPKCs, classical isoforms of PKC; and $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

Figure 32 - Stimuli of Ventricular Myocyte Hypertrophy [204]

GPCR agonists activate several secondary (cytosolic) messengers that include ROS and mitogen-activated protein kinase (MAPK) cascades (ERKs, JNKs, p38-MAPKs) [3, 204]. An alternative pathway also via GPCR is calcineurin activation of NFAT (nuclear factor activated T lymphocytes) [204]. A number of signalling molecules have been proposed, but no signalling molecule has been identified as the “master switch” for the development of hypertrophy suggesting redundant signalling pathways are recruited when a single pathway is supressed [199].

Gene expression has been primarily linked with the expression of foetal cardiac genes that modify motor unit composition and function, energy metabolism and hormonal pathways [199]. ROS dependent activation of tertiary (nuclear) messengers that include nuclear factor-kappaB (NF-κB), activator protein-1 (AP-1), E26 transformation-specific (Ets) factors have been shown to be involved in myocyte hypertrophy and ECM remodelling (Figure 32) and at high levels of ROS apoptosis [3, 73, 81, 210, 211].

1.9.3.1 The Role of Oxidative Stress in Hypertensive LVH

Oxidative stress is an important driver of the development of LVH and in the transition to decompensation (Figure 33). Figure 34 demonstrates the key REDOX sensitive signalling pathways and how ROS influence both adaptive and maladaptive hypertrophy and therefore highlight its potential as a target for therapeutic interventions.

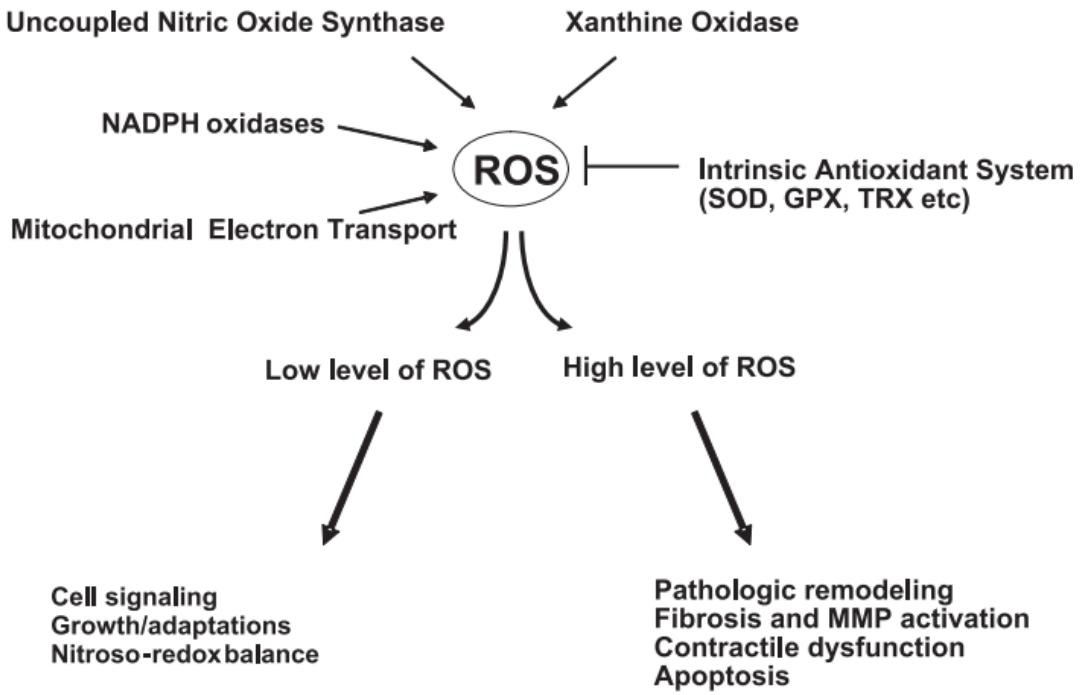


Figure 33 - ROS generation and antioxidant systems in the heart[73]

NADPH oxidase is a major oxidase in vascular and cardiac tissue [212] and is stimulated by G-protein coupled receptor (GCPR) agonists (ATII, ET-1, α-adrenergic agonists), cytokines (tumour necrosis factor-α) and pressure overload to generate superoxide [73, 211, 213]. NADPH oxidase has been demonstrated to have an important role in pressure overload hypertrophy [73]. NADPH oxidase can induce NOS uncoupling

(directly by superoxide or indirectly by the oxidation of BH4) and XO further amplifying ROS generation [73].

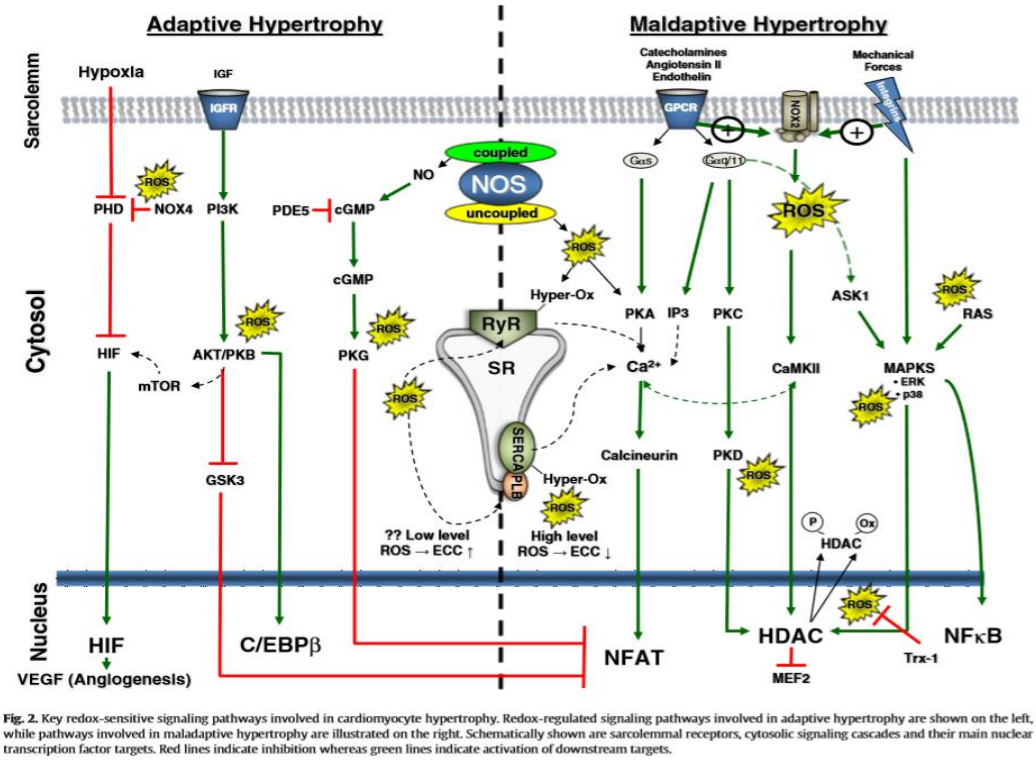


Fig. 2. Key redox-sensitive signaling pathways involved in cardiomyocyte hypertrophy. Redox-regulated signaling pathways involved in adaptive hypertrophy are shown on the left, while pathways involved in maladaptive hypertrophy are illustrated on the right. Schematically shown are sarcolemmal receptors, cytosolic signaling cascades and their main nuclear transcription factor targets. Red lines indicate inhibition whereas green lines indicate activation of downstream targets.

Figure 34 - Redox-sensitive pathways in cardiomyocyte hypertrophy [211]

Increased xanthine oxidase (XO) expression and activity has been demonstrated in a number of cardiovascular conditions including hypertension [31]. Allopurinol inhibits the generation of superoxide and hydrogen peroxide and has been shown to attenuate left ventricular remodelling animal models [73, 214, 215] and regress LVH humans [40, 53, 59].

Nitric oxide (NO) causes post-translation modification of effector molecules (usually via S-nitrosylation) at physiological levels of superoxide which is regulated and reversible. NO signalling via cyclic guanosine monophosphate (cGMP) blunts cardiomyocyte

hypertrophy and fibrosis via transcription regulation and suppression of targeted signalling [73, 211]. Furthermore NO can inhibit activation of xanthine oxidase and NADPH oxidase to maintain superoxide/NO homeostasis [73]. When superoxide is abundant (e.g. pressure overload) NOS is uncoupled, peroxynitrite is formed with the effect of further increasing nitrosative/oxidative stress and reducing NO bioavailability (Figure 35) [73, 211]. Mice lacking the NOS gene had attenuated hypertrophy, dilation, fibrosis and ROS generation compare to wild type controls in response to transverse aortic constriction [90].

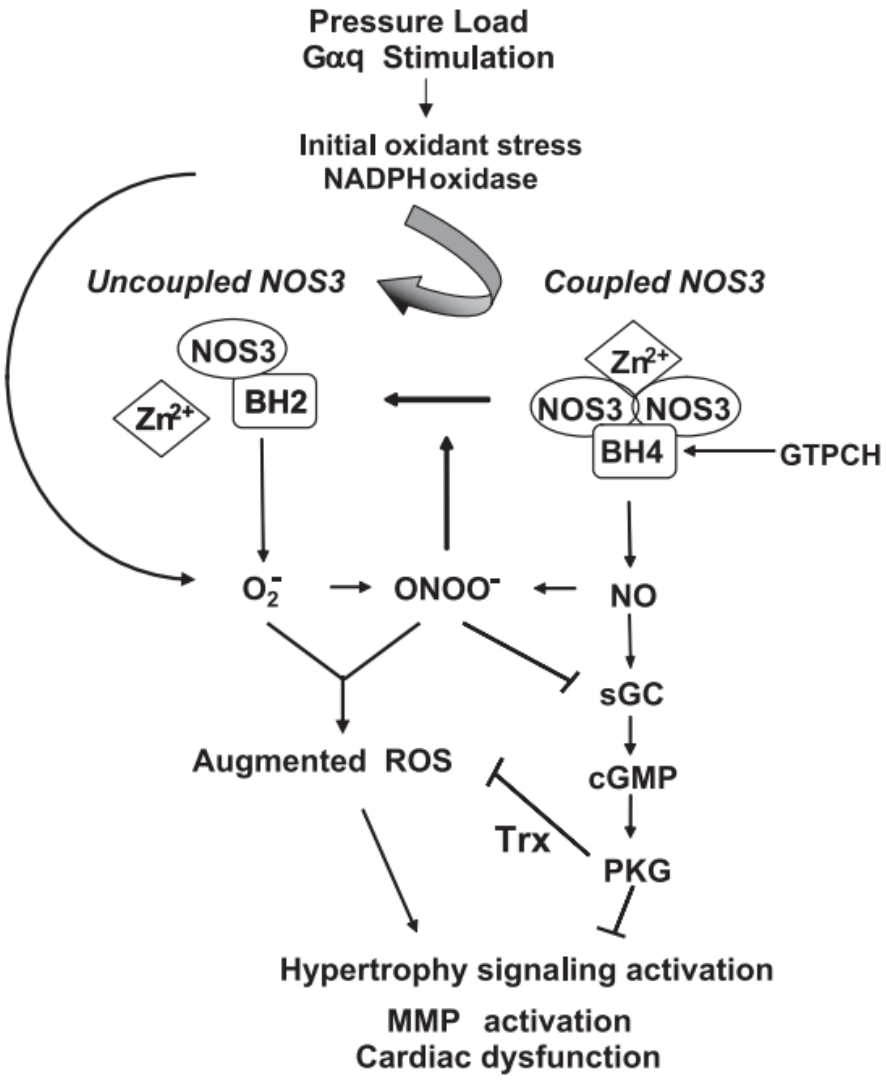


Figure 35 - Coupled/Uncoupled NOS3 involvement in cardiac remodelling [73]

Mitochondrial ROS have been implicated in MI and heart failure and LVH in mouse models [73, 211]. Monoamine oxidases (MAO) located in the mitochondria, are involved in oxidative deamination of catecholamines generating hydrogen peroxide, and has been shown to be a source of ROS in mice subjected to pressure overload [211].

ROS stimulate cardiac fibroblast proliferation and transcription factors promoting MMP expression in addition to post-translational MMP activation leading to fibrosis and matrix remodelling [73].

Impaired contractile function is a feature of disease progression to heart failure, an important component of this via redox modification of excitation/contraction coupling [211]. Modification of ryanodine receptor enhancing its open probability, suppression of L-type calcium channel current and by oxidative/nitrosative interaction with the sarcoplasmic reticular calcium ATPase, inhibiting calcium uptake [73, 211].

1.9.4 Diagnosis

1.9.4.1 ECG

Numerous criteria have been developed to diagnose LVH on ECG, the first of which was the Sokolow-Lyon index. Developed in 1949 LVH was identified if the sum of the S wave in lead V1 plus the R wave in leads V5 or V6 (whichever larger) was ≥ 35mm [216]. Since then more than thirty criteria for the diagnosis of ECG-LVH have been developed and all are limited by their poor sensitivity (Sokolow-Lyon index median specificity 89% and sensitivity of 21%)[217]. A systematic review assessing the use of

multiple ECG criteria for the diagnosis of LVH concluded that the ECG should not be used to rule out LVH in hypertension [217].

1.9.4.2 Echocardiography

The invention of echocardiography allowed direct visualisation of the myocardium and development of geometric models to calculate the LVM e.g. the ASE (Figure 36) and Penn (Figure 37) LV mass cube formulas.

$$\text{LV mass} = 0.8\ (1.04\ ([\text{LVIDD} + \text{PWTD} + \text{IVSTD}]^3 - [\text{LVIDD}]^3)) + 0.6\ \text{g}$$

Figure 36 - ASE LV Mass Cube Formula [1]

$$\text{LV mass} = 1.04\ ([\text{LVIDd} + \text{PWTd} + \text{SWTd}]^3 - [\text{LVIDD}]^3) - 13.6\ \text{g}$$

Figure 37 - Penn LV Mass Cube Formula [2]

Subsequent validation demonstrated very good correlation against direct left ventricular mass measurement post-mortem [218]. Using echo to calculate LVM has advantages in that there is a wealth of published data with demonstrated prognostic value, it is quick and widely available [1]. However, LVM mass calculations are based on the assumptions that the LV is of “normal” geometry (i.e. prolate ellipsoid with a 2:1 long/short axis ratio), beam orientation is perpendicular, hypertrophy is distributed evenly and that good image quality is possible [1]. The standard error of estimate (SEE) for echocardiography is 29 – 97g (95% CI, 57 – 190g) and inter-study reproducibility is

also poor (successive measurement SD 22 – 40g (95% CI, 45 – 78g)), therefore large numbers of patients would be required in clinical trial to assess a change in LVM to overcome this. Echocardiography has been found to overestimate LV mass when compared to CMRI, a study using both methods found the ASE echocardiographic LVM to be 319 +/-21g versus 232+/-11g by cardiac MRI[219]. This observation is consistent with findings from studies conducted at our centre [40, 41, 220]. 3D echo has been shown to be more accurate than linear or 2D techniques [221] and comparable to CMRI (Figure 38)[222] but is still dependent on image quality, which is not possible in around one quarter of patients screened in population studies [223]. An abnormal echocardiographic LVM indexed to body surface area (BSA) is currently defined as >95g/m^2 in females and >115g/m^2 in males by current guidelines [1].

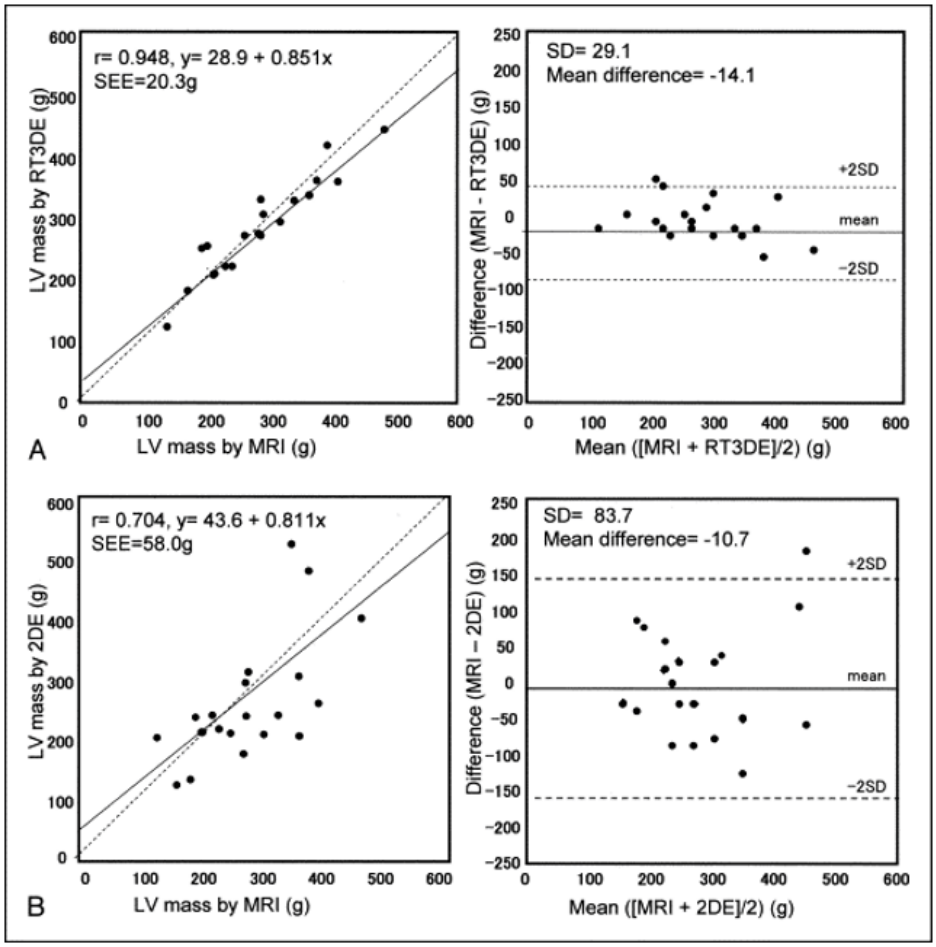


Figure 38- Comparison of 2D, real time 3D echo with CMRI [222]

1.9.4.3 Cardiac MRI

Echocardiography has now been replaced by cardiac MRI as the "gold standard" for non-invasive measurement of LVM [224]. Accurate selection of the imaging plane, good tissue characterisation and consistent image quality allow accurate measurement of LVM independent of geometric assumptions [225]. The area between the epicardium and endocardium is measured on the short axis stack then multiplied by the inter-slice distance to calculate the volume (Figure 39). The volume is multiplied by the myocardial density to calculate the ventricular mass [226].

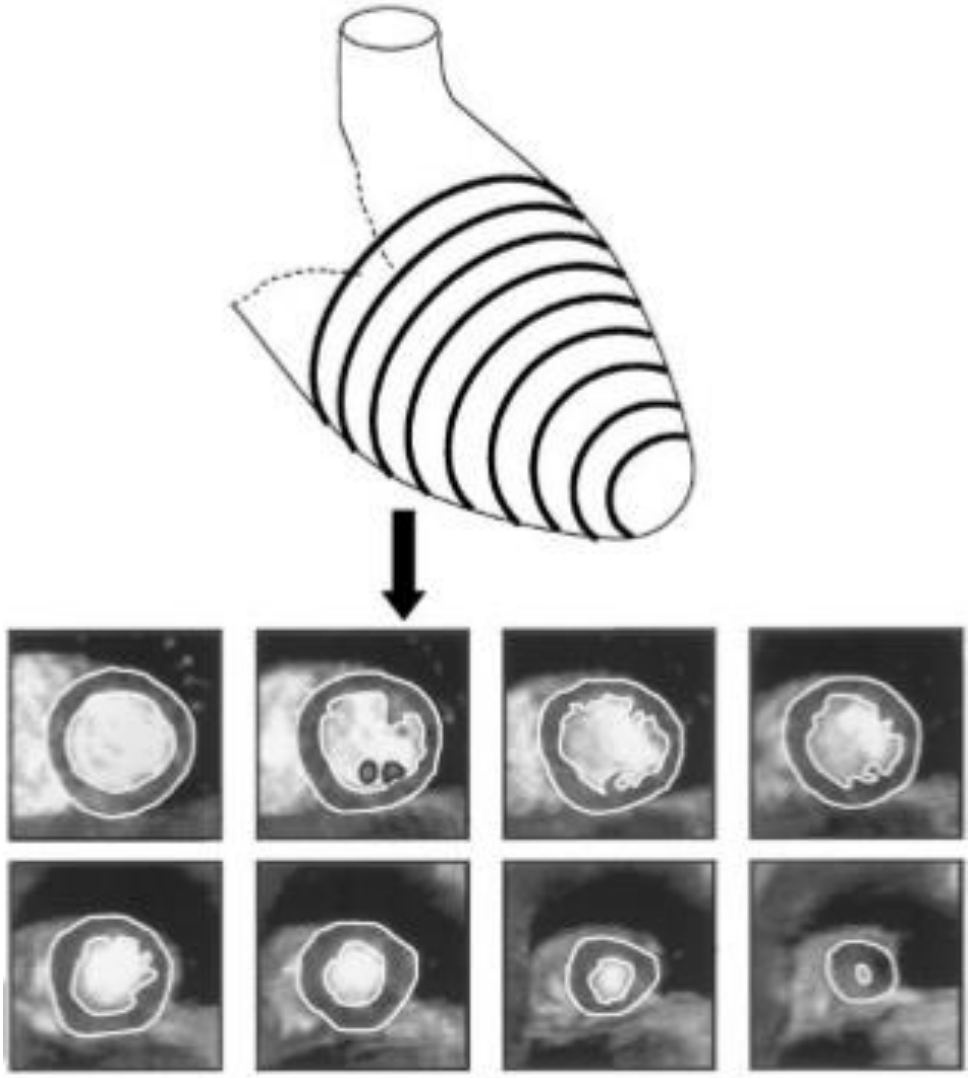


Figure 39- Illustration of LVM measurement on MRI short axis [225]

Gradient-echo sequences (GRE) have now been replaced by steady state free precession (SSFP) as the preferred technique for measurement of LVM. Both techniques have excellent interstudy and interobserver reproducibility [227]. SSFP however uses the tissue to blood T_1/T_2 ratio not through plane blood flow for blood-myocardium contrast [227] and hence has better image quality parameters and significantly shorter acquisition times [228]. Because of differences in the sequences LV volumes measured by SSFP are significantly higher and LVM lower [227, 228] than

those calculated by GRE the normal ranges previously defined are not valid for this technique, see Figure 40 for SSFP normal values.

	Men			Women		
	$mean_p$	SD_p	Lower/ upper limits*	$mean_p$	SD_p	Lower/ upper limits*
EDV [ml]	160	27	106-214	132	23	86-178
EDV /BSA [ml/m^2]	81	12	57-105	76	10	56-96
ESV [ml]	54	14	26-82	44	11	22-66
ESV/BSA [ml/m^2]**	26	6	14-38	24	5	14-34
SV [ml]	108	18	72-144	87	15	57-117
SV/BSA [ml/m^2]**	54	6	42-66	52	7	38-66
EF [%]	67	5	57-77	67	5	57-77
Mass [g]	134	21	92-176	98	21	56-140
Mass/BSA [g/m^2]	67	9	49-85	61	10	41-81

Figure 40 - Normal LV parameters (SSFP 1.5T including papillary muscle)[229]

Steady-state-free-precession (SSFP) CMR has been validated using explanted hearts at the time of cardiac transplant. Hearts imaged ex-vivo demonstrated a very high correlation to the directly measured LV mass (r=0.95, p = <0.001) [224]. Disadvantages of MRI are cost, suitability (i.e. metal implants/injuries etc.), tolerability or availability.

1.9.5 Evidence for LVH Regression

1.9.5.1 Pharmacological Interventions

A meta-analysis (2003) of eighty double-blind, parallel group RCT's included 3767 patients looking at the effect of different antihypertensive medications on echo LVM in essential HTN [230]. The reduction in LVMI decreased by a mean of 13% (95% confidence interval [CI] 8-18%) ARB, 11% (CI 9-13%) CCA, 10% ACE-I (CI 8-12%), 8% diuretics (CI 5-10%), 6% BB (CI 3-8%)[230]. The reduction in LVM among classes

remained significant even after adjustment for diastolic BP change and treatment duration. A pairwise comparison (including Bonferroni correction) found ARB's, CCB and ACE-I reduced the LVM more than BB (Figure 41).

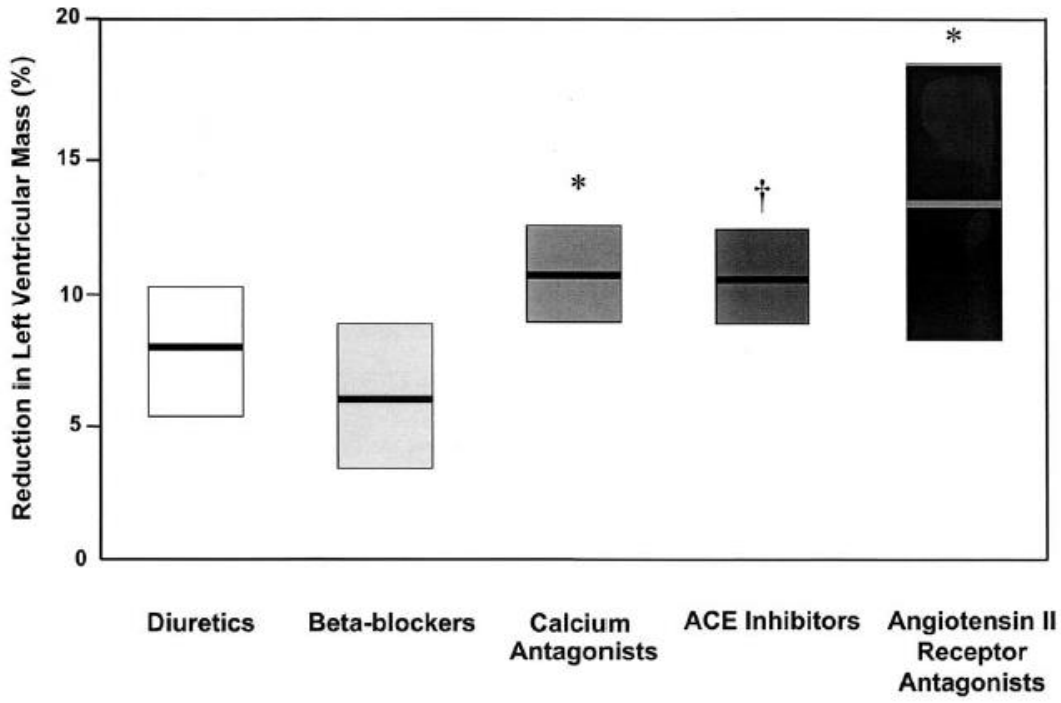


Figure 41- Meta-analysis of antihypertensive class and LV mass change [231]

A more recent meta-analysis (2009) compared pooled pairwise comparisons of the effect on LVM of five antihypertensive drug classes from 78 studies totalling 6001 subjects [232]. An interclass pairwise comparisons found that only ARB's significantly regressed LVM more than BB, 12.5% vs 9.8% (p0.01). Multivariable meta-regression analysis found BB regressed LVM significantly less than the other classes [232].

A meta-analysis of five studies with a total of 3149 patients by Pierdomenico et al (2010) assessed the impact on echocardiographic LVH regression on CV events in hypertensive patients. The risk of a CV event was 46% lower in subjects with LVH

regression/persistently normal LVM compared to those with LVH persistence/LVH development even after adjustment for confounders (HR 0.54, 95%CI 0.35-0.84, p0.007). There was a moderate degree of heterogeneity between the studies (I^2 59%), a random effects meta-analysis of the variables available indicated less benefit in participants with a higher baseline prevalence of diabetes, CV disease and Japanese ethnicity. The majority of trials are in a predominantly white population and a sub group analysis of the CASE trial by Yasuno et al. highlighted that both ethnicity and comorbidities may impact on the benefit from LVH regression[233].

1.9.5.2 The LIFE Study

The Losartan Intervention for End Point Reduction in Hypertension (LIFE) was a large (n=9222 subjects) prospective, randomised, double blind, parallel group study assessing if losartan was superior to atenolol in reducing the cardiovascular morbidity and mortality in a hypertensive population[234]. Losartan significantly reduced the risk by 13% after a mean follow-up period of 4.8 years (adjusted HR 0.87, 95% CI 0.77-0.98, p0.021). This difference was driven by a 25% reduction in stroke (adjusted HR 0.75, 95% CI 0.63-0.89, p0.001). There were no significant differences in BP at the end of the study illustrating that losartan had an effect above and beyond its effect on BP. This could be explained by a significant reduction of both Cornell Product (p<0.001) and Sokolow-Lyon Voltage (p<0.001) in the Losartan arm consistent with LVH regression. A Cox regression analysis was used to explore the relationship between the serum uric acid and the primary end-point in the LIFE study, finding that attenuation of SUA by losartan compared to atenolol accounted for a 29% reduction in the primary end-point [235].

A sub-study of the LIFE trial investigated the effect of LVM changes on the primary composite end-point in 941 subjects who had LV mass measured at enrolment then annually by transthoracic echo [236]. A reduction in LVM by 1SD (25.3g/m^2) reduced the risk of CV mortality by 34% (HR 0.66, 95% CI 0.49-0.90, p0.001) and all-cause mortality by 26% (HR 0.74, 95%CI 0.59-0.93, p0.08) adjusted for baseline LVMI, treatment, BP lowering, age, smoking, diabetes, prior CVA or MI and heart failure. The absence versus presence of LVH corresponded to a 42% lower risk of the adjusted (baseline LVH, treatment, BP lowering) primary end point. Of note the decrease in LVMI was more marked in the highest quintiles of LVMI at baseline (p=<0.01) and the prevalence of LVH fell from 70% to 23% after five years. Although there was no difference in blood pressure between the losartan and atenolol arms it does support LVM regression improving outcomes. Within a sub-population (754 subjects) of the LIFE study changes in LVM with antihypertensive treatment was investigated with serial echocardiographs at baseline, 12 and 24 months [237]. A significant change in mean LVM from 233g at baseline to 206g at (p<0.001 adjusted for change in BP). There was however a smaller but significant reduction in LV mass at 24 months (195g versus 12months p<0.001) despite no significant improvement in BP control after the first year.

1.9.5.3 The HOPE Trial

A sub-group analysis of the Heart Outcomes Prevention Evaluation (HOPE) trial assessed whether treatment with Ramipril prevented/regressed ECG-LVH in patients over 55 years old with cardiovascular risk factors and how a change impacted on prognosis [238]. Treatment with ramipril significantly reduced the

development/persistence of LVH compared to placebo (RR 0.83, 95% CI 0.72-0.95, p0.008) even after adjustment. The composite of death or the development/persistence of LVH again was 20% lower in the Ramipril arm (RR 0.80, 95% CI 0.71-0.89, p<0.001) and what was striking the effect was found to be independent of BP. A further sub-study of the HOPE trial [84] of 506 patients with normal BP at baseline (~35% with hypertension) compared two doses of Ramipril (2.5mg and 10mg) versus placebo on echocardiographic measures of left ventricular mass, volumes and function. LVMI was significantly (p=0.03) reduced in the ramipril 10mg group only (-7.21g/m^2) compared to placebo even after adjustment for age, gender, baseline LVM, LVMI and changes in systolic and diastolic BP (p<0.05) supporting the findings from the sub-group analysis above.

1.9.5.4 LVH Regression in Normotensive Subjects

LVH remains prevalent even in treated hypertension. A cross sectional study by Mancia et al found the prevalence of LVH in those with controlled hypertension was 19% versus 4% in the normotensive subjects of the PAMELA cohort [177]. The blood pressure was significantly higher in the former subjects (128/80mmHg versus 119/77mmHg, p<0.05) and is suggestive that conventional BP targets are not sufficient to completely reverse LVH. A small study by Simpson et al (2010) randomised 51 optimally treated patients with hypertension and LVH to a stepwise escalation of antihypertensive medications versus placebo. For a significant mean BP change of -9.33mmHg in the active arm versus -0.08mmHg placebo (p=0.007) there was a significant corresponding change in LVMI -4.68g/m^2 versus +1.97 g/m^2 respectively (p=0.014). Of note 26% withdrawal from the treatment arm with dizziness suggesting

tolerability could limit this strategy in practice [220]. The findings are consistent with the ACCORD BP trial (2015) that intensive BP therapy in a diabetic population had 39% lower risk of ECG-LVH (odds ratio 0.61, 95% CI 0.43-0.88, p0.008) [239]. However intensive BP lowering failed to reduce the combined rate of fatal and non-fatal CV events [240]. The SPRINT trial (2015) provided evidence for the benefit of more intensive BP lowering in a non-diabetic population with elevated CV risk. A mean systolic BP of 121.4mmHg was achieved in the intensive group versus 136.2mmHg with standard therapy. The intensive group had a significantly lower primary composite event rate 1.65% versus 2.19% (HR 0.75; 95% CI 0.64-0.89; p<0.001. Overall there was no significant difference in SAE between the interventions (p = 0.25) [241]. Although the study didn't assess changes in LV mass it is possible that favourable LV remodelling was at least in part responsible to the improvements in long term outcomes. The reality however is that even current BP targets are not achieved by around 6% of patients[104].

1.9.5.5 Non-pharmacological Interventions

Interventions of weight loss either conventionally (hypocaloric diet +/- increased exercise) or by bariatric surgery has evidence for LVH regression in patients with both normal and elevated BP[242, 243]. An important study by MacMahon et al found that a mean reduction of 8.3kg in weight loss was associated with a 14.8g/m^2 decrease in LVMI (p = 0.018) and this was independent of BP change [242]. Dietary sodium reduction also has also been demonstrated to decrease LVH by itself or in combination with other lifestyle interventions [244, 245]. Current guidelines [47, 96] recommend

lifestyle measures in the management of hypertension however lifestyle measures in the long term are poorly maintained and the prevalence of obesity is increasing [104].

2 METHODS

2.1 Approvals and Trial Registration

2.1.1 Ethical Approval

The "Does **Al**lopurinol regress **L**eft Ventricul**ar** Hypertroph**y** in Patients with Treated Essential Hypertension" (**ALLAY**) trial was approved by the East of Scotland Ethics Service (EoSRES) on the 16th June 2014, research ethics committee (REC) reference number 14/ES/0073.

2.1.2 Medicines & Healthcare products Regulatory Agency (MHRA)

As a clinical trial of investigational medical product (CTIMPs) using Allopurinol versus placebo approval was granted by the MHRA on the 24th June 2014 , EudraCT number 2014-002083-33.

2.1.3 NHS Research and Development

Approval by the local R&D was granted after ethical and MHRA approvals were in place on the 6th August 2014, reference 2012CV15.

2.1.4 Trial Registration

The study was registered with ClinicalTrials.gov, reference number NCT02237339 and with International Standard Randomised Controlled Trial Number Register (ISRCTN), number ISRCTN40476871.

2.2 Funding

The ALLAY trial was funded by a grant from the British Heart Foundation (BHF project grant no. PG/13/67/30444).

2.3 Study design

The ALLAY trial is a randomised, double blind, placebo controlled single-centre study conducted in NHS Tayside to compare allopurinol (300mg once daily for one month, increased to 300mg twice daily if tolerated for a further eleven months) versus placebo (microcrystalline cellulose).

2.4 Investigational Medical Product& Placebo

Both placebo (microcrystalline cellulose) and allopurinol had an identical appearance and were manufactured by Tayside Pharmaceuticals, Ninewells Hospital, Dundee. Medications were stored in the Clinical Trial Pharmacy (Ninewells Hospital) and were dispensed on completion of the relevant forms before a study visit. An initial dose of allopurinol 300mg once daily was taken, increasing if tolerated to 300mg twice daily thereon. According the Electronic Medicines Compendium rash is the most common (≥1% and <10%) side effect followed by hypersensitivity, nausea, vomiting and abnormal LFT's uncommonly (≥0.1% and <1%). Serious side effects such as Steven-Johnson syndrome, toxic epidermal necrolysis or hepatitis occur rarely (≥0.01% and <0.1%). Allopurinol must be used with care in patients with liver or renal dysfunction as this increases the risk of side effects.

2.5 Study Aims

We hypothesised that the inhibition of xanthine oxidase using allopurinol would regress LVM by reducing oxidative stress in patients with optimally treated, well controlled essential hypertension.

2.5.1 Primary endpoint

To determine if allopurinol induces a change in Left Ventricular Mass Index in patients with treated hypertension when compared to placebo, measured by cardiac MRI.

2.5.2 Secondary endpoints

To assess the effect of Allopurinol on;

- Endothelial function as measured by FMD and vascular stiffness measured by PWA and PWV
- Blood Pressure
- Urate, High sensitivity C-Reactive Protein (HsCRP), Thiobarbituric acid reactive substances (TBARs), N-terminal prohormone B-Type Natriuretic Peptide (NT-proBNP), Procollagen type I carboxy-terminal Propeptide (PICP) and soluble ST2 (sST2).
- Changes to absolute LV mass, LV end-systolic volume, end-diastolic volume and ejection factor.
- Cardiac muscle regression independent of scar tissue using gadolinium enhancement.
- Change in LA volumes

2.6 Inclusions Criteria

Participants were eligible if they fulfilled the criteria below;

- are aged over 18 years
- previously diagnosed with essential hypertension
- been on stable antihypertensive therapy for at least 3 months prior to study screening
- have screening ABPM (or home-based BP monitoring if ABPM not tolerated) with daytime average systolic $\underline{<}$135mmHg or 24-hour average systolic ≤ 130mmHg
- have screening echocardiography-based diagnosis of LVH based on ASE criteria (males >115g/m^2, females >95g/m^2)

2.7 Exclusion Criteria

Subjects were excluded if they had any of the following;

- documented intolerance to allopurinol
- left ventricular ejection fraction <45% on echocardiography screening
- severe aortic stenosis on echocardiography screening
- active gout (i.e. gout flare <2yrs) or currently on allopurinol
- severe hepatic disease
- renal disease; CKD class 3B or worse
- on azathioprine, 6 mercaptopurine, or theophylline
- malignancy (receiving active treatment) or other life-threatening diseases
- pregnant or lactating women

- any contraindication to MRI (claustrophobia, metal implants, penetrative eye injury or exposure to metal fragments in eye requiring medical attention)
- patients who have participated in any other clinical trial of an investigational medicinal product within the previous 30 days will be excluded
- patients who are unable to give informed consent
- any other considered by a study physician to be inappropriate for inclusion

2.8 Randomisation

After confirmation of eligibility participants were randomised by the research fellow (CG) to allopurinol or placebo in a double-blind fashion. Randomisation used a web-based good clinical practice (GCP) compliant randomisation system, run by the United Kingdom Clinical Research Network (UKCRN) registered Tayside Clinical Trials Unit (TCTU). Randomisation was minimised for sex and left ventricular mass index (LVMI) i.e. males LVMI >115g/m^2 to <130g/m^2 or ≥130g/m^2, females >95g/m^2 to <115g/m^2 or ≥115g/m^2. Double blind medication (allopurinol/placebo) was prepared, packaged and labelled by Tayside Pharmaceuticals. Medication will come labelled as “Pack No. 001”, “Pack No. 002”, etc. Blinding was maintained until all analysis, data entry/validation was completed, and the data was locked.

2.9 Recruitment

Study subjects were recruited from the following sources;

- NHS Tayside Cardiovascular Risk or Cardiology Clinics

- Previous participants of clinical trials in Cardiology at NHS Tayside and/or in the University of Dundee Department of Clinical Pharmacology
- Local clinical echocardiography databases in NHS Tayside
- Scottish Primary Care Research Network (SPCRN)
- Scottish Health Research Register (SHARE)

2.9.1 Informed consent

All subjects were sent the full participant information sheet a minimum of twenty-four hours before attending for the screening visit. When consent was taken first the trial was explained, risks and benefits discussed, the consent form read and explained follow by an opportunity to ask questions and have them answered before signing (appendix chapter 9.4). Tests of endothelial function (PWA/PWV/FMD), research and genetic bloods could be opted out of.

2.10 Study Visits

Participants had a total seven study visits over a period of twelve to thirteen months described in detail below. Participants continued their usual medications. For a summary table of the visit schedule see appendix (chapter 9.3).

2.10.1 Screening Visit (Visit 1)

- Participant consent
- Height, weight measured
- Screening echocardiogram

- Inclusion/exclusion criteria
- Medical, drug, social and family history were recorded followed by physical examination
- Vital signs (i.e. office BP, pulse)
- Safety/baseline bloods
- 24-hour ambulatory BP or home BP monitoring

2.10.2 Randomisation (Visit 2)

- Adverse event (AE) log completed
- Concurrent medication log updated
- Vital signs
- Electrocardiograph (ECG)
- Pulse wave velocity (PWV)/Pulse wave analysis (PWA)
- Flow medicated dilatation (FMD)
- Cardiac Magnetic Resonance Imaging (CMRI)
- Research, genetic bloods and blinded uric acid
- Randomisation
- Study medications supplied and first dose (300mg allopurinol/placebo) taken

2.10.3 Progress Visits (Visits 3, 4, 5 and 6)

- Adverse event log completed
- Concurrent medication log updated
- Vital signs
- Medication compliance Log

- Safety bloods and uric acid
- Urine pregnancy test (if applicable)
- Study medication supplied

2.10.4 Final Visit (Visit 7)

- Adverse event log completed
- Concurrent medication log updated
- Vital signs
- Medication compliance Log
- ECG
- PWV/PWA
- FMD
- CMRI
- Safety, research, genetic bloods and blinded uric acid
- 24-hour ambulatory BP or home BP monitoring
- Completion of study form

2.11 Blood Pressure Monitoring

Sitting upright after resting for a minimum of five minutes the office BP was recorded using a calibrated Omron 705IT (Omron Healthcare co LTD, Kyoto, Japan). Ambulatory BP monitoring was taken at visit 2 and 7 using a calibrated Spacelabs 90217A (Spacelabs Healthcare, Hertford, United Kingdom) or home BP using the Omron 705IT. The same arm and cuff size appropriate for the patient was used.

2.12 Applanation Tonometry

Augmentation index (AIx) and pulse wave velocity (PWV) was performed at visits 1 and 7 by a blinded single trained operator (CG) using a validated SphygmoCor system (AtCor, Sydney, Australia) and a high fidelity micromanometer. The participant was rested for at least five minutes in a supine position before measurements were taken. For AIx the micromanometer was applied to the distal radial artery to obtain a peripheral pressure waveform. Recordings were made until consistent measurements (minimum of two) were achieved with a minimum operator index (>70). The recording with the highest operator index and most consistent waveform was selected. Augmentation index is calculated by the increment in pressure from the systolic shoulder as a percentage of the peak pulse pressure (Figure 42) [246] and was generated automatically by the software (Figure 43).

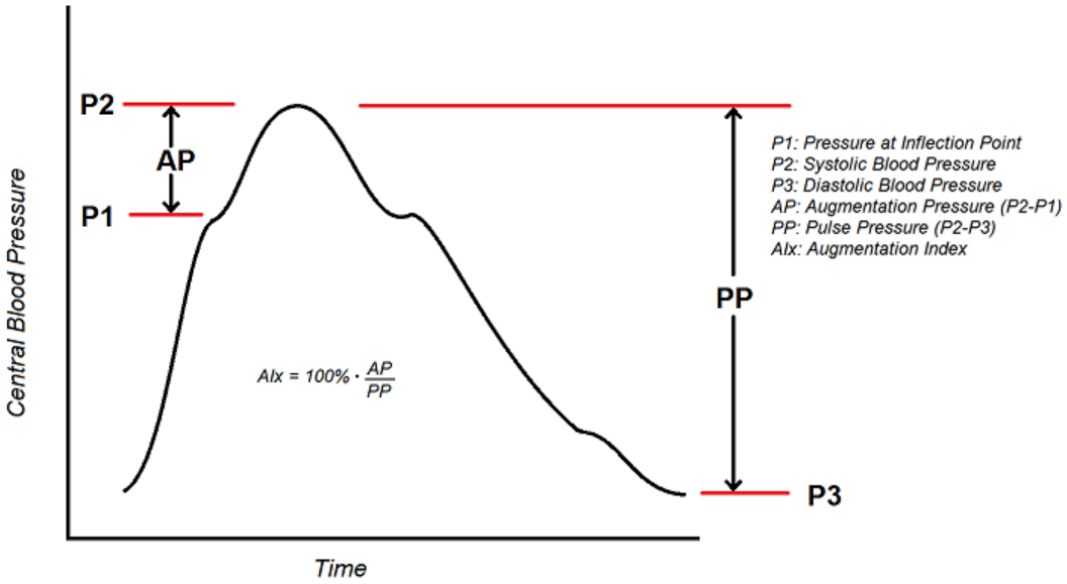


Figure 42 - Central BP waveform and calculation of Augmentation Index [247]

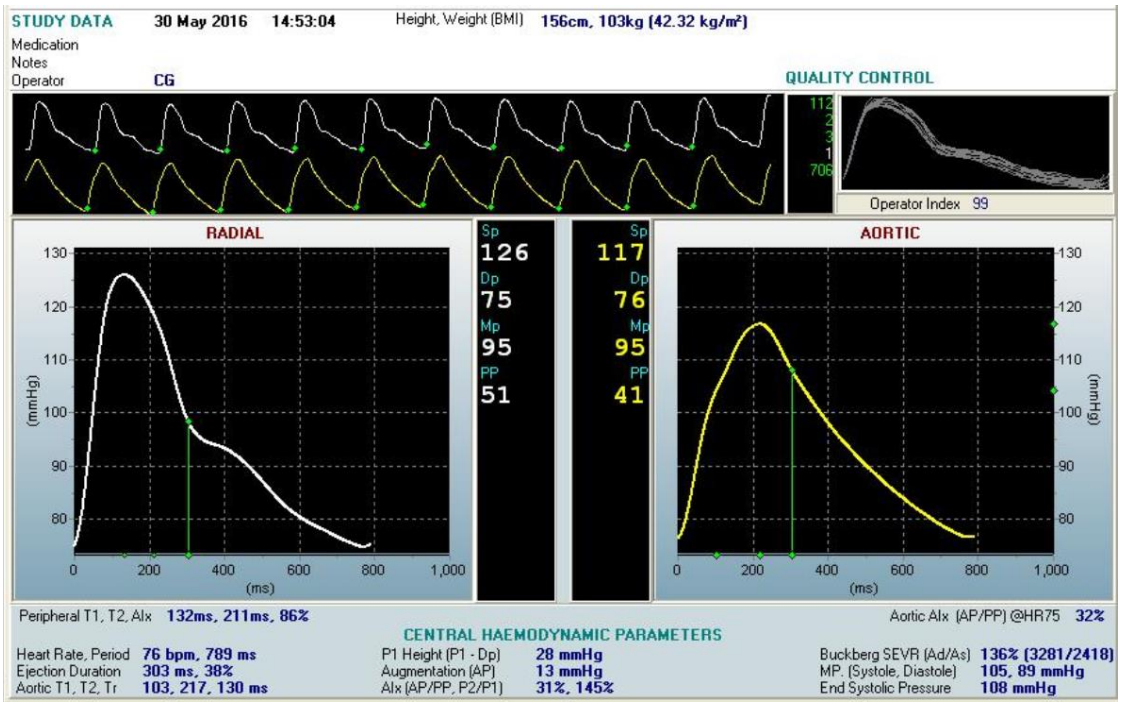


Figure 43 - Pulse Wave Analysis (Own Image)

To calculate PWV, radial-carotid waveforms were obtained with simultaneous ECG gating. Velocity was calculated by the software using the manually measured distance (sternal notch to selected point of carotid pulsation and sternal notch to distal radial pulsation) and time interval from the ECG R-wave and the start of the pressure waveform. Recordings were made (minimum of two) until a consistent PWV result was obtained of sufficient quality. The PWV with the lowest SD and best waveform was selected (Figure 45). Carotid-femoral PWV is the “gold” standard for measuring large artery stiffness [248], however we used carotid-radial due to time constraints.

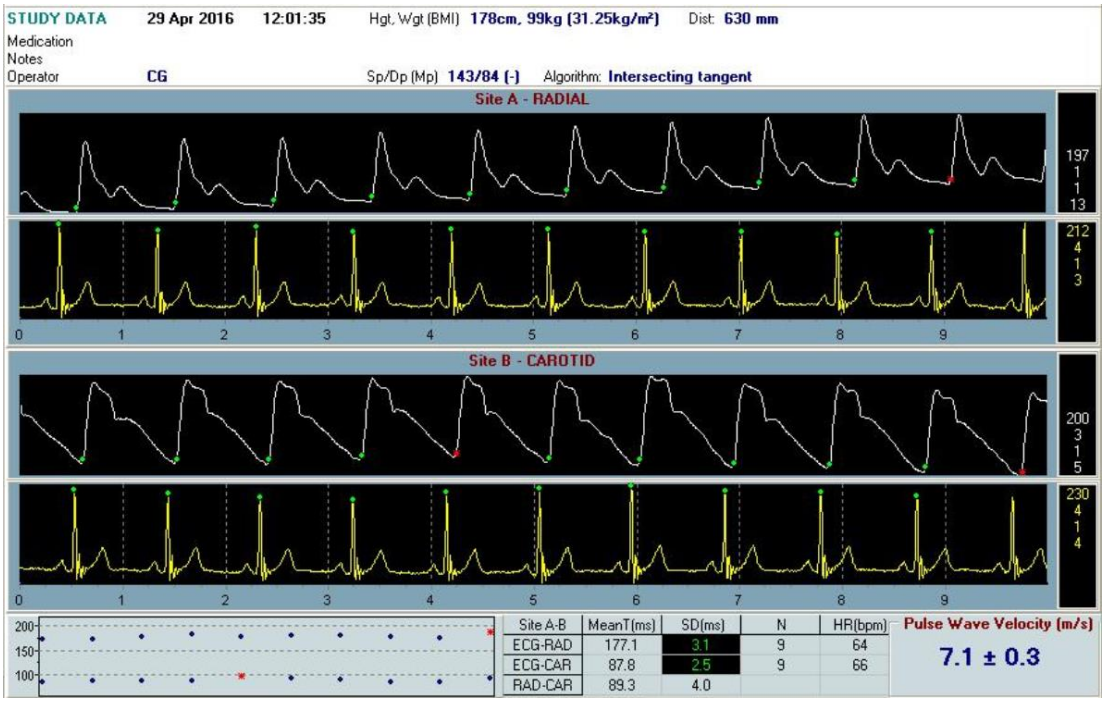


Figure 44 - Pulse Wave Velocity (own image)

2.13 Flow Mediated Dilatation (FMD)

FMD was conducted as per the international brachial artery reactivity task force guidelines [249] at visits 1 and 7 to measure endothelial dependent (hyperaemia) and independent vasodilation (GTN). Brachial FMD was conducted by a blinded single trained operator (CG) using an Acuson Sequoia 512 (Siemens, Camberley, UK) with an 8MHz linear array probe and simultaneous ECG gating.

With the subject rested supine for a minimum of five minutes the brachial artery was imaged in the longitudinal plane above the elbow with the probe mechanically fixed in place when an adequate view of the vessel intima and lumen was acquired, altering 2d gain as necessary (Figure 45).

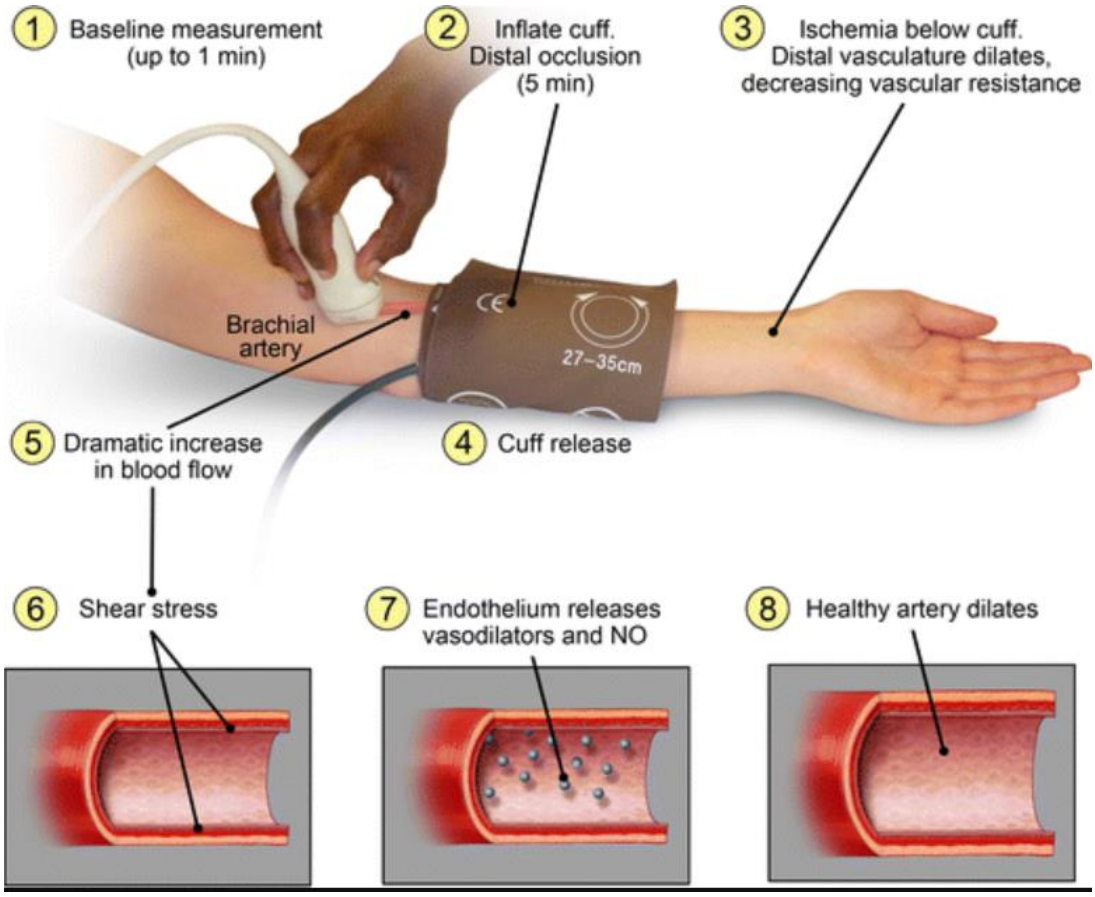


Figure 45 - Flow Mediated Dilation Procedure [250]

A resting 2d recording of the artery was acquired for one minute. Ischaemia was induced with a blood pressure (BP) cuff inflated around the forearm at 50mmHg above systolic pressure or 200mmHg which ever was highest. After five minutes the cuff was rapidly deflated followed by a 2d recording for two minutes. The participant rested the arm for ten minutes and a repeat baseline image was recorded for one minute. Endothelium independent dilation was assessed by giving 400mcg glyceryl trinitrate sub-lingually and the final recording was started after two minutes recording for a total of five minutes.

The FMD was analyzed by a blinded single trained operator (CG) using the Vascular Research Tools software (Medical Imaging Applications LLC, Coralville, IA, USA) to track the diameter of the artery (Figure 46).

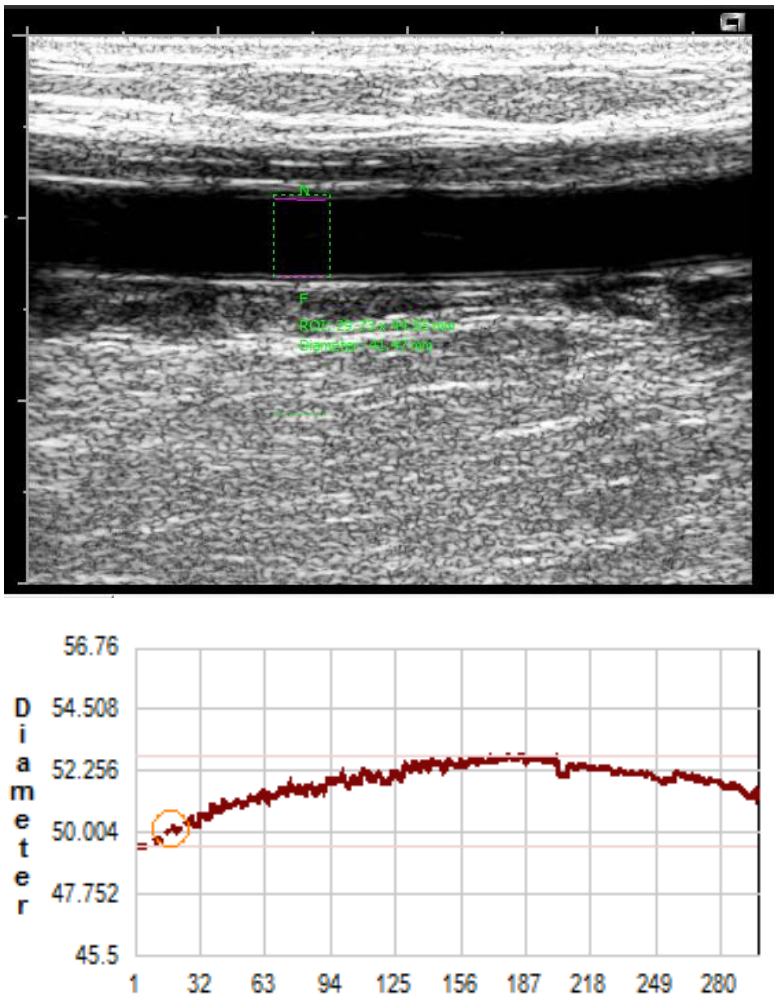


Figure 46- FMD Analysis (own image)

2.14 Echocardiography

Using the joint American Society of Echocardiography and European Association of Cardiovascular Imaging guidelines [1]. Perpendicular 2D guided measurements of the intra-ventricular septum, internal diameter and posterior wall of the LV were acquired

in the parasternal long axis, at the level of the mitral valve leaflets tips at end-diastole by a single operator (CG). LV mass was calculated using the ASE mass cube formula[1]. A Phillips IE33 (Phillips Healthcare, Amsterdam, Netherlands) was used until it was replaced on the 10th of November 2015 with a Phillips EPIQ (Phillips Healthcare, Amsterdam, Netherlands).

2.15 Cardiac MRI

Cardiac MRI was performed on a 3T MAGENTOM Trio-Prisma^{FIT} (Siemens, Erlangen, Germany) using dedicated phased array cardiac coils. Serial contiguous short-axis cine images (electrocardiogram gated, [true fast imaging with steady-state precession (TrueFISP)] were acquired from the AV ring to the apex using the vertical and horizontal long axis of the left ventricle as a guide. The short axis imaging parameters were repetition time (TR) of 2.5ms, echo time (TE) of 1.1ms, flip angle (FA) of 60°, and slice thickness 6mm and 4mm gap.

Late gadolinium enhancement images were acquired approximately 10-15 minutes after the initial bolus injection of contrast agent (Dotarem^{TM}, Guerbet, France). At 10 minutes post-injection, a 2D segmented CINE TrueFISP 'TI scout' sequence (with prospective gating) was applied at a single slice location in the mid-short axis plane in order to identify the correct inversion time (TI) to null the signal from healthy myocardium. The TI scout sequence works by acquiring a selection of images with different TI times, and the scanner operator can then select the TI time that corresponds to the image where the healthy myocardium signal is the lowest. For the TI scout sequence the imaging parameters were TR/TE = 3.13/1.39 ms, flip angle 35°, 8mm slice thickness, field of view 340-380mm and bandwidth 965 Hz/pixel.

After the correct TI had been established, the LGE images were acquired using a 2D ECG prospective gated single-shot segmented phase sensitive inversion recovery (PSIR) TrueFISP sequence. Typically, 10 slices (each 8mm thick) were acquired in the short axis plane (from base to apex) over a field of view of 340-380 mm with in-plane matrix of 192x192 pixels. The TR/TE was 2.55/1.10 ms, with flip angle 40^{o}, parallel imaging (iPAT) factor 2, and bandwidth 1532 Hz/pixel.

Analysis was performed offline by a single trained observer (CG) blinded to the study allocation using Argus software (Version VB15, Siemens Erlangen, Germany). Using the short axis stack 'region-of-interest' contours were placed around the left ventricular endocardial and epicardial borders at end diastole and at end systole to calculate left ventricle ejection fraction (LVEF), left ventricular mass (LVM), end-diastolic (LVEDV), end-systolic (LVESV) and stroke volumes (LVSV). The base and apex were labeled and frames with ≥50% full thickness myocardium were included in the LVM. Papillary muscles were also included in the LVM if the muscle was contiguous with the myocardial wall. Each scan was analyzed twice to ensure consistency, a third measurement was conducted if the LVM varied by >5%.

'Region of interest' contours were placed around the blood pool in left atria (LA) during diastole and systole excluding the LA appendage and pulmonary veins where possible. From these measurements the LA end diastolic volume (LAEDV), end systolic volume (LAESV), stroke volume (LASV) and ejection fraction (LAEF) could be calculated.

Images were screened for LGE using Argus software, any positive or suspected of having LGE were reviewed for inclusion/exclusion by a radiologist (GH) and then analyzed using Circle (Circle Cardiovascular Imaging inc, Calgary, Alberta, Canada).'Region of interest' contours were placed on each of the slices from the short

axis stack to exclude high signal outside the myocardium, areas of enhancement not caused by scar were excluded manually, the software automatically calculated the volume of LGE. LGE positive was defined as cases with a signal intensity above the average of the normal myocardium plus 3 standard deviations.

2.16 Studies of Agreement

A single observer who was blinded to treatment allocation (CG) analysed the CMRI. Intra-observer measurement of LVM had an interclass correlation coefficient of 0.999 (95% CI 0.998 – 0.999; p = <0.0001). Figure 47 shows a Bland Altman plot of LVM measurements.

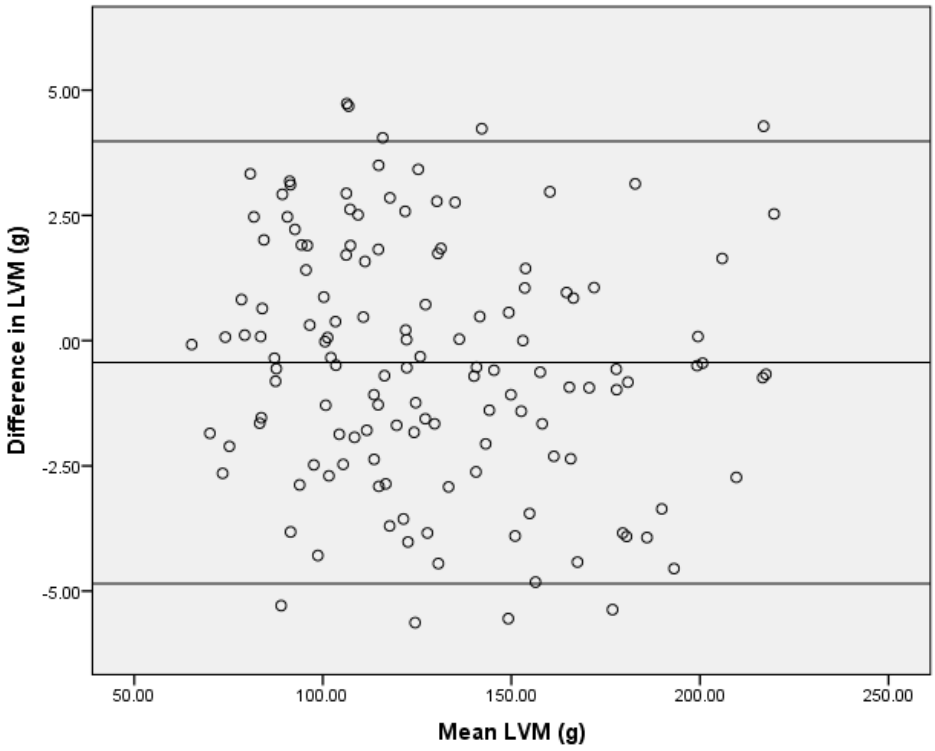


Figure 47 – Bland Altman plot of CMRI LVM Measurement

Middle line mean difference, upper and lower lines mean ± 2SD

Intra-observer measurement of FMD interclass correlation coefficient was 0.813 (95% 0.435 – 0.938; p = 0.002). Intra-observer measurement of AIx interclass correlation coefficient was 0.995 (95% 0.989 – 0.998; p <0.001). Intra-observer measurement of PWV interclass correlation coefficient was 0.860 (0.691 – 0.937; p <0.001).

2.17 Laboratory tests

2.17.1 Biochemistry &Haematology

Urea and electrolytes, liver function tests, lipids, glucose, HBA1c (subjects with diabetes), full blood count and urate were taken with vacutainer tubes and taken immediately for processing in the NHS Tayside laboratory.

2.17.2 Biomarkers

Approximately 10mls of blood was collected in two gold top bottles (SST II advance) and allowed to clot at room temperature for 15 minutes, then spun at 3000rpm at 4^{o}c for ten minutes. Serum was removed and placed in x4 1ml labelled plastic tubes for storage at -20^{o}c and -70^{o}c. A further 4ml sample was taken in a purple top (EDTA) bottle and stored in a -20^{o}c freezer.

2.17.2.1 Uric Acid

Samples were collected in a gold top (SST II advance) tube and processed in the NHS Tayside biochemistry lab throughout the trial using Siemens ADVIA chemistry systems

using the Fossati enzymatic reaction method. The results were not available until the end of the study to preserve the integrity of the blind.

2.17.2.2 Thiobarbituric acid reactive substances

Lipid peroxidation results in a number of intermediate or end products that includes malondialdehyde (MDA) [251]. Thiobarbituric acid reacts with oxidised lipids including the major lipid oxidation product MDA to form thiobarbituric acid reactive substances that are measured by the TBAR assay and hence quantify the degree of lipid peroxidation [251]. There is strong positive correlation between plasma MDA and TBARS concentration ($r = 0.709$) [251]. Serum levels of TBARs have been shown to be strongly, and independently predictive of cardiovascular events in healthy patients and those with stable coronary disease [252, 253]. Other biomarkers for oxidative stress in cardiovascular disease such as F_2-isoprostanes are considered the more reliable in-vivo marker of OS, however analysis is labour-intensive, expensive and therefore not practical for this study [254]. Other promising markers of OS are nuclear factor erythroid 2-related factor 2 (Nrf2) that regulate expression of anti-oxidant defences and show promise in cardiovascular disease [255].

2.18 Data entry and Management

Data was collected on a case report form (CRF) (appendix 9.5) and transcribed to a GCP compliant excel database according to TASC SOP48 and stored on a password protected device. Data validation used single entry (CG) with a second individual

reading aloud whilst the data recorded is checked (CG/RS). All data analysis and entry took place before the database was locked (27th September 2017) according to TASC SOP DOM32 at which time unblinding took place.

2.19 Cohort size & power calculation

For the primary end-point, the study was powered for an absolute change in the in left ventricular mass on cardiac MRI based on previous studies conducted in our department. In those studies (LVH regression using allopurinol in patients with ischemic heart disease) they found that allopurinol significantly reduced LV mass by -5.2± 5.8 grams compared to placebo -1.3±4.5 grams ($p<0.007$) [59].

For an 80% power at a 5% significance level ($p=0.05$), to detect a similar change in LV mass, will require 29 subjects per group. Both our previous studies have shown a 10% drop-out rate. Therefore, accounting for this, a total of 64 patients (32 per group) were required.

The minimum statistically significant improvement that can be detected with intervention is 2% [249]. Based on previous studies we would need 27 participants per arm to detect a 2% change with 80% power at the $p<0.05$ level, assuming a SD of 2.6. Allowing for 20% dropout we plan to randomise a total of 66 patients.

2.20 Statistical analysis

Descriptive statistics are expressed in the form of means and standard deviations for normally distributed continuous variables, non-normally distributed data are presented as median and inter-quartile ranges. Percentages and denominators were

used for categorical variables. Statistical analysis was undertaken using SPSS version 22.0 (SPSS, Chicago, IL). A p value of <0.05 was considered statistically significant.

For the primary analysis a comparison between arms of the trial was assessed by the regression coefficient for treatment arm with final visit LV Mass Index as dependent variable and gender, baseline LV mass Index and blood pressure as covariates in a general linear regression model. The dependent variable will be assessed for approximation to a normal distribution and transformed if necessary. A secondary analysis of the primary end-point used a mixed model to account for missing data was conducted.

Comparison of baseline characteristics, all secondary end-points and sub-group analyses used a student t-test (normally distributed continuous variables) or the Mann-Whitney U test (non-normally distributed continuous variables). Categorical variables were analysed using χ^2 test.

2.21 Adverse events

Adverse events reporting was carried out in accordance with TASC SOP 11 (identifying, recording and reporting adverse events for clinical trials of investigational medical products). All reported AE's will be recorded on the AE page of the CRF. The appropriate investigation, treatment, referral of follow-up will be determined by the investigator. Serious adverse events (SAE), serious adverse reactions (SAR) and suspected unexpected serious adverse reaction (SUSAR) were reported to TASC pharmacovigilance section within twenty-four hours. AEs were coded according to the

medical dictionary for regulatory activities (MedDRA) version 20.0 by preferred term (PT) and system organ class (SOC).

A serious adverse event (SAE), serious adverse reaction (SAR) or suspected unexpected serious adverse reaction (SUSAR) is defined as one that:

- results in death
- is life threatening
- requires hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- Or is otherwise considered serious

3 RESULTS

3.1 Screened Subjects

Of a total of 272 participants who were screened, 200 were excluded for the following reasons:

- No LVH on echocardiography (n=123)
- Uncontrolled hypertension (n=53)
- Insufficient echo quality (n=7)
- Contraindications to MRI (n=7)
    - Previous metal penetrating eye injury (n=4)
    - Claustrophobia (n=3)
- Other (n=10)
    - Gout (n=3)
    - Not hypertensive (n=1)
    - Change in BP medications <3 months (n=1)
    - Severe aortic stenosis (n=1)
    - Taking theophylline (n=1)
    - Active cancer treatment (n=1)
    - Atrial fibrillation (n=1)
    - Decided not to take part (n=1)

3.2 Randomized Subjects

72 participants were randomized, the original target was exceeded as time and the protocol allowed the replacement of subjects who withdrew from the study. 10

participants withdrew from the study, 6 from the placebo group, 4 from the allopurinol group. The breakdown of recruitment is demonstrated in the consort diagram (Figure 48). Adverse events are discussed further in chapter 3.12, including those in the patients who withdrew.

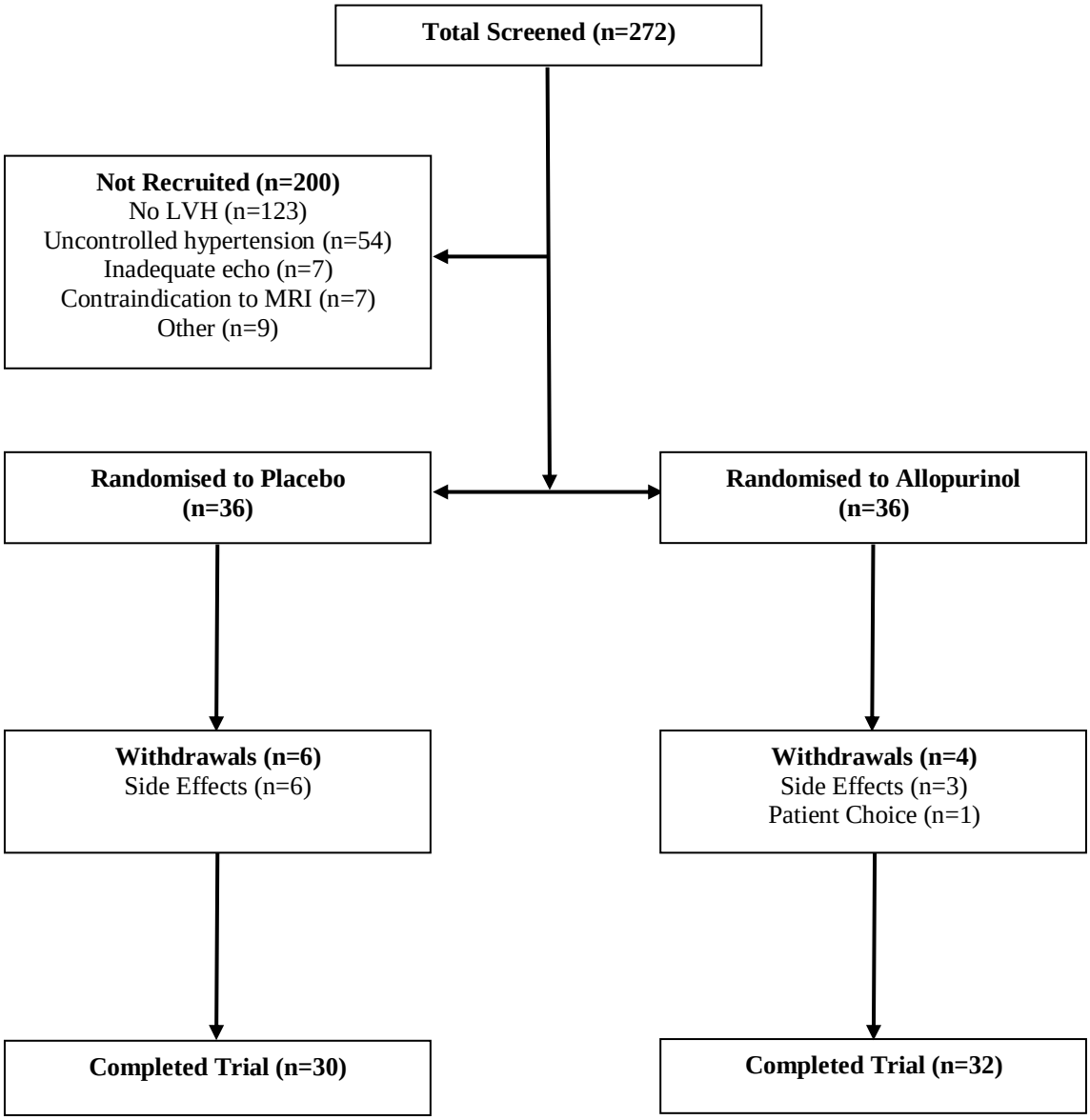


Figure 48 - CONSORT diagram of Study Recruitment

3.3 Baseline characteristics

The baseline characteristics of the recruited patient are demonstrated in *(Table 2)*

There was no significant difference between the groups at baseline, importantly there were no statistically significant differences in gender, BMI, blood pressure, number of antihypertensive medications, urate and LV mass. The average duration since diagnosis of hypertension was 12 years prior to entry into the trial. The majority of patients had 24hour ambulatory BP monitoring, although 19% had home BP monitoring there were no significant differences between the groups (see Table 4 for a detailed breakdown of baseline BP).

Table 2 - Baseline Characteristics

Variable	All Patients	Placebo	Allopurinol	p value
Total Patients	n =72	n = 36	n = 36	
Mean Age (years)	66.2 ± 9.9	65.6 ± 10.4	66.8 ± 9.4	0.611
Male	40 (55.6%)	20 (55.6%)	20 (55.6%)	1.000
BMI	30.6 ± 5.2	30.9 ± 5.1	30.4 ± 5.3	0.686
Daytime SBP AMBP or home monitoring (mmHg)	124.9 ± 8.1	125.6 ± 7.4	124.3 ±8.8	0.557
Daytime DBP AMBP or home monitoring (mmHg)	73.7 ± 8.5	74.5 ± 7.2	72.9 ± 9.6	0.439
Duration of HTN (years)	12.3 ± 8.4	12.4 ± 9.5	12.1 ± 7.3	0.868

IHD	2 (2.8%)	0 (0%)	2 (5.6%)	0.493
Dyslipidaemia	29 (40.3%)	14 (38.9%)	15 (41.7%)	0.810
CVA/TIA	8 (11.1%)	4 (11.1%)	4 (11.1%)	1.000
DM	4 (5.6%)	3 (8.3%)	1 (2.8%)	0.614
PAD	1 (1.4%)	1 (2.8%)	0 (0%)	1.000
Smoker	4 (5.6%)	3 (8.3%)	1 (2.8%)	0.567
Ex-smoker	30 (41.7%)	14 (38.9%)	16 (44.4%)	
Never Smoked	38 (52.8)	19 (52.8%)	19 (52.8%)	
ACE-I	32 (44.4%)	18 (50.0%)	14 (38.9%)	0.343
B blocker	21 (29.2%)	8 (22.2%)	13 (36.1%)	0.195
CCB	48 (66.7%)	26 (72.2%)	22 (61.1%)	0.317
α blocker	16 (22.2%)	7 (19.4%)	9 (25.0%)	0.571
Thiazide diuretic	27 (37.5%)	16 (44.4%)	11 (30.6%)	0.224
Loop diuretic	6 (8.3%)	4 (11.1%)	2 (5.6%)	0.674
MRA	5 (6.9%)	3 (8.3%)	2 (5.6%)	1.000
ARB	29 (40.3)	12 (33.3%)	17 (47.2%)	0.230
Centrally acting anti-hypertensive	2 (2.8%)	1 (2.8%)	1 (2.8%)	1.000
Renin Blocker	1 (1.4%)	1 (2.8%)	0 (%)	1.000
Number of Antihypertensive Medications	2.6 ± 1.2	2.7 ± 1.1	2.5 ±1.3	0.622
Resistant Hypertension	15 (20.8)	7 (19.4)	8 (22.2)	0.772
Haemoglobin (g/L)	139.3 ± 13.0	138.9 ± 12.2	139.75 ± 14.0	0.788
Creatinine (mmol/L)	70.6 ± 13.7	73.7 ± 10.8	67.5 ± 15.7	0.058
Glucose (mmol/L)	5.58 ± 0.90	5.39 ± 0.81	5.77 ± 0.95	0.072

Urate (µmol/L)	360.8 ± 97.9	374.31 ± 85.63	347.28 ± 108.33	0.244
HS-CRP (mg/L)	2.24 ± 3.13	2.34 ± 3.40	2.14 ± 2.88	0.795
TBARs (uM)	2.91 ± 0.95	3.01 ± 1.01	2.81 ± 0.88	0.396
NTproBNP (ρg/mL)	775.97 ± 889.73	657.59 ± 696.49	897.73 ± 1048.96	0.258
PICP (ng/L)	1.62 ± 0.85	1.74 ± 0.99	1.50 ± 0.70	0.269
Soluble ST2 (ng/mL)	19.66 ± 9.42	19.56 ± 7.76	19.76 ± 10.98	0.932
Echo LVM (g)	244.9 ± 57.7	245.0 ± 59.0	244.7 ± 57.2	0.979
Echo LVMI (g/m^2)	124.0 ± 18.3	124.7 ± 20.4	123.3 ± 16.3	0.751
MRI LVM (g)	128.21 ± 37.39	130.56 ±36.26	125.86 ± 38.86	0.597
MRI LVM Height$^{1.7}$($g/m^{1.7}$)	52.5 ± 11.9	53.9 ± 11.9	51.0 ± 11.8	0.288
MRI LVMI (g/m^2)	64.78 ± 14.17	66.43 ± 14.71	63.13 ± 13.61	0.327
MRI LVEDV (mL)	141.56 ± 33.62	142.16 ± 35.82	140.97 ± 31.77	0.882
MRI LVESV (mL)	36.82 ± 15.49	36.94 ± 17.60	36.71 ± 13.29	0.951
MRI LVSV (mL)	104.74 ± 21.80	105.22 ± 22.08	104.26 ± 21.82	0.853
MRI LV Ejection Fraction (%)	74.7 ± 6.3	75.0 ± 7.0	74.5 ± 5.6	0.732
MRI LAEDV (ml)	89.6 ± 23.8	88.6 ± 25.3	90.7 ± 22.4	0.719
MRI LAESV (ml)	42.0 ± 15.8	40.7 ± 15.2	43.4 ± 16.5	0.493
MRI LA Ejection Fraction (%)	54.0 ± 7.3	54.7 ± 6.1	53.2 ± 8.4	0.415
FMD (%) Endothelial Dependent	5.6 ± 4.0	5.4 ± 4.4	5.9 ± 3.7	0.594
FMD (%) Endothelial Independent	15.5 ± 6.0	15.1 ± 5.2	15.9 ± 6.9	0.593
AIx (%)	22.6 ± 14.0	20.4 ± 13.7	24.8 ± 14.2	0.183
PWV (m/s)	8.33 ± 1.17	8.18 ± 1.05	8.49 ± 1.28	0.272

Table 3 - Baseline MRI LVM/LVMI by gender

Variable	All Patients	Placebo	Allopurinol	P value
Male MRI LVM (g)	152.1 ± 31.6	152.7 ± 31.9	151.5 ± 32.2	0.905
Female MRI LVM (g)	98.3 ± 17.0	102.9 ± 17.6	93.8 ± 15.5	0.132
Male MRI LVMI ($g/m^{1.7}$)	58.1 ± 11.9	59.2 ± 12.4	57.9 ± 11.6	0.538
Female MRI LVMI ($g/m^{1.7}$)	45.5 ± 7.4	47.4 ± 7.4	43.6 ± 7.0	0.148

Table 4 – Detailed Breakdown of baseline BP

Variable	All Patients	Placebo	Allopurinol	P value
	n = 14	n = 4	n = 10	
Daytime Home SBP (mmHg)	128.6 ± 4.4	130.8 ± 2.6	127.7 ± 4.8	0.261
Daytime Home DBP (mmHg)	75.3 ± 8.7	77.0 ± 7.3	74.6 ± 9.4	0.658
	n = 58	n = 32	n = 26	
Daytime Ambulatory SBP (mmHg)	124.1 ± 8.5	124.9 ± 7.5	123.0 ± 9.6	0.384
Daytime Ambulatory DBP (mmHg)	73.3 ± 8.4	74.2 ± 7.2	72.3 ± 9.8	0.402
	n = 57	n = 31	n = 26	
24hour Ambulatory SBP (mmHg)	121.1 ± 8.4	121.6 ± 7.6	120.5 ± 9.4	0.635
24hour Ambulatory DBP (mmHg)	70.8 ± 7.8	71.4 ± 6.3	70.2 ± 9.3	0.559

3.4 Effect of Allopurinol on CMRI Parameters

The primary finding of the study was that allopurinol attenuated LVM regression compared to placebo. Those taking allopurinol were found to have a significantly higher final indexed (height$^{1.7}$) and absolute LVM than those taking placebo, after correction for gender, baseline systolic BP and baseline LVM (Table 5).

A large percentage of subjects (14%) withdrew during the study so a secondary analysis of the primary end-point was performed using a mixed model that confirmed the finding from the primary analysis (β 1.49, 95% CI 0.43 – 2.66, p = 0.007). The scatter graph in Figure 49 and Figure 50 shows the change in LVM/LVMI per subject in those in each arm of the trial.

Table 5 - Multiple regression (Adjusted)*

Dependent Variable	β	95% Confidence Interval		p	R^2
		Lower Bound	Upper Bound		
LVMI (g/m$^{1.7}$)	1.36	0.38	2.34	0.007	0.975
Absolute LVM (g)	3.43	0.91	5.95	0.008	0.983

**Gender, baseline systolic BP, baseline LVMI/LVM*

Positive indicates an increased value in the allopurinol cohort

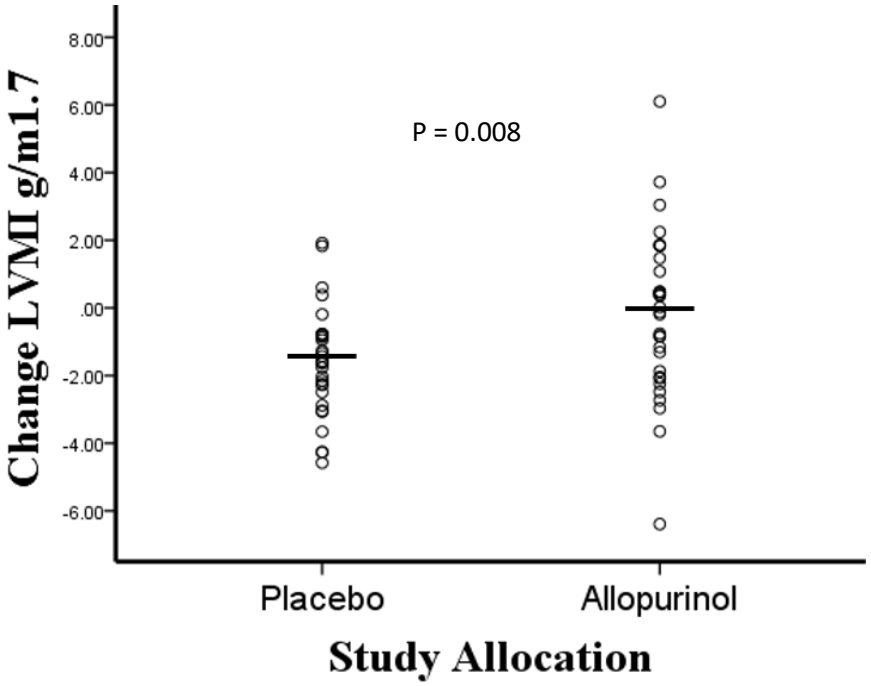


Figure 49 - Change in LVMI height$^{1.7}$ according to study allocation.

Horizontal line indicated mean

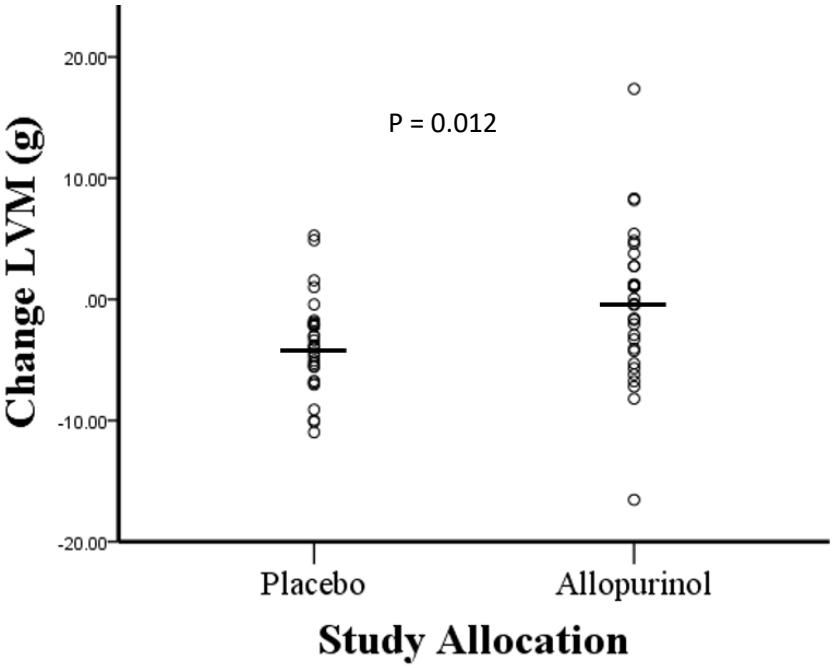


Figure 50 - Change in absolute LVM according to study allocation

Horizontal Line Indicates mean

There was no significant change in other LV or LA parameters measured by CMRI (Table 6, Table 7). Changes in weight can affect the LVM independently of the BP, however there was no significant difference between arms of the trial (placebo -1.61 ± 4.79kg versus allopurinol -0.72 ±3.05kg; p = 0.386).

Table 6 - Effect of Allopurinol on Change in LV MRI Parameters (per-protocol)

Variable	Placebo n = 30	Allopurinol n = 32	p Value
Baseline Indexed LVM ($g/m^{1.7}$)	54.28 ± 11.87	52.19 ± 11.74	0.489
Final Indexed LVM ($g/m^{1.7}$)	52.68 ± 11.73	52.00 ± 11.77	0.823
Change Indexed LVM ($g/m^{1.7}$)	-1.60 ± 1.60	-0.18 ± 2.39	0.009
Baseline Absolute LVM (g)	132.50 ± 35.24	130.26 ± 38.51	0.812
Final Absolute LVM (g)	128.76 ± 35.33	129.89 ± 39.01	0.906
Change Absolute LVM (g)	-3.75 ± 3.89	-0.37 ± 6.08	0.012
Baseline EDV (ml)	142.48 ± 38.03	144.55 ± 31.17	0.815
Final EDV (ml)	144.80 ± 34.93	151.02 ± 38.84	0.511
Change EDV (ml)	2.32 ± 18.26	6.47 ± 16.35	0.349
Baseline ESV (ml)	36.57 ± 18.91	37.29 ± 13.27	0.862
Final ESV (ml)	35.55 ± 17.13	38.87 ± 16.85	0.444
Change ESV (ml)	-1.02 ± 10.64	1.59 ± 10.28	0.331
Baseline SV (ml)	105.91 ± 22.56	107.27 ± 20.94	0.807
Final SV (ml)	109.25 ± 22.42	112.15 ± 25.92	0.641
Change SV (ml)	3.34 ± 10.90	4.88 ± 10.84	0.579
Baseline EF (%)	75.54 ± 7.19	74.73 ± 4.91	0.604
Final EF (%)	76.57 ± 7.67	74.98 ± 6.44	0.379
Change EF (%)	1.03 ± 4.99	0.25 ± 5.49	0.561

Table 7 - Effect of Allopurinol on LA MRI Parameters

Variable	Placebo	Allopurinol	p Value
LAEDV (ml)	3.81 ± 8.86	2.57 ± 8.68	0.605
LAESV (ml)	2.88 ±5.04	2.32 ± 6.78	0.730
LASV (ml)	0.93 ± 6.82	0.26 ± 8.23	0.746
LAEF (%)	-1.36 ± 4.93	-1.07 ± 5.88	0.849

A sub-group analysis by baseline tertile of LVM/LVMI didn't reveal a clear pattern to suggest an effect with low/high baseline LVM (Table 8). A further analysis excluding subjects with diabetes, ischaemic heart disease, cerebrovascular or peripheral vascular disease was consistent with the primary analysis (Table 9). Females had a significant reduction in LVM compared to placebo, in males the vector of change was the same but of a smaller magnitude that failed to reach statistical significance (Table 10).

Table 8 - Change in LVM(I) according to baseline LVM(I) tertile

	1st Tertile			2nd Tertile			3rd Tertile		
	Placebo	Allopurinol	p	Placebo	Allopurinol	p	Placebo	Allopurinol	p
LVM (g)	-3.23 ± 2.61	0.08 ± 4.76	0.114	-4.87 ± 3.09	-1.75 ± 4.49	0.069	-2.85 ± 5.16	0.44 ± 8.42	0.282
LVMI ($g/m^{1.7}$)	-1.56 ± 1.22	-0.20 ± 2.07	0.155	-1.50 ± 1.31	0.52 ± 1.84	0.008	-1.74 ± 2.14	-0.68 ± 3.10	0.361

*Table 9 - Multiple regression (adjusted) excluding those with comorbidities**

Dependent Variable	β	95% Confidence Interval		p
		Lower Bound	Upper Bound	
LVMI ($g/m^{1.7}$)	1.04	0.01	2.06	0.047

**IHD, CVA/TIA, PVD and DM*

Positive indicates an increased value in the allopurinol cohort

Table 10 - Effect of Allopurinol on MRI Parameters by Gender

Variable	Placebo	Allopurinol	p Value
Male Absolute LVM (g)	-2.98 ± 4.24	-0.25 ± 6.8	0.152
Female Absolute LVM (g)	-4.89 ± 3.11	-0.57 ± 4.86	0.016
Male Indexed LVM ($g/m^{1.7}$)	-1.21 ± 1.69	-0.14 ± 2.55	0.141
Female Indexed LVM ($g/m^{1.7}$)	-2.19 ± 1.30	-0.27 ± 2.21	0.018

Table 11 - Change in LVM(I) according to baseline urate tertile

	1st Tertile			2nd Tertile			3rd Tertile		
	Placebo	Allopurinol	p	Placebo	Allopurinol	p	Placebo	Allopurinol	p
LVM (g)	-4.14 ± 3.50	0.64 ± 5.43	0.035	-4.43 ± 2.78	-2.83 ± 5.91	0.426	-2.43 ± 5.39	1.24 ± 6.65	0.208
LVMI ($g/m^{1.7}$)	-1.85 ± 1.48	0.18 ± 2.38	0.039	-1.86 ± 1.18	-1.01 ± 2.31	0.276	-1.01 ± 2.14	0.33 ± 2.49	0.229

Table 12 - Change in LVM(I) according to baseline TBARs tertile

	1st Tertile			2nd Tertile			3rd Tertile		
	Placebo	Allopurinol	p	Placebo	Allopurinol	p	Placebo	Allopurinol	p
LVM (g)	-3.62 ± 3.86	-0.84 ± 4.88	0.148	-3.84 ± 4.23	1.06 ± 7.08	0.810	-3.79 ± 4.0	-0.97 ± 6.94	0.300
LVMI ($g/m^{1.7}$)	-1.48 ± 1.61	-0.38 ± 2.11	0.174	-1.71 ± 1.75	0.36 ± 2.62	0.057	-1.64 ± 1.59	-0.41 ± 2.70	0.253

3.5 Effect of Allopurinol on Blood Pressure

No significant change in blood pressure was found in the study. Although most subjects had twenty-four-hour ambulatory blood pressure monitoring, approximately one fifth had home blood pressure monitoring. No significant difference was found between placebo and allopurinol arms in any of the BP parameters, see

Table 13.

Table 13 - Effect of Allopurinol on Blood Pressure

Variable	Placebo	Allopurinol	p Value
	n = 30	n = 32	
Daytime SBP AMBP or home monitoring (mmHg)	1.6 ± 7.3	-0.9 ± 8.0	0.205
Daytime DBP AMBP or home monitoring (mmHg)	0.1 ± 5.4	0.3 ± 5.7	0.846
	n = 4	n = 9	
Daytime Home SBP (mmHg)	1.5 ± 7.4	-3.4 ± 6.6	0.252
Daytime Home DBP (mmHg)	1.0 ± 5.0	0.4 ± 6.8	0.887
	n = 25	n = 23	
Daytime Ambulatory SBP (mmHg)	1.4 ± 7.6	0.0 ± 8.5	0.549
Daytime Ambulatory DBP (mmHg)	0.1 ± 5.6	0.3 ± 5.4	0.908
	n = 24	n = 23	
24hr Ambulatory SBP (mmHg)	1.2 ± 8.0	0.6 ± 8.0	0.799
24hr Ambulatory DBP (mmHg)	-0.0 ± 5.4	0.7 ± 4.7	0.621

3.6 Effect of Allopurinol on Endothelial Function

No significant difference was detected in endothelial function measured by flow mediated dilation Table 14.

3.7 Effect of Allopurinol on Vascular Stiffness

No significant difference was detected in either augmentation index or pulse wave velocity Table 14. Additionally, there was no correlation between the vascular markers before or after treatment.

Table 14 – Effect of Allopurinol Endothelial Function and Vascular Stiffness

Variable	Placebo	Allopurinol	p Value
FMD (%) Endothelial Dependent	-0.23 ± 3.65	0.14 ± 4.12	0.718
FMD (%) Endothelial Independent	1.07 ± 4.23	0.28 ± 6.51	0.581
AIx (%)	-0.30 ± 13.46	0.06 ± 12.41	0.913
PWV (m/s)	-0.09 ± 1.12	-0.25 ± 1.07	0.581

3.8 Effect of Allopurinol on Biomarkers

There was the expected significant reduction in urate in the cohort treated with allopurinol. TBARS a marker of oxidative stress was also significantly increased in the allopurinol arm but there was no significant change in other biomarkers (Table 15).Sub-group analysis according the tertile of baseline urate (Table 11) and TBARS (Table 12) demonstrated no statistical difference between the lowest and highest tertile.

Table 15 - Effect of Allopurinol on Biomarkers

Variable	Placebo	Allopurinol	p Value
Urate (umol/L)	-1.33 ± 37.04	-189.56 ± 91.95	<0.0001
HsCRP (mg/L)	-0.55 ± 2.10	0.22 ± 1.71	0.122
TBARS (uM)	-0.34 ± 0.83	0.26 ± 0.85	0.007
NTProBNP (pg/mL)	109.08 ± 491.03	-109.03 ± 612.84	0.131
PICP ng/L	-0.18 ± 0.60	-0.05 ± 0.43	0.322
Soluble ST2 ng/mL	-1.02 ± 3.39	-0.61 ± 8.63	0.573

3.9 Gadolinium Enhancement

Thirteen subjects had late gadolinium enhancement at baseline, all except one were assigned to placebo, therefore analysis of the change in LGE between arms of the study therefore could not be assessed.

3.10 Study Drug Compliance

This was assessed by counting the medications returned at each visit during the trial. There was no significant difference in medication compliance between those taking allopurinol and placebo. There was a significant reduction in urate in the allopurinol group compared to placebo (Table 15), with an order of magnitude of a 51.4% reduction in urate levels from baseline consistent with good compliance with allopurinol. The average daily dose of allopurinol in the intervention group was 547mg.

Table 16 - Recorded Compliance

	Placebo	**Allopurinol**	**p**
Compliance (%)	95.5 ± 4.5	94.9 ± 5.3	0.627

3.11 Changes to Antihypertensive Medications

Changes to all anti-hypertensive medications is demonstrated in Table 17. Although there are more alterations to antihypertensives in the allopurinol cohort this wasn't detected as a significant change in BP control over 12months. Table 18 displays changes to RAS inhibitors during the trial as these can affect LVM independently of BP. There is a small number of changes in these medications during the study and therefore unlikely this has altered the LVM significantly.

Table 17 - Changes in Antihypertensives

	Placebo	**Allopurinol**
New/ Up titration of antihypertensive	7	9
Stopped/ Down titration of antihypertensive	5	7

Table 18 - Changes in RAS inhibiting medications

	Placebo	**Allopurinol**
New/ Up titration of RAS inhibitor	1	2
Stopped/ Down titration of RAS inhibitor	1	0

3.12 Adverse Events

Three serious adverse events occurred during the study that required hospitalisation however they were unrelated to the study medications (iatrogenic colonic perforation, infected dog bite requiring debridement, and arthralgia after a fall).

In total there were 166 adverse events, 70 in the placebo group and 82 in the allopurinol arm. Table 19 displays the likely causality to the IMP for each arm of the study based on the summary of product characteristics. Of the three subjects who withdrew due to side effects in the allopurinol arm, two developed nausea and one

had a rash. Table 20 illustrates adverse events by system organ class as per the medical dictionary for regulatory activities (MedDRA) coding. There were two groups with clearly more events in the allopurinol cohort (gastrointestinal disorders and skin and subcutaneous tissue disorders) consistent with the two most common side effects (i.e. rash, nausea and vomiting).

Table 19 - Adverse Event Causality to Study Medication

Causality	**Placebo**	**Allopurinol**
None	28	25
Possible	40	49
Probable	2	3
Definite	0	5

Table 20 - Adverse Event by System Organ Class

System Organ Class	Placebo	Allopurinol
General disorders and administration site conditions	6	6
Infections and Infestations	6	6
Nervous system disorders	10	12
Surgical and medical procedures	2	0
Blood and lymphatic system disorders	0	2
Cardiac Disorders	2	4
Ear and labyrinth disorders	1	0
Eye disorders	0	1
Gastrointestinal disorders	12	17
Injury, poisoning and procedural complications	2	1
Investigations	1	0
Metabolism and nutrition disorders	1	2
Musculoskeletal and connective tissue disorders	10	12
Neoplasms benign, malignant and unspecified	1	0
Psychiatric disorders	2	3
Renal and urinary disorders	3	2
Reproductive system and breast disorders	1	0
Respiratory, thoracic and mediastinal disorders	2	2
Skin and subcutaneous tissue disorders	5	10
Vascular disorders	3	2

4 DISCUSSION

4.1 Main Findings

The main finding of this study is that treatment with high dose allopurinol attenuated LVM regression compared to placebo, in an optimally treated hypertensive cohort, over a twelve-month period. There were no significant changes in other LV, LA volumes or ejection fraction measured by cardiac MRI, nor on measures of haemodynamics (blood pressure, FMD, augmentation index or pulse wave velocity). We found a significant rise in a marker of oxidative stress (TBARS) in the allopurinol arm compared to placebo, but no other significant difference in any other biomarker was detected.

4.2 Possible Mechanisms to Explain Findings

4.2.1 Oxidative Stress

Uric acid (UA) is a major antioxidant in human plasma and an important intracellular free radical scavenger [256]. Previously discussed in chapter 1.3, uric acid can paradoxically become a pro-oxidant in certain conditions i.e. supra normal levels or when other anti-oxidants are depleted [256, 257]. This so called “urate redox shuttle” [26] is a plausible explanation for the unexpected findings in this trial that conflict with previous studies.

Allopurinol has been found to regress left ventricular mass and improve both endothelial function and vascular stiffness in diseases across the cardiovascular spectrum (chronic kidney disease, ischaemic heart disease, diabetes mellitus) [40, 41, 53]. These diseases are associated with high levels of oxidative stress [258] and

therefore lowering both xanthine oxidase generated ROS, and “pro-oxidant” uric acid with allopurinol could shift the redox balance favourably. Although hypertension is associated with increased levels of OS [259], this cohort had well-controlled BP at baseline, had normal or only mildly elevated levels of uric acid (360umol/L) and therefore the baseline levels of XO activity and OS were low. For comparison the uric acid levels in the studies discussed above ranged from 420 - 600µmol/L, but all had a similar baseline indexed MRI LVMI (60 – 71g/m^2) compared to this trial (65g/m^2) [40, 53, 59]. The most likely explanation for the findings from this study is that allopurinol has effectively lowered “antioxidant” uric acid, thereby increasing oxidative stress and attenuating the LV mass regression. An unfavourable change in the redox balance is supported by the significant increase in the marker of OS (TBARS) in the cohort taking allopurinol. There was no clear pattern from sub-group analysis looking at tertile of baseline LVM, TBARS and urate to suggest an effect with higher/lower levels of these. Even in the upper tertile the mean urate is only mildly elevated (placebo 461umol/L, allopurinol 478umol/L).

A sub-group analysis of the OPT-CHF trial found that patients with the highest levels of serum uric acid (>565µmol/L) benefited from oxypurinol, but there was a trend to harm in those with lower levels [260]. Another trial, the CARES study found a significantly higher all-cause and cardiovascular mortality in patients with gout and cardiovascular disease treated with febuxostat compared to allopurinol [69]. Febuxostat is a more potent XO inhibitor and had a greater effect on the serum urate than allopurinol, therefore a possible explanation may be a paradoxical increase in OS from urate lowering. J or U-shaped curves have been described regarding all-cause and cardiovascular mortality in both hypertensive and large general populations associated

with serum UA levels[23-25]. The threshold for uric acid where risk increases varies between studies but the mean final uric acid in this study was 204 ± 68 umol/L in males and 108 ± 33 umol/L in females, lower than any of the gender specific thresholds from the studies above.

Although treatment with allopurinol itself may have a direct adverse effect, independent of uric acid, however this seems implausible and without a mechanistic explanation. Apoptosis is known to be stimulated by high levels of OS [73] therefore it is possible that LVM regression in the previous studies could be explained [40, 41, 53]. This is unlikely in the IHD and CKD cohorts as an improvement in endothelial function and vascular stiffness was demonstrated in both supporting a lowering of OS, but this was not shown in the trial with diabetes.

The PREVENT study demonstrated that serum levels of TBARs have been shown to be strongly, and independently predictive of cardiovascular events in patients with stable coronary disease [252]. Hence both the failure to regress LVM, lowering urate and increasing TBARS may in fact increase cardiovascular risk in this population.

4.2.2 Blood Pressure and Antihypertensive Medications

We studied subjects with treated, well controlled blood pressure, taking evidence-based mediations that included a high percentage of ACE-I and ARBs. Although there are subtle differences in antihypertensive medications between arms no significant differences were found, and overall the number of medications in each arm are similar. No significant differences in blood pressure were found at baseline, nor any significant change over the trial period to explain the results. In fact, the baseline

systolic BP was higher and there was a slight increase in systolic BP in the placebo arm during the trial. Therefore, it is implausible that the results are caused by changes in BP during the study. It would be expected that any changes in antihypertensive medications during the trial would directly influence BP, however RAS inhibiting medication can reduce LVM independent of blood pressure [238]. There were a small number of changes to these medications during the study Table 18 and an analysis excluding these patients had no effect on the findings.

The "placebo" effect on LVM may be due to the regression that has been demonstrated to occur for up to two years with well controlled hypertension [232, 236]. Rekhraj et al noted a similar reduction in LVM within the placebo arm (-1.3± 4.5g) in their study [59]. It is possible that subtle beneficial lifestyle changes were made during the trial by the subjects and these contributed to the result. A major strength of this the study was the blinding of both participants and investigators to study allocation both during the trial and data analysis reducing the risk of bias influencing the results.

4.2.3 Weight

Body mass index (BMI) has been shown to have an independent risk factor for left ventricular hypertrophy [175], and the work by MacMahon et al. demonstrated that a change in weight can have an influence on left ventricular mass independent of blood pressure [242]. At baseline there was no significant difference in the BMI and the weight change overall was greater in the placebo arm than allopurinol but was not found to be statistically significant (placebo -1.61 ± 4.79kg versus allopurinol -0.72

±3.05kg; p = 0.386). It is therefore unlikely that BMI or changes in weight had an influence on the primary finding in this trial.

4.2.4 Endothelial Function and Vascular Stiffness

Allopurinol has been found to improve vascular stiffness and endothelial function in a variety of conditions by reducing vascular oxidative stress [39, 41, 53]. This has been demonstrated by reducing XO generated ROS rather than lowering uric acid in subjects with heart failure [39]. Reduction in cardiac afterload by improvements in vascular function is proposed as one mechanism to explain reductions of LVM seen in previous studies [41, 53]. There were no significant differences in measures of endothelial function nor vascular stiffness at baseline or after twelve months between the groups to suggest an influence of these factors on the LV in this trial. In low vascular oxidative stress, allopurinol may have adversely impacted urate levels and any improvement that might have been seen with XO inhibition or direct anti-oxidant effect has been attenuated.

5 STUDY LIMITATIONS

This was a randomised controlled trial in a single centre with a small sample size and although not captured in the baseline demographics, a completely white population. Hypertension has a wide spectrum of severity, this cohort is on the less severe end of the spectrum and therefore does not represent the disease as a whole.Despite the 10 subjects who withdrew from the study it was adequately powered for the primary end-point. It is possible that there are subtle differences in the baseline demographics between the groups that influenced the results. This study has demonstrated the opposite effect on LVM than previous trials in different cohorts and therefore it is possible the results have occurred by chance.

Although subjects fulfilled criteria for echocardiographic LVH, mean baseline CMRI LVM did not meet criteria for LVH. Screening for LVH using cardiac MRI would be impracticable and prohibitively expensive, echocardiography is an established method to diagnose LVH in clinical practice and was the method used previously in similar studies at our unit. The method for LV contouring excluded partial volume (i.e. <50 full thickness of the myocardium) areas at the basal LV, and the papillary muscles to improve repeatability and hence sensitivity to detect a change but may underestimate overall LV mass as a result. The basal slice has a large cross-sectional area and therefore can have considerable effect on the overall LVM, and the papillary muscles can account for up to 8.9% of the total LVM[261, 262]. Changes in LVM in this study are small and although statistically significant it is unclear whether they are clinically important.

The LIFE study found that while most LVM regression occurs within the first year it can occur for up to two years [237]. Although subjects were enrolled if they had been on

stable anti-hypertensive medications for at least three months, it is possible changes to treatment outside this time frame could already have exerted an effect on LVM. Nineteen percent of subjects had a home blood pressure monitoring rather than 24-hour ambulatory. Mean 24-hour systolic BP correlates best with LVM [178], it is possible that there are differences between arms of the trial in BP not detected in those who had home BP monitoring i.e. nocturnal hypertension.

There are multiple factors however that influence measurements of endothelial function and vascular stiffness such as temperature, time of day, medications, food and smoking [160, 249]. Although we aimed to control factors that affect measurement of vascular stiffness and endothelial function, patients may have eaten, and or smoked prior to testing. Furthermore, it wasn't always practical to repeat the scan at the same time of day because of availability of the patient/equipment or the timing of the MRI scan. It is also recommended that vasoactive medications are withheld before FMD, however this trial was testing the change due to allopurinol versus placebo, so we advised patients to take their usual medication before the tests. Although LVM regression using antihypertensives has established prognostic benefit (chapter 1.9.5), previous studies including this one has used LVM changes as a surrogate end-point for assuming benefit/harm with allopurinol.

6 FUTURE RESEARCH

Large randomised controlled trials to assess hard cardiovascular outcomes with the use of allopurinol are required. One such study the ALL-HEART trial currently in the follow-up phase, is testing allopurinol versus placebo in patients with ischaemic heart disease on the composite end-point of non-fatal MI, non-fatal CVA or CV death[263]. Sub-group analysis of this study specifically looking at baseline UA and outcomes could assess whether there the effect is universal in the population.

It would be unethical to design a trial to assess whether allopurinol increased cardiovascular risk, future studies should select populations with the highest XO activity, oxidative stress or uric acid i.e. resistant hypertension or those with decompensated hypertensive heart disease.

MRI has the advantage of tissue characterisation, the typical pattern of fibrosis in HHD is diffuse reactive rather than focal replacement. Therefore, T1 mapping could be used to quantify changes in the cellular and extracellular compartments over time and provide information on whether changes are occurring to the myocyte, extra-cellular matrix or both.

7 PUBLICATIONS & PRESENTATIONS

Gingles, C.R., et al., *Allopurinol treatment adversely impacts left ventricular mass regression in patients with well-controlled hypertension.* J Hypertens, 2019.

Oral Presentation: European Society of Hypertension 2019, Milan. Lowering uric acid in normouricaemic hypertensive patients has an adverse effect on left ventricular hypertrophy regression.

8 REFERENCES

1. Lang, R.M., et al., *Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging.* Eur Heart J Cardiovasc Imaging, 2015. **16**(3): p. 233-70.
2. Devereux, R.B. and N. Reichek, *Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method.* 1977.
3. Giordano, F.J., *Oxygen, oxidative stress, hypoxia, and heart failure.* J Clin Invest, 2005. **115**(3): p. 500-8.
4. Richette, P. and T. Bardin, *Gout.* Lancet, 2010. **375**(9711): p. 318-28.
5. Johnson, R.J., et al., *Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease?* Hypertension, 2003. **41**(6): p. 1183-90.
6. Sautin, Y.Y. and R.J. Johnson, *Uric acid: the oxidant-antioxidant paradox.* Nucleosides Nucleotides Nucleic Acids, 2008. **27**(6): p. 608-19.
7. Kanbay, M., et al., *The role of uric acid in the pathogenesis of human cardiovascular disease.* Heart, 2013. **99**(11): p. 759-66.
8. Hunter, D.J., et al., *Recent diuretic use and the risk of recurrent gout attacks: the online case-crossover gout study.* J Rheumatol, 2006. **33**(7): p. 1341-5.
9. Pacher, P., A. Nivorozhkin, and C. Szabo, *Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol.* Pharmacol Rev, 2006. **58**(1): p. 87-114.
10. Ames, B.N., et al., *Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis.* Proc Natl Acad Sci U S A, 1981. **78**(11): p. 6858-62.
11. G.F, C., *Targeting urate to reduce oxidative stress in Parkinson disease*. 2017: Experimental Neurology. p. 210-224.
12. Waring, W.S., et al., *Lowering serum urate does not improve endothelial function in patients with type 2 diabetes.* Diabetologia, 2007. **50**(12): p. 2572-9.
13. Waring, W.S., et al., *Hyperuricaemia does not impair cardiovascular function in healthy adults.* Heart, 2004. **90**(2): p. 155-9.
14. WS, W., *Systemic uric acid administration increases serum antioxidant capacity in healthy volunteers*, W. DJ, Editor. 2001: J Cardiovasc Pharmacol. p. 365-71.
15. Waring, W.S., et al., *Uric acid restores endothelial function in patients with type 1 diabetes and regular smokers.* Diabetes, 2006. **55**(11): p. 3127-32.
16. Chamorro, A., et al., *Safety and efficacy of uric acid in patients with acute stroke (URICO-ICTUS): a randomised, double-blind phase 2b/3 trial.* Lancet Neurol, 2014. **13**(5): p. 453-60.
17. Abuja, P.M., *Ascorbate prevents prooxidant effects of urate in oxidation of human low density lipoprotein.* FEBS Letters, 1999. **446**(2): p. 305-308.
18. Sautin, Y.Y., et al., *Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress.* Am J Physiol Cell Physiol, 2007. **293**(2): p. C584-96.
19. Corry, D.B., et al., *Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system.* J Hypertens, 2008. **26**(2): p. 269-75.
20. Santos, C.X., E.I. Anjos, and O. Augusto, *Uric acid oxidation by peroxynitrite: multiple reactions, free radical formation, and amplification of lipid oxidation.* Arch Biochem Biophys, 1999. **372**(2): p. 285-94.
21. Cuspidi, C., et al., *Uric Acid and New Onset Left Ventricular Hypertrophy: Findings From the PAMELA Population.* Am J Hypertens, 2017. **30**(3): p. 279-285.

22. Fang, J. and M.H. Alderman, *Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey.* Jama, 2000. **283**(18): p. 2404-10.
23. Kuo, C.F., et al., *Significance of serum uric acid levels on the risk of all-cause and cardiovascular mortality.* Rheumatology (Oxford), 2013. **52**(1): p. 127-34.
24. Cho, S.K., et al., *U-Shaped Association Between Serum Uric Acid Level and Risk of Mortality: A Cohort Study.* Arthritis Rheumatol, 2018. **70**(7): p. 1122-1132.
25. Verdecchia, P., et al., *Relation between serum uric acid and risk of cardiovascular disease in essential hypertension. The PIUMA study.* Hypertension, 2000. **36**(6): p. 1072-8.
26. Hayden, M.R. and S.C. Tyagi, *Uric acid: A new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: The urate redox shuttle.* Nutr Metab (Lond), 2004. **1**(1): p. 10.
27. Kleber, M.E., et al., *Uric Acid and Cardiovascular Events: A Mendelian Randomization Study*, in *J Am Soc Nephrol*. 2015. p. 2831-8.
28. Hitchings, G.H. and G.B. Elion, *Layer on layer: the Bruce F. Cain memorial Award lecture.* Cancer Res, 1985. **45**(6): p. 2415-20.
29. Kelley, E.E., *Dispelling dogma and misconceptions regarding the most pharmacologically targetable source of reactive species in inflammatory disease, xanthine oxidoreductase.* Arch Toxicol, 2015. **89**(8): p. 1193-207.
30. *Allopurinol 300mg Tablets - Summary of Product Characteristics (SmPC) - (eMC)*. 2018; Available from: https://www.medicines.org.uk/emc/product/6006/smpc.
31. Berry, C.E. and J.M. Hare, *Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications.* J Physiol, 2004. **555**(Pt 3): p. 589-606.
32. Day, R.O., et al., *Clinical pharmacokinetics and pharmacodynamics of allopurinol and oxypurinol.* Clin Pharmacokinet, 2007. **46**(8): p. 623-44.
33. Excellence, T.N.I.f.H.a.C. *BNF: British National Formulary - NICE*. [CorporatePage] 2014 2014-08-05; Available from: https://bnf.nice.org.uk/drug/allopurinol.html.
34. Das, D.K., et al., *Role of xanthine oxidase inhibitor as free radical scavenger: a novel mechanism of action of allopurinol and oxypurinol in myocardial salvage.* Biochem Biophys Res Commun, 1987. **148**(1): p. 314-9.
35. Ricardo, S.D., J.F. Bertram, and G.B. Ryan, *Podocyte architecture in puromycin aminonucleoside-treated rats administered tungsten or allopurinol.* Exp Nephrol, 1995. **3**(5): p. 270-9.
36. Hoey, B.M., J. Butler, and B. Halliwell, *On the specificity of allopurinol and oxypurinol as inhibitors of xanthine oxidase. A pulse radiolysis determination of rate constants for reaction of allopurinol and oxypurinol with hydroxyl radicals.* Free Radic Res Commun, 1988. **4**(4): p. 259-63.
37. Augustin, A.J., et al., *Free radical scavenging and antioxidant activity of allopurinol and oxypurinol in experimental lens-induced uveitis.* Invest Ophthalmol Vis Sci, 1994. **35**(11): p. 3897-904.
38. Knight, T.R., et al., *Vascular and Hepatocellular Peroxynitrite Formation during Acetaminophen Toxicity: Role of Mitochondrial Oxidant Stress.* Toxicological Sciences, 2019. **62**(2): p. 212-220.
39. George, J., et al., *High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid.* Circulation, 2006. **114**(23): p. 2508-16.
40. Szwejkowski, B.R., et al., *Allopurinol reduces left ventricular mass in patients with type 2 diabetes and left ventricular hypertrophy.* J Am Coll Cardiol, 2013. **62**(24): p. 2284-93.
41. Rekhraj, S., *The Effects of Xanthine Oxidase Inhibitors on Left Ventricular Mass and Endothelial Function in Patients with Ischaemic Heart Disease*, in *Clinical Pharmacology*

& Therapeutics, Cardiovascular & Lung Biology. 2014, University of Dundee: http://discovery.dundee.ac.uk/portal/files/4577925/Rekhraj_md_2014.pdf. p. 254.
42. Noman, A., et al., *Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial.* Lancet, 2010. **375**(9732): p. 2161-7.
43. Rajendra, N.S., et al., *Mechanistic insights into the therapeutic use of high-dose allopurinol in angina pectoris.* J Am Coll Cardiol, 2011. **58**(8): p. 820-8.
44. George, J. and A.D. Struthers, *Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress*, in *Vasc Health Risk Manag*. 2009. p. 265-72.
45. Hung, S.I., et al., *HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol.* Proc Natl Acad Sci U S A, 2005. **102**(11): p. 4134-9.
46. Mordi, I. and N. Tzemos, *Is reversal of endothelial dysfunction still an attractive target in modern cardiology?* World J Cardiol, 2014. **6**(8): p. 824-35.
47. Mancia, G., et al., *2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).* Eur Heart J, 2013. **34**(28): p. 2159-219.
48. Farquharson, C.A., et al., *Allopurinol improves endothelial dysfunction in chronic heart failure.* Circulation, 2002. **106**(2): p. 221-6.
49. Doehner, W., et al., *Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies.* Circulation, 2002. **105**(22): p. 2619-24.
50. Tousoulis, D., et al., *Effects of rosuvastatin and allopurinol on circulating endothelial progenitor cells in patients with congestive heart failure: the impact of inflammatory process and oxidative stress.* Atherosclerosis, 2011. **214**(1): p. 151-7.
51. Jalal, D.I., et al., *Vascular Function and Uric Acid-Lowering in Stage 3 CKD.* J Am Soc Nephrol, 2017. **28**(3): p. 943-952.
52. Bayram, D., et al., *The effects of allopurinol on metabolic acidosis and endothelial functions in chronic kidney disease patients.* Clin Exp Nephrol, 2015. **19**(3): p. 443-9.
53. Kao, M.P., et al., *Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease.* J Am Soc Nephrol, 2011. **22**(7): p. 1382-9.
54. Alem, M.M., *Allopurinol and endothelial function: A systematic review with meta-analysis of randomized controlled trials.* Cardiovasc Ther, 2018. **36**(4): p. e12432.
55. Cardillo, C., et al., *Xanthine oxidase inhibition with oxypurinol improves endothelial vasodilator function in hypercholesterolemic but not in hypertensive patients.* Hypertension, 1997. **30**(1 Pt 1): p. 57-63.
56. Mercuro, G., et al., *Effect of hyperuricemia upon endothelial function in patients at increased cardiovascular risk.* Am J Cardiol, 2004. **94**(7): p. 932-5.
57. Kanbay, M., et al., *A randomized study of allopurinol on endothelial function and estimated glomular filtration rate in asymptomatic hyperuricemic subjects with normal renal function.* Clin J Am Soc Nephrol, 2011. **6**(8): p. 1887-94.
58. Khan, F., et al., *Allopurinol treatment reduces arterial wave reflection in stroke survivors.* Cardiovasc Ther, 2008. **26**(4): p. 247-52.
59. Rekhraj, S., et al., *High-dose allopurinol reduces left ventricular mass in patients with ischemic heart disease.* J Am Coll Cardiol, 2013. **61**(9): p. 926-32.
60. Deng, G., et al., *Effects of Allopurinol on Arterial Stiffness: A Meta-Analysis of Randomized Controlled Trials*, in *Med Sci Monit*. 2016. p. 1389-97.
61. Feig, D.I., B. Soletsky, and R.J. Johnson, *Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial.* Jama, 2008. **300**(8): p. 924-32.
62. Soletsky, B. and D.I. Feig, *Uric acid reduction rectifies prehypertension in obese adolescents.* Hypertension, 2012. **60**(5): p. 1148-56.

63. Kanbay, M., et al., *Effect of treatment of hyperuricemia with allopurinol on blood pressure, creatinine clearence, and proteinuria in patients with normal renal functions.* Int Urol Nephrol, 2007. **39**(4): p. 1227-33.
64. Agarwal, V., N. Hans, and F.H. Messerli, *Effect of allopurinol on blood pressure: a systematic review and meta-analysis.* J Clin Hypertens (Greenwich), 2013. **15**(6): p. 435-42.
65. Cingolani, H.E., et al., *The effect of xanthine oxidase inhibition upon ejection fraction in heart failure patients: La Plata Study.* J Card Fail, 2006. **12**(7): p. 491-8.
66. *Adenuric 120 mg film-coated tablets - Summary of Product Characteristics (SmPC) - (eMC)*. of first authorisation: 21 April 2008Date of latest renewal: 20 December 2012; Available from: https://www.medicines.org.uk/emc/product/1925/smpc#POSOLOGY.
67. Becker, M.A., et al., *Febuxostat compared with allopurinol in patients with hyperuricemia and gout.* N Engl J Med, 2005. **353**(23): p. 2450-61.
68. Schumacher, H.R., Jr., et al., *Effects of febuxostat versus allopurinol and placebo in reducing serum urate in subjects with hyperuricemia and gout: a 28-week, phase III, randomized, double-blind, parallel-group trial.* Arthritis Rheum, 2008. **59**(11): p. 1540-8.
69. White, W.B., et al., *Cardiovascular Safety of Febuxostat or Allopurinol in Patients with Gout.* N Engl J Med, 2018. **378**(13): p. 1200-1210.
70. Robbins, N., et al., *The history and future of probenecid.* Cardiovasc Toxicol, 2012. **12**(1): p. 1-9.
71. Lee, M.H., et al., *A benefit-risk assessment of benzbromarone in the treatment of gout. Was its withdrawal from the market in the best interest of patients?* Drug Saf, 2008. **31**(8): p. 643-65.
72. Kim, H.K., et al., *Cardiac Response to Oxidative Stress Induced by Mitochondrial Dysfunction.* Rev Physiol Biochem Pharmacol, 2016. **170**: p. 101-27.
73. Takimoto, E. and D.A. Kass, *Role of oxidative stress in cardiac hypertrophy and remodeling.* Hypertension, 2007. **49**(2): p. 241-8.
74. Vichova, T. and Z. Motovska, *Oxidative stress: Predictive marker for coronary artery disease.* Exp Clin Cardiol, 2013. **18**(2): p. e88-91.
75. Wattanapitayakul, S.K. and J.A. Bauer, *Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications.* Pharmacol Ther, 2001. **89**(2): p. 187-206.
76. Bonomini, F., et al., *Atherosclerosis and oxidative stress.* Histol Histopathol, 2008. **23**(3): p. 381-90.
77. Singh, N., et al., *Oxidative stress and heart failure.* Mol Cell Biochem, 1995. **147**(1-2): p. 77-81.
78. Harrison, R., *Structure and function of xanthine oxidoreductase: where are we now?* Free Radic Biol Med, 2002. **33**(6): p. 774-97.
79. Linder, N., J. Rapola, and K.O. Raivio, *Cellular expression of xanthine oxidoreductase protein in normal human tissues.* Lab Invest, 1999. **79**(8): p. 967-74.
80. Cappola, T.P., et al., *Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy.* Circulation, 2001. **104**(20): p. 2407-11.
81. Seddon, M., Y.H. Looi, and A.M. Shah, *Oxidative stress and redox signalling in cardiac hypertrophy and heart failure.* Heart, 2007. **93**(8): p. 903-7.
82. Suzuki, H., et al., *Xanthine oxidase activity associated with arterial blood pressure in spontaneously hypertensive rats.* Proc Natl Acad Sci U S A, 1998. **95**(8): p. 4754-9.
83. Cave, A., et al., *NADPH oxidase-derived reactive oxygen species in cardiac pathophysiology.* Philos Trans R Soc Lond B Biol Sci, 2005. **360**(1464): p. 2327-34.
84. Lonn, E., et al., *Effects of ramipril on left ventricular mass and function in cardiovascular patients with controlled blood pressure and with preserved left ventricular ejection fraction: a substudy of the Heart Outcomes Prevention Evaluation (HOPE) Trial.* J Am Coll Cardiol, 2004. **43**(12): p. 2200-6.

85. Devereux, R.B., et al., *Regression of hypertensive left ventricular hypertrophy by losartan compared with atenolol: the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial.* Circulation, 2004. **110**(11): p. 1456-62.
86. Cheetham, C., et al., *Losartan, an angiotensin type 1 receptor antagonist, improves endothelial function in non-insulin-dependent diabetes.* J Am Coll Cardiol, 2000. **36**(5): p. 1461-6.
87. Touyz, R.M., *The role of angiotensin II in regulating vascular structural and functional changes in hypertension.* Curr Hypertens Rep, 2003. **5**(2): p. 155-64.
88. Tsai, E.J. and D.A. Kass, *Cyclic GMP signaling in cardiovascular pathophysiology and therapeutics.* Pharmacol Ther, 2009. **122**(3): p. 216-38.
89. Murphy, E., et al., *Signaling by S-nitrosylation in the heart.* J Mol Cell Cardiol, 2014. **73**: p. 18-25.
90. Takimoto, E., et al., *Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load.* J Clin Invest, 2005. **115**(5): p. 1221-31.
91. Sawyer, D.B., et al., *Role of oxidative stress in myocardial hypertrophy and failure.* J Mol Cell Cardiol, 2002. **34**(4): p. 379-88.
92. Sam, F., et al., *Mice lacking inducible nitric oxide synthase have improved left ventricular contractile function and reduced apoptotic cell death late after myocardial infarction.* Circ Res, 2001. **89**(4): p. 351-6.
93. Nordberg, J. and E.S. Arner, *Reactive oxygen species, antioxidants, and the mammalian thioredoxin system.* Free Radic Biol Med, 2001. **31**(11): p. 1287-312.
94. Conrad, M., et al., *Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function.* Mol Cell Biol, 2004. **24**(21): p. 9414-23.
95. Oparil, S., et al., *Hypertension.* Nature Reviews Disease Primers, 2018. **4**: p. 18014.
96. NICE. *Clinical guideline - Hypertension*. 2011 17/10/14].
97. Leoncini, G., et al., *Increased Ambulatory Arterial Stiffness Index Is Associated With Target Organ Damage in Primary Hypertension.* 2006.
98. Calhoun, D.A., et al., *Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research.* Circulation, 2008. **117**(25): p. e510-26.
99. Myat, A., et al., *Resistant hypertension.* Bmj, 2012. **345**: p. e7473.
100. Daugherty, S.L., et al., *Incidence and prognosis of resistant hypertension in hypertensive patients.* Circulation, 2012. **125**(13): p. 1635-42.
101. Cuspidi, C., et al., *Resistant hypertension and left ventricular hypertrophy: an overview.* J Am Soc Hypertens, 2010. **4**(6): p. 319-24.
102. Whelton, P.K., *Epidemiology of hypertension.* Lancet, 1994. **344**(8915): p. 101-6.
103. Kannel, W.B. and T. Gordan, *Evaluation of cardiovascular risk in the elderly: the Framingham study.* Bull N Y Acad Med, 1978. **54**(6): p. 573-91.
104. NatCen Social Research, U.C.L.U., *Health Survey For England*. 2015: http://www.hscic.gov.uk/catalogue/PUB19295/HSE2014-Sum-bklet.pdf.
105. Deschepper, C.F., et al., *In search of cardiovascular candidate genes: interactions between phenotypes and genotypes.* Hypertension, 2002. **39**(2 Pt 2): p. 332-6.
106. Miall, W.E. and P.D. Oldham, *The Hereditary Factor in Arterial Blood-pressure.* Br Med J, 1963. **1**(5323): p. 75-80.
107. Ehret, G.B. and M.J. Caulfield, *Genes for blood pressure: an opportunity to understand hypertension.* Eur Heart J, 2013. **34**(13): p. 951-61.
108. Forman, J.P., M.J. Stampfer, and G.C. Curhan, *Diet and lifestyle risk factors associated with incident hypertension in women.* Jama, 2009. **302**(4): p. 401-11.
109. Stevens, V.J., et al., *Long-term weight loss and changes in blood pressure: results of the Trials of Hypertension Prevention, phase II.* Ann Intern Med, 2001. **134**(1): p. 1-11.

110. Fagard, R.H., *Exercise characteristics and the blood pressure response to dynamic physical training.* Med Sci Sports Exerc, 2001. **33**(6 Suppl): p. S484-92; discussion S493-4.
111. Dickinson, H.O., et al., *Lifestyle interventions to reduce raised blood pressure: a systematic review of randomized controlled trials.* J Hypertens, 2006. **24**(2): p. 215-33.
112. Graudal, N.A., A.M. Galloe, and P. Garred, *Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterols, and triglyceride: a meta-analysis.* Jama, 1998. **279**(17): p. 1383-91.
113. Xin, X., et al., *Effects of alcohol reduction on blood pressure: a meta-analysis of randomized controlled trials.* Hypertension, 2001. **38**(5): p. 1112-7.
114. Whelton, P.K., et al., *Comprehensive Hypertension*. 2007, Philadelphia: Mosby Elsevier.
115. Kumar, R., et al., *The intracrine renin-angiotensin system.* Clin Sci (Lond), 2012. **123**(5): p. 273-84.
116. *Image: The renin-angiotensin-aldosterone system. A I = angiotensin I; Aâ¦*. 2017; Available from: https://expertconsult.inkling.com/read/braunwalds-heart-disease-cardiovascular-bonow-9th/chapter-45/figure-45-8.
117. Rajagopalan, S., et al., *Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone.* J Clin Invest, 1996. **97**(8): p. 1916-23.
118. Paul, M., A. Poyan Mehr, and R. Kreutz, *Physiology of local renin-angiotensin systems.* Physiol Rev, 2006. **86**(3): p. 747-803.
119. Dzau, V.J., et al., *Pathophysiologic and therapeutic importance of tissue ACE: a consensus report.* Cardiovasc Drugs Ther, 2002. **16**(2): p. 149-60.
120. Komuro, I., et al., *Mechanical loading stimulates cell hypertrophy and specific gene expression in cultured rat cardiac myocytes. Possible role of protein kinase C activation.* J Biol Chem, 1991. **266**(2): p. 1265-8.
121. Sadoshima, J. and S. Izumo, *Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: potential involvement of an autocrine/paracrine mechanism.* EMBO J, 1993. **12**(4): p. 1681-92.
122. Sadoshima, J., et al., *Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro.* Cell, 1993. **75**(5): p. 977-84.
123. Mancia, G. and G. Grassi, *The autonomic nervous system and hypertension.* Circ Res, 2014. **114**(11): p. 1804-14.
124. Manrique, C., et al., *The Renin Angiotensin Aldosterone System in Hypertension: Roles of Insulin Resistance and Oxidative Stress.* Med Clin North Am, 2009. **93**(3): p. 569-82.
125. Grassi, G., G. Seravalle, and G. Mancia, *Sympathetic activation in cardiovascular disease: evidence, clinical impact and therapeutic implications.* Eur J Clin Invest, 2015. **45**(12): p. 1367-75.
126. Victor, R.G. and M.M. Shafiq, *Sympathetic neural mechanisms in human hypertension.* Curr Hypertens Rep, 2008. **10**(3): p. 241-7.
127. Grassi, G., et al., *Baroreflex control of sympathetic nerve activity in essential and secondary hypertension.* Hypertension, 1998. **31**(1): p. 68-72.
128. Grassi, G., et al., *Adrenergic and reflex abnormalities in obesity-related hypertension.* Hypertension, 2000. **36**(4): p. 538-42.
129. DiBona, G.F., *The sympathetic nervous system and hypertension: recent developments.* Hypertension, 2004. **43**(2): p. 147-50.
130. Krum, H., et al., *Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study.* Lancet, 2009. **373**(9671): p. 1275-81.
131. Esler, M.D., et al., *Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial.* Lancet, 2010. **376**(9756): p. 1903-9.

132. Bhatt, D.L., et al., *A controlled trial of renal denervation for resistant hypertension.* N Engl J Med, 2014. **370**(15): p. 1393-401.
133. Julius, S., A.V. Pascual, and R. London, *Role of parasympathetic inhibition in the hyperkinetic type of borderline hypertension.* Circulation, 1971. **44**(3): p. 413-8.
134. Julius, S., et al., *Altered cardiac responsiveness and regulation in the normal cardiac output type of borderlind hlpertension.* Circ Res, 1975. **36**(6 Suppl 1): p. 199-207.
135. de Leeuw, P.W., et al., *Sustained Reduction of Blood Pressure With Baroreceptor Activation Therapy: Results of the 6-Year Open Follow-Up.* Hypertension, 2017. **69**(5): p. 836-843.
136. Chen, L., et al., *Regulation of vascular smooth muscle growth by alpha 1-adrenoreceptor subtypes in vitro and in situ.* J Biol Chem, 1995. **270**(52): p. 30980-8.
137. Bevan, R.D. and H. Tsuru, *Functional and structural changes in the rabbit ear artery after sympathetic denervation.* Circ Res, 1981. **49**(2): p. 478-85.
138. *Image: Sympathetic nervous system. Dotted arrows represent inhibitoryâ¦*. 2017; Available from: https://expertconsult.inkling.com/read/braunwalds-heart-disease-cardiovascular-bonow-9th/chapter-45/figure-45-5.
139. Ferrannini, E., et al., *Insulin resistance in essential hypertension.* N Engl J Med, 1987. **317**(6): p. 350-7.
140. Zhou, M.-S., A. Wang, and H. Yu, *Link between insulin resistance and hypertension: What is the evidence from evolutionary biology?* Diabetology & Metabolic Syndrome, 2014. **6**(1): p. 12.
141. Bleakley, C., et al., *Endothelial Function in Hypertension: Victim or Culprit?* J Clin Hypertens (Greenwich), 2015. **17**(8): p. 651-4.
142. Forstermann, U. and T. Munzel, *Endothelial nitric oxide synthase in vascular disease: from marvel to menace.* Circulation, 2006. **113**(13): p. 1708-14.
143. Griendling, K.K. and G.A. FitzGerald, *Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS.* Circulation, 2003. **108**(16): p. 1912-6.
144. Park, J.B. and E.L. Schiffrin, *Small artery remodeling is the most prevalent (earliest?) form of target organ damage in mild essential hypertension.* J Hypertens, 2001. **19**(5): p. 921-30.
145. Olsen, M.H., et al., *Association between vascular dysfunction and reduced myocardial flow reserve in patients with hypertension: a LIFE substudy.* J Hum Hypertens, 2004. **18**(6): p. 445-52.
146. Muiesan, M.L., et al., *Flow-mediated dilatation of the brachial artery and left ventricular geometry in hypertensive patients.* J Hypertens, 2001. **19**(3 Pt 2): p. 641-7.
147. Perticone, F., et al., *Relationship between left ventricular mass and endothelium-dependent vasodilation in never-treated hypertensive patients.* Circulation, 1999. **99**(15): p. 1991-6.
148. Sung, J., et al., *Peripheral endothelium-dependent flow-mediated vasodilatation is associated with left ventricular mass in older persons with hypertension.* Am Heart J, 2002. **144**(1): p. 39-44.
149. Millgard, J., et al., *Left ventricular hypertrophy is associated with an attenuated endothelium-dependent vasodilation in hypertensive men.* Blood Press, 2000. **9**(6): p. 309-14.
150. Yeboah, J., et al., *Endothelial dysfunction is associated with left ventricular mass (assessed using MRI) in an adult population (MESA).* J Hum Hypertens, 2011. **25**(1): p. 25-31.
151. Schiffrin, E.L., *Vascular remodeling in hypertension: mechanisms and treatment.* Hypertension, 2012. **59**(2): p. 367-74.
152. Korsgaard, N., et al., *Histology of subcutaneous small arteries from patients with essential hypertension.* Hypertension, 1993. **22**(4): p. 523-6.

153. Schiffrin, E.L. and R.M. Touyz, *From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension.* Am J Physiol Heart Circ Physiol, 2004. **287**(2): p. H435-46.
154. Whelton, P.K.G., J.P.; Ng, L.L.; Schiffrin, E.L.; MacFadyen, R.J.; Jones, D.W.; Lang, C.C., *Comprehensive Hypertension*. 2007, Philadelphia: Mosby Elsevier.
155. Touyz, R.M., et al., *Differential activation of extracellular signal-regulated protein kinase 1/2 and p38 mitogen activated-protein kinase by AT1 receptors in vascular smooth muscle cells from Wistar-Kyoto rats and spontaneously hypertensive rats.* J Hypertens, 2001. **19**(3 Pt 2): p. 553-9.
156. Intengan, H.D. and E.L. Schiffrin, *Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis.* Hypertension, 2001. **38**(3 Pt 2): p. 581-7.
157. Schiffrin, E.L., *Vascular stiffening and arterial compliance. Implications for systolic blood pressure.* Am J Hypertens, 2004. **17**(12 Pt 2): p. 39s-48s.
158. Boutouyrie, P., et al., *Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study.* Hypertension, 2002. **39**(1): p. 10-5.
159. Laurent, S., et al., *Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients.* Hypertension, 2001. **37**(5): p. 1236-41.
160. Laurent, S., et al., *Expert consensus document on arterial stiffness: methodological issues and clinical applications.* Eur Heart J, 2006. **27**(21): p. 2588-605.
161. Davies, J.I. and A.D. Struthers, *Pulse wave analysis and pulse wave velocity: a critical review of their strengths and weaknesses.* J Hypertens, 2003. **21**(3): p. 463-72.
162. Stamler, J., R. Stamler, and J.D. Neaton, *Blood pressure, systolic and diastolic, and cardiovascular risks. US population data.* Arch Intern Med, 1993. **153**(5): p. 598-615.
163. Nurnberger, J., et al., *Augmentation index is associated with cardiovascular risk.* J Hypertens, 2002. **20**(12): p. 2407-14.
164. Patvardhan, E., et al., *Augmentation index derived from peripheral arterial tonometry correlates with cardiovascular risk factors.* Cardiol Res Pract, 2011. **2011**: p. 1-6.
165. Dart, A.M., et al., *Brachial blood pressure but not carotid arterial waveforms predict cardiovascular events in elderly female hypertensives.* Hypertension, 2006. **47**(4): p. 785-90.
166. Saba, P.S., et al., *Relation of arterial pressure waveform to left ventricular and carotid anatomy in normotensive subjects.* J Am Coll Cardiol, 1993. **22**(7): p. 1873-80.
167. Hashimoto, J., et al., *Enhanced radial late systolic pressure augmentation in hypertensive patients with left ventricular hypertrophy.* Am J Hypertens, 2006. **19**(1): p. 27-32.
168. Snieder, H., et al., *Heritability of central systolic pressure augmentation: a twin study.* Hypertension, 2000. **35**(2): p. 574-9.
169. Grayson, P.C., et al., *Hyperuricemia and incident hypertension: a systematic review and meta-analysis.* Arthritis Care Res (Hoboken), 2011. **63**(1): p. 102-10.
170. Perez-Pozo, S.E., et al., *Excessive fructose intake induces the features of metabolic syndrome in healthy adult men: role of uric acid in the hypertensive response.* Int J Obes (Lond), 2010. **34**(3): p. 454-61.
171. Feig, D.I., *The role of uric acid in the pathogenesis of hypertension in the young.* J Clin Hypertens (Greenwich), 2012. **14**(6): p. 346-52.
172. Feig, D.I., et al., *Uric acid and the origins of hypertension.* J Pediatr, 2013. **162**(5): p. 896-902.
173. Kannel, W.B., T. Gordon, and D. Offutt, *Left ventricular hypertrophy by electrocardiogram. Prevalence, incidence, and mortality in the Framingham study.* Ann Intern Med, 1969. **71**(1): p. 89-105.
174. Levy, D., et al., *Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. The Framingham Heart Study.* Ann Intern Med, 1988. **108**(1): p. 7-13.

175. Schirmer, H., P. Lunde, and K. Rasmussen, *Prevalence of left ventricular hypertrophy in a general population; The Tromso Study.* Eur Heart J, 1999. **20**(6): p. 429-38.
176. Cuspidi, C., et al., *Prevalence of left-ventricular hypertrophy in hypertension: an updated review of echocardiographic studies.* J Hum Hypertens, 2012. **26**(6): p. 343-9.
177. Mancia, G., et al., *Prevalence of left ventricular hypertrophy in hypertensive patients without and with blood pressure control: data from the PAMELA population. Pressioni Arteriose Monitorate E Loro Associazioni.* Hypertension, 2002. **39**(3): p. 744-9.
178. Gosse, P., *Left ventricular hypertrophy--the problem and possible solutions.* J Int Med Res, 2005. **33 Suppl 1**: p. 3a-11a.
179. Post, W.S., et al., *Heritability of left ventricular mass: the Framingham Heart Study.* Hypertension, 1997. **30**(5): p. 1025-8.
180. Arnett, D.K., et al., *Sibling correlation of left ventricular mass and geometry in hypertensive African Americans and whites: the HyperGEN study. Hypertension Genetic Epidemiology Network.* Am J Hypertens, 2001. **14**(12): p. 1226-30.
181. Kapuku, G.K., et al., *Change of Genetic Determinants of Left Ventricular Structure in Adolescence: Longitudinal Evidence from the Georgia Cardiovascular Twin Study.* Am J Hypertens, 2008. **21**(7).
182. Harshfield, G.A., et al., *Genetic and environmental influences on echocardiographically determined left ventricular mass in black twins.* Am J Hypertens, 1990. **3**(7): p. 538-43.
183. Fraser, R., *Studying genes and the development of cardiac hypertrophy: convenient intermediate phenotypes in man.* J Hypertens, 2003. **21**(5): p. 873-4.
184. Vasan, R.S., et al., *Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data.* Jama, 2009. **302**(2): p. 168-78.
185. Drazner, M.H., *The progression of hypertensive heart disease.* Circulation, 2011. **123**(3): p. 327-34.
186. Diamond, J.A. and R.A. Phillips, *Hypertensive heart disease.* Hypertens Res, 2005. **28**(3): p. 191-202.
187. Kurland, L., et al., *Polymorphisms in the angiotensinogen and angiotensin II type 1 receptor gene are related to change in left ventricular mass during antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial.* J Hypertens, 2002. **20**(4): p. 657-63.
188. Levy, D., et al., *Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study.* N Engl J Med, 1990. **322**(22): p. 1561-6.
189. Silberberg, J.S., et al., *Impact of left ventricular hypertrophy on survival in end-stage renal disease.* Kidney Int, 1989. **36**(2): p. 286-90.
190. Koren, M.J., et al., *Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension.* Ann Intern Med, 1991. **114**(5): p. 345-52.
191. Ghali, J.K., et al., *The prognostic role of left ventricular hypertrophy in patients with or without coronary artery disease.* Ann Intern Med, 1992. **117**(10): p. 831-6.
192. Brown, D.W., W.H. Giles, and J.B. Croft, *Left ventricular hypertrophy as a predictor of coronary heart disease mortality and the effect of hypertension.* Am Heart J, 2000. **140**(6): p. 848-56.
193. Vakili, B.A., P.M. Okin, and R.B. Devereux, *Prognostic implications of left ventricular hypertrophy.* Am Heart J, 2001. **141**(3): p. 334-41.
194. Liao, Y., et al., *The relative effects of left ventricular hypertrophy, coronary artery disease, and ventricular dysfunction on survival among black adults.* Jama, 1995. **273**(20): p. 1592-7.
195. Schillaci, G., et al., *Continuous relation between left ventricular mass and cardiovascular risk in essential hypertension.* Hypertension, 2000. **35**(2): p. 580-6.

196. Kahan, T. and H. Persson, *The importance of left ventricular geometry in hypertensive heart disease*, in *J Hypertens*. 2015: England. p. 690-2.
197. Garcia, J.A. and E.K. Incerpi, *Factors and mechanisms involved in left ventricular hypertrophy and the anti-hypertrophic role of nitric oxide.* Arq Bras Cardiol, 2008. **90**(6): p. 409-16.
198. Kahan, T., *The importance of myocardial fibrosis in hypertensive heart disease*, in *J Hypertens*. 2012: England. p. 685-7.
199. Lorell, B.H. and B.A. Carabello, *Left ventricular hypertrophy: pathogenesis, detection, and prognosis.* Circulation, 2000. **102**(4): p. 470-9.
200. Frohlich, E.D., et al., *The heart in hypertension.* N Engl J Med, 1992. **327**(14): p. 998-1008.
201. Imamura, T., et al., *Acute changes in myosin heavy chain synthesis rate in pressure versus volume overload.* Circ Res, 1994. **75**(3): p. 418-25.
202. Matsuo, T., et al., *Mechanisms of cardiac hypertrophy in canine volume overload.* Am J Physiol, 1998. **275**(1 Pt 2): p. H65-74.
203. Gonzalez, A., et al., *Cardiomyocyte apoptosis in hypertensive cardiomyopathy.* Cardiovasc Res, 2003. **59**(3): p. 549-62.
204. Sugden, P.H. and A. Clerk, *Oxidative stress and growth-regulating intracellular signaling pathways in cardiac myocytes.* Antioxid Redox Signal, 2006. **8**(11-12): p. 2111-24.
205. Weber, K.T., *Fibrosis and hypertensive heart disease.* Curr Opin Cardiol, 2000. **15**(4): p. 264-72.
206. Muller-Brunotte, R., et al., *Myocardial fibrosis and diastolic dysfunction in patients with hypertension: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA).* J Hypertens, 2007. **25**(9): p. 1958-66.
207. Hamasaki, S., et al., *Attenuated coronary flow reserve and vascular remodeling in patients with hypertension and left ventricular hypertrophy.* J Am Coll Cardiol, 2000. **35**(6): p. 1654-60.
208. Opherk, D., et al., *Reduction of coronary reserve: a mechanism for angina pectoris in patients with arterial hypertension and normal coronary arteries.* Circulation, 1984. **69**(1): p. 1-7.
209. Kahan, T. and L. Bergfeldt, *Left ventricular hypertrophy in hypertension: its arrhythmogenic potential.* Heart, 2005. **91**(2): p. 250-6.
210. Hirotani, S., et al., *Involvement of nuclear factor-kappaB and apoptosis signal-regulating kinase 1 in G-protein-coupled receptor agonist-induced cardiomyocyte hypertrophy.* Circulation, 2002. **105**(4): p. 509-15.
211. Sag, C.M., C.X.C. Santos, and A.M. Shah, *Redox regulation of cardiac hypertrophy.* Journal of Molecular and Cellular Cardiology, 2014. **73**: p. 103-111.
212. Griendling, K.K., D. Sorescu, and M. Ushio-Fukai, *NAD(P)H oxidase: role in cardiovascular biology and disease.* Circ Res, 2000. **86**(5): p. 494-501.
213. Matsushita, H., K.H. Lee, and P.S. Tsao, *Cyclic strain induces reactive oxygen species production via an endothelial NAD(P)H oxidase.* J Cell Biochem Suppl, 2001. **Suppl 36**: p. 99-106.
214. Engberding, N., et al., *Allopurinol attenuates left ventricular remodeling and dysfunction after experimental myocardial infarction: a new action for an old drug?* Circulation, 2004. **110**(15): p. 2175-9.
215. Chen, C.C., Y.J. Hsu, and T.M. Lee, *Impact of elevated uric acid on ventricular remodeling in infarcted rats with experimental hyperuricemia.* Am J Physiol Heart Circ Physiol, 2011. **301**(3): p. H1107-17.
216. Sokolow, M. and T.P. Lyon, *The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads.* Am Heart J, 1949. **37**(2): p. 161-86.
217. Pewsner, D., et al., *Accuracy of electrocardiography in diagnosis of left ventricular hypertrophy in arterial hypertension: systematic review.* BMJ, 2007. **335**(7622): p. 711.

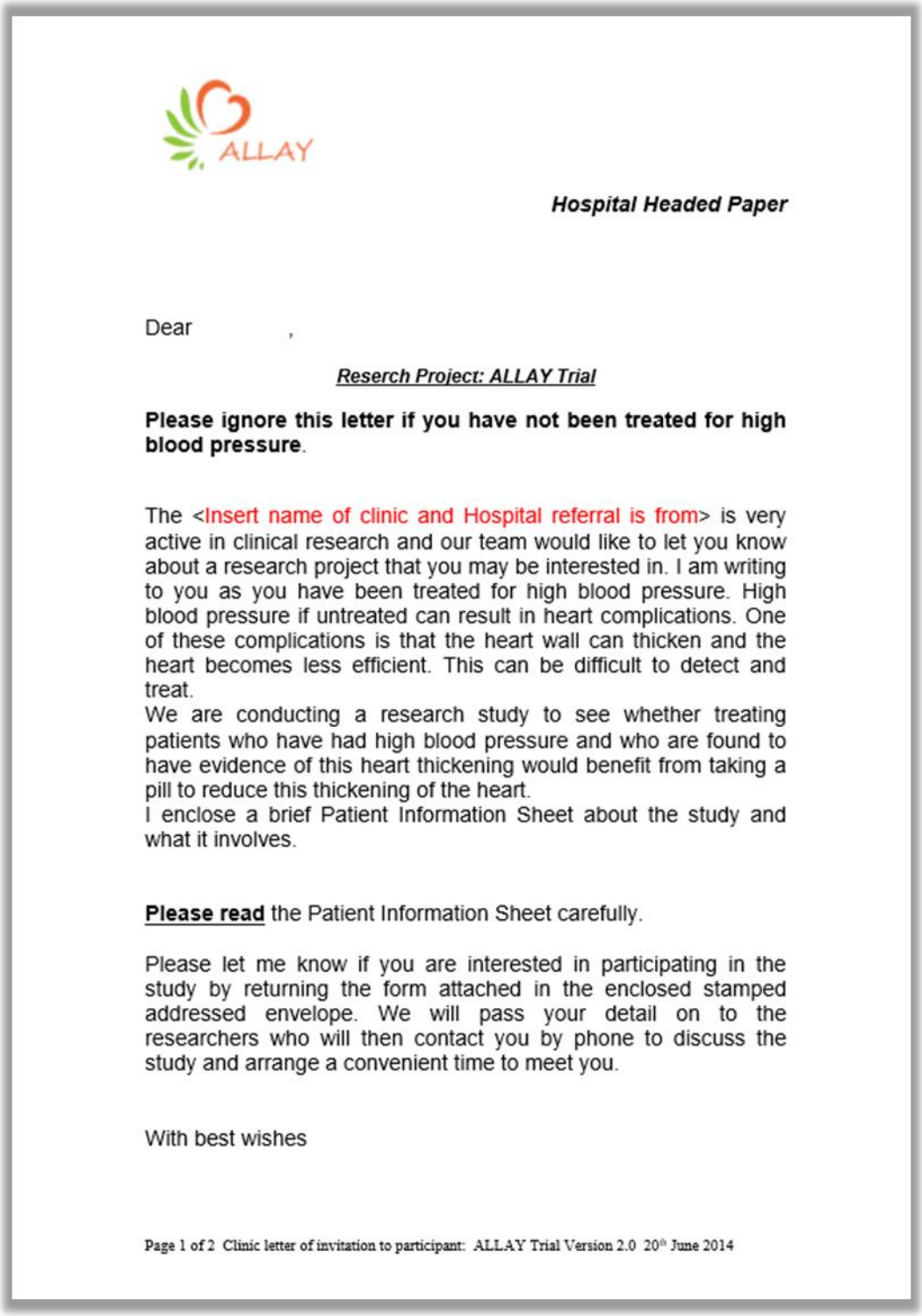
218. Devereux, R.B., et al., *Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings.* Am J Cardiol, 1986. **57**(6): p. 450-8.
219. Missouris, C.G., et al., *Echocardiography overestimates left ventricular mass: a comparative study with magnetic resonance imaging in patients with hypertension.* J Hypertens, 1996. **14**(8): p. 1005-10.
220. Simpson, H.J., et al., *Left ventricular hypertrophy: reduction of blood pressure already in the normal range further regresses left ventricular mass.* Heart, 2010. **96**(2): p. 148-52.
221. Gopal, A.S., et al., *Three-dimensional echocardiography: in vitro and in vivo validation of left ventricular mass and comparison with conventional echocardiographic methods.* J Am Coll Cardiol, 1994. **24**(2): p. 504-13.
222. Oe, H., et al., *Comparison of accurate measurement of left ventricular mass in patients with hypertrophied hearts by real-time three-dimensional echocardiography versus magnetic resonance imaging.* Am J Cardiol, 2005. **95**(10): p. 1263-7.
223. Foppa, M., B.B. Duncan, and L.E. Rohde, *Echocardiography-based left ventricular mass estimation. How should we define hypertrophy?*, in *Cardiovasc Ultrasound*. 2005. p. 17.
224. Farber, N.J., et al., *Ex vivo cardiovascular magnetic resonance measurements of right and left ventricular mass compared with direct mass measurement in excised hearts after transplantation: a first human SSFP comparison.* J Cardiovasc Magn Reson, 2014. **16**: p. 74.
225. Myerson, S.G., N.G. Bellenger, and D.J. Pennell, *Assessment of left ventricular mass by cardiovascular magnetic resonance.* Hypertension, 2002. **39**(3): p. 750-5.
226. Armstrong, A.C., et al., *LVM Assessed by Echocardiography and Cardiac Magnetic Resonance, Cardiovascular Outcomes, and Medical Practice.* JACC Cardiovasc Imaging, 2012. **5**(8): p. 837-48.
227. Moon, J.C., et al., *Breath-hold FLASH and FISP cardiovascular MR imaging: left ventricular volume differences and reproducibility.* Radiology, 2002. **223**(3): p. 789-97.
228. Plein, S., et al., *Steady-state free precession magnetic resonance imaging of the heart: comparison with segmented k-space gradient-echo imaging.* J Magn Reson Imaging, 2001. **14**(3): p. 230-6.
229. Kawel-Boehm, N., et al., *Normal values for cardiovascular magnetic resonance in adults and children.* J Cardiovasc Magn Reson, 2015. **17**: p. 29.
230. Klingbeil, A.U., et al., *A meta-analysis of the effects of treatment on left ventricular mass in essential hypertension.* American Journal of Medicine, 2003. **115**(1): p. 41-46.
231. Klingbeil, A.U., et al., *A meta-analysis of the effects of treatment on left ventricular mass in essential hypertension.* The American Journal of Medicine, 2003. **115**(1): p. 41-46.
232. Fagard, R.H., et al., *Regression of left ventricular mass by antihypertensive treatment: a meta-analysis of randomized comparative studies.* Hypertension, 2009. **54**(5): p. 1084-91.
233. Yasuno, S., et al., *Clinical significance of left ventricular hypertrophy and changes in left ventricular mass in high-risk hypertensive patients: a subanalysis of the Candesartan Antihypertensive Survival Evaluation in Japan trial.* J Hypertens, 2009. **27**(8): p. 1705-12.
234. Dahlof, B., et al., *Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol.* Lancet, 2002. **359**(9311): p. 995-1003.
235. Hoieggen, A., et al., *The impact of serum uric acid on cardiovascular outcomes in the LIFE study.* Kidney Int, 2004. **65**(3): p. 1041-9.
236. Devereux, R.B., et al., *Prognostic significance of left ventricular mass change during treatment of hypertension.* JAMA, 2004. **292**(19): p. 2350-6.

237. Devereux, R.B., et al., *Progressive hypertrophy regression with sustained pressure reduction in hypertension: the Losartan Intervention For Endpoint Reduction study.* J Hypertens, 2002. **20**(7): p. 1445-50.
238. Mathew, J., et al., *Reduction of cardiovascular risk by regression of electrocardiographic markers of left ventricular hypertrophy by the angiotensin-converting enzyme inhibitor ramipril.* Circulation, 2001. **104**(14): p. 1615-21.
239. Soliman, E.Z., et al., *Effect of Intensive Blood Pressure Lowering on Left Ventricular Hypertrophy in Patients With Diabetes Mellitus: Action to Control Cardiovascular Risk in Diabetes Blood Pressure Trial.* Hypertension, 2015. **66**(6): p. 1123-9.
240. *Effects of Intensive Blood-Pressure Control in Type 2 Diabetes Mellitus.* N Engl J Med, 2010. **362**(17): p. 1575-85.
241. Wright, J.T., Jr., et al., *A Randomized Trial of Intensive versus Standard Blood-Pressure Control.* N Engl J Med, 2015. **373**(22): p. 2103-16.
242. MacMahon, S.W., D.E. Wilcken, and G.J. Macdonald, *The effect of weight reduction on left ventricular mass. A randomized controlled trial in young, overweight hypertensive patients.* N Engl J Med, 1986. **314**(6): p. 334-9.
243. Cuspidi, C., et al., *Effects of bariatric surgery on cardiac structure and function: a systematic review and meta-analysis.* Am J Hypertens, 2014. **27**(2): p. 146-56.
244. Jula, A.M. and H.M. Karanko, *Effects on left ventricular hypertrophy of long-term nonpharmacological treatment with sodium restriction in mild-to-moderate essential hypertension.* Circulation, 1994. **89**(3): p. 1023-31.
245. Liebson, P.R., et al., *Comparison of five antihypertensive monotherapies and placebo for change in left ventricular mass in patients receiving nutritional-hygienic therapy in the Treatment of Mild Hypertension Study (TOMHS).* Circulation, 1995. **91**(3): p. 698-706.
246. Kelly, R., et al., *Noninvasive determination of age-related changes in the human arterial pulse.* Circulation, 1989. **80**(6): p. 1652-9.
247. *Augmentation-index.PNG (706Ã—392)*. 2019; Available from: https://upload.wikimedia.org/wikipedia/commons/6/6d/Augmentation-index.PNG.
248. *ESC/ESH Arterial Hypertension (Management of) Guidelines.* 2019.
249. Corretti, M.C., et al., *Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery - A report of the International Brachial Artery Reactivity Task Force.* Journal of the American College of Cardiology, 2002. **39**(2): p. 257-265.
250. Weissgerber, T.L., *Flow-mediated dilation: can new approaches provide greater mechanistic insight into vascular dysfunction in preeclampsia and other diseases?* Curr Hypertens Rep, 2014. **16**(11): p. 487.
251. Moselhy, H.F., et al., *A specific, accurate, and sensitive measure of total plasma malondialdehyde by HPLC[S]*, in *J Lipid Res*. 2013. p. 852-8.
252. Walter, M.F., et al., *Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the PREVENT study.* J Am Coll Cardiol, 2004. **44**(10): p. 1996-2002.
253. Tanaka, S., et al., *Serum levels of thiobarbituric acid-reactive substances are associated with risk of coronary heart disease.* J Atheroscler Thromb, 2011. **18**(7): p. 584-91.
254. Frijhoff, J., et al., *Clinical Relevance of Biomarkers of Oxidative Stress.* Antioxid Redox Signal, 2015. **23**(14): p. 1144-70.
255. Ooi, B.K., et al., *The Role of Natural Products in Targeting Cardiovascular Diseases via Nrf2 Pathway: Novel Molecular Mechanisms and Therapeutic Approaches.* Front Pharmacol, 2018. **9**.
256. Lippi, G., et al., *The paradoxical relationship between serum uric acid and cardiovascular disease.* Clin Chim Acta, 2008. **392**(1-2): p. 1-7.

257. Bagnati, M., et al., *When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid.* Biochem J, 1999. **340 (Pt 1)**: p. 143-52.
258. Liguori, I., et al., *Oxidative stress, aging, and diseases*, in *Clin Interv Aging*. 2018. p. 757-72.
259. Oparil, S., et al., *Hypertension.* Nat Rev Dis Primers, 2018. **4**: p. 18014.
260. Hare, J.M., et al., *Impact of oxypurinol in patients with symptomatic heart failure. Results of the OPT-CHF study.* J Am Coll Cardiol, 2008. **51**(24): p. 2301-9.
261. Vogel-Claussen, J., et al., *Left ventricular papillary muscle mass: relationship to left ventricular mass and volumes by magnetic resonance imaging.* J Comput Assist Tomogr, 2006. **30**(3): p. 426-32.
262. Clay, S., et al., *Normal range of human left ventricular volumes and mass using steady state free precession MRI in the radial long axis orientation.* Magma, 2006. **19**(1): p. 41-5.
263. Mackenzie, I.S., et al., *Multicentre, prospective, randomised, open-label, blinded end point trial of the efficacy of allopurinol therapy in improving cardiovascular outcomes in patients with ischaemic heart disease: protocol of the ALL-HEART study.* BMJ Open, 2016. **6**(9): p. e013774.

9 APPENDICIES

9.1 Participant Invitation letter (clinic)



ALLAY

Hospital Headed Paper

Dear ,

Reserch Project: ALLAY Trial

Please ignore this letter if you have not been treated for high blood pressure.

The <Insert name of clinic and Hospital referral is from> is very active in clinical research and our team would like to let you know about a research project that you may be interested in. I am writing to you as you have been treated for high blood pressure. High blood pressure if untreated can result in heart complications. One of these complications is that the heart wall can thicken and the heart becomes less efficient. This can be difficult to detect and treat.
We are conducting a research study to see whether treating patients who have had high blood pressure and who are found to have evidence of this heart thickening would benefit from taking a pill to reduce this thickening of the heart.
I enclose a brief Patient Information Sheet about the study and what it involves.

Please read the Patient Information Sheet carefully.

Please let me know if you are interested in participating in the study by returning the form attached in the enclosed stamped addressed envelope. We will pass your detail on to the researchers who will then contact you by phone to discuss the study and arrange a convenient time to meet you.

With best wishes

Page 1 of 2 Clinic letter of invitation to participant: ALLAY Trial Version 2.0 20th June 2014

Local Clinical Lead or PI Signature

Research Project: ALLAY Trial

Please only complete and return if you wish to participate in the study

Name……

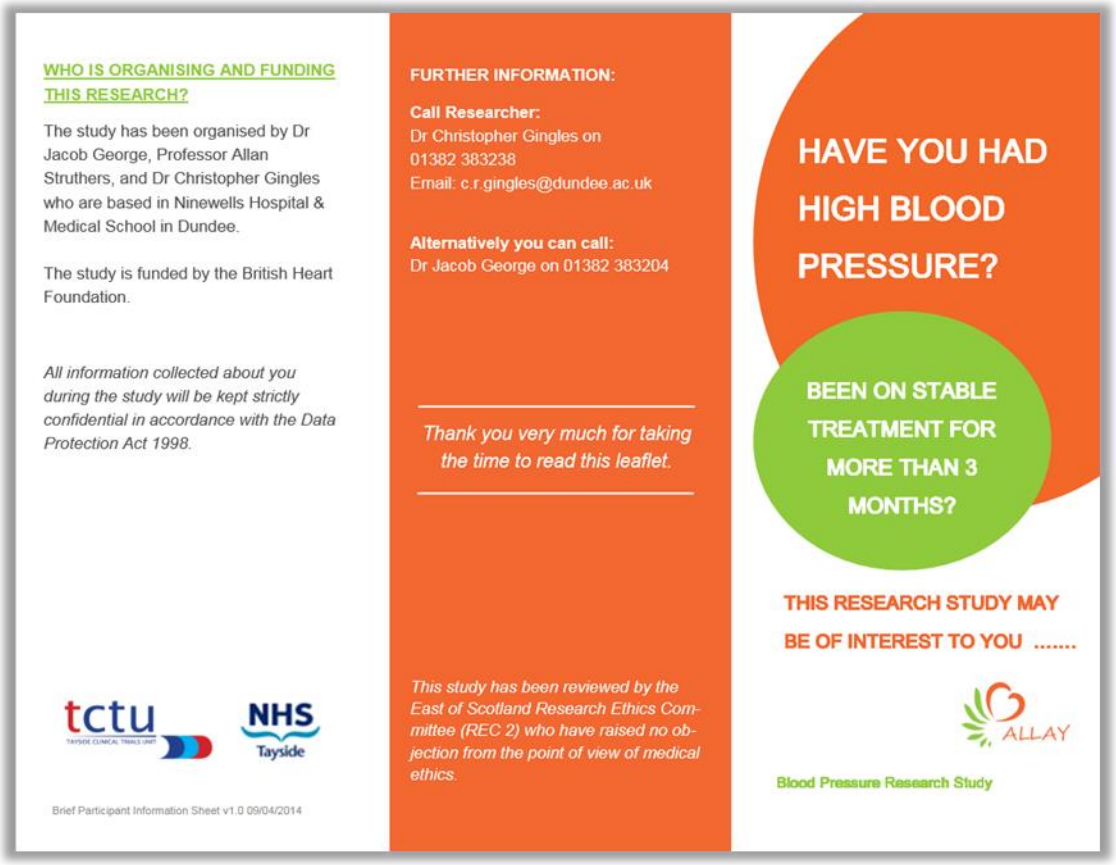
Address……

…… Postcode ……

Telephone number……

Best time to contact……

9.2 Brief PIS



WHO IS ORGANISING AND FUNDING THIS RESEARCH?

The study has been organised by Dr Jacob George, Professor Allan Struthers, and Dr Christopher Gingles who are based in Ninewells Hospital & Medical School in Dundee.

The study is funded by the British Heart Foundation.

All information collected about you during the study will be kept strictly confidential in accordance with the Data Protection Act 1998.

tctu TAYSIDE CLINICAL TRIALS UNIT

NHS Tayside

Brief Participant Information Sheet v1.0 09/04/2014

FURTHER INFORMATION:

Call Researcher:
Dr Christopher Gingles on
01382 383238
Email: c.r.gingles@dundee.ac.uk

Alternatively you can call:
Dr Jacob George on 01382 383204

Thank you very much for taking the time to read this leaflet.

This study has been reviewed by the East of Scotland Research Ethics Committee (REC 2) who have raised no objection from the point of view of medical ethics.

HAVE YOU HAD HIGH BLOOD PRESSURE?

BEEN ON STABLE TREATMENT FOR MORE THAN 3 MONTHS?

THIS RESEARCH STUDY MAY BE OF INTEREST TO YOU .....

ALLAY

Blood Pressure Research Study

WHY ARE WE DOING THIS STUDY?

High blood pressure can cause thickening of the heart wall. This thickening of the heart wall can cause an increased risk of heart attack or stroke. It can be reversed by improving BP control but controlling BP does not always improve this condition. This study will examine patients to see if they have this heart wall thickening and if they do will invite them into a drug trial to test if one years treatment with allopurinol can improve it.

WHO CAN TAKE PART?

- Aged 18 or over
- Being treated for high blood pressure
- Been on BP medications for at least three months that have not changed

WHAT IS BEING TESTED?

Allopurinol

Allopurinol is a medicine usually given for treating gout. It has been used by doctors for over 50 years.

Other research suggests that allopurinol might be able to improve thickening of the heart wall.

WHAT WILL HAPPEN IF I TAKE PART?

We will do an ultrasound scan of your heart to check if the heart wall is thickened and will ask about your general health and the medicines you take to confirm the study is suitable for you.

If you are suitable to take part an MRI scan of your heart will be arranged to get a more accurate measurement of the extent of this heart wall thickening and after a year of treatment this will be repeated to see if it has improved.

You will be in the study therefore for around one year. It will involve seven to eight visits to the hospital lasting approximately 30 mins to 2 hours. A taxi can be arranged to take you to the hospital visits or we will reimburse your travel expenses.

You will be asked to take one capsule of the study medicine twice daily. This will contain either allopurinol or a placebo (dummy) medicine. This will be chosen at random.

You can take all your usual medicines during the study.

- We will take blood samples and check your BP also.

WHAT ARE THE RISKS AND BENEFITS OF TAKING PART?

Allopurinol can sometimes cause a rash. If this does occur your study medications will be stopped.

You will be closely monitored for side effects and have regular blood tests to ensure your kidneys and liver are functioning well.

The study may not immediately benefit you but the results may lead to further research aimed at improving care of patients with high BP in future.

Some people may not be able to take part due to specific medical conditions or medications. To find out more please call the researchers.

British Heart Foundation

UNIVERSITY OF DUNDEE

9.3 Detailed Participant Information Sheet

PARTICIPANT INFORMATION SHEET

Title of Study

Does Allopurinol regress Left Ventricular Hypertrophy in Patients with Treated Essential Hypertension? The ALLAY Trial.

Name of Researcher

Chief Investigator: Dr Jacob George

Principal Investigator: Dr Christopher Gingles

Details of Study

You are being invited to participate in a clinical trial being sponsored by the University of Dundee and NHS Tayside. This study will form part of a doctorate in medicine degree for Dr Gingles. Before you decide whether or not to take part it is important for you to understand why the research is being carried out and what it involves. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is unclear or if you would like more information. Take your time to decide whether or not you would like to take part.

Background

People who have had high blood pressure can develop heart disease, which is why it is essential that your blood pressure is controlled to a normal level. One of the biggest potential problems is that the muscle wall of the heart thickens if the blood pressure remains persistently high. The medical term for this is Left Ventricular Hypertrophy (LVH). LVH makes the heart less efficient and patients with LVH are at a 10 times greater risk of heart complications than those without it.

A goal of treating high blood pressure is to reduce the strain on the heart and to try to decrease this thickening of the heart wall. However, even when blood pressure is treated and is under control, LVH can persist, and as there are no symptoms to LVH it can go undetected. Currently the only way to reduce LVH would be to lower blood pressure (BP) even further. This can cause side-effects from low BP such as dizziness and nausea.

It has previously been shown that a drug allopurinol, which is usually used to treat gout had a beneficial side effect of being able to reduce this thickening of the heart wall in patients who had kidney disease or diabetes. The aim now is to see if patients with high blood pressure and LVH may also benefit from treatment with allopurinol. If LVH can be reduced using allopurinol, this might be a new way to reduce the risk of a cardiac event in these patients without needing to lower BP even further.

In this study the aim is to recruit patients who have treated and well controlled blood pressure but may still have LVH. They will be screened for LVH by doing an ultrasound scan of the heart and then that will be confirmed with a Magnetic Resonance Imaging (MRI) scan, which is a special scan of the heart using an MRI machine to measure the extent of thickening of the heart muscle before they start on treatment of allopurinol or placebo.

Do I have to take part?

Participation in this study is entirely voluntary and you are free to refuse to take part or withdraw from the study at any time (without having to give a reason) and without this in any way affecting your future medical care or your relationship with medical staff looking after you. Some insurance companies consider that participation in medical research such as this is a "material fact" which should be mentioned in any proposal for health-related insurance or which could influence their judgement in consideration of claims under existing policies. You should check that participation in this research does not affect any policy you might be thinking about taking out or any existing policy.

What is involved in the study?

This study takes between 12 to 13 months for you to complete with visits for the study scheduled to take place at your convenience. This study is a randomised, double blinded, placebo controlled single centre study to be conducted at Ninewells Hospital & Medical School, Dundee. You will be given a tablet which contains the medication we are testing (called allopurinol) or an inactive tablet (called a placebo). Before you start on any study medication the doctor will do a screening visit to check you are eligible for the study. A summary of the tests done is included in a diagram at the end of this information sheet.

Visit 1 Screening

The screening period may take between 1 and 4 weeks whilst the doctor assesses your suitability for the trial. Typically this may involve one to a max of three visits.

At the first screening visit the doctor will do an ultrasound scan of your heart to check if you have evidence of LVH. This is a painless procedure where the doctor will put some gel on your chest wall and use an ultrasound scanner to get a picture of your heart. If you **do not have LVH** you will be **ineligible to participate** in this study further.

Should you have LVH the doctor or research nurse will check your medical history, do a clinical examination, check your blood pressure and pulse readings and do some routine blood tests to assess your full blood count (to check you are not anaemic), kidney and liver function, random glucose, haemoglobin A1C (if diabetic), cholesterol and urate to be sure that it is safe for you to participate. The total volume of blood taken at this visit will be approx 17ml (3 teaspoons full).

If you have LVH on the ultrasound scan you will also have a 24 hour BP monitor test to assess your BP over a 24 hour period during the screening period when convenient for you or if you are unable to tolerate 24hr BP you will be given a home BP monitor and instructions on how to record this information by the doctor. If you blood pressure is controlled and all your blood tests tell the doctor it is safe for you to proceed you will be asked to return for an MRI Scan- a special scan of your heart (further details below).

Baseline MRI Scan

For the MRI Scan you will receive an appointment either by telephone call, letter or e-mail and be sent directions to attend the MRI department of the Clinical Research Centre, Ninewells Hospital, Dundee. We plan that the MRI scan should be conducted at the baseline visit (visit 2) but could occur up to two weeks before or after this. If you are a woman of child bearing potential you will be asked to provide a urine specimen which you can either bring with you (a specimen bottle will be provided) or you could provide a sample at the beginning of your MRI clinic visit. A pregnancy test will be performed to ensure your safety. A positive result will exclude you from having an MRI scan.

During the screening visit you will meet one of the research team who will check that you are eligible to have the scan and who will obtain your written consent. On the day of the MRI you will then be seen by the radiographer, the person taking your scan, and she/he will help you to complete a checklist about matters that might prevent you from having the scan. If at Visit 1 you are found to have a history of a penetrative eye injury or exposure to metal fragments in your eye(s), that required medical

attention, you will be advised that it is unsafe for you to continue further in this study as there is a risk that the magnetic field in the MRI scan could move the metal fragment which may cause harm to your eye. With your consent, we will write to your GP informing them of your MRI safety status, as this information may be of benefit for your future health care needs.

If at Visit 1 you are found to have NO history of a penetrative eye injury and have had NO exposure to metal fragments in your eye(s), or otherwise the radiology staff establish that it is SAFE to scan you, will may proceed to have your MRI whereupon you will then be asked to change into a gown for the scan. A cannula will be placed in your arm by one of the radiology staff to inject a contrast agent, gadolinium, into your veins. This contrast agent is required to enhance the images on the MRI scan so they can be viewed. Having the cannula placed in your arm may be a bit uncomfortable but no more so than having a blood test done. There is a very rare risk that the contrast dye can cause side effects in people with kidney disease or damaged kidneys. Your kidney function will be assessed in the screening visit to ensure it is safe for you to have this test. After being prepared for your scan, you will be asked to lie up on the scanning table and then will be moved into the centre of the scanner (the scanner is shaped like a big doughnut). During the scan, which takes around 45 minutes, you will be able to speak to the radiographer. The scan will take pictures of your heart and blood vessels. As the scan is noisy you will be wearing hearing protection. After you have completed the scan you are free to go home. You can drive if you need to or if you prefer a taxi can be arranged to take you to and from the hospital. A specialist will examine your scan at a later date for any signs of disease and will measure your left ventricular mass.

This MRI visit should take no longer than one and a half hours.

Visit 2 Baseline

This visit will take place up to four week after the screening visit. It will include the following investigations:

Vital signs- checks of your BP, pulse.

Research bloods will be taken and stored for analysis after the trial has ended along a blood test to check your urate level will also be taken but results will not be made available until after the trial. Total blood taken will be 37ml at each visit where all bloods are taken (approx. 7 teaspoons) and 17 ml when safety bloods only are taken. Following completion of the trial we may test for additional markers of interest on any left-over blood which will be stored anonymously in the secure Dundee University laboratory in the division of Cardiovascular and Diabetes Medicine.

Genetic analysis blood test. You will be asked to provide one 10ml blood sample for storage. The sample will be fully anonymised and will be subject to approval of a Research Ethics Committee prior to future DNA analysis. The results of any future genetic tests would not be linked to your records and you would not receive any information about the results. You can opt not to have this done without affecting your participation in the study. This sample will be stored in the secure laboratory within the Division of Cardiovascular and Diabetes Medicine at Ninewells Hospital.

ECG You will also have an ECG (electrocardiogram) done to measure the electrical activity in your heart.

These extra tests are optional

Please note that you may decline the following Pulse Wave Analysis (PWA) and Flow Mediated Dilatation (FMD) measurements without it affecting your participation in the rest of the trial or stop them if they are too uncomfortable for you to tolerate.

Pulse Wave Analysis -We will measure the elasticity of the blood vessels in your neck, wrist and thigh by measuring your blood pressure, and then using a small pencil-like device or ultrasound probe placed over the blood vessel to measure your pulse. At the same time, the electrical activity of your heart will be monitored using an ECG machine.

Flow-mediated dilatation- We will look at a blood vessel in your arm (the brachial artery) using an ultrasound scanner. The function of this artery will be assessed by measuring its diameter before and after stopping blood flow through it with an inflated blood pressure cuff for 5 minutes. Occluding the artery can cause some tingling in the fingers (like when you have fallen asleep on your arm), but that should be the only discomfort. The diameter of the artery will again be measured before and after a spray of Glyceryl Trinitrate (GTN) is given under the tongue. GTN is a medicine that is normally used in the treatment of angina and can cause a mild headache. It is being used here to help dilate the brachial artery. Throughout these measurements, the electrical activity of your heart will be monitored using an ECG machine.

At the end of this first drug dosing visit you will be randomly assigned to either low dose allopurinol (300mg) or placebo so that the tablets allocated to you are decided in a random way (a bit like tossing a coin) such that neither you nor the research staff will know which tablet you are taking at any time until after the study is completed. This ensures that the study results cannot be influenced by knowing

whether you are receiving the medication or not. You will be given enough study drug to take once daily for one month.

Visits 3

Visit 3 will occur approx. 1 month after visit 2. At this visit you will have blood tested for safety measures, have your vital signs measured and the doctor will assess if you have had any problems on the study medications. At the end of the visit you will receive a higher dose of allopurinol or placebo (300mg twice daily) for the duration of the study and will get two months supply to take home with you.

Visits 4, 5 & 6

Visits 4, 5 and 6 will occur at approx. three months, six months and nine month respectively from the baseline visit. At these visits safety blood tests will be taken and you will be checked by the doctor to ensure there are no adverse events whilst on the study medication. Study medication will be dispensed to you at visits 4, 5 & 6. If you have tolerated a lower dose of medication and then develop problems tolerating a higher dose the doctor will discuss with you returning to the lower dose with your agreement.

Final Visit 7

This is the final study visit. At this visit your, the final urate, safety and research blood tests will be done, you will have an ECG, MRI scan (within 2 weeks of the visit) and have your 24 hour BP recorded again (or do home BP monitoring again) which will ultimately be compared with the baseline measurements taken before you started on study medication. With your permission we will also take an extra 20ml sample of blood for research purposes. This sample will be stored in the secure laboratory within the Division of Cardiovascular and Diabetes Medicine at Ninewells Hospital. At this visit you can have the FMD and PWA tests repeated, if you agreed to them at baseline.

For all visits noted above the doctor will assess you for any side effects of the medication and will check your vital signs and do blood tests to assess if the allopurinol has caused any problems.

Medication being tested

The medication used in this study is called allopurinol. It was been around for about 50 years now although mainly for the treatment of gout. It has a good safety record and is generally well tolerated. However, like most medicines, allopurinol occasionally causes side effects. The most common side effect is nausea and some abdominal discomfort which affects less than one in ten of patients on allopurinol. This can be minimised by taking the tablets with food.

Allopurinol causes a skin rash in one in a hundred or less of patients. This may be associated with fever, swollen glands, joint pains, unusual blistering or bleeding. Were any of these symptoms to develop, you should stop taking the tablet immediately and contact the study doctor as soon as possible. You may also seek advice from your GP.

Reports of other side effects of allopurinol are very rare (less than 1 in 10,000 people) and it is not always clear if they are truly related to the treatment. The complete range of reported side effects is set out in a Patient Information Leaflet, a copy of which will be given to you at your screening visit for your information, but include headache, stomach upset, drowsiness, anaemia. This will be further discussed with you before you make a final decision about taking part in this study.

Contraceptive Advice

Anyone who is pregnant cannot take part in this study. If you are a woman of childbearing age we will need to do a pregnancy test before the study. It is also important that you do not become pregnant during the study. We will do a urine pregnancy test at all clinic visits if you are a women of childbearing potential and not practicing one of the types of contraception or abstinence noted below.

Here is some advice on contraception. To avoid getting pregnant, not having sex at all is obviously effective. If you follow this strictly, no contraception is needed. If not, these are effective types of contraception:

- Combined Oral Contraceptive Pill
- EVRA-osetrogen and progestogen: 'Transdermal Patch'
- Progestogen only pill: 'mini pill'
- Depoprovera injection (medroxyprogesterone acetate)
- Implanon Implant (Etonogestrel)
- Mirena Coil (Intra-Uterine System)
- IUD-copper containing intrauterine device
- Female sterilisation

Male vasectomy is also a good form of contraception but only if the procedure has been checked afterwards by your doctor to make sure it has worked.

No contraception method is 100% reliable by itself. Even surgical sterilisation in men and women has been known to fail very occasionally. We advise using additional contraception from the start of the study.

You may normally use 'barrier methods' such as the condom, diaphragm or cap. There is no definite proof that using a spermicide with a 'barrier method' gives extra protection but some condoms are manufactured with spermicide on them. If you require further advice on contraception, please ask.

What are the discomforts, risks and side effects?

The side effects of the allopurinol are discussed under the 'medication' section above.

Having blood tests taken can cause some mild bruising. The flow mediated dilatation may cause temporary numbness in the arm as noted above.

MRI scanning: This type of scan is very safe and does not use radiation. Some people, when being scanned, may feel a bit closed in but you will be in constant contact with the person performing the scan and you can come out at any time. The scanner is a bit noisy but you will be given ear protection which also plays music. Your kidney function will be assessed before the scan to ensure it is safe to give you the contrast agent described above.

What are the benefits of taking part in the study?

You will be monitored closely during the study and will be seen by a doctor with a special interest in cardiology at each of your study visits. Besides having tests that have already been mentioned, your medication will be reviewed on a regular basis. The tests will give us information about the function of your heart, kidneys and blood circulation. If any of these investigations, including information from the MRI scan of your heart reveal any new abnormality we will either discuss this with your hospital consultant or refer you to a specialist clinic (whichever seems most appropriate). The study will not immediately benefit you, but if the results of the study are positive it may change the practice of managing patients with treated high blood pressure but may still have LVH, like you and potentially will have a great impact on other such patients in the future. If so, you may gain eventually from our discovering a new treatment for your condition.

Complaints, Insurance and indemnity

Right to raise concerns

If you have any concerns about your participation in the study you have the right to raise your concern with a researcher involved in conducting the study or a doctor involved in your care.

Right to make a complaint

If you have a complaint about your participation in the study, you should first talk to a researcher involved in the study. However you have the right to raise a formal complaint. You can make a complaint to a senior member of the research team or to the Complaints Officer for NHS Tayside.

Complaints and Claims Manager
Complaints and Advice Team
Level 7, Ninewells Hospital
Dundee DD1 9SY
Freephone: 0800 027 5507
Email: nhstaysidecomplaints@thb.scot.nhs.uk

Right to make a claim

In the event that you think you have suffered harm as a result of your participation in the study there are no automatic financial compensation arrangements. However, you may have the right to make a claim for compensation against the University of Dundee or NHS Tayside. Where you wish to make a claim, you should consider seeking independent legal advice but you may have to pay for your legal costs.

Insurance

The University of Dundee maintains a policy of professional negligence clinical trials insurance which provides both legal liability cover and no fault compensation in respect of accidental injury. Tayside Health Board is a member of the Clinical Negligence and Other Risks Insurance Scheme which provides legal liability cover.

Genetic testing

You should be aware that if you apply for health insurance you may be asked questions about your health, including medical history, pre-existing medical conditions and if you have had any genetic test. If you have a diagnosed medical condition, even where the condition is diagnosed as part of a clinical research study, the insurer may take this in to consideration when deciding whether to offer insurance to you.

Participation in this study DOES NOT constitute a "genetic test" as defined by insurance companies. Your data will remain confidential unless we are legally required to disclose information by Court order or by statute.

Will the research influence the treatment I receive?

The research will not immediately alter the regular treatments you currently receive.

Will my taking part in the study be kept confidential?

Your personal data will be kept confidential. With your permission, identifiable information about you and data collected during the study will be held securely by the University of Dundee and under the control of the Chief Investigator. All data collected in this study will be coded and stored on a computer system protected by a password only available to the researchers. No one outside the research team will have access to any identifiable information and all identifiable information and data will be kept securely. Your data will be archived securely for at least five years after the end of study as this is a legal requirement for drug studies. With your permission, we will inform your GP of your participation in this study. It is a requirement of the regulatory authority for clinical trials that your records in this study, together with any other relevant medical records, be made available for scrutiny by appropriate staff from NHS Tayside, University of Dundee (or their appointed third party) and the regulatory authority themselves.

Additionally there will be two sets of information obtained after you have had your MRI scan. One set will be the MRI scan images and the other, the research data obtained from those images. The MRI images obtained will be stored indefinitely using your name and unique hospital record number within the NHS clinical system and can be made available to specialist doctors for your future health care needs. Your research data will be stored using a unique study code which is non-identifiable and held on password protected University of Dundee secure databases. Only individuals directly involved with the study will have access to this information.

Will I continue to receive the medication used in this study after it finishes?

No. The study is designed to give an indication of possible benefit from the medicine being tested and it may be some time before we can be sure about how useful it actually is.

Expenses

Taxi transport, or reasonable costs to cover your travel costs, will be provided for any extra visits to the hospital for the purposes of this study.

Who has reviewed this study?

The East of Scotland Research Ethics Committee (REC 2), which has the authority to scrutinise proposals for medical research on humans, has examined this study and has raised no objections from the point of view of medical research.

It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from The University of Dundee, NHS Tayside and by the Regulatory Authorities, whose role it is to check that research is properly conducted and the interests of those taking part are adequately protected.

Contact details for the study Doctor.

If you are worried at any time about the research or wish to discuss things generally further, please do not hesitate to contact:

Dr Christopher Gingles
Clinical Research Fellow
University of Dundee
Division of Cardiovascular and Diabetes Medicine
Medical Research Institute
Mail Box 2, Ninewells Hospital and Medical School
Dundee DD1 9SY
Tel: (01382) 383238
Email: c.r.gingles@dundee.ac.uk

Contact Numbers if unwell during the trial

If during the study you become unwell or are concerned, you can contact the study team during normal working hours on (01382) 383013 If you are unwell and need urgent advice or assistance do not delay in seeking further advice or treatment as usual through the NHS services such as NHS24 (Tel: 111) or by contacting your GP who will have received details of your participation in this study should you agree to them being informed.

Thank you for reading this information sheet and considering taking part in this study. If you would like more information or want to ask questions about the study please contact the study team on the number above.

What will happen to me during the study?

The following diagram is the programme of visits involved in this study.

CLINIC VISIT	Visit 1 Screening	Visit 2 Baseline/ Randomisation	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7 Final Visit
	-1 to -6 weeks	Day 0	1 month (+/- 1 week)	Month 3 (+/- 1 week)	Month 6 (+/- 2 weeks)	Month 9 (+/- 2 weeks)	Month 12 (+/- 2 weeks)
Daily drug dose if increasing		300mg/ placebo	600mg/placebo				
Informed Consent	X						
Doctor to check suitability for trial	X						
Medical & Social History, Family History	X						
Doctor to do Clinical Examination	X						
Check BP, Pulse + Weight, Height *	X*	X	X	X	X	X	X*
Echocardiography to check for LVH	X						
ECG		X					X
MRI#		X					X
Safety Blood Tests & Urate	X		X	X	X	X	X
Research Bloods & urate*		X *					X
Genetic blood sample (if consented)		X					
24hr BP monitoring or Home BP monitoring	X						X
Urine Pregnancy Test (if applicable)	X	X	X	X	X	X	X
Dispense Study medication		X	X	X	X	X	
FMD* PWV/PWA*		X					X
Doctor to check any problems whilst on medications		X	X	X	X	X	X
Record or Review other Medications	X	X	X	X	X	X	X

#:- Screening MRI and 24 hour BP to be done only if echo LVH criteria fulfilled. Note MRI can be done (+/- 2 weeks of scheduled study visit date) * if participant agrees to FMD, PWV/PWA

9.4 Participant consent

Study Number: (EudraCT) 2014 -002083-33

Participant Identification Number for this trial: .....

PARTICIPANT CONSENT FORM

Title of Study: **Does Allopurinol regress Left Ventricular Hypertrophy in Patients with treated Essential Hypertension (The ALLAY Trial)**

Name of Researcher:

Chief Investigator: Dr Jacob George
Principal Investigator: Dr Christopher Gingles

Please initial box

1. I confirm that I have read and understand the information sheet dated_____(version___) for the above study. I have had the opportunity to consider the information, to ask questions, and have had them answered satisfactorily. ☐

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

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by the research team or from the regulatory authorities, NHS Tayside, or the University of Dundee (or their appointed third party), where it is relevant to my taking part in this study. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in this study.
YES/NO (*please circle*) ☐

5. I understand and agree that the information that I provide will be gifted by myself, and analysed by members of the study team or stored (anonymised) for up to 15 years and can be used for future, as yet unspecified, medical research into health, illness and medical treatment. ☐

6. I agree to have a pregnancy test pre and post study medication (if applicable) ☐

7. I agree to use an adequate form of contraception as discussed in the information sheet ☐

8. ***OPTIONAL***
I agree to undertake the Pulse Wave Analysis and Flow Mediated Dilatation tests described in the information sheet ☐
YES/NO (*please circle*)

9. ***OPTIONAL***
I agree to donate an additional 10ml of blood for genetic research purposes and understand that this may be stored indefinitely for future research use. ☐
YES/NO (*please circle*)

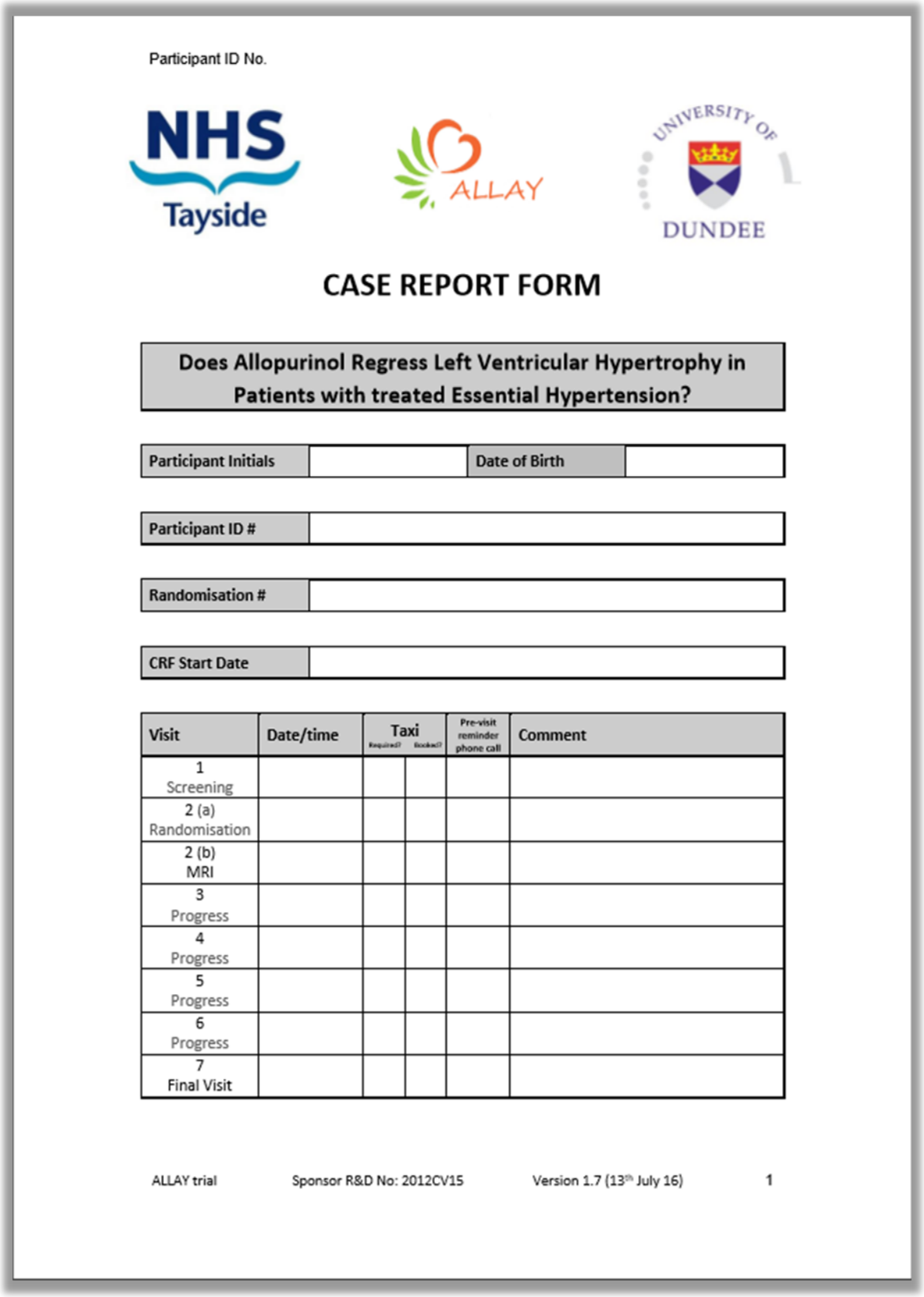
10. ***OPTIONAL***
I agree to donate an additional separate 20ml blood sample at the start and end of the study for research purposes and understand that these may be stored indefinitely for future research use. ☐
YES/NO (*please circle*)

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

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

Original to be kept with TMF, 1 copy for participant; 1 copy for hospital notes

9.5 Case Report Form



Participant ID No.

NHS Tayside

ALLAY

UNIVERSITY OF DUNDEE

CASE REPORT FORM

Does Allopurinol Regress Left Ventricular Hypertrophy in Patients with treated Essential Hypertension?

Participant Initials		**Date of Birth**	

Participant ID #	

Randomisation #	

CRF Start Date	

Visit	Date/time	Taxi Required?	Taxi Booked?	Pre-visit reminder phone call	Comment
1 Screening					
2 (a) Randomisation					
2 (b) MRI					
3 Progress					
4 Progress					
5 Progress					
6 Progress					
7 Final Visit					

Participant ID No.

Visit 1

	Yes	No
Has a consent form been completed & filed in the TMF?	☐	☐

Date of informed consent ___/___/___

Inclusion Criteria

Aged between > 18	☐	☐
Previously diagnosed with essential hypertension	☐	☐
Stable antihypertensive therapy for 3months before study screening	☐	☐
Screening ABPM (home based monitoring if not tolerated) daytime average ≤ 135mmHg or 24-hour average systolic ≤ 130mmHg	☐	☐
Screening echo LVH (males >115g/m^2, females > 95g/m^2)	☐	☐

Exclusion Criteria

Documented intolerance to allopurinol	☐	☐
LV ejection fraction <45%	☐	☐
Severe aortic stenosis	☐	☐
Active gout (i.e. flare up <2yrs) or currently on allopurinol	☐	☐
Severe hepatic disease	☐	☐
Renal disease; CKD 3B or worse	☐	☐
On azathioprine, 6-metacaptopurine or theophylline	☐	☐
Malignancy (receiving active treatment) or other life threatening diseases	☐	☐
Pregnant or lactating woman	☐	☐
Contraindication to MRI	☐	☐
In another clinical trial of an investigational medical product within last 30 days	☐	☐
Unable to give informed consent	☐	☐
Any other serious illness or significant abnormalities that may compromise their safety or successful participation in the study	☐	☐
Is the subject eligible to participate in this study?	☐	☐

Reason if ineligible? _____

Signed		Name		Date	

Participant ID No.

Visit 1

Past Medical History:

1. **Angina/MI** ☐
2. **HTN** ☐ **Duration (yrs)**_____
3. **Dyslipidaemia** ☐
4. **Stroke/TIA** ☐
5. **Diabetes** ☐
6. **PAD** ☐
7. **Other(s) relevant** ☐

Medications (Record in medications Log check when completed) ☐

Social History:

Smoking status: 1. Current smoker ☐ 2. Never ☐ 3. Ex-smoker ☐ 4. E-Cigarette ☐

cigarettes/day (A) _____ # years smoked (B) _____pack years _____ (AxB/20)

Average weekly alcohol intake: _____units

SIMD 2012 Quintile_____
SIMD 2012 Decile_____

Q risk 2 (2016) Score_____%

Family History:

1st degree relative with MI/CVA, F <65yrs, male <55yrs: 1. Yes ☐ 2. No ☐

Examination:

1. Normal ☐ 2. Abnormal (not significant) ☐ 3. Abnormal significant ☐

Comments_____
_____

Age _____yrs Height _____ m BMI _____ kg/m^2
Male/Female Weight _____ kg BSA _____m^2

Blood Pressure Log (updated) ☐

	N/A	Yes	No
Patient female of child bearing potential?	☐	☐	☐

Participant ID No.

Visit 1

If yes what is the result of a pregnancy test (+/-)? _____

Venepuncture (safety/baseline bloods) ☐

Screening Echo Completed ☐

LVIDd: cm PWTd: cm SWTd: cm
LV Mass g LV Mass/BSA g/m^2

LV Systolic function: 1. Normal/Mild (EF ≥ 45%) ☐ 2. Mod/Sev (EF ≤44%) ☐

Severe Aortic stenosis 1. Yes ☐ 2. No ☐

Recruitment Source Code_____

Recruitment Source_____

* Recruitment Source Code
1 = GP
2 = Area 6 Clinic
3 = XX Clinic (specify)
4 = Website
5 = Advert
6 = Patient registry
7 = Other (specify)

Ambulatory BP/home monitoring + instructions ☐

Give patient copy of consent form ☐

CMRI safety checklist/form completed and sent (+copy of consent) ☐

Screening Log completed ☐

Inform GP of screening failure (if applicable) N/A ☐ ☐

Visit 2 booked and recorded on front of CRF ☐

Signed		**Name**		**Date**	

Data recorded on ALLAY excel. ☐

- Date_____ Signature_____ Print Name_____

Participant ID No.

Visit 2

Pre Visit 2 phone-call **Check as completed**

- Confirm patient appointment time/date/transport ☐
- Remind them of FMD requirements: ☐
  - not eating for four hours before attending
  - no tea/coffee/caffeine for four hours before attending
  - no cigarettes for four hours before attending
- Visit one baseline/safety bloods reviewed and recorded in bloods Log ☐
- 24 BP/home monitoring result recorded in log ☐
- Complete exclusion/inclusion criteria ☐

Visit 2

Adverse Event Log (updated) ☐

Medication Log (updated) ☐

Blood Pressure Log (updated) ☐

ECG (report/file in case notes) ☐

FMD

- Completed N/A ☐ ☐
- Reported + recorded in FMD log ☐

PWV/PWA

- Completed N/A ☐ ☐
- Reported + recorded in PWV/PWA log ☐

CMR

- Completed ☐
- Reported 1 ☐
- Reported 2 ☐
- Reported 3 N/A ☐ ☐

Venepuncture Research ☐ Genetic ☐

Urine pregnancy testing Result (+/-) ____ N/A ☐ ☐

Participant ID No.

Visit 2

Randomisation + Log Completed ☐

Sex	Male	☐	Female	☐
LVMI	116g/m^2 – 129g/m^2	☐	96g/m^2 – 114g/m^2	☐
	≥ 130g/m^2	☐	≥115g/m^2	☐

Randomisation Number _____

Medications dispensed ☐

Letter sent to GP to inform of enrolment in trial ☐

Copy of GP letter/Consent/PIS/trial sticker/trial divider placed in case notes ☐

Visit 3 booked (record on front of CRF) ☐

Signed		**Name**		**Date**	

Data recorded on ALLAY excel. ☐

- Date_____ Signature_____ Print Name_____

Participant ID No.

Visit 3

Pre Visit 3 phone-call **Check as completed**

- Confirm patient appointment time/date/transport ☐

Visit 3

Adverse Event Log (updated) ☐

Medication Log (updated) ☐

Blood Pressure Log (updated) ☐

Medication compliance Log (updated) ☐

Safety bloods and uric acid ☐

Urine pregnancy testing Result (+/-) _____ N/A ☐ ☐

Study medication supplied with instructions (up-titration of medications if tolerated) ☐

Visit 4 booked (record on front of CRF) ☐

Signed		Name		Date	

Data recorded on ALLAY excel. ☐

- Date_____ Signature_____ Print Name_____

Participant ID No.

Visit 4

Pre Visit 4 phone-call **Check as completed**

- Confirm patient appointment time/date/transport ☐
- Visit 3 safety bloods reviewed and recorded in bloods Log ☐

Visit 4

Adverse Event Log (updated) ☐

Medication Log (updated) ☐

Blood Pressure Log (updated) ☐

Medication compliance Log (updated) ☐

Safety bloods and uric acid ☐

Urine pregnancy testing Result (+/-) _____ **N/A** ☐ ☐

Study medication supplied with instructions ☐

Visit 5 booked (record on front of CRF) ☐

Signed		**Name**		**Date**	

Data recorded on ALLAY excel. ☐

- Date_____ Signature_____ Print Name_____

Participant ID No.

Visit 5

Pre Visit 5 phone-call	**Check as completed**
• Confirm patient appointment time/date/transport	☐
• Visit 4 safety bloods reviewed and recorded in bloods Log	☐

Visit 5

Adverse Event Log (updated)			☐
Medication Log (updated)			☐
Blood Pressure Log (updated)			☐
Medication compliance Log (updated)			☐
Safety bloods and uric acid			☐
Urine pregnancy testing	Result (+/-) _____	**N/A** ☐	☐
Study medication supplied with instructions			☐
Visit 6 booked (record on front of CRF)			☐

Signed		**Name**		**Date**	

Data recorded on ALLAY excel. ☐

- Date_____ Signature_____ Print Name_____

Participant ID No.

Visit 6

Pre Visit 6 phone-call **Check as completed**

- Confirm patient appointment time/date/transport ☐
- Visit 5 safety bloods reviewed and recorded in bloods Log ☐

Visit 6

Adverse Event Log (updated) ☐

Medication Log (updated) ☐

Blood Pressure Log (updated) ☐

Medication compliance Log (updated) ☐

Safety bloods and uric acid ☐

Urine pregnancy testing Result (+/-) _____ **N/A** ☐ ☐

Study medication supplied with instructions ☐

Visit 7 booked (record on front of CRF) ☐

Signed		**Name**		**Date**	

Data recorded on ALLAY excel. ☐

- Date_____ Signature_____ Print Name_____

Participant ID No.

Visit 7

Pre Visit 7 phone-call **Check as completed**

- Confirm patient appointment time/date/transport ☐
- Remind them of FMD requirements: ☐
  - not eating for four hours before attending
  - no tea/coffee for four hours before attending
  - no cigarettes for four hours before attending
- Visit 6 safety bloods reviewed and recorded in bloods Log ☐

Visit 7

Weight Visit 7_____kg

Adverse Event Log (updated) ☐

Medication Log (updated) ☐

Blood Pressure Log (updated) ☐

Medication compliance Log (updated) ☐

Safety/baseline/research bloods & uric acid taken ☐

Urine pregnancy testing Result (+/-) _____ N/A ☐ ☐

ECG (report + file in case notes) ☐

FMD

- Completed N/A ☐ ☐
- Reported + recorded in FMD log ☐

PWV/PWA

- Completed N/A ☐ ☐
- Reported + recorded in PWV/PWA log ☐

CMR

- Completed ☐
- Reported 1 ☐
- Reported 2 ☐
- Reported 3 N/A ☐ ☐

Ambulatory BP/home monitoring + instructions ☐

Completion of Study form ☐

Signed		**Name**		**Date**	

Data recorded on ALLAY excel. ☐

Participant ID No.

- Date_____ Signature_____ Print Name_____

Medications Log

Code	*Medication*	*Dose*	*Frequency*	*Start date (if known)*	*End date (if discontinued)*

Medication Coding:

1. ACEI	5. Thiazide diuretic	9. Statin	13. Oral hypoglycaemic
2. β Blocker	6. Loop diuretic	10. Aspirin	14. Other
3. Ca channel Blocker	7. Aldosterone Blocker	11. Clopidogrel	15. Centrally acting anti-HTN
4. α Blocker	8. ARB	12. Insulin	16. Renin inhibitor

Participant ID No.

Baseline/Safety Blood

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Haemoglobin (g/L)							
WBC x10^9/L							
Neutrophils x10^9/L							
Platelets x10^9/L							
Sodium (mmol/L)							
Potassium (mmol/L)							
Urea (mmol/L)							
Creatinine (mmol/L)							
eGFR (mL/min)							
ALT (U/L)							
Bilirubins (µmol/L)							
Alk Phos (U/L)							
Albumin (g/L)							
Glucose mmol/L							
Total Chol mmol/L							
HbA1c mmol/ml (diabetics)							
Initial/Date							

Participant ID No.

PWA/PWV & FMD Results Log

Check if patient declined FMD ☐ or PWA/PWV ☐

Visit 2

Pulse wave	Radial
Pulse (bpm)	
Tr (ms)	
Aug (mmHg)	
AIx (%)	
AIx @ 75bpm (%)	
SEVR (%)	
Aortic BP (mmHg)	
PWV (m/s)	

Visit 7

Pulse wave	Radial
Pulse (bpm)	
Tr (ms)	
Aug (mmHg)	
AIx (%)	
AIx @ 75bpm (%)	
SEVR (%)	
Aortic BP (mmHg)	
PWV (m/s)	

Participant ID No.

Visit 2

Pulse wave	Integral 1	Max Veloc 1	Integral 2	Max Veloc 2	Integral 3	Max Veloc 3	Integral Average (m)	Max Veloc Average (m/s)
Pre-cuff								
Post-cuff								
% change	[redacted]							

Diameter	FMD Cuff	FMD GTN
Pre (mm) ave		
Post (mm) max		
% change		

Visit 7

Pulse wave	Integral 1	Max Veloc 1	Integral 2	Max Veloc 2	Integral 3	Max Veloc 3	Integral Average (m)	Max Veloc Average (m/s)
Pre-cuff								
Post-cuff								
% change	[redacted]							

Diameter	FMD Cuff	FMD GTN
Pre (mm) ave		
Post (mm) max		
% change		

Participant ID No.

Bloods Pressure Log

	Visit 1	Visit 2	Visit3	Visit 4	Visit 5	Visit 6	Visit 7
Arm (L/R)							
Office Systolic Pressure (mmHg)							
Office Diastolic Pressure (mmHg)							
24 hour systolic (average)		[redacted]					
24 hour diastolic (average)							
Daytime Systolic (average)							
Daytime Diastolic (average)							
Pulse (bpm)							

Note – The same arm should be used throughout trial. Take office BP >5mins after subject arriving twice sitting at 45 degrees at rest.

Participant ID No.

Medication Compliance Log

	A	B	C	D	E
Visit	Tablets Dispensed No.	Tablets Expected to take No.	Tablets Expected to return No.	Tablets Actual Returned No.	Compliance %
3					
4					
5					
6					
7					

Tablets expected to return = Tablets dispensed – Tablets expected to take

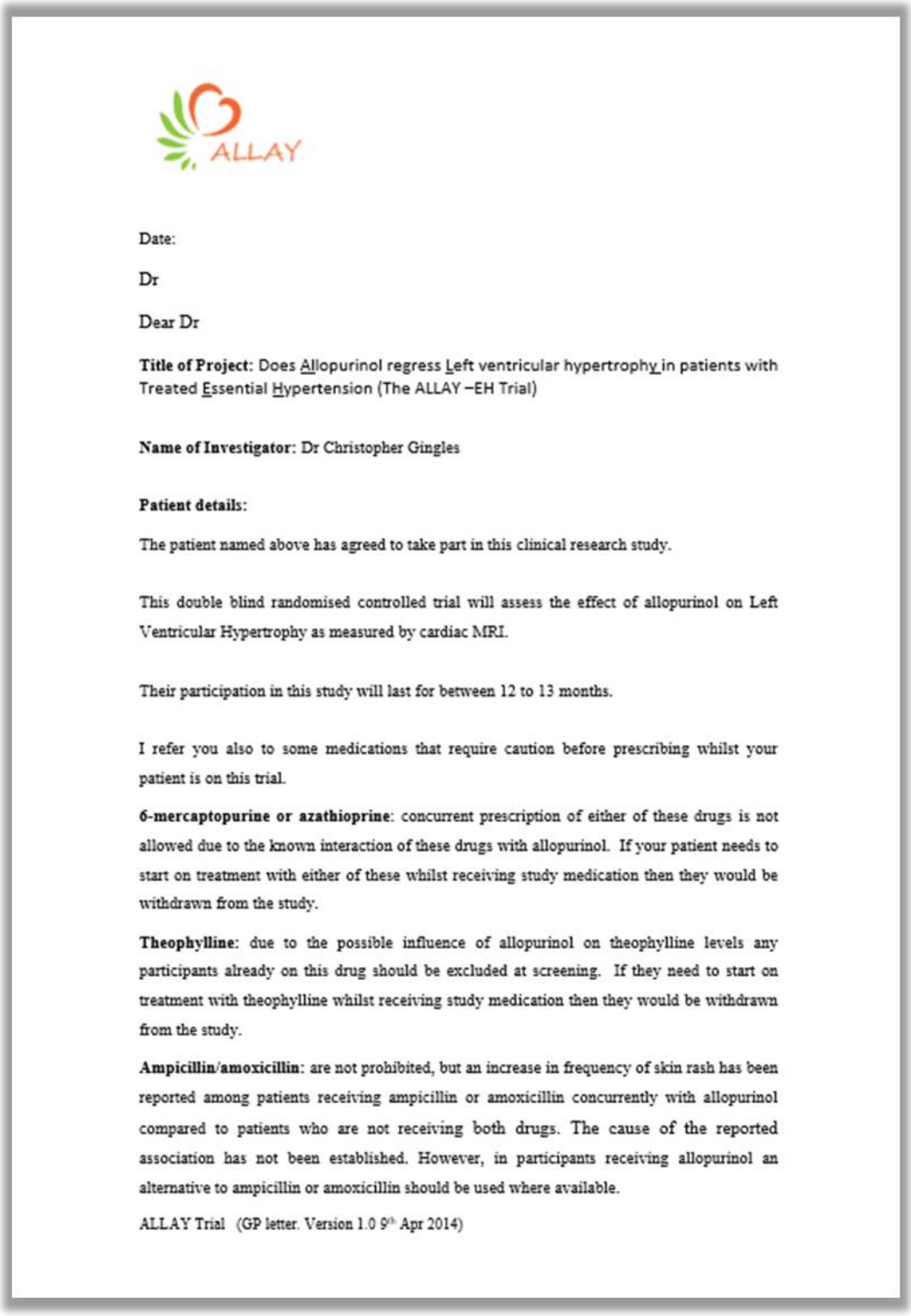
C = A – B

Compliance = Tablets expected to take – (Tablets actually returned – Tablets expected returned)/Tablets expected to take x100

E = B – (D-C)/B x100

Overall IMP compliance _____%

9.6 GP Letter



Date:

Dr

Dear Dr

Title of Project: Does Allopurinol regress Left ventricular hypertrophy in patients with Treated Essential Hypertension (The ALLAY –EH Trial)

Name of Investigator: Dr Christopher Gingles

Patient details:

The patient named above has agreed to take part in this clinical research study.

This double blind randomised controlled trial will assess the effect of allopurinol on Left Ventricular Hypertrophy as measured by cardiac MRI.

Their participation in this study will last for between 12 to 13 months.

I refer you also to some medications that require caution before prescribing whilst your patient is on this trial.

6-mercaptopurine or azathioprine: concurrent prescription of either of these drugs is not allowed due to the known interaction of these drugs with allopurinol. If your patient needs to start on treatment with either of these whilst receiving study medication then they would be withdrawn from the study.

Theophylline: due to the possible influence of allopurinol on theophylline levels any participants already on this drug should be excluded at screening. If they need to start on treatment with theophylline whilst receiving study medication then they would be withdrawn from the study.

Ampicillin/amoxicillin: are not prohibited, but an increase in frequency of skin rash has been reported among patients receiving ampicillin or amoxicillin concurrently with allopurinol compared to patients who are not receiving both drugs. The cause of the reported association has not been established. However, in participants receiving allopurinol an alternative to ampicillin or amoxicillin should be used where available.

ALLAY Trial (GP letter. Version 1.0 9th Apr 2014)

I have enclosed a patient information sheet which gives full study details, however if there are any questions you may have regarding the study, I would be happy for you to contact me.

Yours faithfully

Dr Christopher Gingles
BHF Clinical Research Fellow
01382 383238